

**Rapid test of urinary tract infection due to amoxicillin susceptible *E. coli* using triple real-time PCR done on fresh, uncultured urine**

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**Objectives:** Empirical therapy of pyelonephritis is based on fluoroquinolone or 3<sup>rd</sup> generation cephalosporin, with subsequent de-escalation to amoxicillin if the causative bacterium is susceptible to amoxicillin. These broad spectrum antibiotics exert a strong selection pressure. Diagnosing rapidly the causative bacterium and its resistance profile to amoxicillin would allow to start amoxicillin without preliminary broad spectrum empirical treatment. Our goal was to develop and validate a triple real-time PCR for the diagnosis of UTI due to *Escherichia coli* susceptible to amoxicillin.

**Methods:** We developed a real time PCR for Amoxicillin Susceptible *E. coli* (ASEC) applied after DNA extraction from 1 ml of fresh, uncultured, urine. The ASEC PCR detect an *E. coli* specific gene *ycct*, and both *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes, encoding for the main beta-lactamases causing amoxicillin resistance in French *E. coli* isolates. The ASEC PCR was considered positive for ASEC when the PCR was positive for *ycct* gene whereas PCR for *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes were negative. We first developed the ASEC PCR on 50 positive and sterile urines. Then we analyzed prospectively the ASEC PCR on patients hospitalized for less than 3 days in a French hospital from Mars to August 2014, suspected to have UTI, in whom an empirical treatment was started after urine sampling. Routine identification and susceptibility tests were used as gold standard.

**Results:** Among 124 patients, routine tests isolated *E. coli* (n=71, 58%), including 36 amoxicillin susceptible isolates, other bacterium (n=28), or proved sterile (n=25). The ASEC PCR result was obtained in 3 hours and 10 minutes (CI 95% 3:07-3:14). The ASEC PCR was positive on 20 (16%) patients. The specificity of ASEC PCR was 98,9% (CI 95% 97,0-100), the sensibility was 52,8% (CI 95% 44,0-61,5), the positive predictive value (PPV) was 95,0% (CI 95% 91,2-98,8) and the negative predictive value was 83,7% (CI 95% 77,2-90,1). The DNA extraction performance was not influenced by leucocyturia or hematuria. Finally, the use of ASEC PCR would have reduced by 16% the prescription of fluoroquinolones and 3<sup>rd</sup> generation cephalosporins.

**Conclusion:** The ASEC real time PCR allowed the diagnosis of UTI due to amoxicillin susceptible *E. coli* in 3 hours after urine sampling. According to its high specificity and PPV, ASEC PCR may be used to treat UTIs immediately with amoxicillin. The ASEC PCR is a promising tool to save fluoroquinolones and 3<sup>rd</sup> generation cephalosporins.