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Short Communication

Rapid detection of amoxicillin-susceptible *Escherichia coli* in fresh uncultured urine: a new tool to limit the use of broad-spectrum empirical therapy of community-acquired pyelonephritis

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ABSTRACT

Because of the high prevalence of amoxicillin resistance among uropathogens, amoxicillin is not recommended as an empirical treatment of urinary tract infections (UTIs). Quick detection of an amoxicillin-susceptible *Escherichia coli* (ASEC) would allow prescribing amoxicillin without preliminary broad-spectrum empirical treatment in uncomplicated pyelonephritis. To quickly diagnose UTIs due to ASEC, we developed a real-time PCR that detects in fresh uncultured urine the *E. coli*-specific gene *yccT* as well as the bla_{TEM} and $bla_{CTX\text{-}M}$ genes. The ASEC rapid test was considered positive if the PCR was positive for the *yccT* gene but negative for bla_{TEM} and $bla_{CTX\text{-}M}$. The test was compared with culture and susceptibility testing. Among 200 patients with a suspected community-acquired UTI, 61 (30.5%) had a monobacterial UTI due to ASEC. The ASEC rapid test result was obtained in 3 h 13 [95% confidence interval (CI) 3 h 12–3 h 15] and was positive for 43 patients (21.5%). Specificity and sensitivity were 97.8% (95% CI 95.8–99.8%) and 65.6% (95% CI 59.0–72.1%), respectively. Positive and negative predictive values were 93.0% (95% CI 89.5–96.5%) and 86.6% (95% CI 81.9–91.3%), respectively. Owing to its high specificity and positive predictive value, the ASEC rapid test allows the diagnosis of UTI due to ASEC only 3 h after urine sampling. A positive ASEC rapid test may be used to treat uncomplicated pyelonephritis with amoxicillin from the start, without preliminary broad-spectrum empirical treatment. The ASEC rapid test is a promising tool to spare fluoroquinolones and third-generation cephalosporins in UTIs.

1. Introduction

Escherichia coli causes 85% of community-acquired urinary tract infections (UTIs) [1,2]. Broad-spectrum antibiotics such as third-generation cephalosporins and fluoroquinolones are recommended for the empirical treatment of community-acquired pyelonephritis [2]. Conversely, aminopenicillins are not recommended as an empirical treatment of community-acquired pyelonephritis owing to the high prevalence of amoxicillin resistance [2–4]. Ampicillin resistance in *E. coli* is mainly mediated by TEM and SHV penicillinases, inhibitor-resistant TEMs, and CTX-M and TEM extended-spectrum β-lactamases (ESBL) [5–7]. Cephalosporins and fluoroquinolones are associated with a major risk of development of antibiotic resistance, especially resistance mediated by ESBLs, and their use should be limited to preserve their effectiveness [2,8,9]. Conversely, the use of amoxicillin may not be associated with ESBL resistance [10]. A rapid test aimed at detecting aminopenicillin-susceptible uropathogens would make it possible to treat community-acquired pyelonephritis with an aminopenicillin from the start. In this study, the performance of a rapid test to detect amoxicillin-susceptible *E. coli* (ASEC) in uncultured urine was assessed.

2. Materials and methods

2.1. Patients

This prospective study was conducted in 2014 in Centre Hospitalier Universitaire de Nantes, a 2600-bed tertiary, university-affiliated centre in Nantes, France. All urines sampled in the emergency department (ED) and in medical wards were screened daily. Patients were included if they met the following criteria:

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(i) acute or recent onset of urinary symptoms suggestive of UTI (Appendix A); (ii) urine strip test positive for leukocyte esterase and/or nitrite; and (iii) empirical antibiotic treatment for UTI initiated on the day of urine sampling. Exclusion criteria were refusal or inability to give informed consent, pregnancy, urine sampling >48 h after hospital admission and indwelling urinary catheter. The study was approved by the Institutional Review Board (Groupe nantais d'éthique dans le domaine de la santé).

2.2. Standard microbiology tests

Urine samples were collected in BD Vacutainer® Plus C&S Preservative Tubes (Becton Dickinson, Sparks, MD). A 1 mL aliquot was frozen (–20 °C) for the ASEC rapid test. The remaining sample was analysed in a standardised procedure: erythrocyte and leukocyte measurements (iQ®200; Iris Diagnostics, Chatsworth, CA); chromogenic agar culture (Uriselect4; Bio-Rad, Marnes-la-coquette, France); bacterial identification by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (VITEK® MS; bioMérieux, Marcy-l'Étoile, France); and antibiotic susceptibility (VITEK®2 ASTN233 card; bioMérieux). The gold standard was urine culture combined with blood culture (BD BACTEC™ detection system; Becton Dickinson) in patients who had a sterile urine sample. Susceptibility tests were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [11]. The time to gold standard results was defined as the difference between receipt of the urine sample in the laboratory and susceptibility test results made available to the clinician.

2.3. *Amoxicillin-susceptible* E. coli *(ASEC) rapid test*

The ASEC rapid test was based on a triplex real-time PCR targeting the *yccT* gene specific for *E. coli* [12] as well as the bla_{TEM} and $bla_{CTX\text{-}M}$ genes [13]. The PCR primers are listed in Table 1. Whole-cell DNA was extracted using a NucleoSpin® Tissue Kit (Macherey-Nagel, Hœrdt, France). PCR was performed in a final volume of 20 µL comprising 10 µL of the SYBR® Premix Ex Taq™ for SYBR Green detection (Takara Bio Inc., Otsu, Japan), 4 µL of sterile distilled water, 0.5 µL of each primer (concentration of 20 mM) and 5 µL of DNA extracted from the urine sample. Three positive controls (bla_{TEM}, $bla_{CTX\text{-}M}$ and *yccT*), one negative control and one internal control were included [14]. The methodology is detailed in Appendix B. DNA extracts were amplified using a Rotor-Gene 6000® (QIAGEN, Courtaboeuf, France) as follows: 40 cycles of 94 °C for 3 min, 60 °C for 30 s and 72 °C for 15 s, followed by a melting curve analysis. Amplification was monitored at 530 nm. Simultaneous presence of *yccT* but absence of bla_{TEM} and $bla_{CTX\text{-}M}$ genes was interpreted as a positive ASEC rapid test (i.e. UTI caused by ASEC). Every other combination was interpreted as a negative test. The time to the ASEC rapid test result was defined as the difference between urine thawing and the result. Regarding the standard microbiology test, time of transportation of urine samples from the bedside to the laboratory was not taken into account.

Table 1
Primers used to detect the *Escherichia coli*-specific *yccT* gene as well as bla_{TEM} and $bla_{CTX\text{-}M}$ genes from fresh uncultured urine samples.

Gene	Primers
$bla_{CTX\text{-}M}$ [13]	CTX-MA1, 5′-SCSATGTGCAGYACCAGTAA-3′ CTX-MA2, 5′-CCGCRATATGRTTGGTGGTG-3′
bla_{TEM} [13]	TEM-A, 5′-GACTGGATGGAGGCGGGA-3′ TEM-B, 5′-CAATGCTTAATCAGTGAGGC-3′
yccT [12]	yccTRT-F, 5′-GCATCGTGACCACCTTGA-3′ yccTRT-R, 5′-CAGCGTGGTGGCAAAA-3′

2.4. Statistical analysis

The prevalence of UTI caused by *E. coli* and the proportion of amoxicillin susceptibility among *E. coli* isolates were assumed to be 80% and 50%, respectively, in accordance with previous studies [1,2]. To obtain a specificity of 95% with a 5% precision, it was calculated that 183 patients were required. Spearman rank correlation was used to assess the association between the number of cycle thresholds (Ct) necessary to detect the *yccT* gene by real-time PCR and the urine concentration of leucocytes and erythrocytes. A *P*-value of <0.05 was considered significant. Analyses were performed using R software v.3.1.0 (http://CRAN.R-project.org).

3. Results

3.1. Patient characteristics and culture results

From March to November 2014, up to 200 patients (mean ± standard deviation age, 44.5 ± 23.0 years) were included in the study (Fig. 1). Among 155 female patients, 31 and 124 had cystitis and pyelonephritis, respectively. The mean delay of culture results was 56 h [95% confidence interval (CI), 53–59 h]. Urine and blood cultures were sterile for 37 patients (18.5%). Urine cultures grew 165 bacteria, including 118 *E. coli* (results are detailed in Appendix C). Among 157 monobacterial-positive urine cultures, the causative organism was *E. coli* in 116 cases (73.9%), for which the amoxicillin resistance prevalence was 52%. An additional patient treated with ceftriaxone for 2 days before urine sampling had a positive blood culture for ASEC and a negative urine culture and was classified as UTI due to ASEC. Hence, 61 patients (30.5%) had a UTI due to ASEC. Cultures were indicative of UTI due to bacteria other than ASEC for 102 patients, including a patient who had been treated with ceftriaxone and amikacin for 1 day before urine sampling with a positive blood culture for amoxicillin-resistant *E. coli* and sterile urine culture. Four patients had a polymicrobial UTI, including *E. coli* in two cases, and were classified as UTI due to bacteria other than ASEC.

3.2. *Number of Ct for* yccT *gene detection*

The *yccT* gene was detected in 196 patients (98.0%). Sensitivity and specificity of the *yccT* gene PCR were dependent on the number of Ct (ranging from 7.26 to 34.78). Using *E. coli* growth as the reference, the area under the receiver operating characteristic (ROC) curve was 0.96 (95% CI 0.95–0.99) (Fig. S1). At Ct = 18, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for *E. coli* growth were, respectively, 87.3% (95% CI 82.7–91.9%), 96.2% (95% CI 93.6–98.9%), 97.2% (95% CI 94.9–99.5%) and 83.5% (95% CI 78.4–88.7%). Hence, we considered that a positive *yccT* PCR was a marker for *E. coli* UTI if the number of Ct was ≤18. The number of Ct for *yccT* gene detection was not significantly associated with the urine erythrocyte concentration ($r = 0.18$, 95% CI –0.01 to 0.36; $P = 0.054$) but was significantly correlated with the urine leucocyte concentration ($r = 0.30$, 95% CI 0.12–0.467; $P = 0.001$).

3.3. PCR results and ASEC rapid test interpretation

The mean result delay of the ASEC rapid test was 3 h 13 (95% CI 3 h 12–3 h 15). The test was positive in 43 patients (21.5%) of the whole cohort and in 30 (24.2%) of 124 females with pyelonephritis (Table 2; Table S1). Specificity, sensitivity, PPV and NPV were 97.8% (95% CI 95.8–99.8%), 65.6% (95% CI 59.0–72.1%), 93.0% (95% CI 89.5–96.5%) and 86.6% (95% CI 81.9–91.3%), respectively. Results of each PCR are detailed in Table S2. Among the 37 patients with negative urine and blood cultures, 8 (22%) had a positive PCR for the bla_{TEM} gene and none for $bla_{CTX\text{-}M}$ gene.