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Predict Urinary Tract Infection and to Estimate Causative Bacterial Class in a Philippine Subspecialty Hospital

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Abstract

Objective: Urinary tract infection diagnosis by urine culture can be time- and labor- consuming. In the National Kidney and Transplant Institute, up to 75% of urine culture samples sent to the laboratory yield no growth or insignificant growth. A rapid and reliable screening method to rule out bacterial UTI could reduce culture workload, turnaround time of negative results and also unnecessary antibiotic prescription. Sysmex UF-1000i is urine flow cytometry analyzer which is capable of quantifying urine particles including bacteria and leukocytes. We evaluated the UF-1000i performance for ruling out UTIs and its bacterial scattergram feature to estimate the causative bacterial group, using standard urine culture results as reference method.

Methods: 293 urine samples were analyzed using Sysmex UF-1000i analyzer and compared with urine culture results. Bacterial cluster distribution in the bacterial scattergram of positive cultures was also analyzed for causative bacterial class estimation.

Results: Out of 293 urine samples, 104 (35.5%) samples had bacterial growth of more than 10^3 CFU/ml on culture and most common organism isolated was Escherichia coli (28.8%). The optimum bacterial cut off value of 55/µl and/or leukocytes cut-off value of 27/µl were observed using ROC analysis to rule out UTI. The bacterial scattergram analysis showed 82.7% concordance with the results of the urine culture, with only 5.8% discordant results, and 11.5% had results showing non-specific or wide distribution patterns near the 30° angle.

Conclusions: Urine samples can be routinely screened for UTI using the Sysmex UF-1000i, and this can improve the overall turnaround time of negative results and reduce laboratory culture workload..

Keywords: Automated urinalysis; Bacterial scattergram; Causative organism; Urinary tract infection; Urine flow cytometer

Introduction

Urinary tract infection (UTI) is a common medical problem and may range from asymptomatic bacteriuria to severe infection that can lead to serious complications when left untreated. Women are more likely to be affected than men [1]. Specific subpopulations are at increased risk of UTI, including infants and children, pregnant women, diabetic and immunocompromised patients, the elderly and those with underlying urologic abnormalities [1]. Thus, prompt diagnosis is important for proper and timely management.

Urine culture is considered to be the gold standard to diagnose UTI. However, the performance of urine culture is laborious and time consuming, with results usually released after 3 to 4 days. Moreover, studies have proven that up to 80% of urine samples subjected to urine culture are reported negative for bacteria which make urine culture less cost-effective [2].

Several other methods have been used to evaluate urine for the presence of leukocytes or pyuria to screen for UTI. The dipstick leukocyte esterase test is a rapid and inexpensive test that detects esterase, an enzyme released by white blood cells. However, this test has low sensitivity and a high false-positivity since this can be affected by contamination, often by vaginal secretions [3]. Microscopic examination of urine samples, on the other hand, is used to look for formed cellular elements, casts, bacteria, yeast, parasites and crystals. However, the procedure is time consuming and the results of this method of detection vary with each observer [2].

Sysmex urine flow cytometer UF-1000i (Sysmex Corporation, Kobe, Japan) uses a red semiconductor laser that can quantitatively measure red blood cells (RBC), white blood cells (WBC), epithelial cells, casts and bacteria in non-centrifuged urine in 72 seconds. The instrument has separate sediment and bacteria channels and uses a single polymethine dye for both counting channels. Specific diluents dissolve disturbing salts in the reaction chamber of the sediment channel and allow sensitive staining of urinary formed elements. In the reaction chamber of the bacteria channel, specific diluents also prevent non-specific staining of non-bacterial particles (e.g. red blood cells and casts) by lysing, while the polymethine dye stains bacterial nucleic acids. This provides an accurate quantitative bacterial count without interference of cell debris. The stained particles pass through the center of the fluidic stream one by one at a high velocity. As the stained urine particles cross the laser beam, fluorescent light is emitted and the signals are analyzed according to appropriate particle types. Particle characterization and identification are based on detection of fluorescence (Fl), forward-scatter light (Fsc) and side-scatter light (Ssc) and are graphically supplemented with scattergrams. At the medical laboratory of the National Kidney and Transplant Institute (NKTI), a tertiary subspecialty hospital for renal diseases in the Philippines, urine samples account for majority of the specimens analyzed for routine

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tests, especially among UTI suspected cases. Quantitative evaluation and bacterial identification by urine culture is usually used to diagnose UTI, however, only about 25% of urine samples yield positive results. An accurate and reliable screening method that readily detects negative urine samples is needed to avoid unnecessary culture tests and to minimize total expenditure on urine sample processing. In order for a true negative urine sample to be correctly classified as such, the screening method should have a high sensitivity and negative predictive value. This can translate in improved detection efficiency and reduction of laboratory costs. The rapid reporting of negative results can also aid the clinicians in prompt and proper management.

Various studies have been reported using UF-1000i as a screening tool for negative UTI results and different cut-off values have been purposed for UTI detection [4-6]. No study has been reported till now to establish optimum cut-off values for the Philippine population.

Thus, the study was designed to determine the utility of Sysmex UF-1000i in the tertiary sub-specialty hospital as a rapid screening test in predicting negative UTI. The study also aimed to determine optimum cut-off values for leukocytes and bacteria to select urine samples for bacterial cultures, and to evaluate if the distribution of the bacteria cluster on the scattergram can presumptively differentiate bacteria as gram-positive or gram-negative as reported previously [7,8].

Materials and Methods

From October 2013 to December 2013, we prospectively studied 293 urine samples submitted for urine culture to the Clinical Microbiology Section of the NKTI Medical Laboratory. Both male and female non-transplant out-patients from 18 to 80 years of age were included in the study. Post-transplant patients, patients with urinary diversions, orthotopic neobladder with bowel segments, indwelling urinary catheters, stents and other foreign body devices inserted in the urinary bladder and along the urinary tract, and urine samples obtained via suprapubic bladder aspirate were excluded, as well as patients with current history of antibiotic use. The study was reviewed and approved by the NKTI Technical Review Board and written consent and pertinent clinical data were obtained using an informed consent form (IFC) and questionnaire approved by the NKTI Research Ethics Committee.

Two hundred and ninety-three clean-catch mid-stream urine samples collected in a wide-rimmed preservative-free sterile container were processed within thirty minutes after collection. Both male and female participants were given instructions on proper urine collection. The urine samples were first subjected to urine culture according to the current Microbiology section work instruction manual to avoid contamination by manipulation. After gentle inversion, 1 µL (0.001 ml) of urine was inoculated onto 1/3 of a blood agar plate (BAP) by making a straight line down for primary streak and making a series of uninterrupted streak procedure using a calibrated loop; and another 1 µL of the same sample was inoculated to 1/3 of a MacConkey agar plate (MAC) by interrupted streak procedure. Both agar plates were incubated at 35-37°C for 18-24 hours, then colony counting was carried out and expressed as number of colony forming units (CFU)/ml. The culture plates were interpreted based on the current Clinical and Laboratory Standards Institute (CLSI) guidelines. A culture was considered positive if there is growth of more than 10^2 CFU/ml in a symptomatic female, 10^3 CFU/ml in a symptomatic male or 10^5 CFU/ml in voided urine of an asymptomatic patient. A sample was considered negative for UTI if there was no growth or there was $<10^3$ CFU/ml bacterial growth, which is considered as insignificant growth. For a positive urine culture, isolated microorganisms were identified using conventional biochemical procedures. *Lactobacillus*, diphtheroids, *Bacillus* species, other types of coagulase negative *Staphylococci* and *Streptococcus viridans* group isolates were considered to be contaminants [9] and were excluded. Specimens yielding mixed growth of gram-positive and negative bacteria, fungi and mycobacteria were also excluded.

The following bacteria were considered to be uropathogens: *Enterobacteriaceae*, non-fermentative gram-negative bacilli, *Staphylococcus saprophyticus*, *Enterococcus* species, *Staphylococcus aureus*, Group A, B, C or G *Streptococci*, *Streptococcus pneumoniae*, *Corynebacterium urealyticum* or *Aerococcus* species (i.e. >65 years of age) and yeasts [9].

Within 10 minutes after inoculation of the culture, the urine samples were transferred to the Clinical Microscopy section and were immediately analyzed using the urine particle analyzer Sysmex UF1000i (quantitative) and bacterial and leukocytes counts were obtained. Manual microscopic examination was done to verify abnormal samples flagged by the laboratory's flagging criteria for pathological casts, yeast-like organisms, sperm, etc. The bacterial scattergrams of urine samples with positive urine cultures were printed and their angle of bacterial distribution were classified as having greater than, less than or wide angle distribution in reference to the 30° angle [8].

Data analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for different cut-off values for leukocyte and bacterial counts were calculated using the urine culture as the reference. Receiving operator characteristics (ROC) curves for bacteria and leukocytes were plotted to assess the cut-off values using the SPSS 19.0 software. The Area Under the ROC (AUROC) curves were also computed. The number of urine cultures that could have been avoided using the optimum cut-off values was determined. Bacterial scattergram results were analyzed using descriptive statistics.

Results

There were 189 female and 104 male participants in the study. Among the 293 urine samples, 104 (35.5%) were positive for bacterial growth of more than 10^3 CFU/ml in symptomatic patients and more than 10^5 CFU/ml in asymptomatic patients, 130 (44.4%) were negative and 59 (20.1%) were excluded due to growth of contaminants, polymicrobial growth (>3 microorganisms) and fungi. The majority of the positive urine samples (73/104; 70.2%) and excluded samples that yielded contaminants, polymicrobial infection or yeasts (54/59; 91.5%) were from female participants. Seventy-seven percent (80/104) of the positive cultures yielded gram-negative bacteria and 24 (23%) grew gram-positive bacteria. *Escherichia coli* was the most common organism isolated (28.8%), followed by *Staphyloccocus spp.* (13.5%), *Klebsiella pneumoniae* (9.6%), *Escherichia coli*-ESBL producer (7.7%), *Klebsiella pneumonia*-ESBL producer (3.8%), *Acinetobacter lwoffii* (2.9%), *Enterococcus faecalis* (2.9%), *Klebsiella* and *E. coli* co-infection (2.9%) and *Pseudomonas aeruginosa* (2.9%) (Table 1).

The receiving operator characteristic (ROC) curves for leukocytes and bacterial counts are shown in Figure 1. The area under the ROC (AUROC) curve of the UF-1000i for bacterial count (0.918) was higher than the AUROC for the leukocytes count (0.808) when greater than 10^3 CFU/ml was considered as the standard for a positive urine culture (Table 2). Tables 3 and 4 lists the summary of the sensitivities,

Bacteria	Frequency
Escherichia coli	30 (28.8%)
Staphylococcus spp.	14 (13.5%)
Klebsiella pneumonia	10 (9.6%)
Escherichia coli (ESBL)	8 (7.7%)
Klebsiella pneumoniae (ESBL)	4 (3.8%)
Acinetobacter lwoffii	3 (2.9%)
Enterococcus faecalis	3 (2.9%)
Klebsiella pneumoniae, Escherichia coli	3 (2.9%)
Pseudomonas aeruginosa	3 (2.9%)
Enterobacter aerogenes	2 (1.9%)
Enterococcus spp.	2 (1.9%)
Klebsiella ozanae	2 (1.9%)
Streptococcus agalactiae	2 (1.9%)
Acinetobacter baumanii, Serratia marcescens	1 (0.96%)
Enterobacter agglomerans	1 (0.96%)
Enterobacter agglomerans (AmpC+) , Escherichia coli	1 (0.96%)
Escherichia coli (AmpC+)	1 (0.96%)
Escherichia coli, Enterobacter aerogenes (AmpC+)	1 (0.96%)
Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae	1 (0.96%)
Escherichia vulneri	1 (0.96%)
Klebsiella ozanae (AmpC+)	1 (0.96%)
Klebsiella pneumoniae (AmpC+)	1 (0.96%)
Klebsiella pneumoniae (ESBL), Escherichia coli (ESBL)	1 (0.96%)
Proteus mirabilis, Escherichia coli	1 (0.96%)
Proteus vulgaris	1 (0.96%)
Pseudomonas aeruginosa (AmpC+)	1 (0.96%)
Pseudomonas aeruginosa (AmpC+), Escherichia coli (ESBL)	1 (0.96%)
Shigella sonnei	1 (0.96%)
Staphylococcus aureus (MRSA)	1 (0.96%)
Staphylococcus aureus, Gamma streptococci	1 (0.96%)
Staphylococcus saprophyticus	1 (0.96%)
Total	104

Table 1: List of Bacteria found in 104 Urine Cultures

specificities, PPV and NPV of different cut off values for the bacterial and leukocytes counts, respectively.

The optimum cutoff value for the bacterial count was 55/µl on the ROC curve, achieving a sensitivity of 94.2% and specificity of 72.3%, PPV of 77.3% and NPV of 92.6%. With this cut off, 39% of unnecessary cultures could be avoided with a 2.56% false-negative rate.

The cutoff value for the leukocytes count, on the other hand, was 27/µl on the ROC curve with a sensitivity of 84.6%, specificity of 65.4%, PPV of 71% and NPV of 81% and allows the elimination of 40.4% of unnecessary cultures but with a 6.84% false negative rate.

If we consider the bacterial count and leukocytes count cut-off values together, there was an increase in sensitivity to 95.2%, specificity 82.3%, PPV 81.2%, NPV 95.5% and the number of unnecessary cultures that could be avoided improved to 43.6% with only a 2.14% false negative rate.

The bacterial scattergram of the 104 urine samples with positive cultures revealed that 86 (82.7%) scattergram results were in agreement with the results of the urine culture, while 6 (5.8%) results were discordant. Seven (6.7%) cases had a wide distribution pattern or had dots distributed above and below the 30° reference angle and 5 (4.8%) cases showed scattered, non-specific bacterial distribution. Examples

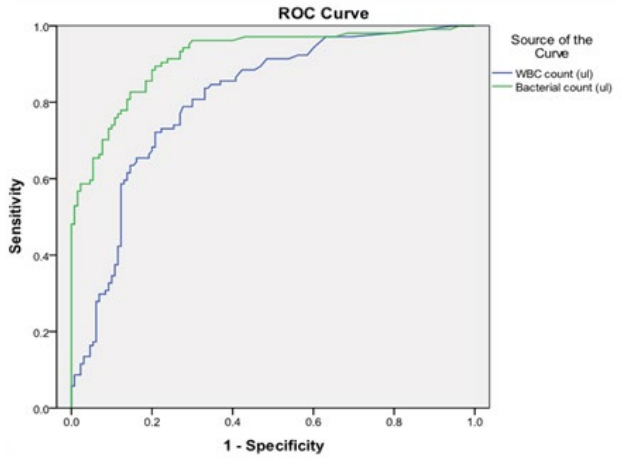

Figure 1: ROC Curve of Bacterial and WBC Count

Test Result Variable	Area	Standard Error	p-value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
WBC (µl)	0.808	0.029	0	0.752	0.864
BAC (µl)	0.918	0.018	0	0.882	0.954

Table 2: Area Under the Curve (AUC) for Bacteria (BAC) and White Blood Cell (WBC) Counts

of the different bacterial distribution patterns are shown in Figure 2. Among the concordant results, 69 (66.3%) samples had bacterial distribution of less than 30° on the scattergram and grew gram-negative bacteria on culture and 17 (16.3%) samples had a bacterial distribution of greater than 30° and yielded gram positive bacteria on culture. Of the 6 discordant results, 4 samples showed bacterial distribution with an angle less than 30° but yielded *Staphylococci spp,* with one case yielding *S. aureus (MRSA),* while 2 samples both had bacterial distribution of more than 30° but grew gram-negative bacteria, one grew *E. coli* and the other yielded a co-infection of *Klebsiella pneumonia*e (ESBL) and *E. coli* (ESBL) (Table 5).

Discussion

Urinary tract infection is a common disease that requires prompt diagnosis and treatment. Unfortunately, urine culture, the gold standard for the diagnosis of UTI, is time-consuming, expensive and often yields negative results. This not only affects physicians' decision making and patients' treatment, but laboratory workload and management as well. The new generation fully automated Sysmex UF-1000i urine analyzer allows a more accurate analysis of RBC, leukocytes, bacteria, epithelial cells and casts in urine in a very short time, and can thus contribute to the screening of UTI [7] in contrast to other urine screening methods such as the dipstick leukocyte esterase test and urinary sediment microscopy which show varied and inconsistent results [2]. In this study, we explored the utility of the automated urine particle analyzer Sysmex UF-1000i as a rapid screening tool for UTI and investigated the ability of its bacterial scattergram to predict bacterial class in order to help physicians in choosing the appropriate empiric treatment.

The study had more female subjects diagnosed with UTI compared to males [1]. This can be attributed to the short urethra in females and its proximity to the external female genitalia and anus. Furthermore, the women in this study are within the reproductive age group and

Bacterial Cut-off (µl)	Sensitivity or True Positive(TP)	Specificity or True Negative(TN)	False Negative(FN) = 1-Sensitivity	False Positive(FP) = 1-Specificity	PPV	NPV	Reduction of Cultures
-1.0	1.000	0.000	0.000	1.000	0.500	1.000	0.000
10.5	0.971	0.462	0.029	0.538	0.643	0.941	0.245
25.0	0.962	0.677	0.038	0.323	0.749	0.946	0.358
55.0	0.942	0.723	0.058	0.277	0.773	0.926	0.390
85.0	0.913	0.762	0.087	0.238	0.793	0.898	0.424
103.0	0.885	0.792	0.115	0.208	0.810	0.873	0.454
213.0	0.808	0.854	0.194	0.146	0.847	0.816	0.523
301.5	0.769	0.885	0.231	0.115	0.870	0.793	0.558
400.0	0.702	0.923	0.298	0.077	0.901	0.756	0.611
528.0	0.654	0.931	0.346	0.069	0.904	0.729	0.638
737.0	0.625	0.946	0.375	0.054	0.921	0.716	0.661
1019.0	0.587	0.977	0.413	0.023	0.962	0.703	0.695
1461.0	0.519	0.992	0.481	0.008	0.985	0.674	0.737
2139.0	0.481	1.000	0.519	0.000	1.000	0.658	0.760

Table 3: Summary of Sensitivity, Specificity, Negative Predictive Value, Positive Predictive Value and Reduction of Cultures of UF-1000i according to bacterial count cut-off values in 234 specimens

WBC Cut-off (µl)	Sensitivity or True Positive(TP)	Specificity or True Negative(TN)	False Negative(FN) = 1-Sensitivity	False Positive(FP) = 1-Specificity	PPV	NPV	Reduction of Cultures
-1	1	0	0	1	0.5	1	0
10.5	0.913	0.5	0.087	0.5	0.646	0.852	0.293
22.5	0.856	0.615	0.144	0.385	0.69	0.81	0.38
27	0.846	0.654	0.154	0.346	0.71	0.81	0.404
50.5	0.76	0.731	0.24	0.269	0.738	0.752	0.486
105	0.654	0.823	0.346	0.177	0.787	0.704	0.585
200	0.596	0.869	0.404	0.131	0.82	0.683	0.637
322.5	0.481	0.877	0.519	0.123	0.796	0.628	0.698
422	0.423	0.885	0.577	0.115	0.786	0.605	0.731
500	0.375	0.892	0.625	0.108	0.777	0.588	0.759
712.5	0.327	0.908	0.673	0.092	0.78	0.574	0.79
1012	0.298	0.931	0.702	0.069	0.812	0.57	0.816
1581	0.173	0.938	0.827	0.062	0.738	0.532	0.883
2013.5	0.144	0.954	0.856	0.046	0.758	0.527	0.905

Table 4: Summary of Sensitivity, Specificity, Negative Predictive Value, Positive Predictive Value and Reduction of Cultures of UF-1000i according to WBC count cut-off values in 234 specimens

Bacterial distribution on bacterial scattergram	Positive Urine Culture (n=104)	
	Gram Positive Bacterial Growth	Gram Negative Bacterial Growth
Greater than 30° angle	17	2
Less than 30° angle	4	69
Wide distribution pattern	2	5
Non-specific distribution pattern	3	2
TOTAL	26	78

Table 5: Summary of Sensitivity, Specificity, Negative Predictive Value, Positive Predictive Value and Reduction of Cultures of UF-1000i according to WBC count cut-off values in 234 specimens

may be sexually active or pregnant, making them more susceptible to UTI [1].

Setting a low bacterial colony growth (>10^3 CFU/ml) for the diagnosis of UTI is important in our hospital setting to avoid exclusion of critical patients (i.e. pregnant, immunocompromised, recurrent UTI patients) for whom a low colony count is considered significant [4]. The cut-off values set for bacteria and leukocytes counts of 55/µl and 27/µl, respectively, were set as such, taking in consideration the prerequisite of a good screening test which has to have a high sensitivity and negative predictive value, without compromising specificity. The total number of cultures that could be eliminated using these cut-off values was also considered, such that false-negative rates obtained were acceptable for our laboratory. The high sensitivity and negative predictive values of bacterial counts of the UF-1000i in this study are comparable with values obtained by other laboratories around the world [5-7,10, 11]. However, the specificity obtained was lower compared to the studies of Wang et al. and Broeren et al. who set higher bacterial cut-off values, >100/µl and 230/µl, respectively [5,6].

On the ROC analysis, the UF-1000i showed better AUROC for bacteria than leukocytes. This can be due to the efficiency of the dedicated bacteria channel of the analyzer that allowed the exclusion of other cell fragments, debris and mucus from the urine particles.

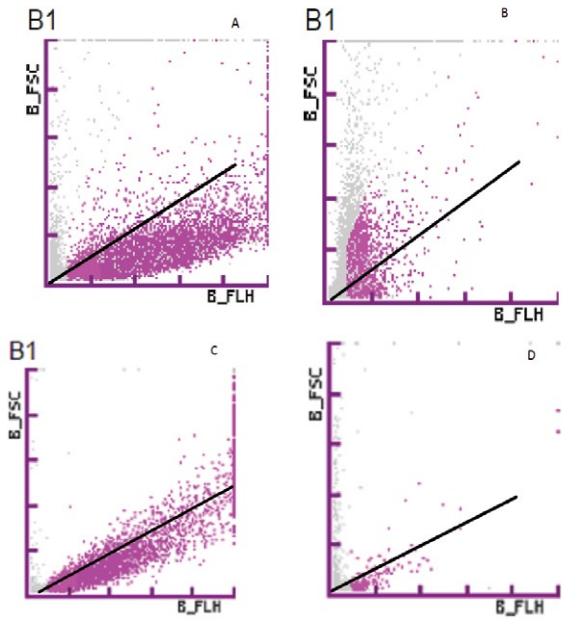

Figure 2: Examples of Different Bacterial Distribution Patterns in the Bacterial Scatter gram: A. Less than 30° angle; B. More than 30° angle; C. Wide distribution pattern; D. Non-specific distribution pattern

- B_FSC: Forward scattered light intensity (size)
- B_FLH: Fluorescent light intensity (depth of staining)

The study had a lower sensitivity, specificity, PPV and NPV values obtained from the set leukocytes cut-off value compared to that of the bacterial count. According to the study by Foxman in 2003, the presence of leukocytes in the urine may not be a reliable indicator of UTI alone as this could be due to factors, such as presence of female genital tract infections and persistence of leukocytes after antibiotic treatment of UTI [1], and this could explain why some patients had a high leukocytes count but the culture results was negative. However, by collectively using the bacteria and leukocyte cut-off values, the specificity and PPV of the instrument significantly improved and more unnecessary urine cultures (43%) can be eliminated with less than 3% false negative rate, which is acceptable for our hospital's out-patient non-transplant population.

We observed that most of the gram-positive bacteria tend to be distributed above 30° and that gram-negative bacterial distribution is noted below 30° in the bacteria scattergram, further supporting the aptness of setting the reference angle at 30° [8]. There were 4 samples noted with bacterial distribution below the 30° angle but showed *Staphylococci* growth including 1 case of *S. Aureus* (MRSA) growth, while the other 2 samples with bacterial distribution above the 30' angle showed growth of *E. coli* and a co-infection of two gram-negative ESBL producing organisms. Also, the 7 samples that showed a wide-angle distribution pattern near the 30° angle yielded *Enterococcus faecalis, Staphylococcus saprophyticus, Streptococcus agalactiae, Shigella sonnei, Acinetobacter lwoffii, Escherichia vulneri* and *AmpC+ Klebsiella ozonae*. The discordance in results and the wide distribution pattern in these cases may be caused by varied reasons as observed in different studies [8,12]. Several studies have shown that *Enterococcus spp.* may have bacterial distribution patterns similar to gram-negative bacteria due to their formation of short chains [12]. Previous antibiotic treatment and probable development of resistance are also seen to affect bacterial distribution due to filament formation in gram-negative bacilli [8]. Furthermore, the causative bacteria mentioned are commonly observed among complicated and recurrent UTI patients. It is therefore suggested that in patients whose bacterial scattergram show a wide distribution pattern, the presence of complicated UTI should be considered and urine culture is warranted. Further investigations on the factors causing the wide distribution pattern is also suggested. Meanwhile, five samples with a non-specific bacterial distribution had low colony counts (10^3-10^4 CFU/ml) on culture and low bacteria counts (less than 55/µl) determined by the UF-1000i that resulted in insufficient number of dots to be analyzed.

Not all laboratories offer bacterial culture in Philippines, and if they do, the results of the urine culture are usually available only after 2-5 days from the time of collection. In the majority of cases, the physician at the out-patient department already prescribes empiric antibiotics in patients with signs and symptoms of UTI even before culture results are seen. The utility of the fully automated UF-1000i in rapidly screening urine samples and predicting positive urine culture outcomes with results available within the same day of collection allows the physician to obtain reproducible and reliable results which can help avoid costly and unnecessary laboratory tests and the prescription of unnecessary antibiotics. Moreover, the laboratory is able to reduce culture workloads and laboratory costs by concentrating manual efforts in true positive samples, thus improving workflow and efficiency.

Conclusion

Analysis of bacterial counts and/or leukocytes counts generated by the UF-1000i may be a useful screening method to exclude UTI and reduce laboratory workload and unnecessary urine cultures. In our setting, we suggest the use of the bacterial count of 55/µl and/or leukocytes count of 27/ µl in order to eliminate 43.6% of unnecessary

cultures. Also, the use of bacterial scattergram allows a rapid and accurate estimation of the causative organism that can be a helpful guide in the selection of empiric antimicrobial treatment for UTI.

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