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Research Article

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Full Length Research Paper

Evaluation of Sysmex UF-1000i and Alifax Alfred and HB&L in rapid diagnosis of acute urinary tract infections in a hub and spoke setting

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This paper compares, in a hub and spoke (H&S) setting, the performance of two automated methods in rapid diagnosis of urinary tract infections (UTI). 2335 midstream urine samples obtained from adult patients were considered. In the spoke laboratory, rapid diagnosis of UTI was performed by using bacteria quantification with a Sysmex UF-1000i analyzer. In the hub laboratory, rapid diagnosis of UTI was performed by using Alifax Alfred and HB&L analyzer. Moreover, in the hub laboratory, a quantitative culture was performed in all samples. Using UF-1000i with a cut-off at 175 bacteria/µl, sensitivity was (SE) 0.95, specificity (SP) 0.80, negative predictive value (NPV) 0.98, positive predictive value (PPV) 0.64, and diagnostic accuracy (DA) 0.84. Using Alifax Alfred and HB&L with a cut-off at 30000 bacteria/ml, SE was 0.99, SP 0.99, NPV 0.99, PPV 0.98 and DA 0.98. In an H&S setting, UTI screening with UF-1000i is acceptable for routine applications. In our setting, after implementation of an UF-1000i based UTI screening, the number of bacterial cultures was thought to be reduced to 50%. Therefore, using the Alifax Alfred and HB&L system, with a higher SP, it was assumed that there is need to carry out further urine microbiological tests, allowing to perform reliable samples of about 70%. Another relevant positive aspect may be the availability of the negative results within 9 to 10 h after samples collection.

Key words: Bacteriuria, rapid diagnosis, urinalysis, urinary tract infection.

INTRODUCTION

Urinary tract infections (UTI) are second in frequency only to upper respiratory tract infections (Linhares et al., 2013; Nicolle, 2013). However, the request for microbiological examination of urine samples exceeds those for detection of respiratory pathogens and urine culture is the commonest microbiological test in diagnostic laboratories (Schifman et al., 1984; Wu et al., 1985). Thus, diagnosis and management of urinary tract infection represent a significant burden for the Italian National Health Service. The “gold standard” for
diagnosis is still bacterial culture, but a large proportion of samples are negative, microbiological tests are time and resources consuming, and also the results, although negative, are available no earlier than 24 h after sample collection. So, many rapid tests were proposed in these years to avoid unnecessary cultures. Assays used for rapid diagnosis of UTI can be based on direct detection of bacteria and leukocytes by microscopic observation or automated instrumentation, or on the detection of metabolites related to the presence of leukocytes (that is, esterase) or bacteria (that is, nitrites). Alternatively, cultural tests with detection of bacterial growth kinetics in liquid medium are available (Kass, 1956; Lipsky et al., 1987; Pappas, 1991; Pezlo, 1988, 1992; Wu et al., 1985).

In Italy, only small laboratories do not use a rapid method of screening in diagnosis of UTI. Laboratories with increased workload use screening methods based on the identification of bacteria and leukocytes with equipment dedicated to the analysis of the corpuscular fraction of urine (Sysmex UF-1000i, Iris iQ200, sediMAX/UriSed, etc.), or in alternative, rapid cultural methods in liquid medium which detects the kinetics of bacterial growth (UroQuick, Alfred and HB&L, etc). Considering non cultural test for rapid diagnosis of UTI, in literature, there are data supporting the possibility to perform a rapid and affordable diagnosis of UTI by using flow cytometry and quantitative evaluation of bacteria and leukocytes in urine samples (Broeren et al., 2011; Koken et al., 2002; Munoz-Alagarra et al., 2013; Yasuma et al., 2012). In previous reports, it was suggested that, using a Sysmex UT-1000i cytometer, quantification of urine bacteria alone without quantification of leukocytes may be sufficient for a rapid diagnosis of UTI (Manoni et al., 2009). Considering cultural test for rapid diagnosis of UTI in Italy, the system Alifax Alfred and HB&L is largely diffused (Ballabio et al., 2010; Ilik et al., 2010). This system was used also for non urinary samples (Barocci et al., 2010; Cavallaro and Squarzon, 2009; Fontana et al., 2009; Tessari et al., 2010).

In the Venetian area, in the time elapsed from our initial reports (Manoni et al., 2009), many changes in our operativity were introduced: adoption of new collection systems with possibility to perform samplings with vacuum tubes and centralization of microbiological diagnostics following a Hub and Spoke model, with the need to take tubes added with preservatives.

The hub and spoke model for microbiology laboratories is not derived from a technical choice, but it was a political and administrative decision that has forced the closure of some sections of microbiology in peripheral hospitals and the gradual centralization of diagnostics in tertiary care hospitals. The technical component, had only the task and the ability to assess the impact of this decision on the diagnosis and to choose the way of change management best suited to different local realities. For example, in this paper we considered primarily transports (there are 50 kilometers between hub and spoke) and after the costs because the two hospitals belong to two different administrative entities (Local Health Authority). Last but not least, it is very important for us to make available the negative reports on the same day of collection of the samples to avoid unnecessary antibiotic therapies.

This paper therefore has two purposes: to validate our new operating procedure and to compare results obtained using Sysmex UF-1000i (performed in the spoke laboratory) and Alifax Alfred and HB&L in comparison with urine culture test (both performed in the hub laboratory) for rapid diagnosis of UTI.

MATERIALS AND METHODS

Patient’s selection

2335 consecutive samples of midstream urine collected in Chioggia were considered. For outpatients, general practitioners and patients were aware of correct procedures for urinary samples collection, and together with the sterile container, written instructions showing the correct methods for sample collection for midstream urine culture were handed to patients.

For inpatients, only samples obtained from adult subjects were considered, sterile containers and written instruction were available; moreover, nurses were trained about the importance of preanalytical phase in laboratory assays (Manoni et al., 2011).

Samples collection and transport conditions

These urine samples were obtained by using clean catch midstream technique. All these samples were collected in a sterile container of capacity of about 100 ml, equipped with screw cap and an integrated system for sampling the vacuum tubes (Vacutest Kima, Arzergrande, PD, Italy). Each sample was divided into two sterile tubes with borate as preservative. The first tube was shipped to the hub laboratory in Mestre (about 50 km from Chioggia), for Alifax Alfred and HB&L screening and for urine culture; in this laboratory, screening tests and culture were performed within 8 h from samples collection. Transport of samples between spoke laboratory and hub laboratory was performed using adiabatic containers with eutectic plates, at a temperature between 4 and 10°C with tracking temperature and time of transport. The second tube was stored at room temperature (20 to 24°C) in the spoke laboratory. Analysis with Sysmex UF-1000i was performed within 4 h from samples collection (Broeren et al., 2011; Manoni et al., 2009; Munoz-Alagarra et al., 2013).

Quantitative culture

All samples were manually dispensed in a BD CHROMagar Orientation Medium (Becton Dickinson GmbH, Heidelberg, Germany) and the colonies identified with Vitek2 (bioMerieux, Marcy-l’Etoile, France). With the same system, the antimicrobial susceptibility tests of the growth bacteria were performed (Chatzigeorgiou et al., 2011).

Sysmex UF-1000i

Sysmex UF-1000i analyzes 1.0 ml of sample in batch by combining flow cytometry with fluorochrome (polymethine dyes) and impedance
analysis. The results are available in 1 min for one sample. The application of these technologies allows the discrimination and quantification of bacteria, erythrocytes, leukocytes, epithelial cells, casts, crystals, fungi, etc., in urine samples (Broeren et al., 2011; Manoni et al., 2009; Munoz-Alagarr et al., 2013).

Alifax Alfred and HB&L (Alifax, Polverara, PD, Italy)

It is a system based on a light-scattering technique that detects reliably microbial growth in fluid samples, providing real-time growth curves and bacterial counts as colony forming units/milliliter (CFU/ml). Alifax Alfred unit dispenses 500 µl of sample into vials containing eugonic broth constantly stirred and thermostated (37°C). After dispensation, the vials are incubated in HB&L unit. During incubation, every 5 min, a laser beam passes through the vials allowing detection of changes in turbidity. Light scatter was collected by two detectors (at 30 and 90°), processed and displayed graphically. Bacterial growth detected by the instrument is then determined exclusively by live and replicants bacteria (Ballabio et al., 2010; Ikki et al., 2010).

Screening assays comparison

Data obtained with Sysmex UF-1000i and Alifax Alfred and HB&L were compared with those obtained in culture. A sample was considered culture-positive if it contained a pure culture of ≥10^5 CFU/ml, and when pure growth or predominant growth of one or two organisms were observed, unless diptheroids or Lactobacillus species were identified. More than two different organisms growing in equal numbers was interpreted as contamination (negative for pathogens) (Aspevall et al., 2001; ECLM, 2000).

Statistical analysis

Statistical evaluation of obtained results was performed with parametric and nonparametric methods, as appropriate. Cohen's correlation coefficient was evaluated too. Methods' diagnostic performance was evaluated by determining sensitivity (SE), specificity (SP), positive predictive value (PPV), and diagnostic accuracy (DA). The evaluation of statistical data was carried out with the software Analyze-it (release 2.20). ROC curves were obtained by plotting Sensitivity and Specificity; the best cut-off value was calculated using the Youden index ((SE+SP)-1).

RESULTS

Samples

2335 consecutive samples collected from adult patients between 15 and 91 years old in Chioggia were considered. Of these, 989 (41%) were collected from inpatients and 1346 (59%) from outpatients; 801 samples (34%) came from males and 1534 (66%) from females. Analyzing the distribution of samples based on age, different frequency peaks were observed, particularly from 20 to 45 and 60 to 85 years old patients.

Microbiological examination

A total of 1695 (72%) samples were negative at urine culture (no bacterial growth or a bacterial count < 10^5 CFU/ml), 42 (2%) were considered contaminated because they showed 3 or more types of colonies without any dominant species, and 598 (26%) were positive (with a bacterial count ≥10^5 UFC/ml). The analysis of positive samples stratified by sex showed a frequency of positivity of 33% in samples from female subjects and 21% in samples from male subjects. In the considered population, the pre-test possibility of disease or prevalence of UTI was 26%. The prevalence of observed bacteria strains is reported as shown in Table 1.

UF-1000i

In analysis of Sysmex UF-1000i results, the following bacteria counts were considered: 19, 130 175 and 345 µl-1 corresponding, respectively to performance sensitivity equal to 100, 99, 95, and 90% (these data are reported as shown in Table 2). As shown in Figure 1, the ROC curve is reported. Considering that a screening system in the diagnosis of urinary tract infections should ensure the best performance in terms of sensitivity and negative predictive value; the optimal cut-off for counting bacteria UF1000i appears to be 175 bacteria/µl in order to ensure effective recovery of diagnostic specificity with a minimal loss in specificity and this cut-off value ensure the better Youden index (0.75). By using a cut-off at 175 bacteria/µl in this patients' series, a sensitivity of 0.95, specificity of 0.80, negative predictive value of 0.98, positive predictive value of 0.64, diagnostic accuracy of 0.84 and the Youden index of 0.75 were observed. By using this experimental cut-off, we observed 22 false negative (FN).

These FN results were mainly represented by Gram positive bacteria: Enterococcus faecalis (5), Staphylococcus species (5), Streptococcus agalactiae (4), Yeast: Candida species (3), and various (5) slow-growing Gram negative isolates: Acinetobacter species (4) and Stenotrophomonas maltophilia (1).

Alifax Alfred and HB&L

In analysis of Alifax Alfred and HB&L results were considered the following bacteria counts: 10000, 20000, 30000, and 50000 ml^{-1}, respectively to performance sensitivity equal to 100, 99, 95, and 90% (these data are reported as shown in Table 3). In Figure, 2 the ROC curve is reported. In this study, a cut-off value at 30000 CFU/ml after 3 h of incubation was adopted. Using these cut-off values, in these patients' series, an SE of 0.99, SP of 0.98, NPV of 0.99, PPV of 0.95 and a DA of 0.98 were observed and the Youden index was 0.97. These results were reported as shown in Table 3. By using this experimental cut-off, four false negative were observed: Candida species (2) and slow-growing Gram negative bacteria (2); Acinetobacter spp. caused the four false
Table 1. Prevalence of bacteria strains observed in this patients series.

<table>
<thead>
<tr>
<th>Bacteria strains</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>58.90</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>7.10</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>5.50</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>4.90</td>
</tr>
<tr>
<td><em>Proteus</em> spp.</td>
<td>4.20</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>3.20</td>
</tr>
<tr>
<td><em>Serratia</em> spp.</td>
<td>2.50</td>
</tr>
<tr>
<td><em>Providencia</em> spp.</td>
<td>2.10</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp.</td>
<td>2.10</td>
</tr>
<tr>
<td><em>Candida</em> spp.</td>
<td>2.10</td>
</tr>
<tr>
<td>Others</td>
<td>6.30</td>
</tr>
</tbody>
</table>

Table 2. Evaluation of Sysmex UF-1000i performance at different cut-off values for bacteria.

<table>
<thead>
<tr>
<th>BACT/µl</th>
<th>SE</th>
<th>SP</th>
<th>NPV</th>
<th>PPV</th>
<th>DA</th>
<th>Youden index</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>1.00</td>
<td>0.37</td>
<td>1.00</td>
<td>0.41</td>
<td>0.54</td>
<td>0.41</td>
</tr>
<tr>
<td>130</td>
<td>0.99</td>
<td>0.75</td>
<td>0.99</td>
<td>0.59</td>
<td>0.81</td>
<td>0.73</td>
</tr>
<tr>
<td>175</td>
<td>0.95</td>
<td>0.80</td>
<td>0.98</td>
<td>0.64</td>
<td>0.84</td>
<td>0.75</td>
</tr>
<tr>
<td>345</td>
<td>0.90</td>
<td>0.84</td>
<td>0.96</td>
<td>0.72</td>
<td>0.87</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 3. Evaluation of Alifax performance at different cut-off values for bacteria after 180 min.

<table>
<thead>
<tr>
<th>BACT/µl</th>
<th>SE</th>
<th>SP</th>
<th>NPV</th>
<th>PPV</th>
<th>DA</th>
<th>Youden index</th>
</tr>
</thead>
<tbody>
<tr>
<td>10000</td>
<td>1.00</td>
<td>0.23</td>
<td>0.25</td>
<td>1.00</td>
<td>0.75</td>
<td>0.23</td>
</tr>
<tr>
<td>30000</td>
<td>0.99</td>
<td>0.98</td>
<td>0.99</td>
<td>0.95</td>
<td>0.98</td>
<td>0.97</td>
</tr>
<tr>
<td>40000</td>
<td>0.95</td>
<td>0.98</td>
<td>0.99</td>
<td>0.92</td>
<td>0.95</td>
<td>0.93</td>
</tr>
<tr>
<td>50000</td>
<td>0.90</td>
<td>0.99</td>
<td>0.99</td>
<td>0.88</td>
<td>0.93</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Screening assays comparison

Using Cohen’s K between the two methods used, a correlation analysis was also performed. The coefficient K measures the degree of agreement between two variables. It presents a maximum of 1, when the agreement between two variables is perfect, 0, when agreement is no better than chance, and negative values, when agreement is worse than chance. We found a value for coefficient K of 0.49 (P < 0.0001).

DISCUSSION

Laboratory diagnosis of UTI is based on the detection and quantification of bacteria and leukocytes in urine. The presence of bacteria in the urine does not necessarily diagnose a UTI, as bacteriuria may also result from samples’ contamination from normal urethral bacterial flora. The presence of urinary leukocytes is often associated with UTI, but may also derive from vaginal contamination in women (Pappas, 1991; Pezlo, 1988, Pezlo et al., 1992).

Analyzing the distribution of samples based on age, in this patient’s series, two different frequency peaks were
observed from 20 to 45 and from 60 to 85 years old patients. This bimodal distribution is exactly what one would expect regarding to urine cultures in adult patients received in the laboratory in a multifaceted center (Broeren et al., 2011; Pieretti et al., 2010; Yasuma et al., 2012).

For interpretation of quantitative culture, criteria recommended from European Urinalysis guidelines were adopted (Aspevall et al., 2001; ECLM, 2000).

Usually, a screening test is used for testing a population with a low prevalence of positivity. In the case of this study, the prevalence of positive urine cultures was 26%. Thus, an ideal screening test might be able to reduce the number of samples examined by urine culture by about 70 to 75%.

**Sysmex UF-1000i**

In this study, a cut-off value for bacteria quantification was adopted at 175 BACT/µL. Using this value, SE was 0.95, SP was 0.80, NPV was 0.98, PPV was 0.64 and DA was 0.84. The adoption of this cut-off value improved the performance of the screening process and allowed a significant reduction of 51% (1256 samples) in bacterial culture, while maintaining an acceptable level of FN (22 samples: 0.8%). Using the Sysmex UF-1000i analyzer, most of the false negatives observed resulted due to infections caused by Gram-positive bacteria. The lower sensitivity of flow cytometry in the detection of Gram positive bacteria is already reported in the literature and it would seem depending on the fact that the bacteria are often gathered in small aggregates formed from multiple bacterial cells and are counted as a single element of large size (De Rosa et al., 2011; Wang et al., 2010). On the other hand, the SP observed in this study was still sub-optimal because of the relatively high FPs (467 samples: 21.25%). The number of false positives observed in this series of patients was higher than data previously reported in our laboratory in a different setting characterized by not operating a hub-and-spoke model.
In fact, in different series of patients, the observed SP values remained between 0.88 and 0.94 (Manoni et al., 2009). Moreover, some authors reported values of SE and SP similar to those observed in this paper. For example, Broeren et al. (2011) reported SE of 0.95 with SP of 0.80 with a 52% reduction in the need for microbiological examination of urine. Pieretti et al. (2010) reported an SE of 0.98 with SP of 0.62 with a 43% reduction in the need for microbiological examination of urine. Wang et al. (2010) reported an SE of 0.97 with SP of 0.79 with a 50% reduction in the need for microbiological examination of urine.

In this study, for Alfred and HB&L system, a cut-off value at 30000 UFC/mL after 180 minutes of incubation was adopted; this result was in good agreement with data from the literature (Ballabio et al., 2010; Ilki et al., 2010). The analytical performance of the instrumentation Alifax Alfred and HB&L, in comparison with the quantitative culture was extremely satisfactory in terms of SE (0.99) and SP (0.98). In particular, the very low number of FN observed (4, 0.2%), associated with a low number of FP (33, 1.4%), could afford to avoid manual plates inoculation for 70% of required urine microbiological examination with a very high percentage of confidence.

On the other hand, the screening of UTI was performed using the Sysmex UF-1000i analyzer available in seconds and requires no additional equipment other than analyzers normally used for performing a standard urinalysis, without additional costs or the need of further resources. Conversely, screening of UTI was carried out using Alifax Alfred and HB&L analyzer requires 180 min to be able to offer the result and requires a dedicated staff and equipment. Moreover, the Alifax Alfred and HB&L system have some interesting potential applications in microbiological diagnostics of other fluids (Barocci et al., 2010; Cavallaro and Squarzon, 2009; Fontana et al., 2009; Tessari et al., 2010). Also, it can be used to quickly make the direct test of chemosensitivity to antibacterial drugs (Barocci et al., 2010).

This type of sequence in the two screening tests was imposed by logistics and organizative considerations. Urine samples arrive at the spoke laboratory around 11.00 a.m. Sysmex UF-1000i is the opportunity to perform at the spoke laboratory (not equipped with a micro-
and HB&L, there would be two orders of problems: first of all, the need to acquire a further instrumentation that requires dedicated personnel; secondly, the results would not be available before 03:00 pm. At this point, positive samples must be subjected to culture in the hub laboratory. Samples transport could be performed, theoretically, the next day; however, this would be incompatible with the TAT recommended by European guidelines (Aspevall et al., 2001; ECLM, 2000).

Alternatively, there would be the need to implement a new dedicated shuttle for transport to the hub laboratory of positive samples, obviously with increasing costs and complexity of the logistical aspects related to transport.

The hub laboratory microbiology section is operative up to 06:00 p.m. Urine samples from spoke laboratory arrive before 01:30 p.m. Then urine samples can be immediately tested with the system Alifax Alfred and HB&L, that is able to provide results within 05.00 p.m., allowing the report in the first day of a further aliquots negative samples (about another 30%). In this way, the results for the negative samples are available via Web for patients and general practitioners within 06:00 p.m., the same day of collection of the sample with the possibility of a better management of the diagnostic and therapeutic approaches to diagnosis of UTI. In fact, the availability of a negative result within a few hours of sample collection allows, on one hand, to consider other diagnoses and to avoid the onset of inappropriated antibiotic therapy. Moreover, the rapid availability of a negative result, in case of strong suspicion of UTI diagnosis on clinical grounds, may allow the prompt collection of a second sample. On the other hand, the lack of evidence of a negative result, in a clinical ground suggestive of UTI, can support the decision to start an empirical antibiotic therapy. Positive samples are subjected to culture and the next morning, after 18 h of incubation, and are available for a full microbiological evaluation: growth quantification, biochemical identification and study of susceptibility to antibacterial drugs. Usually, a complete positive microbiological report is available within 48 h after samples collection.

In this paper, it is suggested that, in a hub and spoke setting, using a Sysmex UF-1000i analyzer in the spoke laboratory, a routine screening of bacteriuria should be useful in rapid rule out samples without any sign of urinary tract infections. Based on the series of these patients, the cut-offs for bacteriuria (175/μl) allowed a reduction in sample’s shipping to the hub laboratory of 43% and a corresponding decrease in the need for microbiological examination of urine. These results are important, because they allowed a reduction in urine culture costs and make available laboratory resources for other activities. In a hub and spoke setting, the laboratory hub is guaranteed the presence of staff in the area of diagnostic microbiology for about 12 h at least. Samples results were found not negative at screening with Sysmex UF-1000i, which appeared burdened with a relatively high number of FP. Therefore, an evaluation of these samples using the system Alifax Alfred and HB&L characterized by a high SP were made. In this way, we found out that it is possible to reduce further the need to carry out microbiological analysis of urine allowed to perform a reliable rule out of about 70% of the samples, using only the Alifax system, which ensures a better performance.

In the spoke laboratory, the Sysmex UF-1000i analyzer is used for the evaluation of urine particles after the physico-chemical examination performed using a dip-stik automated reader. The total cost of a urine test is ca. 0.85 Euro; the system has an output of 60 tests/h. So, in our laboratory, the screening of UTI with flow cytometry is not a separate test that needs dedicated staff and analyzers but lies within standard urine assay. In the hub laboratory screening, the Alifax Alfred and HB&L analyzer is used for the rapid microbiological evaluation of urine samples. The total cost of a test is around 2.5 Euros.

Obviously, this study has some limits, for example, only samples obtained, with clean catches midstream technique, from adult patients were considered. No data were available for pediatric samples. In this paper, we considered mainly outpatients, so only a cut-off at 10^5 UFC/ml was adopted without differentiation between females and males. Moreover, routine samples were considered, so, unfortunately, no information was found about antimicrobial therapy previously taken by patients. However, it is believed that this model may present some aspects of interest. It is validated, in a large series of patients from the daily routine, a hub and spoke organization between two separate local health authorities that collaborate each other, while being administratively separate. We had multiple needs: to ensure a satisfactory diagnostic quality, to reduce the number of tubes to carry, to use the existing logistics, to make available the negative results on the day of sample collection, and finally to find a solution compatible with available economic resources. It is believed that the proposed model can be an adequate response to these items.

Conflict of interests

None of the authors have a conflict of interest to declare.

REFERENCES


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