Indigenous rapid diagnostic technology for antibiotic susceptibility testing in urinary tract infection: from bench side to bedside

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ABSTRACT
Infectious diseases are a significant burden on global economies and public health. There are indeed more deaths due to infections than cancer demonstrating that it is high time to pay attention to the escalating bacterial enemy. Urinary tract infections (UTIs) pose a serious health problem affecting millions of people each year with total cost for treatment being in billions of dollars. A large share of that expense and inappropriate use of antimicrobials comes from a 48 hours wait for the infected sample to be cultured in the laboratory and tested for antibiotic sensitivity (AST). The current study presents an ultra-rapid point-of-care system for determining AST of pathogens found in human urine leading to UTI. Sensitivity and specificity of the new test for antibiotic susceptibility was found to be 0.94 and 0.97, respectively. The results of AST using the new test are available in 4 hours as compared with 48–72 hours of conventional methods. Availability of this rapid assay will obliterate the need for empirical treatment in case of UTI-like infectious diseases and lead to specific and most appropriate treatment at the earliest ensuring healthcare to all. Further as the result of cultures are available in ~4 hours this test facilitates de-escalation necessary for the containment of bacterial resistance, favours targeted patient therapy with reduced antimicrobial spectrum of action of prescribed antibiotics and, consequently, lowers the cost of treatment significantly.

BACKGROUND
Infectious diseases are a significant burden on global economies and a major public health threat. The fight against bacterial infection represents one of the highest challenges of modern medicine. Urinary tract infection (UTI), the second most common infectious disease, is almost exclusively caused by bacteria. UTI occurs more commonly in women than men (four time higher frequency in women), with 50% of women having infection at least once at some point in their lives. Nearly 10% women develop UTIs every year. Recurrences are common, with nearly half of people getting a second infection within a year. Antibiotics are the mainstay of treatment of diseases caused by bacterial infection. In uncomplicated cases, UTIs are easily treatable with a short course of antibiotics. However, due to widespread and inappropriate use of antibiotics, bacteria have developed resistance against these antimicrobial agents. Regrettably, the use of these wonder drugs has been accompanied by the rapid appearance of resistant strains. The most prevalent Gram-negative pathogens, such as Escherichia coli and Klebsiella pneumoniae, the main causing agents of UTI in humans have developed antimicrobial resistance (AMR) over the past half-century. These new, resistant and stronger bacteria pose a significant threat to general health and welfare and a challenge to researchers. Not only is AMR responsible for longer lasting infections and higher risk of death, it also reduces the effectiveness of the available antibiotics. Moreover, resistant infections require more advanced and costlier therapies, which result in increased healthcare costs and financial burden on patients, their families and society as a whole. Thus, there is an urgent need for having...
an improved rapid point-of-care diagnostic method that substantially reduces the time and cost in conducting the test. This urgent need if addressed would greatly help in selecting, or even confirming, a proper treatment regime for UTIs in lesser time and help managing the menace of escalating AMR in bacteria. Overall, the aim of the present invention is to promote evidence-based prescription of antibiotics and minimise the irrational/empirical use of antibiotics thus inhibiting the rate at which bacteria are becoming resistant to antimicrobial agents and promoting healthcare.

METHODS
A prospective study on 426 urine samples was conducted. All pathogens isolated from urine specimen of suspected UTI patients (both men and women; age 14–72 years) who attended the outpatient departments in Birla Sarvajank Hospital, Pilani (Rajasthan) and local diagnostic laboratories in Punjab and Mediciti Institute of Medical Sciences, Hyderabad (Telangana) were included in the study. Patients were informed by the doctor about the test prior to collection of samples and the test for culture and sensitivity was conducted (based on prescription and doctor’s advice). All patients who had significant bacteriuria (>10^5 cfu/mL) were included for further microbiological analysis. Only one specimen per patient was included.

Conventional urine analysis
The widely used method to determine the susceptibility of microorganisms to antimicrobial agents is the disc agar-diffusion method known as Kirby-Bauer disc diffusion method. The principle of this method is dependent on the inhibition of microorganism growth on the surface of a solid medium by an antimicrobial agent which diffuses into the medium from a filter paper disc. The isolates were tested for antimicrobial susceptibility using standard Luria Broth (LB) agar plates after inoculation with microorganisms and placement of antibiotic discs. After 24-hour incubation at 37°C, the zone of inhibition around the disc impregnated with the antimicrobial agent were measured. Results were interpreted based on the diameter of the observed zone of inhibition. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines; the obtained results were categorised into three groups namely sensitive (S); intermediate (I); resistant (R) and interpreted accordingly.

New rapid method
This novel test replicates the basic tenets of clinical microbiology, namely (1) growth of bacteria in a specialised medium and (2) measuring the inhibition of growth of bacteria in the presence of an antibiotic. Detection is based on chromogenic endpoints. The output is analysed using laboratory-developed algorithm-based software which reports the sensitivity of the pathogen to the panel of antibiotics tested. The urine sample is collected in a sterile container. To harvest the bacteria, urine sample is filtered with the help of a filter attached to 10 mL syringe and the filtrate is discarded. After that, BITGEN, a specially designed medium for accelerated growth of uropathogens, is pushed through the filter in the vial, shaken well and then closed with the dropper cap. This is then left dormant for about 5 min. To identify the susceptibility of pathogens, two preloaded antibiotic strips are used. Each of the antibiotic strips has about 8 compartments and except the first compartment (or reference well) of each of the 2 antibiotic strips, all the remaining 14 compartments are subjected to preloading by 14 distinct antibiotics listed down in the table. It is to be noted that the concentration and composition of the below listed antibiotics is chosen as per the established CLSI guidelines. Four drops from the vial are loaded into each well of antibiotic strips and incubated at 37°C for 4 hours. In case the urine sample has pathogens it would be reflected in the antibiotic strips, at least in the reference well of both the antibiotic strips as it does not inhibit bacterial growth. The remaining 14 compartments may show varied levels of bacterial growth depending on the bacterial susceptibility to those antibiotics. The bacterial growth within the preloaded antibiotic compartment will be represented by a change in colour of the BITGEN, measurable at 450 nm. The intensity of the colour is a measure of the number of growing cells in the presence and absence of a particular antibiotic. The antibiotics used are amoxicillin, gentamicin, amikacin, cefepime, ofloxacin, ciprofloxacin, ceftiraxone, piperacillin, cefotaxime, kanamycin, cefuroxime, tobramycin, levofloxacin, ampicillin.

RESULTS
A total of 426 urine samples from patients clinically suspected with UTI were analysed. Of these, 243 (57.04%) samples were found to be culture positive showing significant bacteriuria and the remaining 183 (42.95%) samples showed negative or were sterile. Conventional method shows 243 samples as UTI-positive and 183 as UTI-negative samples, whereas, rapid culture method identified 234 samples as UTI-positive and 192 UTI-negative results. Figure 1 shows the distribution of positive and negative results obtained with both methods. By comparing with conventional method the false-positive and false-negative percentages are 4.91% and 3.70%. But the nine samples which were reported positive in conventional and negative in rapid method had <10^5 cells/mL of urine. The antibiotic profile obtained from the new method was compared with the standard Kirby-Bauer disc diffusion done in clinical hospital laboratories. The number of isolates tested for antimicrobials vary (table 1).
For the calculation of sensitivity and specificity of new rapid test, the Kirby-Bauer disc diffusion method was considered as the gold standard method. A comparison of both methods was performed by the calculation of the positive and negative predictive value and Cohen’s \( \kappa \) coefficient. Table 2 shows the distribution of positive and negative test results obtained with both methods. This showed a sensitivity of 0.93 and a specificity of 0.96 for the new rapid test. The overall Cohen’s \( \kappa \) coefficient for measuring agreement between the two methods was 0.9 and the positive and negative predictive values were 0.97 and 0.92, respectively. The \( \kappa \) value has a range from 0 to 1.00, with larger values indicating better reliability. Generally, a \( \kappa > 0.70 \) is considered satisfactory. The obtained \( \kappa \) in our study is >0.70 which concludes that the inter-rater reliability is satisfactory.

The positive predictive value indicates the probability that a patient with a positive test actually has disease. A test with higher specificity (fewer false positives) will have a higher positive predictive value in a given population. The negative predictive value indicates the probability that a patient without a positive test actually or with a negative test. A test with higher sensitivity (fewer false negatives) will have a higher negative predictive value in a given population. The \( \kappa \) statistic is used to assess inter-rater reliability. The \( \kappa \) value is considered to be an improvement over using per cent agreement to evaluate this type of reliability.

### DISCUSSION

The use of microbiological culture method, that is, Kirby-Bauer disc diffusion is well established in the diagnosis of infectious diseases. However, the major drawback is the time consumption of this method. However, initial antibiotic therapy in UTIs is mostly empirical. Therefore, an alternative method for antibiotic sensitivity and subsequent decrease of time will be beneficial to patient care as well as curtailing unnecessary antibiotic prescriptions. Vitek (BioMerieux) and MicroScan Walkaway (Diamond Diagnostics) are two of the most commonly used automated antimicrobial susceptibility test systems. A study evaluating the susceptibility of Gram-negative bacilli to 11 antibacterials using MicroScan Rapid Neg MIC/Combo panels (Diamond Diagnostics) and auto SCAN-W/A (Baxter MicroScan, West Sacramento, California, USA) showed that the results were available between 3.5 and 7 hours in 92.7% of the isolates and overall agreement with the standard test was 94% with a 3.4% major error rate.\(^8\) Comparison of Vitek and Cobas Micro Systems (Roche Diagnostics, Basel, Switzerland) with a semiautomated conventional microsystem MIC2000 (Dynatech, McLean, Virginia, USA), for the susceptibility testing of Gram-negative bacilli revealed 86% overall agreement with 3% major discrepancies for Vitek and 90% overall agreement with 2% major discrepancies for Cobas Micro
The most widely used conventional assay for AST is a Reporting of intermediate sensitivity assumes significant The present newly developed test showed a sensitivity of 0.93 and a specificity of 0.96 for the new rapid test. The overall Cohen’s $\kappa$ coefficient for measuring agreement between the two methods was 0.9 and the positive and negative predictive values were 0.97 and 0.92, respectively. The $\kappa$ value has a range from 0 to 1.00, with larger values indicating better reliability and provides following clinical and financial benefits over all other available tests:

- While conventional method takes 48–72 hours (manual) and 18–24 hours (automated), this test provides results in ~4 hours.
- The most widely used conventional assay for AST is a manual test priced between 280 and 350 rupees per sample and the automated tests are priced at 800–1200 rupees per sample. The new rapid assay is expected to cost ~280–350 rupees per sample.
- It does not need any other equipment (autoclave, laminar air flow, dedicated space) or trained personnel as required for the conventional assays.
- As the kit contains a preset panel of antibiotics (14 in number) no user/laboratory can decide to save cost by running a lower number of antibiotics.
- Reporting of intermediate sensitivity assumes significant importance in chronic cases of UTI (due to bacteria resistant to more than one antibiotics), asymptomatic pregnant women who develop recurrent urinary infections and in old age patients who are already on more than one drug for other comorbid conditions.

Further in an era of spiralling healthcare costs and limited resources, policymakers and healthcare payers are concerned about the cost-effectiveness of antibiotics. A study by Simoens indicated that the cost-effectiveness of antibiotics is influenced by factors relating to the characteristics and the use of antibiotics such as diagnosis, comparative costs and comparative effectiveness, resistance, patient compliance with treatment, and treatment failure and by external factors such as funding source, clinical pharmacy interventions and guideline implementation interventions. Physicians need to take into account these factors when prescribing an antibiotic and assess whether a specific antibiotic treatment adds sufficient value to justify its costs. In America alone an extra $31 million a year was spent on healthcare costs for using antibiotics when they are not required!

If first-line treatment fails due to resistance, additional costs are incurred due to the need for second-line treatment or hospitalisation, or both. A study by Simoens and Decramer revealed that the cost of antimicrobials in an institution, especially in the intensive care unit, represents 30–50% of total drug costs and at least 50% of patients make use of antibiotics on hospitalisation. Hospitalisation costs accounted for more than 45% of healthcare costs and drug costs made up between 6% and 21% of costs. The authors compared comparative costs and the comparative effectiveness of first-generation antibiotics (aminopenicillins, macrolides and tetracyclines) and second-generation antibiotics (eg, fluoroquinolones). Fluoroquinolones generally had higher acquisition costs than first-generation antibiotics, but both macrolides and fluoroquinolones were equally effective. In total, 38.8% of antibiotics were more effective and less costly than the comparator; 45.9% of antibiotics improved effectiveness but also increased costs; 15.3% of antibiotics were less effective and more costly than the comparator. Antimicrobial discontinuation reduced the spectrum of action of the antibiotic prescribed, and the treatment costs from 2673.12 to 727.03 rupees, $p=0.001$.

In instances where prescriptions were written before microbiological confirmation of the infection causative agent and resistance profile an increasing use of broad-spectrum antibiotics action in the empirical treatment is one of the major factors leading to widespread resistance to antibiotics. De-escalation is highlighted as a measure, allowing the adequacy of drugs prescription, according to the causative agent of infection, promoting rational use of antibiotics and thereby minimising the selection of multiresistant bacteria. Studies have reported that this can reduce costs of antimicrobial treatment and it has been reported that de-escalation reduces cost statistically ($p=0.000$). De-escalation, after the result of cultures, necessary for the containment of bacterial resistance, favoured retargeting patient therapy, reducing antimicrobial spectrum of action prescribed and, consequently, antimicrobial costs significantly ($p=0.01$) for Methicillin Sensitive Staphylococcus Aureus (MSSA) and Methicillin Resistant Staphylococcus Aureus (MRSA) patient groups studied. In another study in outpatient episodes among 50,000 patients, antibiotics accounted for 23% of the total cost of care. Antibiotics cost, on average, was $9.91 for each episode of care in outpatient office visits totalling to a whopping $1.62 million in a year for upper respiratory infections of the Medicaid programme.

**CONCLUSION**

Affordability of healthcare is not only an issue in India, but pervades through the globe. In the USA, UTIs alone account for nearly seven million office visits, one million emergency department visits, and 100,000 hospitalisations every year. The cost of these infections is significant both in terms of lost time at work and costs of medical care mounting to a direct cost of treatment being US$1.6 billion/year. In India, 10,000,000 people suffer from UTI every year, which poses a heavy economic toll. Availability of economical, rapid and reliable diagnostic test would
make healthcare access to everyone. Since most UTIs are treated empirically the selection of antimicrobial agent should be determined by the most likely pathogen and by its confirmed susceptibility pattern. Therefore, periodic monitoring of aetiological agents of UTI, and their resistance pattern in the community is essential for prudent empirical antibiotic therapy to control the menace of increasing AMR so as to maintain efficacy of available antibiotics. The choice of antimicrobials to be used after microbiological confirmation should be performed evaluating five basic principles, such as: efficacy, safety, ease of administration, cost of antibiotic selected and, above all, the action spectrum, which should be as low possible, thus emphasising the importance of antimicrobial de-escalation as essential in containing bacterial resistance. The rapid assay described here does just that—determines the efficacy of an antibiotic tested, in the shortest possible time, and that too in every possible setting where healthcare services are provided, Primary healthcare centre, doctor’s office, hospital wards and even in the sanctity of the long-term sick confined patients’ home. The new rapid test will enable evidence-based prescription to manage the menace of growing antimicrobial resistance.

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