Performance of Direct Rapid Antimicrobial Susceptibility testing on urine

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Performance of Direct Rapid Antimicrobial Susceptibility testing on urine

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DEDICATIONS

I dedicate this dissertation to the Almighty God for the protection, guidance and giving me a healthy life.

I also dedicate it to my Mother and family members for their love, patience and encouragement.

Lastly, I dedicate this dissertation to friends and any other person who supported me in one way or another throughout the process.

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GLOSSARY

UTI	Urinary tract infection
DST	Direct susceptibility testing
AST	Antimicrobial susceptibility testing
dRAST	Direct rapid antimicrobial susceptibility testing
CFU	Colony forming unit
ESBL	Extended spectrum beta lactamase
ICU	Intensive care unit
ID	Identification
CLSI	Clinical and laboratory standard institute
EUCAST	European committee for antimicrobial susceptibility testing
FDA	Food and drug administration
ATCC	American type culture collection
MHA	Muller Hinton agar
MALDI-TOF	Mass spectrometry, matrix-assisted laser desorption/Ionization-Time of flight
MIC	Minimal inhibitory concentration

ABSTRACT

Urinary tract infections (UTIs) are a medical threat with the high morbidity of 150 million people worldwide a year. UTIs like other infections are treated with the broad-spectrum antibiotics which consequently leads to the increase of resistances and the conventional method for the diagnosis of urinary tract infections takes 48-72h for the identification of the bacteria and antimicrobial susceptibility testing (AST) results to be available to the physicians. Hence the rapid AST would increase the adequate choice of antibiotics for the better outcome of the patients. Therefore, our study aimed to demonstrate that AST can be performed directly on urine samples using the disk diffusion method within 8 hr' incubation.

A total of 103 urine samples containing $\geq 2.10^4$ bacterial/ml and monobacterial (*Enterobacteriaceae*) after gram staining was tested using disk diffusion method with 8 hr' incubation. And the results were compared to the standardized VITEK-2 AST. We compared the categorical agreements and the correlation between both methods.

Over the 1545 tested drug combinations in all isolates studied, the overall categorical agreement of the direct rapid disk diffusion and the standardized susceptibility testing using VITEK-2 was 92.9%, with 4.9% minor errors, 0.9% major error 1.2% very major errors. Our results showed an excellent categorical agreement and correlations between diameters for dRAST and VITEK-2 susceptibility. The direct rapid AST on urine containing monobacterial *Enterobacteriaceae* can predict the reliable result of AST within 8 hr. The rapid AST reports by using DST in UTIs can facilitate the timely and adequate antimicrobial treatment.

I. INTRODUCTION

Urinary tract infections (UTIs) are a medical threat with the high morbidity of 150 million people worldwide a year [1]. Among the bacteria and fungi causing UTIs *Enterobacteriaceae* is the most common [2]. Especially, *Escherichia coli* cause 80% of the uncomplicated UTI [3]. UTIs like other infections are treated with broad spectrum antibiotics which consequently leads to the increase of resistance. Hence the rapid AST would increase the adequate choice of antibiotics for the better outcome of the patients [4]. Disk diffusion (standard method) and commercial broth microdilution kits are widely used for antimicrobial susceptibility testing in many clinical settings [5]. The commercial kit requires much time and effort to perform, and it is expensive as well [6].

To ensure accurate and timely antimicrobial therapy we need rapid methods for identification since it takes 2 to 3 days for the conventional method from the time of sample collection till the time when the results are available to the physician [7]. Many other methods which are reliable for rapid AST have been developed such as Vitek Classic (bioMe´rieux, Marcy L'E`toile, France), the more automated VITEK 2 (bioMe´rieux) and Microscan Walkaway (Dade-Behring Microscan, Sacramento, CA, USA), and Phoenix system (BD Diagnostic Systems, Sparks, MD,USA) [8].

Despite the reliability and fastness of results from these methods, many laboratories from low-income settings cannot afford due to their high cost [9]. Currently EUCAST published a guideline for rapid AST in blood using disk diffusion methods. But, neither EUCAST nor CLSI has approved yet the guideline for rapid AST in the urine. Many papers on reducing the turnaround time of antimicrobial susceptibility testing have been published [10]. However, limited studies have been done on short incubation time of 8hr using disk diffusion directly on urine samples.

Our study aimed at evaluating the performance of direct rapid disk diffusion AST on urine samples within 8 hr' incubation with the standard antimicrobial susceptibility testing by VITEK2 using CLSI AST breakpoints.

II. LITERATURE REVIEW

Urinary tract infections are a public health concern in all age groups of men and women by which annually encounters 7 million office visits and more than 1 million emergency visits along with 100, 000 hospitalizations [11]. Compared to males, females are more exposed to urinary tract infections which is explained by 40 to 50% of women experience at least one UTI during their lifetime [1]. Bacterial identification and antimicrobial susceptibility testing results on routine analysis take normally 2 to 3 days, where most of the laboratories use disc diffusion test, E-test and broth dilution using microtitration plates for antimicrobial susceptibility testing [12].

In a time of increasing antimicrobial resistance, an inexpensive method that will shorten the turn around time of identification and antimicrobial susceptibility testing of pathogens responsible for urinary tract infections would play an important role in proper and timely antimicrobial therapy [3, 7]. The high usage of unnecessary antibiotics in urinary tract infections has led to the emergence of resistance which is not only a problem among nosocomial and complicated UTIs but also in community-acquired, simple UTIs [11]. In the USA during the year 2014 more than 266 million antimicrobial prescriptions provided, 30% of them were given unnecessarily [2]. Rapid sensitivity test directly on urine is reliable in monobacterial gram-negative infections and it is useful in the proper management of UTI and reduces the use of broad-spectrum antimicrobials [1].

A rapid diagnostic method provides the real image of patients prior to the treatment with significant number of pathogens in urine to avoid the needless antibiotic therapy in patients with non-microbial urinary tract symptoms and helps in selecting the proper antibiotic for instance though 60% of *E. coli* strain in community maybe ampicillin-resistant but patients found to have been infected by *E. coli* strain susceptible to ampicillin they will be treated by ampicillin [11].

Table 1. Clinical manifestation of urinary tract infections [13]

Type	Clinical Manifestations
Asymptomatic	Patients do not present any local or systemic symptom referring to
Symptomatic	1. Uncomplicated UTI a. Acute Cystis: dysuria, urinary frequency, and urgency. Nocturia, hesitancy, suprapubic discomfort. b. Acute Pyelonephritis: low-grade fever with or without lower-back. c. Prostatitis for men only. 2. Complicated UTI Symptomatic episodes of cystis and pyelonephritis in men and women.

Asymptomatic bacteriuria is commonly seen in elderly people and is characterized by the presence of a positive urine culture specimen, bacteria count of $\geq 10^5$ CFU/ml (with or without pyuria) but with no signs or symptoms of infection. It does not necessitate antimicrobial therapy unless for the exceptional conditions [13, 14].

2.1 incidence and antimicrobial resistance trends of urinary tract infections

Globally we estimate 150 million urinary tract infections annually [15]. Around 2007, there was an estimation of 10.5 million office visits for UTI symptoms in which 0.9% were outpatients and 2-3 million visits at the emergency in the USA [16]. Urinary tract infection is a common contagion among men and women but the incidence is quite high among women due to their physiology [17]. As the number of antimicrobials resistant

to outpatient therapies has risen, the number of hospitalizations for UTIs has increased also, Between 2000 and 2009, hospitalizations for UTIs increased dramatically [18].

The indwelling of catheters in hospitalized patients due to comorbidities such as old age, diabetes, spinal cord injury, and urologic abnormalities usually leads to urinary tract infections [19]. UTIs are categorized in clinical settings as either uncomplicated or complicated whereby Uncomplicated UTIs affect the so-called healthy individuals with no remarkable structural or neurological urinary tract abnormalities [16]. *E. coli* is considered as the pathogen responsible for the most incidence of UTIs with 80-85% of the cases followed by *Staphylococcus saprophyticus* which occupies 5-10% [17]. Most of the times UTIs are not life-threatening by which its management only requires simple antibiotic therapy and the infection is limited to the lower urinary tract. However, recurrences are common and may evolve into an upper UTI (pyelonephritis) requiring a heavier antibiotic treatment and more extensive management [20].

The management of acute complicated UTI by empirical antibiotics especially in women requires full comprehensive knowledge of current pathogens involving in most cases of infections and their drug susceptibility [21]. Patients with symptomatic UTI usually receive antibiotic therapy, which consequently can lead to multidrug-resistant strains due to the long-term disruption of the vagina and gastrointestinal normal flora [16]. The prescription of antibiotics in UTIs increases the risk of antibiotic resistance that persists for at least twelve months after therapy [22]. Different antibiotics such as nitrofurantoin monohydrate, trimethoprim-sulfamethoxazole, fosfomycin trometamol, pivmecillinam, fluoroquinolones, and beta-lactams are recommended by international guidelines for the management of uncomplicated UTIs and pyelonephritis [23]. The growing of CTX-M ESBLs in *E. coli*, as well as *Klebsiella* species in the community, have made the management of UTI became complicated. Differently to before 2003 when most of the mutations were in *Klebsiella* species (TEM-ESBL, SHV penicillinase-ESBL) [24].

The UTI caused by ESBL–producing *Enterobacteriaceae* is common in all health facilities mostly *E. coli*. Therefore, before selecting the antibiotic prior to the treatment of the known pathogen, the activity of the drug and duration of therapy must be taken into consideration as the antimicrobial resistance differs depending on the continent, country or institution [25]. The increase *of E. coli* in hospitalized patients and in fecal carriage among healthy people in France is leading to the high antimicrobial resistance prevalence in urinary tract infections [26]. The pathogens responsible for Urinary tract infections in Korea are proven to be higher resistant to trimethoprim-sulfamethoxazole (TMP-SMX) than those in the United States and Europe where this antibiotic is given as primary treatment for UTIs, it is in this regard that in Korea they recommend fluoroquinolones as a primary antibiotic for UTIs. However, the failure rate of fluoroquinolone treatments is high [27].

The study done in Switzerland on the analysis of *E. coli* in urinary samples collected from a university hospital between 1997 and 2007 showed the increasing trend in resistance to the following antibiotics trimethoprim-sulfamethoxazole, ciprofloxacin and amoxicillin-clavulanic acid (from 17.4% to 21.3%, 1.8% to 15.9%, and 9.5% to 14.5%, respectively) [28].

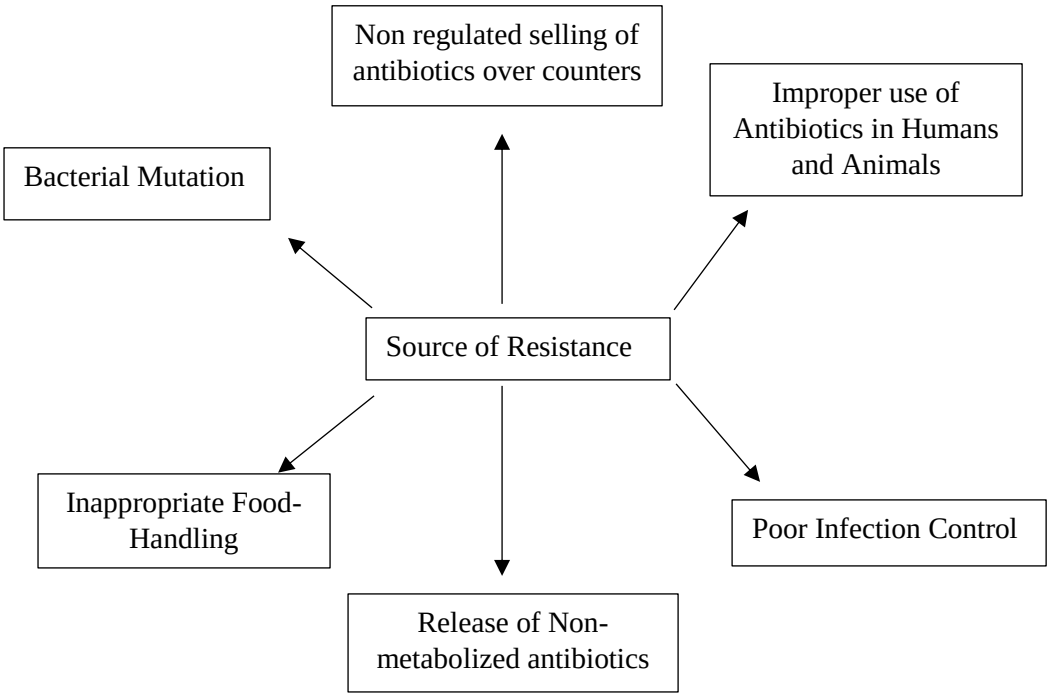


Figure 1**.** Main roots causes of antimicrobial resistance *[29]*

The Antibiotics administration in many countries is not regulated whereby they are easily accessible by anyone over counters that are consequently overused unnecessarily [30]. The antibiotics prescribed in ICUs 30% to 60% of them are wrongly and needlessly given, which definitely play an important role in the development of problems caused by antimicrobial resistance, moreover, the epidemiological studies proved the association between the intake of antibiotics and the spreading of resistance in the hospitals and ICU [31]. The hospital stay gets longer, delayed recovery and extensive disability and the public healthcare costs increase as well, whereby in 2013 the estimated health cost linked to antimicrobial resistance in the USA was 55 billion USD and every year they encountered

2 million people who were sick and 23,000 deaths cause of antimicrobial resistance infections [32].

2.2 Diagnosis for UTIs

The diagnosis of UTI in medical settings like other bacterial infections depends on culturing the clinical sample which has the delay of two to three days from the time of collection up to the delivery of results, this delay is due to the time of sample transportation from ward to the laboratory and the time necessary for the bacteria to grow on a culture media for identification [33]. Despite the incidence of urinary tract infections, their diagnosis is complicated as well. For some UTIs exhibit, symptoms and others are asymptomatic thus require various diagnostic criteria for the Clinicians to be able to differentiate UTIs from other diseases [34].

Standard diagnostic examination for UTI begins with the presentation of clinical symptoms, which commonly include dysuria, urinary frequency, and urgency. Clinicians often order screening by colorimetric dipstick testing for nitrites and leukocyte esterase, which detect bacteriuria and pyuria, respectively. However, urine dipsticks can give false-negative results in the case of non-nitrite-producing pathogens, such as *Enterococcus* and *Staphylococcus* species, or in dilute urine samples [35]. The midstream urine culture is necessary for the identification and detection of the pathogen and reveals the level of bacteriuria. However, neither medical laboratory nor any scientific paper has standardized the minimum level of bacteriuria signifying a urinary tract infection but many laboratories use 10^5 CFU/ml urine, though it sometimes misses many important infections [36].

Table 2. Interpretation and tests of urine culture [37]

Definitions	Bacteria count	Interpretation and tests
Asymptomatic	$\leq 10^4$ CFU/ml	Probable Absence of UTI
	10^4-10^5 CFU/ml	Repeat the Test (Request for the second Urine)
	$\geq 10^5$ CFU/ml	Identification and AST
Symptomatic	10^4 CFU/ml	Probable Absence of UTI
	10^4-10^5 CFU/ml	Identification and AST
	$\geq 10^5$ CFU/ml	Identification and AST
		If more than two pathogens are present in the urine sample it is reported as probable contamination.

Flow cytometry identifies samples that can be cultured from those that can be reported as negative without culture, having that urine is among the most processed sample in microbiological laboratories, it requires a method that can rapidly identify positive samples appropriate for direct ID and AST to shorten the time of results [38]. The clinical symptoms give a general indication of the infections but many infections don't manifest any symptom or exhibit the same symptoms with others. Therefore, any patient suspected to have UTIs must be examined on a culture basis where we use semi-quantitative or quantitative cultures and their susceptibility to antimicrobial agents is determined which many resistant strains are associated with hospital-acquired infection and cause a serious infection of the urinary tract [39]. CLSI and EUCAST have set the breakpoints of antibiotics for the bacteria which serve in classification of bacteria to susceptible, intermediate or resistant and it helps clinicians to know the right antibiotic and dose to give

to the patient accordingly, however, it is crucial to have both identification and AST for the proper antibiotic therapy [40].

2.3 Therapeutic options in UTIs

Antibiotics such as fluoroquinolones, β-lactams, trimethoprim-sulfamethoxazole, and nitrofurantoin are generally used in clinical settings to treating UTI, however, the dose differs depending on the condition of the patients [41]. Due to the side effects caused by many antibiotics, clinicians should check on patients after 2 days since they have started the therapy and change the regimen for where it is necessary to reduce the risk of effects [42]. To ensure the effectiveness of oral antibiotics therapy in UTIs patients, they must have no renal malfunction to retain the minimum concentration capable of killing the pathogen. However, in multidrug resistance, intravenous medication is recommended [43]. The longer antibiotic therapy (≥7 days) has been proved to be effective in pyelonephritis or febrile UTI. However, in cystis the shorter treatment is recommended [44].

In vitro experiment done has proved the combination of ESBL and β-lactamase inhibitors to be effective in infections caused by carbapenem-resistant *Enterobacteriaceae*. The ceftazidime combined with β-lactamase inhibitor avibactam has shown the potential in treating the ESBL and Carbapenemase-producing gram-negative bacilli [45]. Pathogens presenting resistance to amoxicillin and ampicillin are high, therefore, they are not recommended to be used for empirical treatment [46].

Table 3. Antibiotic therapy in acute uncomplicated cystis [27]

Antimicrobial agent	Regimen Dosage	Duration of Therapy (days)
Fosfomycin	3 g, x1	1
Ciprofloxacin	300 mg or 250 mg, x2	3
Cefpodoxime proxetil	100 mg, x2	5
Cefdinir	100 mg, x3	5
Cefditoren pivoxil	100 mg, x3	3
Cefcapene pivoxil	100 mg, x3	5
Cefexime	400 mg, x1	3
Nitrofurantoin	100 mg, x2	5
Pivmecillinam	400 mg, x3	3
Amoxicillin-Clavulanate	500 or 150 mg, x2	7
Trimethoprim-sulfamethoxazole	160 /800 mg, x2	3

Table 4. Treatment for acute pyelonephritis [46]

Antimicrobial agent	Regimen Dosage	Duration of Therapy (days)
Ciprofloxacin	300 mg or 250 mg, x2	7
Levofloxacin	750 mg, x1	5
Where fluoroquinolone resistance is thought to exceed 10%, the initial long-acting dose of ceftriaxone (1g) is recommended.		
Trimethoprim-sulfamethoxazole	160/800 mg, x2	14

The resistance rate of antibiotics in vitro changes overtime differently from a place to another. Therefore, it is better to identify the individual predictors of resistance to help physicians in administering better empirical therapy [46]. In a study done from Korea showed that in uncomplicated cystitis, fluoroquinolones are the better option for empirical antibiotic therapy in regions where resistances are high [27]. To combatting bacterial infections a lot of remedies such as bacteriophages, immune stimulator, vaccine, antimicrobial peptides, antibodies, and lysine are being developed in addition to the former antibiotics for better management and control of the high resistance globally [29]. Despite the great role that antibiotics play in treating bacterial urinary tract infections, they cause serious side effects. For instance, nitrofurantoin may be the cause of respiratory distress and liver injury, and FDA has proved that the intake of fluoroquinolones may lead to permanent neuropathy and increased retinal detachment as well [47].

III. MATERIALS AND METHODS

3.1 Specimen collection

The study was carried out in the clinical microbiology laboratory of Severance Hospital from August to November 2019. The first phase consisted of setting the quality control ranges (Table 5), using the culture-negative urine samples spiked with *E. coli* ATCC 25922 to different bacterial load. The infected urine was processed by flow cytometry (Sysmex UF-1000i, TOA Medical Electronics, Kobe, Japan), to establish a cut-off value of bacterial count to select samples for direct antimicrobial susceptibility testing. The second phase consisted of performing direct AST to every urine received in the clinical microbiology laboratory. The samples which fulfilled the inclusion criteria (≥ 20,000 bacteria/ml plus the presence of gram-negative bacteria on microscopy).

3.2 Sample processing

103 Urine samples received in the microbiology laboratory contained ≥ 20,000 bacterial cells/ml and monobacterial (gram-negative) after gram stain were processed immediately or kept at 4°C for no longer than 24 hours before testing. The control strain was *E. coli* ATCC 25922.

3.3 Species identification

The identification of all isolates included in our study was performed after the sufficient number of colonies on overnight incubated agar plates and it was determined using MALDI- TOF MS system, ASTA Micro IDSys (ASTA Inc., Suwon, Korea) according to manufacturer recommendations.

3.4 Direct susceptibility testing

Direct susceptibility testing was done on infected urine for quality control as well as all urine specimens received in the laboratory fulfilling the criteria within the study period. For the quality control, infected urine samples with *E. coli* strain were diluted 100 and 1000 fold to achieve the starting concentrations of 10^6 & 10^5 CFU/ml. 100 µl of the mixture was inoculated on the MHA plate using a spreader. Commercial antimicrobial disks (ampicillin, cefazolin, ertapenem, cefepime, cefotaxime, ceftazidime, cefoxitin, aztreonam, trimethoprim-sulfamethoxazole, amikacin, gentamicin, piperacillin-tazobactam, tigecycline, ciprofloxacin, imipenem (Becton, Dickinson and Company, Sparks, MD, USA) were applied and pressed firmly into the agar surface with a sterile forceps.

For the clinical samples, the volume suggested by EUCAST for direct rapid AST in blood [48]. Depending on plates size. Hence, 130 µL and 350 µL of sample were taken and spread on the 90 mm and 150 mm-diameter MHA using a rotator, respectively. The commercial antibiotics were loaded by using disk dispenser and pressed firmly into agar surface using forceps. The plates were incubated at 35±1°C and read on the same day after 8 hr of incubation. The images of inhibition zones were taken and recorded using a digital scanner (Giles scientific, DC, USA, bio-MIC vision analyzer).

3.5 Conventional urine culture, species identification and AST

The urine sample was inoculated and spread on both blood agar and Mac Conkey agar plates using a wire loop. Plates were incubated at 35±1 °C overnight. The colonies on agar plate were counted and identified using MALDI- TOF MS system, ASTA Micro IDSys. A standardized 0.5 McFarland suspension was prepared and N224 VITEK® 2 cards (Biomerieux, Inc.NC, USA) for AST were inoculated following the manufacturer recommendation.

3.6 Comparison of DST and conventional AST

For comparison, the antimicrobial susceptibility testing of isolates from standard cultures was determined using VITEK-2. *E. coli* ATCC 25922 was included for quality control. Interpretation of Susceptibility testing (susceptible, intermediate, or resistant) for each bacteria and antibiotic was done following the criteria of the Clinical and Laboratory Standards Institute [13], to compare the concordance of the two methods.

Results from direct and standard tests were compared by testing the correlation coefficient r using the Pearson test analyzed by SPSS 25. And discrepancies were classified as very major errors (VME), major errors (ME), or minor errors (mE). A Very major error was a susceptible result by the direct method and a resistant result by the standard method. A Major error was a resistant result by the direct method and a susceptible result by the standard method. A minor error was any discrepancy involving an intermediate result. Samples were kept in freezer -70 ℃ for the testing of MIC by E-test (bioMe'rieux SA, Marcy-L'E'toile, France) retrospectively for the discrepancies (Major and very major errors) seen between both methods.

IV. **RESULTS**

We have preselected 128 urine samples with *Enterobacteriaceae* during the study period and 103 were included as presented in Figure 2. They were distributed as 68 (66%) *E. coli*, 17 (16.5%) *Klebsiella pneumonia*, 5 (4.9%) *Citrobacter freundii*, 2 (1.9%) *Citrobacter braaki*, 2 (1.9%) *Enterobacter cloacae*, 2 (1.9%) *Klebsiella ocytica*, 2 (1.9%) *Proteus vulgaris*, 2 (1.9%) *Enterobacter aerogenes*, 1 (1%) *Raoultella ornithinolytica*, 1 (1%) *Proteus mirabilis*, 1 (1%) *Morganella morgani*.

Concerning 25 excluded, 17 had more than one bacteria, 8 exhibited poor growth after 8 hr and were unreadable.

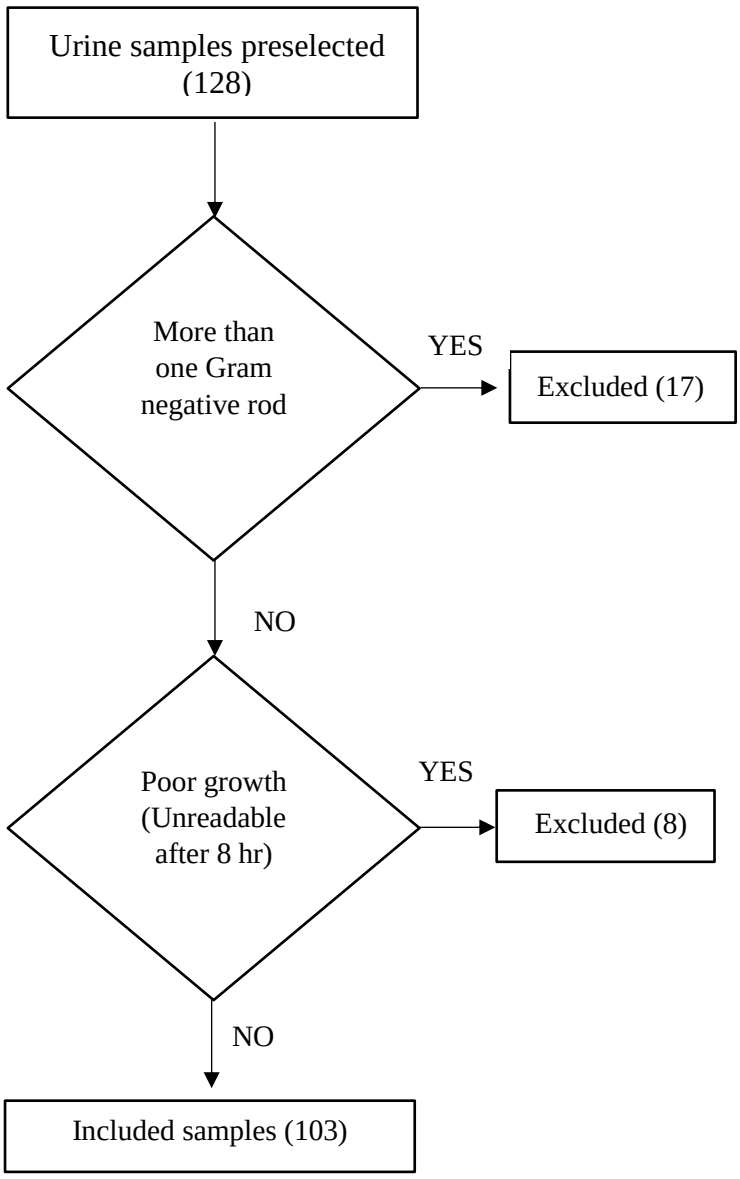


Figure 2. Urine samples studied

Over the 1545 tested combinations in all isolates studied, the overall categorical agreement of the direct rapid disk diffusion and the standard susceptibility testing using VITEK-2 was 1436 (92.9%), with 76 (4.9%) minor errors, 14 (0.9%) major errors and 19 (1.2%) very major errors (Table 6). minor errors were mainly reported in ciprofloxacin 15 (14.6%), ceftazidime 13 (12.7%), cefoxitin 9 (8.7%). According to the result from the comparison between dRAST and VITEK-2 susceptibility, All Antibiotics used, have correlation coefficient $r \geq 0.90$ except from piperacillin-tazobactam, amikacin, ertapenem, imipenem, cefepime, tigecycline which have 0.74, 0.79, 0.78, 0.59, 0.84, 0.58 respectively.

Concerning majors errors observed , 4 (3.9%) cefepime, 2 (1.9%) aztreonam, 2 (1.9) cotrimoxazole, 1 (1%) amikacin, 1 (1%) cefotaxime, 1 (1%) gentamycin, 1 (1%) cefazolin, 1 (1%) ceftazidime, 1 (1%) tigecycline. Out of 19 VME observed 5 (4.9%) were due to tigecycline, 4 (3.9%) due to cefazolin, 3 (2.9%) due to piperacillin-tazobactam, 2 (1.9%) due to cotrimoxazole, 2 (1.9%) due to imipenem 1 (1%) due to ampicillin,1 (1%) due to aztreonam, 1 (1%) due to ertapenem. For some of the discrepancies, inhibition zones were close to clinical breakpoints, where a slight difference in mm zone of diameter would yield to a major or very major error.

Table 5. Proposed QC range for *E. coli* ATCC 25922 in comparison with Standard disk diffusion

Antibiotics	DST	Standard disk diffusion
	QC Range	QC Range
FOX	22-29	23-29
AMP	18-24	15-22
CIP	31-38	29-37
TZP	27-33	24-30
AN	25-31	19-26
ATM	31-37	28-36
CTX	32-38	29-35
GM	24-29	19-26
IPM	29-36	26-32
MI	24-31	19-25
FEP	32-39	31-37
CZ	25-33	21-27
SXT	24-33	23-29
CAZ	28-35	25-32
ETP	31-38	29-36
TGC	23-30	20-27

Abbreviations: SD: Standard deviation, FOX: cefoxitin, AMP: ampicillin, CIP: ciprofloxacin, TZP: piperacillin-tazobactam, AN: amikacin, ATM: aztreonam, CTX:

cefotaxime, GM: gentamicin, IPM: imipenem, MI: minocycline, FEP: cefepime, CZ: cefazolin, SXT: trimethoprim-sulfamethoxazole, CAZ: ceftazidime, ETP: ertapenem, TGC: tigecycline, QC: Quality control.

Table 6. Comparison of susceptibility between Direct rapid AST and VITEK-2 Results

Antibiotics	**Total**	**CA**		**Minor error**		**ME**		**VME**		**r**
		n	%	n	%	n	%	n	%	
Cefoxitin	103	94	91.2	9	8.7	0	0	0	0	0.93
Ampicillin	103	99	96.1	3	2.9	0	0	1	1	0.92
Ciprofloxacin	103	88	85.4	15	14.6	0	0	0	0	0.92
Piperacillin-Tazobactam	103	94	91.3	6	5.8	0	0	3	2.9	0.74
Ertapenem	103	100	97.1	2	1.9	0	0	1	1	0.78
Amikacin	103	101	98.1	1	1	1	1	0	0	0.79
Aztreonam	103	95	92.2	5	4.8	2	1.9	1	1	0.90
Cefotaxime	103	97	94.2	5	4.8	1	1	0	0	0.95
Gentamycin	103	100	97.1	2	1.9	1	1	0	0	0.96
Imipenem	103	95	92.2	6	5.8	0	0	2	1.9	0.59
Cefepime	103	93	90.3	6	5.8	4	3.9	0	0	0.84
Cefazolin	103	98	95.1	0	0	1	1	4	3.9	0.90
Cotrimoxazole	103	98	95.1	1	1	2	1.9	2	1.9	0.92
Ceftazidime	103	89	86.4	13	12.6	1	1	0	0	0.90
Tigecycline	103	94	91.3	2	1.9	1	1	5	4.9	0.58
TOTAL	1545	1435	92.9	76	4.9	14	0.9	19	1.2	

Abbreviations: CA: Categorical agreement, ME: major error, VME: Very major error, r: correlation coefficient.

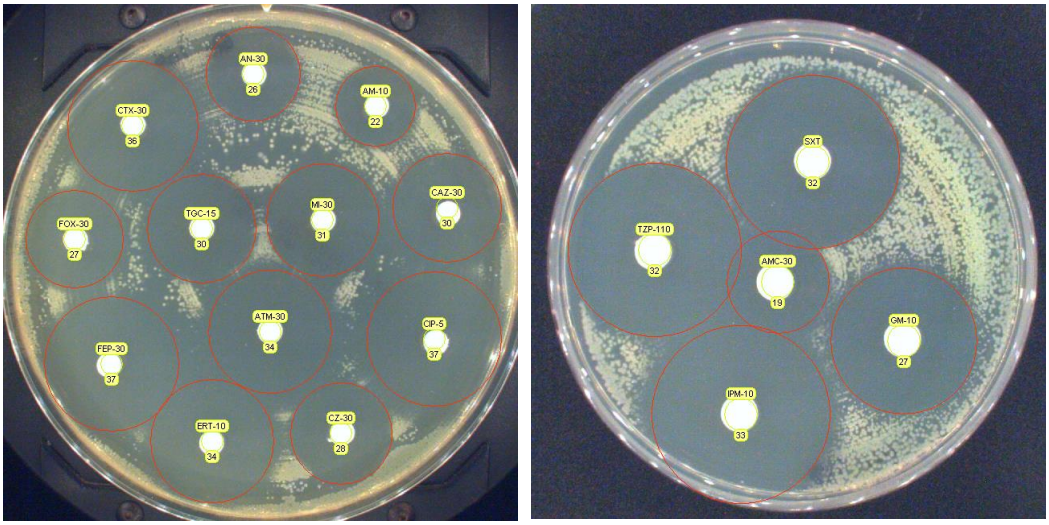
Table 7. Bacterial response to antimicrobial agents used in both methods

Antimicrobial agents	**Direct susceptibility testing**			**VITEK-2**			**No. of discrepancies**
	S	I	R	S	I	R	
Amikacin	101	1	1	103	0	0	2
Ampicillin	12	3	88	12	0	91	4
Aztreonam	65	5	33	67	0	36	6
Ciprofloxacin	34	21	48	45	10	48	15
Cefoxitin	77	3	23	70	8	25	9
Cefepime	79	0	24	80	6	17	10
Cefazolin	44	0	58	41	0	62	5
Ceftazidime	61	14	26	69	1	33	14
Ertapenem	100	2	1	98	1	4	3
Gentamycin	75	1	27	75	1	27	3
Imipenem	99	2	2	93	6	4	8
Tazobactam-Piperacillin	91	8	4	86	8	9	9
Cotrimoxazole	56	1	46	57	0	46	5
Tigecycline	96	0	7	92	2	9	9
Cefotaxime	50	6	47	54	3	46	6

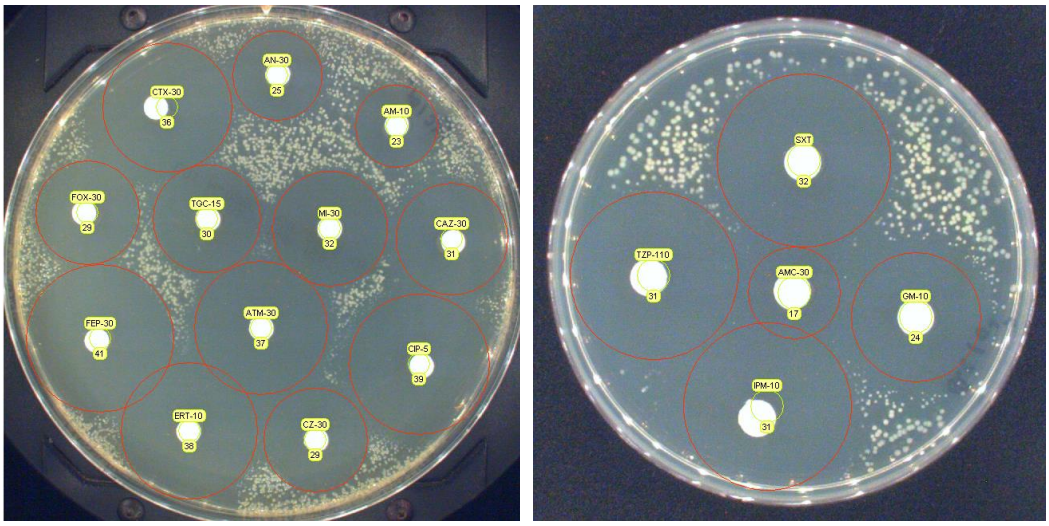
S: Susceptible, I: Intermediate, R: Resistance

Table 8. Discrepancy Analysis by E-TEST

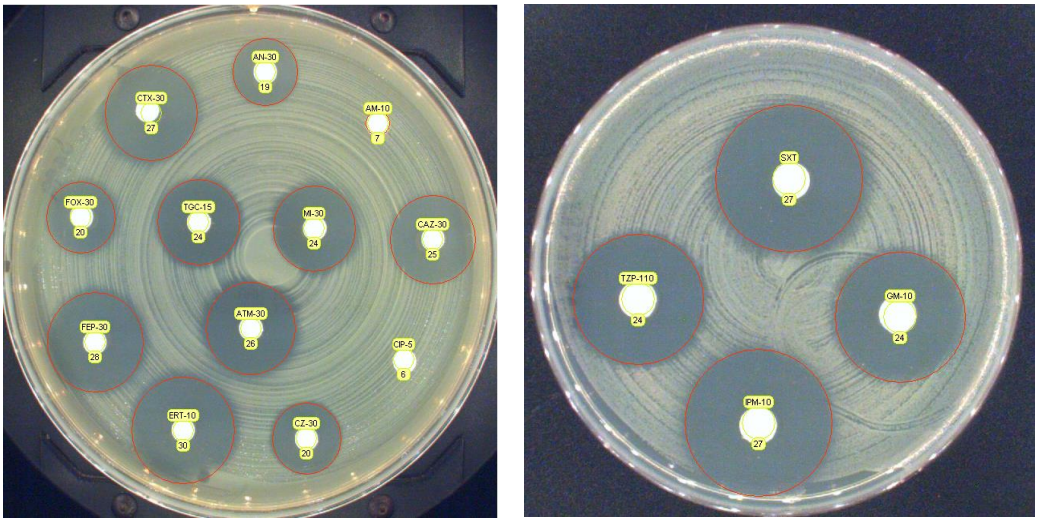
Strain	Antibiotic	dRAST	VITEK-2	difference	E-test result (mg/ml)	interpretation	Correct method
E. coli	FEP	R	S	ME	4	S	Vitek-2
K. pneumoniae	FEP	R	S	ME	12	R	DST
	GM	R	S	ME	32	R	DST
	ATM	R	S	ME	48	R	DST
E. coli	ATM	R	S	ME	0.5	S	Vitek-2
P. mirabilis	IPM	S	R	VME	1.0	S	DST
	TGC	S	R	VME	24	R	Vitek-2
E. coli	AN	R	S	ME	4	S	Vitek-2
E. coli	SXT	S	R	VME	1.5	R	Vitek-2
K. pneumoniae	TGC	S	R	VME	2	R	Vitek-2
P. vulgaris	TGC	S	R	VME	16	R	Vitek-2
M. morganii	TGC	S	R	VME	1.5	R	Vitek-2
P. vulgaris	TGC	S	R	VME	2	R	Vitek-2
E. coli	FEP	R	S	ME	8	S	Vitek-2
E. coli	SXT	R	S	ME	≥32	R	DST
E. coli	SXT	R	S	ME	48	R	DST



a. *E. coli* ATCC 2592 with 10^6 CFU/ml



b. *E. coli* ATCC 2592 with 10^5 CFU/ml



c. Clinical *E. coli* isolate

Figure 3. Inhibition zone diameters by Digital Scanner image

V. DISCUSSION

The main purpose of this study was to evaluate the performance of rapid direct AST on urine compared to the standard method using VITEK-2. Our study showed excellent results compared to the susceptibility of VITEK-2. According to Jorgensen criteria the rates of VME and the combination of ME and mE should be < 3%, <7% respectively [50]. And for our study VME was 1.2% and the combination of ME and mE were 5.8%. The observed discrepancies between DST and VITEK-2 AST were compared using the results of MIC by E-test. Out of 26 discrepant strains, only 13 strains were available and tested. Therefore, in 16 combination drug tested, found that the correct method for 37.5% of the drug tested was DST and 62.5% for Vitek-2 (Table 8). When analyzed the concordance between both methods, the interpretations of AST results were done following the published breakpoints from CLSI, however, for tigecycline we used EUCAST breakpoint since there is no published breakpoint by CLSI for this antibiotic in *Enterobacteriaceae.*

The agreement for each antibiotic exceeds 85% in all isolates studied. Most of the strains studied were resistant to ampicillin, cefazolin, ciprofloxacin, cefotaxime, trimethoprim-sulfamethoxazole (85.4, 56.3, 46.6, 45.6, 44.7%) respectively. Direct antimicrobial susceptibility testing of urine form UTI samples ($\geq 2.10^4$ bacteria/ml) showed that the most effective agents were amikacin (98.1%), ertapenem (97.1%), imipenem (96.1%), tigecycline (93.2%), and piperacillin-tazobactam (88.3%). A great number of urine samples (16.5%) have been excluded from this study due to the mixed infection of more or equal than two *Enterobacteriaceae*. However, its susceptibility results would be still useful in choosing the better option of empirical treatment. In a study done by Perillaud et al, the DST was compared to the standard disk diffusion AST and the categorical agreement of 97.9%, 1.5% of minor errors, 0.3% ME, and 0.3% VME were found [51].

Though the susceptibility of rapid method seems to be incomplete, the phenotypic result generated in a short period compared to the conventional method can help the physician give the appropriate empirical therapy [10]. On the other hand, the direct susceptibility testing gives the real image of population susceptibility to antibiotics for it is done on the whole sample rather than from a single colony, therefore, the results are likely to be clinically relevant [10]. Studies done on DST on urine have all proved the reliability of this method in gram-negative, however, the errors in the case of polymicrobial are quite high. Thus, it is suggested to be done in monobacterial only [52]. The limitation of our study is the spectrum of test strain. We included only *Enterobacteriaceae*. DST is not reliable in polymicrobial urine samples, either. Further, bacteria that have slow growth capacity do not allow the reading of inhibition zone diameters after 8 hr of incubation. Multiple bacterial infections with similar colonies characteristics in a sample are also big huddle to this rapid method because though it is rare, discrimination of different isolates on a plate by its' colonial morphology is not easy , thus it can make an error in inhibition zone diameter measure.

In conclusion, despite some errors, DST on urine is reliable in *Enterobacteriaceae*. Furthermore, the overall agreement and errors rates were within the acceptable limits which gives it the credential to be adopted into routine microbiology laboratory workflow since it helps us to save more than 24 hr. *E. coli* and *K. pneumoniae* were the most predominant isolates in UTI with a prevalence of 66%, 16.5%, respectively. Resistant rates to ampicillin, cefazolin and ciprofloxacin were the highest in the tested strains, whereas amikacin, ertapenem, imipenem, tigecycline were found to be the most effective drugs. DST and standard AST methods almost give the same results. The rapid AST reports by using DST in UTIs can facilitate timely and adequate antimicrobial treatment.

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