Introduction

Co-amoxiclav is widely used in France to treat different infectious diseases, in particular community-acquired otitis, sinusitis and pneumonia, and occasionally urinary tract infections (UTIs). While the mechanisms of resistance to co-amoxiclav have been well defined, particularly in Escherichia coli, the influence of co-amoxiclav treatment on the in vivo selection of E. coli resistant to co-amoxiclav has never clearly been demonstrated; however, ampicillin–sulbactam has been shown to select for resistance in E. coli responsible for various nosocomial infections.1–6

In order to prove that co-amoxiclav treatment can be a risk factor for selecting co-amoxiclav-resistant E. coli in vivo, we conducted a study of patients who had a bacteriologically confirmed E. coli UTI, and for whom the history of co-amoxiclav consumption in the month before the reported infection was documented. The β-lactam susceptibility of the urinary E. coli isolates of patients who had been given co-amoxiclav in the month before diagnosing the infection was compared with that of the urinary E. coli isolates of patients who had not been given co-amoxiclav. We also determined the frequency of co-amoxiclav-resistant isolates in the faeces of patients treated with amoxicillin or co-amoxiclav for the reported UTI, and compared it with that for patients treated with other antibiotics, such as third-generation cephalosporins or fluoroquinolones. Hence, individual exposure to co-amoxiclav is a risk factor for UTIs caused by co-amoxiclav-resistant E. coli or for carrying co-amoxiclav-resistant Gram-negative bacilli in the digestive tract.

Patients and methods

Patients

One hundred and six in-patients who were hospitalized in different wards of the A. Paré Hospital between November 1997 and February 1998, and two healthcare workers, were included in the study after diagnosis of E. coli lower UTI (≥10⁴ leucocytes/mL and ≥10⁵ cfu/mL). Patients with urinary catheters were excluded.

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Antibiotic consumption

The possible consumption of co-amoxiclav in the month before diagnosing the UTI and any treatment given for the reported infection were recorded for all patients. Information about co-amoxiclav consumption in the month before the diagnosis of UTI was obtained from the patients’ hospital files, from the prescribing form provided by the general practitioner, or on occasion by questioning patients or their families.

Screening for co-amoxiclav-resistant E. coli isolates in faeces

As soon as the E. coli UTI was diagnosed, quantitative screening for co-amoxiclav-resistant E. coli isolates in stool samples was undertaken. One gram of faeces was suspended in 5 mL of sterile water, and 10 μL of this suspension and of its 10⁻³ dilution were spread on Drigalski plates (bioMérieux, Marcy l’Étoile, France), one without antibiotic, and one containing amoxicillin 200 mg/L (SmithKline Beecham, Nanterre, France) with clavulanate 20 mg/L (SmithKline Beecham, Nanterre, France). The concentration of amoxicillin used to screen for co-amoxiclav-resistant E. coli (200 mg/L) was chosen because the MIC of amoxicillin is always ≥200 mg/L for E. coli resistant to co-amoxiclav.7 The fixed concentration of clavulanate 20 mg/L was chosen to be high enough to select co-amoxiclav-resistant E. coli, but lower than the concentration that has intrinsic activity against E. coli.8

Lactose-positive and lactose-negative cfu were counted on each Drigalski plate and their concentration per gram of faeces was determined.

Bacterial identification and antibiotic susceptibility

Bacterial identification was carried out with the API system (bioMérieux). The antibiotic susceptibility of E. coli isolates from urine and of those selected from faeces on Drigalski plates containing co-amoxiclav was determined using the agar disc diffusion method, and interpreted according to the recommendations of the French Committee of Antibiogramme.9 The following antibiotics were tested: amoxicillin, co-amoxiclav, cefalothin, cefuroxime, cefotaxime, kanamycin, gentamicin, chloramphenicol, tetracycline, sulphonamides, trimethoprim, nalidixic acid and pefloxacin.

Molecular analysis

Co-amoxiclav-resistant E. coli from faeces were compared with E. coli isolates from urine from individuals in terms of β-lactam susceptibility and clonal relatedness. The clonal relatedness was assessed by the DNA profile obtained by the random amplified polymorphic DNA (RAPD) typing system as described previously.10 The two primers singly used for RAPD typing were the following: HLWL74 5’TACGTATCTGC-3’ and A3 5’TGGACCCGGC-3’.11,12 When faeces and urinary E. coli isolates of a patient displayed the same RAPD profile, the underlying mechanism of β-lactam resistance was defined by using amplification reactions specific for blaTEM genes and blaOXA genes, and by sequencing as described previously.13

Statistical analysis

Statistical analyses were performed with Epi Info software (v. 6.04). Relative risks (RR) and 95% confidence intervals (95% CI) were calculated for binomial variables; P values were calculated by Fisher’s exact test for binomial variables, and a P value of ≤0.05 was considered significant.

Results

Co-amoxiclav-resistant urinary E. coli isolates and consumption of co-amoxiclav

The categories of in-patients with E. coli UTIs included in this study were as follows: medical patients, n = 71; paediatric patients, n = 22; urology patients, n = 9; orthopaedic patients, n = 3; patients on medical intensive care unit, n = 1. Paediatric patients included 85% who were <2 years old and adult patients included 70% who were >70 years old.

Amongst the 108 patients included in this study, 16% (n = 17) had E. coli isolates resistant to co-amoxiclav (inhibition zone diameter <14 mm). The percentage of patients with community-acquired UTI due to co-amoxiclav-resistant E. coli (16.7%, n = 78) was not significantly different from that of patients with hospital-acquired UTI due to co-amoxiclav-resistant E. coli (13.3%, n = 30).

Twelve (11%) of the 108 patients had received co-amoxiclav in the month before diagnosis of their UTI. Among these patients, four were babies (3–6 months old) who had been given co-amoxiclav 50 mg/kg/day po for 7–10 days in three cases, and co-amoxiclav 75 mg/kg/day iv for 1 day in one case. The eight remaining patients were adults (average age 70 years, range 47–89 years) who, with one exception, had been given co-amoxiclav 2–3 g/day po for 1–45 days (average treatment duration 13.5 days). One of the adult patients had been given co-amoxiclav 3 g/day iv for 4 days.

Fifty per cent (6/12) of urinary E. coli isolates obtained from these 12 patients were resistant to co-amoxiclav, whereas only 11.5% (11/96) of the isolates from patients who had not been given co-amoxiclav were co-amoxiclav-resistant. Co-amoxiclav consumption in the month before a UTI due to E. coli is a risk factor associated with the isolation of co-amoxiclav-resistant E. coli from urine (RR = 4.36; 95% CI 1.97–9.65; P < 0.001).
Co-amoxiclav exposure and co-amoxiclav-resistant *E. coli*

**Table 1.** Distribution of co-amoxiclav-resistant *E. coli* in faeces according to the treatment for *E. coli* UTI

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AMC-resistant E. coli</th>
<th>other bacilli</th>
<th>AMC-susceptible flora</th>
<th>no Gram-negative bacilli</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Cefotaxime or ceftriaxone</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Other molecules</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>8</td>
<td>17</td>
<td>11</td>
<td>46</td>
</tr>
</tbody>
</table>

AMC, co-amoxiclav.

**Screening for co-amoxiclav-resistant *E. coli* isolates in faeces**

Stool could be sampled from only 46 (42.6%) of the 108 patients, on average, 3.3 days (0–16 days) after the beginning of the treatment of the UTI. As indicated in Table 1, no Gram-negative bacilli were isolated from the stools of 11 of these 46 patients (24%), whereas 17 (37%) had a Gram-negative flora fully susceptible to co-amoxiclav; 18 (39%) had a Gram-negative flora resistant to co-amoxiclav. For 10 of the latter patients, co-amoxiclav-resistant organisms were *E. coli* species, and in eight others they were bacterial species inherently resistant to co-amoxiclav, such as *Enterobacter* spp., *Citrobacter freundii* and *Pseudomonas aeruginosa* (data not shown). Overall, 10 of the 46 patients (22%) carried co-amoxiclav-resistant *E. coli* in their digestive tract.

The great majority of patients (41/46) were receiving antibiotic treatment for an *E. coli* UTI when stool samples were taken (Table 1). The most common treatment consisted of third-generation cephalosporins (n = 20), followed by fluoroquinolones (n = 9), co-amoxiclav (n = 6) and amoxicillin (n = 5) (Table 1). Treatment with other antibiotics not active against *E. coli* was given for a concurrent infection in one case. Among the five patients not receiving antibiotics on the day of stool sampling, one was never treated, and four were treated immediately after specimen collection.

Amoxicillin treatment appeared to be a risk factor associated with the isolation of co-amoxiclav-resistant Gram-negative bacilli (RR = 4.29; 95% CI 2.24–8.20; *P* = 0.002), especially *E. coli* (RR = 6; 95% CI 2.18–16.51; *P* = 0.006), in the digestive tract in comparison with all treatments except co-amoxiclav. A similar risk factor was found with co-amoxiclav treatment in comparison with all other treatments except amoxicillin, in terms of isolation of all Gram-negative species resistant to co-amoxiclav (RR = 2.86; 95% CI 1.21–6.76; *P* = 0.05), but not for the isolation of co-amoxiclav-resistant *E. coli* alone (RR = 2.5; 95% CI 0.58–10.70; *P* = 0.26). Treatment with third-generation cephalosporins was associated with the absence of Gram-negative bacilli in the digestive tract in comparison with all other treatments (RR = 4.72; 95% CI 1.16–19.25; *P* < 0.03) and amoxicillin treatment (RR = 2.5; 95% CI 0.58–10.70; *P* = 0.05). Treatment with third-generation cephalosporins was associated with the absence of Gram-negative bacilli in the digestive tract in comparison with all other treatments (RR = 4.72; 95% CI 1.16–19.25; *P* < 0.03) and amoxicillin treatment (RR = 2.5; 95% CI 0.58–10.70; *P* = 0.05).

**Comparison of co-amoxiclav-resistant *E. coli* in faeces and urinary *E. coli* isolates in individuals**

For the 10 patients with co-amoxiclav-resistant *E. coli* in faeces, five had β-lactam-susceptible *E. coli* in urine, one had an isolate resistant to amoxicillin but susceptible to co-amoxiclav, and four had amoxicillin- and co-amoxiclav-resistant *E. coli* (Table 2). By comparing RAPD profiles (data not shown) of faeces and urinary isolates for each patient, it was found that the profiles were different for the two types of isolate when these isolates displayed different β-lactam phenotypes (patients A, B, C, D, E and F in Table 2), but identical when both were resistant to co-amoxiclav (patients G, H, I and J in Table 2). Moreover, we demonstrated that the two isolates from the four latter patients displayed, in addition to the RAPD profile identity, an identical molecular mechanism of resistance to co-amoxiclav: OXA-1 for patient G, and TEM-40, TEM-78 and TEM-30 for patients H, I and J, respectively. It must be noted that the rate of co-amoxiclav resistance in *E. coli* among the lactose-positive flora was generally higher in the faeces of patients for whom the *E. coli* RAPD profile was identical for faeces and urinary isolates in comparison with the patients carrying *E. coli* with different RAPD profiles (Table 2).

**Discussion**

The aim of this study was to assess whether co-amoxiclav consumption is a risk factor for the selection of co-amoxiclav-resistant *E. coli*.
V. Leflon-Guibout et al. clav-resistant E. coli isolates in vivo. The analysis, performed on 108 patients who had an E. coli UTI demonstrated that those who had taken co-amoxiclav in the month before diagnosing the infection had a four- to five-fold greater risk of having a co-amoxiclav-resistant urinary E. coli isolate. These patients ($n = 12$), who represented 11% of the patients studied, were mostly elderly people ($> 60$ years old, $n = 5$) and babies ($< 6$ months old, $n = 4$), two population categories for whom co-amoxiclav prescription is likely, particularly for the season of the study (autumn and winter). Co-amoxiclav is one of the recommended antibiotics for otitis, sinusitis and lower respiratory tract infections, which frequently occur in these population categories.\textsuperscript{14,15} The frequency of patients with urinary co-amoxiclav-resistant E. coli isolates (16%) is much higher than that previously determined by a French multicentre study performed between 1996 and 1998 (5%).\textsuperscript{5} This discrepancy might be due to a high proportion of children and elderly people in our population. The percentage of patients with co-amoxiclav-resistant E. coli UTI acquired in hospital was only 13.3%, and 16.7% for community-acquired infections. The percentages published by Henquell et al.\textsuperscript{16} were 25% and 10%, respectively, for the hospital and private laboratories of Clermont-Ferrand. Although our method is different from that of Kaye et al.,\textsuperscript{6} our assessment of risk factors is very similar to theirs. Thus, they demonstrated by a case-control study that individual exposure to ampicillin–sulbactam (an antibiotic combination comparable to co-amoxiclav) was a significant and independent risk factor associated with the isolation of nosocomial ampicillin–sulbactam-resistant E. coli isolates from different specimens. They demonstrated that this same risk factor also correlated with previous ampicillin treatment. In our study, we showed that the treatment of a current E. coli UTI with amoxicillin was associated with intestinal carriage of co-amoxiclav-resistant E. coli isolates. This risk was not statistically significant for co-amoxiclav in our study because of the very small population. Nevertheless, co-amoxiclav treatment of the reported infection was a risk factor for the isolation of co-amoxiclav-resistant Gram-negative bacilli from faeces. We did not examine the consumption of amoxicillin in the month before diagnosing the current UTI. Nevertheless, antibiotics may have been prescribed and hence some patients ($n = 11$) not known to have taken co-amoxiclav had a co-amoxiclav-resistant E. coli UTI.

### Table 2. Quantification of co-amoxiclav-resistant E. coli in faeces of 10 patients, and comparison of their $\beta$-lactam resistance phenotypes, mechanisms of resistance and RAPD profiles with urinary E. coli isolates

<table>
<thead>
<tr>
<th>Patient</th>
<th>$\beta$-lactam R marker or mechanism</th>
<th>RAPD type</th>
<th>$\beta$-lactam R marker or mechanism</th>
<th>RAPD type</th>
<th>AMC-R E. coli/lactose positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>no</td>
<td>I</td>
<td>AMX AMC CEF FOX CXM</td>
<td>II</td>
<td>$10^7$/10^6 0.1</td>
</tr>
<tr>
<td>B</td>
<td>no</td>
<td>III</td>
<td>AMX AMC CEF FOX CXM</td>
<td>IV</td>
<td>$10^7$/10^6 0.1</td>
</tr>
<tr>
<td>C</td>
<td>no</td>
<td>V</td>
<td>AMX AMC CEF FOX CXM</td>
<td>VI</td>
<td>$10^7$/10^6 0.1</td>
</tr>
<tr>
<td>D</td>
<td>no</td>
<td>VII</td>
<td>AMX AMC CEF FOX CXM</td>
<td>VII</td>
<td>$10^7$/10^6 0.01</td>
</tr>
<tr>
<td>E</td>
<td>no</td>
<td>IX</td>
<td>AMX AMC CEF FOX CXM</td>
<td>X</td>
<td>$1.5 \times 10^7$/10^6 15</td>
</tr>
<tr>
<td>F</td>
<td>AMX</td>
<td>XI</td>
<td>AMX AMC CEF FOX CXM</td>
<td>XII</td>
<td>$10^7$/10^6 10</td>
</tr>
<tr>
<td>G</td>
<td>OXA-1</td>
<td>XIII</td>
<td>OXA-1</td>
<td>XIII</td>
<td>$10^7$/5.10^6 20</td>
</tr>
<tr>
<td>H</td>
<td>TEM-40</td>
<td>XIV</td>
<td>TEM-40</td>
<td>XIV</td>
<td>$10^7$/10^6 10</td>
</tr>
<tr>
<td>I</td>
<td>TEM-78</td>
<td>XV</td>
<td>TEM-78</td>
<td>XV</td>
<td>$10^7$/10^6 100</td>
</tr>
<tr>
<td>J</td>
<td>TEM-30</td>
<td>XVI</td>
<td>TEM-30</td>
<td>XVI</td>
<td>$10^7$/10^6 1</td>
</tr>
</tbody>
</table>

Abbreviations: R, resistance; AMX, amoxicillin; AMC, co-amoxiclav; CEF, cefalothin; CXM, cefuroxime; FOX, cefoxitin.
there was a tendency toward a higher percentage in patients who had the same strain in urine and stools. In one patient in particular, this percentage was as high as 100%.

The four previously published mechanisms responsible for co-amoxiclav resistance in clinical isolates of *E. coli* were all identified in this study in the co-amoxiclav-resistant *E. coli* carried in the patients’ digestive tract. These mechanisms are: OXA-1 and inhibitor-resistant TEM (TEM-30, -40 and -78) enzymes or the β-lactamase resistance phenotype for both hyperproduction of TEM enzymes (resistance to penicillins alone or associated with clavulinate, cefalothin and cefuroxime), and chromosomal cephalosporinase (resistance to amoxicillin alone or associated with clavulanate, cefalothin and cefoxitin).1,2,5,17,18

In conclusion, this study confirms that it is clinically relevant to ask patients suffering from UTI if they have recently been treated with co-amoxiclav in order to avoid another course of it, since such patients have a significantly higher risk of co-amoxiclav-resistant *E. coli* infections.

References


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