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Combination of quantitative bacterial and WBC count from urine flow cytometry to estimate the success of urine culture in symptomatic urinary tract infections

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ABSTRACT

Aims: Urinary tract infection (UTI) is a common infection that can occur in inpatient and outpatient setting. The current guideline is using urine culture as standard for UTI diagnosis. Recently, urine flow cytometry method with dedicated bacterial channel is intended as useful tools for UTI diagnostic. In our study, we determine the cut-off value of white blood cell (WBC) and bacterial count of fluorescence flow cytometry as an estimation of the successful of urine culture in symptomatic UTI population.

Methodology and results: The study was held from January until April 2015 at secondary and tertiary hospital in Bandung, Indonesia. A number of 215 UTI patients were enrolled. Urine specimens were analyzed using automated flow cytometry urine analyzer (UX2000, Sysmex Corp., Japan) and results were compared with urine culture, as gold standard. The cut-off value of WBC and bacterial count were determined using ROC to generate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The combination of bacterial count ≥ 277.4 bacteria/uL, WBC count ≥ 300.7 cells/uL, in symptomatic UTI patients, achieved sensitivity of 82.7%, specificity of 87.5%, PPV of 96.6% and NPV of 53.8%. This combination can be use as estimation of positive culture as high as 96.6%.

Conclusion, significance and impact of study: In microbiology aspect, quantitative bacterial count and WBC count of urine flow cytometry can be useful to estimate the success of culture-proven UTI, which can help in clinician decision-making and better patient management.

Keywords: symptomatic UTI, flow cytometry, cut-off, urine culture, quantitative count

INTRODUCTION

Urinary tract infection (UTI) is a condition where pathogens enter the urinary tract and cause infection. In fact, urinary tract infection has received relatively little attention. Tests available for detecting UTI include the urine test strip analysis of nitrite and leukocyte esterase, urine sediment analysis, and urine culture for bacterial testing (Yasutake *et al.*, 2013). Unfortunately, urine culture, the gold standard for the diagnosis of UTI, is still time-consuming, expensive and often yields negative results. This not only affects clinician decision-making and patient management, but laboratory workload as well (Agpaoa *et al.*, 2015). Recently, newest diagnostic device, a fluorescence flow cytometry, which is intended for urinalysis purposes, provides new analytical features that seem particularly suitable for microbiological diagnostics (De Rosa *et al.*, 2010; Yamaguti *et al.*, 2013).

Our laboratory is placed in one of the biggest tertiary hospital and act as the reference laboratory for West Java Province, with approximately 900–1000 patients per day.

We received approx. 57,116 urine specimens per year and of those, 41% underwent culture. The positive rate of urine culture, regardless to clinical manifestation was approximately 87%, with the turn-around time between 3 and 5 days. Various studies have reported the utilization of fluorescence flow cytometry as a screening tool for UTI (Manoni *et al.*, 2009; De Rosa *et al.*, 2010; Jolkkonen *et al.*, 2010; Pieretti *et al.*, 2010; Broeren *et al.*, 2011; Giesen *et al.*, 2013; Dai *et al.*, 2014; Agpaoa *et al.*, 2015). The rapid reporting of results can aid the clinicians in prompt and proper management (De Rosa *et al.*, 2010). In view of these facts, however, no studies has shown the usefulness of this method combining with symptomatic UTI patient which can increase its diagnostic value for UTI and also estimate the chance of successful of urine culture. The purpose of this study is to determine the cut-off value of white blood cell (WBC) and bacterial count of fluorescence flow cytometry as an estimation of the successful of urine culture from symptomatic UTI population.

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MATERIALS AND METHODS

Study population

This study was conducted in tertiary hospital Dr. Hasan Sadikin Bandung and its satellite hospital, M. Salamun Bandung from January until April 2015 and consecutively screened for symptomatic UTI patient from the outpatient (urology clinic) and inpatient (internal medicine). The screening process used the US Centers for Disease Control and Prevention (CDC) criteria for community-UTI and catheter-associated-UTI (Horan *et al.*, 2008; CDC, 2012) as a reference. Both male and female subjects above 18 years old, with or without any comorbidities or other underlying disease were included. Subject with indwelling urinary catheter presenting in the outpatient and subject with unconscious state were excluded from this study.

We targeted 200 participants with positive urine dipstick analysis and clinical symptoms, based on the preliminary UTIs screening study. This study was limited by time period for three months. Inclusion continued until the 200 participants were reached or at the end of the data collection period. The sample size was calculated for both two contributing hospitals. Study staff was trained on data collection procedures and participated since a preliminary study before the commencing actual study activities.

Specimen collection

In outpatient setting, the mid-stream urine was collected into a clean sterile urine container; both male and female participants were given instructions of proper urine collection by the study staff. For inpatient setting, urine sample was obtained from a catheterized patient, using clamps on the soft rubber connector between urine catheter and the collecting bag, until urine is ready to collect for 5-10 mL (Vandepitte *et al.*, 2003; Wilson and Gaido, 2004). All urine specimens were immediately stored at 2-8 °C, and transferred to the laboratory in the tertiary hospitals for culture and susceptibility testing within 24 h.

Laboratory procedure

The urine samples were analyzed for routine urinalysis and bacterial identification. Urine samples were assessed by dipstick analysis (Combur 10, Roche Diagnostic - Germany) according to the instruction of the manufacturer. All samples with a positive dipstick (based on at least one positive nitrite reaction or leukocyte esterase reaction), were processed for urine flow cytometry and bacterial culture. The collected urine was inoculated onto blood agar plate and MacConkey agar plate using a calibrated loop (10 µL), according to the standard guidelines. Both agar plates were incubated at 35-37 °C for 18-24 h and inspected for colony morphology. Suspected fungi colonies were excluded. Colony counting was carried out and expressed as number of colony-forming units (CFU) per mL. The culture plates were interpreted based on the current guidelines (Vandepitte *et al.*, 2003). A growth of culture positive was defined if the semi-quantitative colony counting resulted in at least 10^3 CFU/mL until more than 10^5 CFU/mL. The purpose of include bacterial culture with a colony counting less than 10^5 CFU was to screen as low as possible the number of bacteria in the urine (Manoni *et al.*, 2009). Bacterial colonies were identified using automatic microbiology analyzer (Vitek2 Compact, Biomérieux - France). An automated flow cytometer urinalysis analyzer (UX2000, Sysmex - Japan) was used for urine sediment analysis (FCM). The automatic urine analyzer with flow cytometry use of a 635 nm semi-conductor diode laser and two separated analytical channels with polymethine-based fluorescent dyes with specific temperature and incubation time. In dedicated bacteria channel of flow cytometry, the urine specimen is mixed at a controlled temperature of 42 °C with a special diluent which increases the permeability of the bacterial cell membrane and facilitates the absorption of fluorescent dye. The particles are then classified and counted on the basis of their size and staining characteristics, using the forward scatter and fluorescence light intensity emitted by each cell, and presented in specific histogram and scattergram patterns generated from the bacteria channel. Two additional parameters available from this channel are the B_FSC (bacteria forward scatter) and the B_FLH (bacteria fluorescent light intensity), expressed in arbitrary units (analytical channel, ch), provide information on the size and the nucleic acid content, respectively, of the bacteria (Sakai *et al.*, 2007; Yuno and Kawakami, 2008; De Rosa *et al.*, 2010; Yamaguti *et al.*, 2013). The bacterial count, WBC counts, and bacteria scattergram were obtained for analysis. Quality controls for agar plate and automatic microbiology analyzer were performed on a weekly basis according to CLSI guidelines (CLSI, 2010). Quality control for automated urinalysis analyzer was performed on a daily basis according to the instruction of the manufacturer.

Data analysis

Clinical history and culture result were collected daily and entered into pre-designed excel files. The bacterial count, WBC count and bacterial scattergram results were collected automatically from the Sysmex UX database instrument and outputted into excel files. All of these excel files were merged together. Data were analyzed using the STATA 12.0 (Stata Corp, Texas - USA). The sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were presented for WBC cut-off value and bacterial counts cut-off value, against culture result as the reference. The receiving operator characteristics (ROC) curves for bacteria and WBC count were plotted to assess the cut-off values with Youden Index Method. The results of the study were presented as diagram figure and table with number and percentage.

The primary outcome was the cut-off of bacterial count and WBC count as estimation of the successful urine

culture among symptomatic UTI population, which is presented from Sp value and PPV. In addition, we also presented the result of bacterial scattergram pattern and urinalysis among the positive urine culture samples to evaluate if combination between distribution of bacteria pattern on scattergram and dipstick might improve the UTI diagnostic, which is presented as percentage of bacterial scattergram pattern and dipstick, stratified by bacterial type, Gram-negative and Gram-positive, separately.

Ethical

This study was approved by the Universitas Padjadjaran Faculty of Medicine Ethics committee and Dr. Hasan Sadikin General Hospital Research committee and Medical Committee of M. Salamun Hospital. Written informed consent was obtained from every recruited patients and confidentiality was maintained at every stage of the research.

RESULTS

From January to April 2015, there were 287 participants screened from community and hospital population as UTI case. Of those, 72 participants were excluded because they failed to give consent or urine specimen. Rest 215 participants (75%) were included in the study. The positivity growth rate of culture was around 81%. The bacterial identification of the culture showed 86% were Gram-negative bacteria and 14% was Gram-positive bacteria (Figure 1).
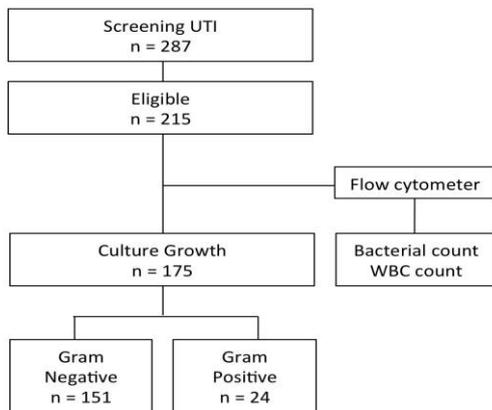

Figure 1: The study population diagnostic workup.

The age group for this population was dominantly more than 55 years old, with one or two clinical sign and symptoms of UTI were more often present. Among inpatient and outpatient participants, the positivity of leukocyte esterase was above 55% and the positivity of both nitrite and leukocyte esterase were above 35% (Table 1). In this study, of those 175 positive cultured urine, 78 specimens (44.6%) had both positive nitrite and leukocyte esterase; 5 specimens (2.3%) show only positive for nitrite and 92 specimens (42.7%) showed only

positive leukocyte esterase. Of those 175 positive cultured urine samples, the distribution of bacteria was predominantly *Escherichia coli* (40%) and *Klebsiella* spp. (21.1%) (Table 2).

Table 1: Demography of subjects.

Variable	Inpatient (n = 106)		Outpatient (n = 109)	
	n	%	n	%
Gender				
Male	67	63.2	72	66.1
Female	39	36.8	37	33.9
Age (year)				
18 - 24	14	13.2	3	2.8
25 - 34	9	8.5	13	11.9
35 - 44	10	9.4	13	11.9
45 - 54	26	24.5	22	20.2
55 - 64	28	26.4	27	24.6
≥ 65	19	17.9	31	28.4
Number of UTI symptoms*				
One symptoms	25	23.6	45	41.3
Two symptoms	71	67.0	48	44.0
Three symptoms	9	8.5	16	14.7
Four symptoms	1	0.9	0	0.0
Dipstick				
Nitrite(+)	4	3.8	3	2.8
Leukocyte esterase(+)	62	58.5	64	58.7
Nitrite(+) and Leukocyte esterase(+)	40	37.7	42	38.5

*Clinical symptoms of UTI using CDC criterion (Horan *et al.*, 2008; CDC, 2012)

Table 2: Bacteria strains identified from 175 culture-positive urine samples.

Bacteria Gram-Negative	n	%	Bacteria Gram-Positive	n	%
E. coli	70	40.0	*Staphylococcus coagulase negative*	12	6.9
Klebsiella spp.	37	21.1	*S. aureus*	6	3.4
Citrobacter freundii	10	5.7	*Streptococcus viridans*	2	1.1
Enterobacter cloacae	8	4.6	*Micrococcus*	2	1.1
Acinetobacter spp.	7	4.0	*Enterococcus faecalis*	2	1.1
Pseudomonas spp.	5	2.9			
Morganella spp.	4	2.3			
Proteus spp.	3	1.7			
Others	7	4.0			

In this study, the bacterial count and WBC count showed the area under the ROC (AUC) of 0.878 and 0.723, respectively (Figure 2). According to the AUC, we used cut-off of bacterial count for ≥ 277.4 bacteria/uL and

achieved sensitivity of 85.7% and specificity of 75% (95% CI: 80.6 - 95). The cut-off of WBC count was used at ≥ 300.7 cells/uL and achieved sensitivity of 53.1% and specificity of 89.3% (95% CI: 62.3 – 82.2). Using the combination of bacterial count ≥ 277.4 bacteria/uL, WBC count ≥ 300.7 cells/uL, and positive UTI criterion, achieved sensitivity of 82.7%, specificity of 87.5%, PPV of 96.6% and NPV of 53.8%. This combination showed positivity estimation of culture growth based on these criterions was 96.6% (Table 3).
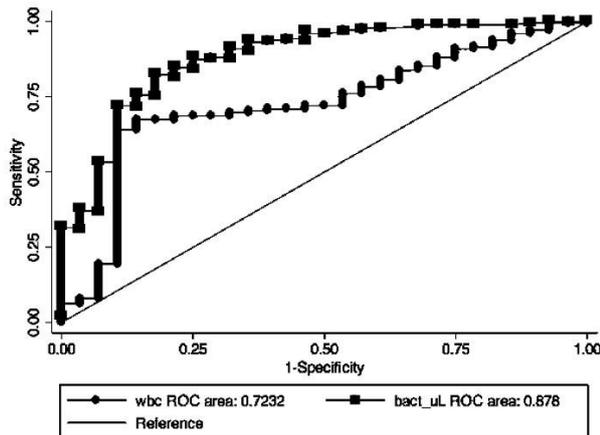

Figure 2: The ROC curve for bacterial count and WBC count.

Table 3: Sensitivity-specificity for bacterial count and WBC count from culture-proven UTI.

Variable	Sn (%)	Sp (%)	PPV(%)	NPV(%)
Only BACT	85.7	75	95.5	45.7
Only WBC	84.6	75	95.5	43.8
BACT + WBC	82.7	87.5	96.6	53.8

BACT, bacterial count (≥ 277.4 bacteria/uL); WBC, leukocyte count (≥ 300.7 cells /uL)

In flow cytometry, bacteria aggregate and display as a single dot in the bacterial scattergram. Gram-positive bacteria (cocci) are positioned in an area of the scattergram as highly stained and relatively large. As a result, they are plotted above the 30° angle line from the x-axis (high angle scattergram). Gram-negative bacteria (bacilli) are seen on the scattergrams as an area that contains plots of a smaller size and a more lightly stained area compared to cocci. Eventually, these dots are distributed below the 30° angle line from the x-axis (low angle scattergram). On the other hand, if more than one type of bacteria present (cocci and bacilli) in the area of scattergram, they are plotted above and below the 30° angle line from the x-axis (wide pattern) (Yamaguti *et al.*, 2013; Agpaoa *et al.*, 2015). In this study, the bacterial scattergram pattern was evaluated and classified into high angle pattern, low angle pattern, wide pattern and non-specific pattern (Figure 3). The bacterial scattergram from 175 positive urine culture revealed that 89 (50.9%) were
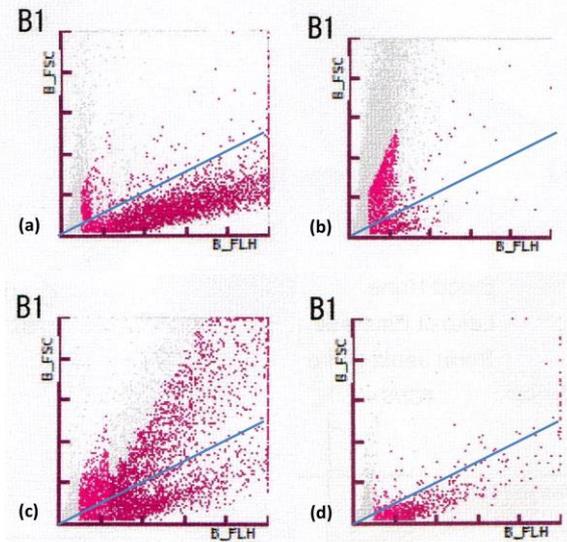

Figure 3: Bacterial Scattergram Pattern. a, low angle pattern; b, high angle pattern; c, wide pattern; d, non specific pattern. B1 (bacteria flow cytometry channel) and B_FSC (bacteria forward scatter) indicate the size bacteria while B_FLH (bacteria fluorescent light intensity) indicates the depth of staining.

Table 4: Bacterial scattergram pattern from positive urine culture samples.

Scattergram	Dipstick	Gram (n=175)			
		Negative		Positive	
		n	%	n	%
Low angle pattern (Less than 30° angle)	NIT+	3	1.7	0	0.0
	LE+	34	19.4	5	2.9
	NIT+ and LE+	52	29.7	6	3.4
High angle pattern (Greater than 30° angle)	NIT+	0	0.0	0	0.0
	LE+	5	2.9	4	2.3
	NIT+ and LE+	3	1.7	0	0.0
Wide pattern	NIT+	0	0.0	0	0.0
	LE+	22	12.6	2	1.1
	NIT+ and LE+	7	4.0	0	0.0
Non-specific pattern	NIT+	2	1.1	0	0.0
	LE+	13	7.4	7	4.0
	NIT+ and LE+	10	5.7	0	0.0

NIT,nitrite; LE, leukocyte esterase

Gram-negative bacteria, which had positive nitrite and/or leukocyte esterase with low distribution pattern. Four (2.3%) were Gram-positive bacteria, which had positive leukocyte esterase with high distribution pattern. There were 19 discordant results, 8 results showed high

bacterial distribution with Gram-negative bacteria and 11 results showed low bacterial distribution with Gram-positive bacteria (Table 4).

DISCUSSION

Urinary tract infection is a common disease but often-neglected in daily clinical practices. Urine culture with semi-quantitative colony counting still remains gold standard for diagnosis UTI. However, since the urine culture needs time and technical experience, many clinicians still rely on patient symptoms and routine urine examination to diagnose the UTI (Silver *et al.*, 2009). According to previous reported studies (Manoni *et al.*, 2009; De Rosa *et al.*, 2010; Jolkkonen *et al.*, 2010; Pieretti *et al.*, 2010; Broeren *et al.*, 2011; Giesen *et al.*, 2013; Dai *et al.*, 2014; Agpaoa *et al.*, 2015), this was the first study conducted based on patient clinical symptoms for evaluating bacterial count and WBC count in UTI. Our study has shown the additional value of urine flow cytometry (WBC count and bacterial count) to improve the UTI diagnostic. For many years, the combination of nitrite and/or leukocyte esterase was common and widely used as screening of UTI, besides sign and symptoms in the clinical practices. In this study, we found the positivity of nitrite was low (2.3%), compared with positivity of leukocyte esterase (42.7%). This finding is similar with Krongvorakul study, which describes about the useful of the urine dipstick as screening test. A positive result from either leukocyte esterase or nitrite can be used for screening of UTI. However, leukocyte esterase may show a false negative result in specimens with an elevated specific gravity, protein or glucose and the nitrite test alone has high specificity but low sensitivity, which can be used for diagnostic purposes rather than screening (Krongvorakul *et al.*, 2012).

The purpose of this study was to determine the optimal cut-off for culture-proven UTI (diagnostic feature). From this study, we found the cut-off of bacterial count and WBC count is higher than other reported studies. We compared several studies from the last five years. In the last 5 studies, there were various cut-off value for bacteria ranging from 55 bacteria/uL to 288.9 bacteria/uL, and for WBC cut-off value, the range was from <24 cells/uL to 150 cells/uL (Manoni *et al.*, 2009; De Rosa *et al.*, 2010; Jolkkonen *et al.*, 2010; Pieretti *et al.*, 2010; Broeren *et al.*, 2011; Giesen *et al.*, 2013; Dai *et al.*, 2014; Agpaoa *et al.*, 2015). The possible explanations for those wide ranges were difference in study population and definition of UTI. Most of the studies population used laboratory-based method instead of clinical patient complains. This finding is similar as stated by Krongvorakul study (Krongvorakul *et al.*, 2012).

During our study, we use the clinical definition of UTI, which is defined by CDC and widely used in the clinical practices and confirmed with urine culture, as gold standard. From Table 3, the sensitivity and specificity of the cut-off of both bacterial count and WBC count are improving as UTI diagnostic rather than UTI screening. Compared with previous reported studies, our specificity

was higher than other reported, as well as PPV. But the sensitivity and NPV were lower than the other studies report. This can be explained due the specific population that we use in the study. Previous reported studies were use the combination of bacterial count and WBC count as screening criteria in UTI, therefore, most of the studies were choose and show the cut-off that could improve the sensitivity and NPV, instead of specificity and PPV (Manoni *et al.*, 2009; De Rosa *et al.*, 2010; Jolkkonen *et al.*, 2010; Pieretti *et al.*, 2010; Broeren *et al.*, 2011; Giesen *et al.*, 2013; Dai *et al.*, 2014; Agpaoa *et al.*, 2015). Reported study from Agpaoa *et al.* which was used the combination of population between clinically diagnosis UTI with laboratory diagnosis of UTI, showed higher value of specificity (82.3%) and PPV (81.2%) among all reported studies (Agpaoa *et al.*, 2015). This could explain the important population determination for establishing the cut-offs of those parameters.

In our understanding from this study, the high specificity and PPV from this combination can be interpreted as the positive estimation of culture growth, which is achieved as high as 96.6% among the study population. Silver et al. stated the culture-proven UTI among of UTI population for symptomatic UTI group was reported as high as 25%, while the rest were population without clinical symptoms associated UTI but the culture-proven as UTI (Silver *et al.*, 2009). This means that result of positive estimation of culture growth gives an opportunity to urine flow cytometry to play a role in microbiology aspects, clinician decision-making and also patient management. However, false positive and false negative result can be seen for both bacteria and WBC count cut-off. The false result for WBC and bacteria count can be due to many factors, such as persistence of WBC after antibiotic treatment, dead bacteria or any debris that could be counted in the flow cytometer, although the dedicated bacteria channel of the analyzer allows the exclusion of other fragments (Okada *et al.*, 2007; Ozawa *et al.*, 2012; Yamaguti *et al.*, 2013; Agpaoa *et al.*, 2015).

From Table 4, we observed that the positivity of nitrite and/or leukocyte esterase with bacterial scattergram might improve UTI diagnostic interpretation. The concordance between low angle scattergram with nitrite and/or leukocyte esterase can contribute to Gram-negative bacteria detection (Fujinami *et al.*, 2013). The discordance of scattergram pattern with culture result may be caused by varied reasons as observed in different studies (Okada *et al.*, 2007; Ozawa *et al.*, 2012; Yasutake *et al.*, 2013; Agpaoa *et al.*, 2015). Some bacteria might be not sufficiently stained with the polymethine dye, as basic detection in flow cytometry. Bacterial might be not stained in condition where the nitrate-reducing bacteria proliferate and produce a large amount of nitrite ions that might decompose the polymethine dye, although additional substance is added to reduce this process. Previous antibiotic treatment and probable development of resistance are also considered to affect bacterial distribution pattern (Sakai *et al.*, 2007; Muratani *et al.*, 2013; Yasutake *et al.*, 2013; Agpaoa *et al.*, 2015). Furthermore, among the wide distribution pattern, it is

known that most of the participant was having complicated UTI, and consider involvement of multidrug resistant (Ozawa *et al.*, 2012; Agpaoa *et al.*, 2015). However, further investigation to explain the discordance of distribution pattern in Gram-negative and Gram-positive bacteria is still needed.

The limitation of this study is different education stratum from the subject population. During the screening and inclusion process, our study staff had teach to every included subject the procedure how to collect mid-stream urine, but different education stratum can lead to misunderstand which is unavoidable for this study. Difficulties of tracking previous treatment given are also limitation during this study, which could interfere to bacterial distribution pattern, as reported in previous study (Sakai *et al.*, 2007; Muratani *et al.*, 2013; Yasutake *et al.*, 2013; Agpaoa *et al.*, 2015).

CONCLUSION

In microbiology aspect, quantitative bacterial count and WBC count of urine flow cytometry can be useful to estimate the success of culture-proven UTI, which can help in clinician decision-making and better patient management. Furthermore, cut-off of bacterial count and WBC count should be considered and adjusted properly, depending on the clinical population settings.

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