Rapid Sensor Based Technology: A Novel Tool for Direct Antimicrobial Susceptibility Testing in Urinary Tract Infection

Suman Kapur, Shivani Gupta, Padmavathi DV, Anuradha Pal, Jitendra Pant, Rashi Jain

Department of Biological Sciences, Birla Institute of Technology and Science (BITS Pilani), Hyderabad campus, Jawahar Nagar, Shameerpet Mandal, R.R District, Hyderabad- 500078, India

Corresponding author: Suman Kapur

Abstract

Urinary Tract Infection (UTI) is the second most common infectious disease in humans. Increasing antimicrobial resistance emphasizes the urgent need for quick and reliable diagnostic tests for evidence based antibiotic use/therapy. Increasing trend in resistance to common antibiotics used for treatment of UTI due to polymicrobial infection as opposed to monomicrobial infection makes it important to reconsider the standard diagnostic procedure. All the available automated and manual systems for direct susceptibility testing of microbial culture have the limitation of prolonged incubation time. The present study describes a newly developed device for rapid and direct antibiotic sensitivity testing of uropathogens in 3 hours as against the usual duration of 48-72 hours.

Keywords: Urinary tract infection, Polymicrobial, antibiotic resistance diagnostics.

Background

Infectious diseases are a significant burden on global economies and a major public health threat. The fight against bacterial infections represents one of the key challenges of modern medicine. UTI, the second most common infectious disease [1], is almost exclusively caused by bacteria. Most of the UTI cases are caused by *Escherichia coli* (*E.coli*) and *Enterococcus faecalis* (*E. faecalis*), while *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas sp*. accounts for the remaining cases [2]. Antibiotics are the mainstay of treatment of diseases caused by bacterial infection [3]. A short course of antibiotic usually cures uncomplicated infections caused by a single type of bacteria. But unfortunately, most bacteria have developed resistance to commonly available antibiotics, leading to ineffective treatment and disease severity [4]. A clinical microbiology laboratory usually doesn't report more than one microorganisms present in mixed culture from patient urine samples. As a

result many cases of UTIs go under/mistreated, particularly when polymicrobial infections are present. Moreover, it is very likely that the antibiotic resistance may be a misinterpretation of empirical prescription made on the of basis clinical judgement due to lack of availability of rapid diagnostic test and due to the concomitant presence of more than one type of bacteria contributing to the infection. There are several conditions when polymicrobial bacteriuria is not only frequently significant but its overall clinical impact seems to be substantial [5]. Chances of polymicrobial UTIs are further exacerbated by conditions like ileal conduit, ne urogenic bladder, or vesico-colic fistula, complications due to stones, chronic renal abscesses, or long term indwelling urinary catheters [6, 7]. Unfortunately, very few studies have evaluated the clinical significance of polymicrobial or mixed growth from urine. Furthermore, the frequency with which polymicrobial growth truly represents mixed infection is still unknown. It has recently been reported that more than 30% of samples depict polymicrobial infections [8]. Therefore, there is an urgent need to interpret the polymicrobial infections cautiously and reconsider the standard diagnostic procedure for UTIs caused by more than one bacteria type while prescribing any antibiotic for effective treatment.

Overall aim of the present study is to promote evidence-based prescription of antibiotics in case of both monomicrobial and polymicrobial UTI for successful and timely clinical outcome. The selection of antimicrobial agent should not only be determined on the basis of most likely pathogen but also by confirming its susceptibility pattern. Early diagnosis, periodic monitoring of etiological agents and their resistance pattern in co-existence is essential for effective antibiotic therapy in order to control the increasing global problem of antibiotic resistance.

Therapeutic importance of doing antibiotic susceptibility testing on mixed cultures

Polymicrobial UTIs impose a heightened threat to the health and well-being of the population. The empirical prescription of antibiotics, due to lack of a rapid diagnostic test, result in over prescription/misuse of antibiotics as compensatory behaviour to pacify patients' expectations of treatment. Many researchers believe that primary and direct susceptibility testing can play a significant role in diagnosis. For example, 47% of National Health Service laboratories in the United Kingdom surveyed has adopted this practice [9]. Direct methods for urine cultures have been evaluated by a number of studies [10-13].

The organisms present in polymicrobial UTIs possess increased resistance to common front-line antibiotics like ciprofloxacin and trimethoprim used for UTI treatment as compared to their monomicrobial counterparts [8]. Bacteria of different species may influence the pathogenicity of each other when they co-exist in a certain environment. Due to the complexities involved in the diagnosis and treatment in these infections many patients may receive inadequate antibiotic treatment or indeed a lack of treatment altogether. It has been reported that the majority of organisms isolated from polymicrobial cultures also exhibited increased human pathogenic potential as evidenced by *in vitro* cell infection assays [8]. The frequent co-isolation of *E. coli* and *Enterococcus faecalis* from the clinical UTI samples emphasizes on performing direct susceptibility for antibiotic sensitivity testing. Also, the routine practice of direct susceptibility testing on urine samples permits the availability of results on the following day, i.e., a day earlier than those of tests on pure subcultures, and often helps patterns of susceptibility to the antimicrobial agents tested. Moreover, the additional time, labour and costs of performing subculture are often avoided. Shortened time in microbiological diagnosis of UTI is important to enable patients to receive pathogen based antimicrobial therapy adequately at an early stage for appropriate treatment. In this study, the most common clinical isolates from infected urine samples were compared for their antibiotic susceptibility when present alone and in combination (Table 1). The preliminary data shows that mixtures of resistant and sensitive species appeared either as "resistant" or "sensitive" depending upon the organisms and the drug used. A number of “sensitive” species, as determined by the classical antibiotic sensitivity test emerged as “resistant” when tested in combination, confirming that the growth pattern and drug metabolising behaviour of microbes changes in the presence of another microbe.

Diagnostic Methods for detecting UTI: Historical and Current technologies

Traditional method of bacterial culture and sensitivity tests includes pathogen growth, purification and isolation, identification and drug susceptibility test. The whole method needs two to three days to finish the report. Clinical and financial benefits of early reporting of antibacterial susceptibility results have been shown in many studies from time to time. In recent years two new automated systems have become available across the world. These include the Vitek2 (BioMérieux) and the Phoenix systems (BD), based on broth susceptibility with specific cards [14]. MicroScan AST is based on conventional micro-broth dilution minimum inhibitory

Table 1: Comparison of antibiotic susceptibility of clinical isolates from infected urine samples when present alone and in combination [S: Sensitive, MS: Moderately Sensitive, R: Resistant]

Antibiotic	E. coli	Klebsiella pneumoniae	E.coli + Klebsiella pneumoniae
GEN	S	R	R
CIP	S	MS	S
PIT	S	R	R
CTX	S	MS	R
KAN	S	R	S
LEV	S	R	S
AMP	R	S	MS
Antibiotic	E. coli	Enterococcus faecalis	E.coli + Enterococcus faecalis
AMX	R	S	R
GEN	S	S	R
PIT	S	S	R
CTX	S	R	MS
AMP	R	R	S

[GEN: Gentamicin; CIP: Ciprofloxacin; PIT: Piperacillin/Tobazactam; CTX: Ceftriaxome; KAN: Kanamicin; LEV: Levofloxacin; AMP: Ampicillin; AMX: Amoxicillin, S-sensitive, R-resistant and MS-moderately sensitive]

concentration. Another test, Sensilatest Antibiotic susceptibility tests is based on the break point system according to the EUCAST standard. Although several studies have reported the reliability of these automated systems for identification and susceptibility testing directly from the culture systems but the protocols include long incubation times [15]. Briefly, plates are inoculated with the test cultures for 18-24 hours at 37ºC. Isolated colonies are used to prepare a suspension of the bacteria which is used for the further testing. Microscan/ Vitek 2 /Phoenix have similar overall performance [16] and on an average it still takes 18-24 hr leading to a 24 hours wait before the medication can be prescribed to a patient based on the real/evidence based diagnosis. But, clinicians need quick and reliable

results for initiating appropriate antibiotic therapy or taking other necessary preventative steps. Thus, there is a crucial need of faster and reliable technology which could guide antibiotic therapy more accurately, and reduce patients' exposure to ineffective or unnecessary antibiotic(s) while awaiting susceptibility test results from bacterial culture and sensitivity tests.

Novel technology for assessing antibiotic sensitivity of mixed cultures

The newly developed technology has been optimized for direct antibiotic sensitivity testing of uropathogens found in infected human urine. The device comes with a ready to use kit for rapid culture of pathogens present in the infected sample and tests a panel of antibiotics for their bactericidal/ bacteriostatic effect on the pathogens present in the sample. A small portable, battery operated instrument provides results in a ready to use format in 3 hours time from the start of the assay as against the usual wait of 48 to 72 hours for a sample to be cultured and tested in a lab using the conventional clinical microbiology assays. The components of the test are: (i) in house specially designed medium for accelerated growth of uropathogens (ii) specially fabricated readout-machine, which gives an alphanumeric display of results on a screen and (iii) pre-functionalized antibiotic panel in strip format allowing screening for multiple antibiotics. Our new technology replicates the basic tenets of clinical microbiology including growth of bacteria in a specialized medium and measurement of inhibition of growth of bacteria. The detection is based on enzymatic hydrolysis of specific cocktail of substance by the UTI causing bacteria. Detection is based on chromogenic endpoints. The intensity of the colored end product is a measure of number of growing cells in the presence or absence of a particular antibiotic and this is measured using sensitive optical sensors. The output is analyzed using an indigenous software, based on a lab-developed algorithm which reports the sensitivity of the pathogen to the chosen panel of antibiotics tested. This novel test and accompanying device offers six major advantages over conventional methods which are: (i) ease of operation and on spot analyses of results; (ii) rapid results at the bedside or in doctors chambers/lab or in the field in three hours; (iii) reliability comparable to conventional disc assay for antibiotic sensitivity of pathogens; (iv) affordable low cost per test; (v) and is the fastest antibiotic finder available till date.

Conclusions

It is estimated that 150 million UTI cases occur yearly on a global basis, resulting in more than 6 billion dollars in direct health care expenditures [17]. A large share of that expense and misuse of antimicrobials comes from a) 48 to 72 hours wait for the infected sample to be cultured in the lab and tested for antibiotic sensitivity, b) misinterpretation resulting from the presence of polybacterial infection. This in turn leads to empirical prescription of antibiotics (symptomor clinical judgment based) or inappropriate antibiotic prescription (based on antimicrobial susceptibility of pure cultures). Availability of this rapid point of care diagnostic test for urinary tract infections will have a significant gain on clinical management of the UTI cases particularly who are highly susceptible to polymicrobial infections and at the same time also obliterate the need for empirical antibiotic therapy, thus leading to specific, early and most appropriate treatment.

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Conflict of Interest: None

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