Original Article

Comparison of different urine culture methods in urinary tract infection

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Background: Midstream urine specimen cultures play an important role in assisting clinicians to choose antibiotics to remove urinary tract pathogens. At present, there are many culture methods for obtaining midstream urine specimens. In this article, different urine culture methods were compared to improve the detection rate of pathogenic bacteria in patients with urinary tract infections (UTIs).

Methods: Urina sanguinis specimens were collected from 90 outpatients and inpatients who had UTI symptoms, had been clinically diagnosed with UTI between February and March 2021, and had been tested within 2 h using the conventional 1-μL culture method, the modified 100-μL culture method, and the centrifugal urine sediment culture method. After incubation at 35 ℃ with 5% carbon dioxide (CO_2) for 48 h, the detection rates of pathogenic bacteria in UTIs were compared among the three methods.

Results: A total of 75 strains of pathogenic bacteria were detected in the 90 specimens. The positive detection rates of pathogenic microorganisms were 23.33%, 73.33%, and 75.56% for the conventional 1-μL culture method, the modified 100-μL culture method, and the centrifugal urine sediment culture method, respectively. Among the patients who used antibiotics before the collection of the urine specimens, the positive detection rates of pathogenic microorganism were 16.00%, 66.00% and 66.00% for the conventional 1-μL culture method, the modified 100-μL culture method, and the centrifugal urine sediment culture method, respectively. In the specimens grown aseptically using the conventional 1-μL culture method, a similar average number of colonies was found using the modified 100-μL culture method and the centrifugal urine sediment culture method. Among the specimens with bacterial growth in the conventional 1-μL culture method, the average colony numbers of the three methods were similar.

Conclusions: The modified 100-μL culture method and the centrifugal urine sediment culture method greatly improved the positive detection rates of pathogenic bacteria in patients with UTIs.

Keywords: Urinary tract infection (UTI); urine culture; centrifugation; modification

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Introduction

Urinary tract infections (UTIs) are common infectious disease and pose a serious health risk. If left untreated, chronic UTIs can develop, which can eventually affect kidney function. The culturing of the midstream specimen of urine plays a crucial role in assisting clinicians to select antibacterial drugs to remove pathogenic bacteria from the urinary tract. Currently, midstream urine specimen cultures are usually performed using 1- or 10-μL inoculation,

and most are inoculated with blood agar medium and MacConkey agar medium only (1). However, for patients with obvious clinical symptoms and repeated negative cultures, the conventional urine culture method may fail to detect bacteria. Thus, we sought to compare the conventional 1-μL culture method, the modified 100-μL culture method, and the centrifugal urine sediment culture method to determine the most suitable method for clinical operation and increase the positive detection rate of UTIs. We present the following article in accordance with the MDAR reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-22-73/rc).

Methods

Materials

A total of 90 urina sanguinis specimens were collected from outpatients and inpatients with UTI symptoms, who had been diagnosed with a UTI and had urine leukocytes ≥5/high magnification field for men, and ≥10/high magnification field for women from February to March 2021 at The Second Hospital of Tianjin Medical University. Of the patients, 54 were male and 36 were female. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Human Ethics Review Committee of The Second Hospital of Tianjin Medical University (No. KY2021K172) and informed consent was taken from all the patients.

Study methods

Specimen collection

The urina sanguinis specimens retained in the bladder for at least 4 to 6 h were collected, and operated in strict accordance with the National Clinical Laboratory Practice (4th ed.). Before collection, the female patients were required to wash the pudendum and disinfect the urethral orifice, and the male patients were required to wash the urethral orifice and prepuce. A midstream urine specimen was then collected by aseptic methods. No less than 10 mL of urine was collected in a special sterile container. Urine specimens were transported to the laboratory within 2 h of collection.

Culture methods for midstream urine specimen

All patient specimens were tested using the following three methods: (I) the conventional 1-μL culture method; (II) the modified 100-μL culture method; and (III) the centrifugal urine sediment culture method. All three methods were inoculated with blood agar medium (Oxoid, UK), MacConkey agar medium (Oxoid), chocolate agar medium (Oxoid) and hypertonic L medium (Oxoid) for quantitative culture.

- Under the conventional 1-μL incubation method, 1 μL each of urine specimen was scribed on 4 types of agar media of blood (Oxoid), MacConkey (Oxoid), chocolate (Oxoid) and hypertonic L-plates (Oxoid) using a quantitative inoculation loop, and placed in a 35 ℃, 5% carbon dioxide (CO_2) incubator (Thermo Fisher Scientific, Waltham, MA, USA) for 48 h. The number of colonies grown per ml were counted and recorded, and multiplied by a dilution of 1,000, and the routine identification of the drug sensitivity results were reported.
- Under the modified 100-μL culture method, 100 μL each of urine from the urine culture tube after routine incubation was taken and coated onto 4 types of agar media of blood (Oxoid), MacConkey (Oxoid), chocolate (Oxoid) and hypertonic L-type plate (Oxoid) in a 35 ℃, 5% CO_2 incubator (Thermo Fisher Scientific) for 48 h. The number of colonies grown per mL were counted and recorded, and multiplied by a dilution of 10.
- Under the centrifugal urine sediment culture method, after the inoculation of the urine specimen using the first 2 methods, 4 mL of urine was retained and divided into 4 sterile centrifuge tubes (1 mL per tube), and centrifuged at 3,000 r/min for 10 min. The supernatant was then discarded, and all the sediment (about 0.1 mL) was taken and inoculated in the 4 kinds of agar medium [i.e., blood (Oxoid), MacConkey (Oxoid), chocolate (Oxoid) agar medium and hyperosmotic L-plates (Oxoid)], and incubated at 35 ℃ in a 5% CO_2 incubator (Thermo Fisher Scientific) for 48 h. The number of colonies per ml of growth was calculated directly from the number of colonies counted on the plates and recorded.

Identification of pathogenic bacteria

All pathogenic bacteria with colony counts ≥100 colony-forming units (CFU)/mL under the three incubation methods were purified, and strains were identified using the VITEK II compact fully automated microbial

identification system (Bio Mérieux, France). Any strains that could not be identified by that afore-mentioned system were identified using the Mérieux VITEK MS mass spectrometry identification system (Bio Mérieux). The quality control strains were *Enterococcus faecalis* ATCC700327, *Enterobacter cholerae* ATCC700323, *Klebsiella aerogenes* ATCC13048, and *Pseudomonas slickum* ATCCMYA-2950.

Statistical methods

All the data were collected and entered into an EXCEL database, and analyzed using the statistical package of IBM SPSS Statistics V21.0. The detection rates of the pathogenic bacteria among the three culture methods were compared using the paired chi-square test (McNemar). A P value <0.05 indicated a statistically significant difference.

Results

Among the 90 specimens, 22 cases without bacteria growing were found, and 75 strains of bacteria and fungi were detected using all 3 methods. In the conventional 1-μL culture method, 18 strains were detected by blood agar medium, 18 strains by chocolate agar medium, 13 strains by MacConkey agar medium, and 19 strains by L-plate. In the modified 100-μL culture method, 66 strains were detected by blood agar medium, 65 strains by chocolate agar medium, 19 strains by MacConkey agar medium, and 55 strains by L-plate. In the centrifugal urine sediment culture method, 69 strains were detected by blood agar medium, 69 strains by chocolate agar medium, 20 strains by MacConkey agar medium, and 57 strains by L-type plate. The specific results for each method are shown in *Table 1*.

Comparison of the detection rates of pathogenic bacteria in 90 specimens among the three urine culture methods

The positive detection rate was 23.33% (21/90) for the conventional 1-μL culture method, 73.33% (66/90) for the modified 100-μL culture method, and 75.56% (68/90) for the centrifugal urine sediment culture method. The analysis was performed using the IBM SPSS Statistics V21.0 statistical package, and the detection rates of pathogenic bacteria in the three culture methods were compared by paired chi-square tests (McNemar).

(I) In relation to the comparison of the conventional 1-μL culture method and the modified 100-μL culture method, the difference was statistically significant (P<0.001).
(II) In relation to the comparison of the conventional 1-μL culture method and the centrifugal urine sediment culture method, the difference was statistically significant (P<0.001).
(III) In relation to the comparison of the modified 100-μL culture method and the centrifugal urine sediment culture method, the exact probability was P>0.05, and the difference was not statistically significant.

Comparisons of the detection rates of pathogenic bacteria among the three urine culture methods for specimens from a population premedicated with an antimicrobial drug

After searching the electronic medical record system of our hospital, 50 patients who had used antimicrobial drugs before the collection of the middle urine specimens were identified. These patients had positive detection rates of 16.00% (8/50) for the conventional 1-μL culture method, 66.00% (33/50) for the modified 100-μL culture method, and 66.00% (33/50) for the centrifugal urine sediment culture method. The analysis was performed using the IBM SPSS Statistics V21.0 statistical package, and the comparisons of the detection rates of pathogenic bacteria among the three culture methods were performed using paired chi-square tests (McNemar).

(I) In relation to the comparison of the conventional 1-μL culture method and the modified 100-μL culture method, the difference was statistically significant (P<0.001).
(II) In relation to the comparison of the conventional 1-μL culture method and the centrifugal urine sediment culture method the difference was statistically significant (P<0.001).
(III) In relation to the comparison of the modified 100-μL culture method and the centrifugal urine sediment culture method, the exact probability was P>0.05, and the difference was not statistically significant.

Comparison of the number of positive detection of various pathogenic bacteria

The number of positive detection cases for the various types of pathogenic bacteria among the different methods using different culture media were compared (see *Table 1*).

Table 1 Number of positive detections of various pathogenic bacteria in different media using different methods

Isolated strain	Number of positive detection (strains)	Conventional 1-μL culture method (strains)				Modified 100-μL culture method (strains)				Centrifugal urine sediment culture method (strains)			
		BAP	Chocolate	MAC	L-form	BAP	Chocolate	MAC	L-form	BAP	Chocolate	MAC	L-form
Candida albicans	2	0	0	0	0	2	2	0	2	2	2	0	2
Staphylococcus epidermidis	6	0	0	0	1	6	6	0	3	6	6	0	2
Enterobacter aerogenes	1	1	1	1	1	1	1	1	1	1	1	1	1
Klebsiella oxytoca	1	0	0	0	0	1	1	1	1	1	1	1	1
Escherichia coli	12	7	7	6	7	10	10	10	11	12	12	11	12
Enterococcus faecalis	17	3	3	1	4	17	17	1	16	17	17	1	16
Citrobacter freundii	1	1	1	1	1	1	1	1	1	1	1	1	1
Candida glabrata	1	0	0	0	0	1	1	0	1	1	1	0	1
Streptococcus mitis	5	0	0	0	0	5	5	0	2	5	5	0	2
Corynebacterium tuberculostearicum	2	0	0	0	0	1	1	0	1	1	1	0	2
Corynebacterium urealyticum	2	0	0	0	0	2	2	0	1	2	2	0	1
Corynebacterium glucuronolyticus	2	0	0	0	0	2	1	0	1	2	1	0	1
Actinomyces meyeri	1	0	0	0	0	0	1	0	0	0	1	0	0
Candida tropicalis	1	0	0	0	0	1	1	0	1	1	1	0	1
Staphylococcus haemolyticus	2	0	0	0	0	1	1	0	1	2	2	0	1
Actinomyces schaalii	2	0	0	0	0	1	0	0	1	1	1	0	0
Enterococcus faecium	7	5	5	3	4	7	7	3	6	7	7	3	6
Stenotrophomonas maltophilia	1	0	0	0	0	1	1	0	1	1	1	0	1
Pseudomonas aeruginosa	1	1	1	1	1	1	1	1	1	1	1	1	1
Staphylococcus capitis	1	0	0	0	0	0	0	0	0	0	0	0	1
Corynebacterium amycolatum	4	0	0	0	0	2	2	0	2	2	2	0	3
Streptococcus sanguis	1	0	0	0	0	1	1	0	0	1	1	0	0
Streptococcus anginosus	1	0	0	0	0	1	1	0	0	1	1	0	0
Enterobacter cloacae	1	0	0	0	0	1	1	1	1	1	1	1	1
In total	75	18	18	13	19	66	65	19	55	69	69	20	57

BAP, blood agar medium; MAC, MacConkey agar medium.

Comparison of average bacterial colony number between modified 100 μL culture method and centrifuge urine sediment method

The average number of colonies of pathogenic bacteria grown using the modified 100-μL culture method and the centrifuged urine sediment culture method for the 69 specimens grown aseptically using the conventional 1-μL culture method were compared (see *Figure 1*).

Comparison of mean colony number of pathogenic bacteria in three different methods

The average counts of pathogenic bacteria grown using the

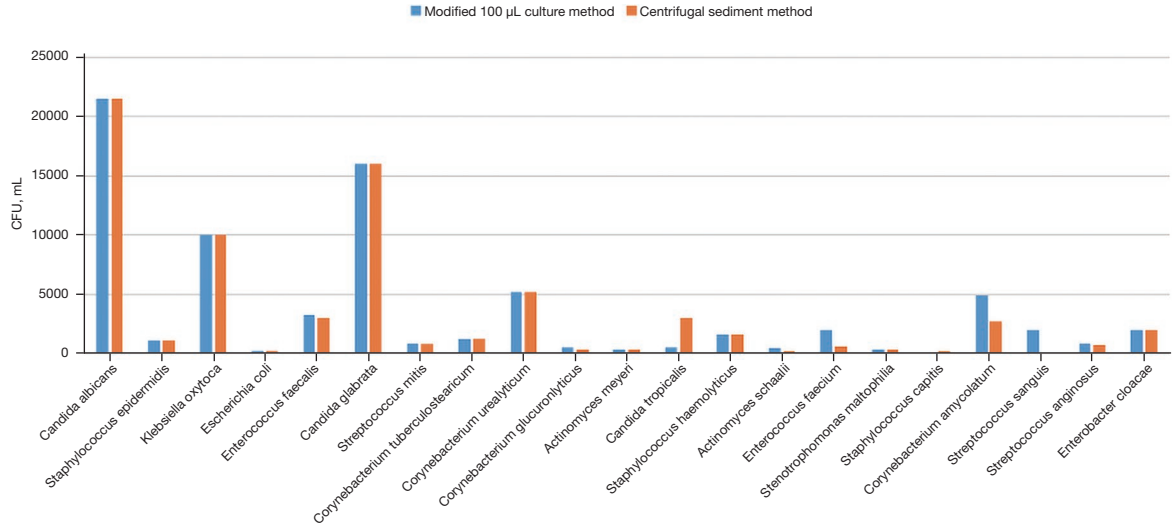

Figure 1 Comparison on the average colony count of the other methods. CFU, colony-forming units.

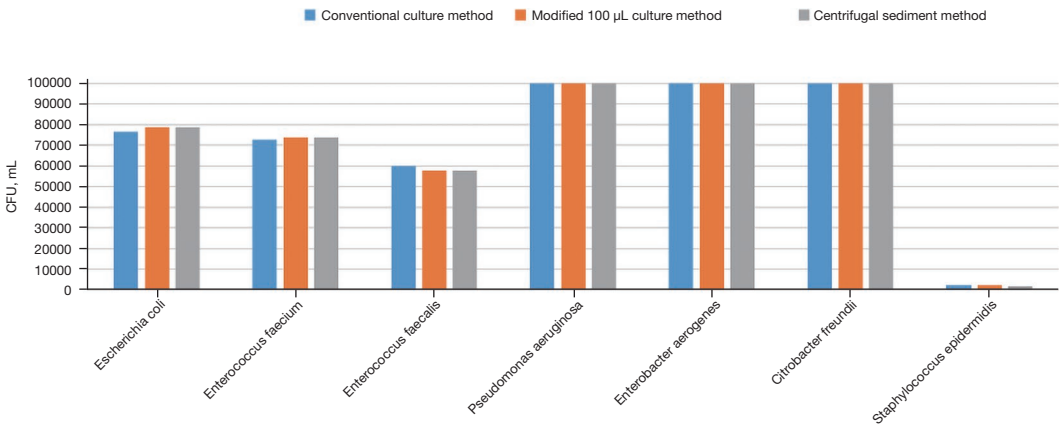

Figure 2 Comparison on the average colony count of three methods for conventional bacteria-growing specimens. CFU, colony-forming units.

three methods for the 21 specimens grown with bacteria using the conventional 1-μL culture method were compared (see *Figure 2*).

Discussion

Urine cultures are essential for the diagnosis of UTIs. Methods of urine collection include suprapubic cystocentesis, a direct indwelling catheter, and the independent retention of a clean midstream urine specimen. Among these methods, a clean midstream urine specimen is the easiest to obtain clinically. At present, most microbiology laboratories use 1 or 10 μL of urine for inoculation. After 24–48 h of cultivation, the number of colonies in the medium is multiplied by the corresponding dilution time to generate the measurement results (2). However, some patients with UTIs have low positive urine culture rates, as the hospital does not have the conditions for urine culture detection, and the patients have used antibacterial drugs or drank a great deal of water before admission, causing urine dilution (3,4). The concentration of pathogenic bacteria in urine decreases due to antibacterial drugs or the dilution of urine, and may exceed the lower limits of detection of conventional urine culture methods. This study increased the inoculation volumes using the modified 100-μL culture method, and increased

the concentrate of specimens using the centrifugal urine sediment culture method to improve the positive detection rate of UTIs, and thus ensured the selection of the most suitable method for clinical operation.

By comparing the detection rate of pathogenic bacteria in the urinary tract using the conventional 1-μL culture method, the modified 100-μL culture method, and the centrifugal urine sediment culture method, we found that the differences between the conventional 1-μL culture method and the modified 100-μL culture method, and the centrifugal urine sediment culture method were statistically significant, and the detection rates of pathogenic bacteria in the latter two methods were significantly higher than that of the conventional method. The differences between the modified 100-μL culture method and the centrifugal urine sediment culture method were not statistically significant, and had similar detection rates of pathogenic bacteria.

When the positive detection rates of pathogenic bacteria for the three methods among the population who used antimicrobial drugs before urine collection were compared, we found that the detection rates of the modified 100-μL culture method and the centrifugal urine sediment culture method were still significantly higher than that of the conventional 1-μL culture method. Compared to the conventional method, these methods increased the detection rate of pathogenic bacteria from <20% to >60%, which is significant for clinicians who are eager to find pathogenic bacteria. Thus, the modified 100-μL and centrifugal urine sediment culture methods substantially increased the positive detection rate of pathogenic bacteria in patients with UTIs and reduced the leakage of pathogenic bacteria, especially of bacteria that are rarely detected by conventional methods, but are closely associated with UTIs, such as *Corynebacterium tuberculos tearicum*, *Corynebacterium urealyticum*, *Corynebacterium glucuronolyticum*, *Actinomyces schaalii*, and *Corynebacterium amycolatum* (5).

The average colony counts of the modified 100-μL culture method and the centrifugal urine sediment culture method were found to be similar in specimens grown aseptically using the conventional 1-μL culture method, and mostly ranged from several hundred to several thousand CFU/mL. In the research group, the lower limit of detection of the conventional 1-μL culture method and the inhomogeneity of the urine resulted in the pathogenic bacteria of the above order of magnitude being unable to be detected in the inoculum volume of 1 μL, causing missed detections. Thus, compared to the conventional 1-μL culture method, the modified 100-μL culture method and centrifugal urine sediment culture method achieved more accurate bacterial culture results, which could prevent delays in treatment, and assist clinicians to target the pathogenic bacteria.

A comparison of the detection of pathogenic bacteria in blood agar medium, chocolate agar medium, MacConkey agar medium, and L-plates by the modified 100-μL culture method and centrifugal urine sediment culture method showed that the species of the pathogenic bacteria and the positive detection rates were almost equal in the blood agar medium and chocolate agar medium, which were significantly higher than those of the MacConkey agar medium and L-plates; however, the average number of the colonies of pathogenic bacteria grown in both methods did not differ significantly. There was no significant difference in the average number of colonies of pathogenic bacteria between the two methods. As a weakly selective medium, MacConkey agar medium is mainly used for the isolation of gram-negative bacteria. Thus, only blood agar medium and MacConkey agar medium should be selected for inoculation in routine cases.

In addition, there were 3 cases in which the pathogenic bacteria only grew on L-type plates with small fried egg-like colonies, which were identified as *Corynebacterium tuberculostearicum*, *Corynebacterium amycolatum*, and *Actinomyces schaalii* after their repeated incubation in blood agar media. A review of the medical records revealed that all 3 patients had used β-lactam antimicrobial drugs in combination with multiple antimicrobial drugs before the collection of the midstream urine specimens, and had multiple documented UTIs. Due to the hypertonic renal medullary environment and the history of multiple antimicrobial medications for UTIs, the cell walls of the bacteria in the urine were damaged and formed L-shaped bacteria, which persisted in the urinary tracts of the patients. Thus, it is recommended that L-type plates be added to the specimens of patients with chronic UTIs who have experienced recurrent clinical episodes, undergone persistent treatment, and engaged in the long-term use of antimicrobial drugs to avoid the missed detection of L-type bacteria.

After comprehensive comparisons, we found the conventional 1-μL culture method is more convenient for colony counting, and the colony count is more intuitive

and easy to determine at a dilution of 1,000 times, but there are too many growing colonies and these colonies are difficult to determine directly in the modified 100-μL culture method and the centrifugal urine sediment culture method. In terms of operability, the centrifugal urine sediment culture method is more tedious than the other two methods, as it requires that multiple portions of urine be centrifuged for a long time, and thus takes significantly longer operation time than the other two methods, and thus is not suitable for routine clinical operation. The modified 100-μL culture method did not differ significantly to the centrifugal urine sediment culture method in terms of the pathogenic bacteria species, number of positive cases, and average colony count.

Given the ease of interpretation, low leakage rate, and high operability, the following method for the culturing of midstream urine specimens is recommended: inoculate 1 μL of urine in blood agar and MacConkey agar medium, respectively, and inoculate 100 μL of urine in the additional blood agar medium. If a caustic bacterial infection is highly suspected, inoculate 100 μL of urine in the additional chocolate agar medium. For patients who have repeatedly undergone the routine 1-μL culture method to detect aseptic growth or for those who have previously used antimicrobial drugs, the modified 100-μL culture method should be used, and an L-type plate should be added to avoid the missed detection of L-type bacteria, which could further provide more accurate and rapid test results to support the diagnosis, treatment, and prognosis of clinical UTIs (6,7).

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-22-73/rc

Data Sharing Statement: Available at https://tau.amegroups.com/article/view/10.21037/tau-22-73/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-22-73/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Human Ethics Review Committee of The Second Hospital of Tianjin Medical University (No. KY2021K172) and informed consent was taken from all the patients.

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