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RESEARCH NOTE

Susceptibility of *Escherichia coli* to the amoxycillin–clavulanate combination: which recommendations should be used to provide relevant information to clinicians?

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ABSTRACT

This study compared MIC distributions of amoxycillin–clavulanate obtained with NCCLS and French (Comité de l'Antibiogramme de la Société Française de Microbiologie; CA-SFM) methodologies for *Escherichia coli* isolates from urine that were non-susceptible to amoxycillin–clavulanate by the disk diffusion method. With the NCCLS and CA-SFM methods, 74% and 13%, respectively, of these isolates were susceptible to amoxycillin–clavulanate. Therefore, the apparent relatively poor efficacy of amoxycillin–clavulanate against *E. coli* in French hospitals probably reflects a methodological difference rather than a localised resistance problem. This implies that amoxycillin–clavulanate could be used as an alternative to fluoroquinolones for treatment of *E. coli* urinary tract infections. Susceptibility tests for amoxycillin–clavulanate should be standardised worldwide.

Keywords Amoxycillin–clavulanate, CA-SFM, *Escherichia coli*, MICs, NCCLS, susceptibility testing

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Escherichia coli is the bacterial pathogen isolated most frequently from clinical samples taken for

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diagnostic purposes in hospitals and in the community [1–5]. This species is naturally susceptible to a large number of antibiotics, but the emergence and spread of various resistance mechanisms now limits the prescription of a number of first-line antibiotics. In the hospital studied, the frequencies of amoxycillin and amoxycillin–clavulanate resistance within *E. coli* have reached 45% and 40%, respectively (personal unpublished observations). However, the distribution of isolates between non-susceptible categories (i.e., 'intermediate' or 'resistant') varied considerably for these two antimicrobial agents, in that all the *E. coli* isolates that were non-susceptible to amoxycillin were resistant, whereas 86.5% of the amoxycillin–clavulanate-non-susceptible isolates were classified as intermediate. Meanwhile, the proportion of *E. coli* isolates resistant to fluoroquinolones increased from 5% in 1999 to 12% at present, probably following an increase in the consumption of this class of antibiotics [5]. Fluoroquinolones are currently the recommended empirical treatment for uncomplicated acute bacterial cystitis in women [6,7].

In exploring therapeutic alternatives to fluoroquinolones, the present study aimed to characterise the resistance of *E. coli* to amoxycillin–clavulanate. The combination of two active agents in amoxycillin–clavulanate makes it more difficult to establish clinically relevant breakpoints for in-vitro susceptibility tests [8]. Many laboratories worldwide follow the NCCLS guidelines [9], but French laboratories follow the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM, or Antibiogram Committee of the French Society of Microbiology) [10]. The CA-SFM and NCCLS guidelines differ in two ways, namely the susceptibility breakpoints and the concentration of clavulanate used. The objective of this study was to examine the effects of these two sets of guidelines on the reported susceptibility of urinary isolates of *E. coli* to amoxycillin–clavulanate.

In November and December 2003, 100 non-replicate consecutive *E. coli* isolates from urine that were non-susceptible to amoxycillin–clavulanate according to the CA-SFM disk diffusion method were collected. The susceptibility of each isolate to amoxycillin–clavulanate (GlaxoSmithKline, Evreux, France) was determined by the agar dilution method according to NCCLS and CA-SFM guidelines [9,10]. Both guidelines recommend testing with a bacterial inoculum of 10^4 CFU/spot. The differences for the determination of the MIC of amoxycillin–clavulanate between the two sets of guidelines are listed in Table 1. *E. coli* strain ATCC 25922 was included in each test run as an internal control.

The amoxycillin–clavulanate MIC distributions determined by the NCCLS and CA-SFM methods were markedly different (Fig. 1), although the MIC of amoxycillin–clavulanate for *E. coli* ATCC 25922 was 4 mg/L by both methods. The MIC for 13 isolates classified as non-susceptible by the routine disk diffusion test was 4 mg/L; thus, these isolates were susceptible by both agar dilution methods. However, when the amoxycillin concentration in the amoxycillin–clavulanate combination was between 8 and 32 mg/L, MICs were lower with the NCCLS method, with a further 60 isolates classed as susceptible by the NCCLS method only. Only 12 isolates were resistant to amoxycillin–clavulanate (MICs

Table 1. Differences between guidelines for the determination of MICs of amoxycillin–clavulanate by the agar dilution method

Detail of method	NCCLS	CA-SFM
Clavulanate concentration	2:1 ratio of amoxycillin : clavulanate	Fixed 2 mg/L
MIC breakpoints (mg/L)		
Susceptible	≤ 8/4	≤ 4/2
Intermediate	16/8	8/2-16/2
Resistant	≥ 32/16	> 16/2
Number of colonies for endpoint	≤ 1	≤ 3

CA-SFM, Comité de l'Antibiogramme de la Société Française de Microbiologie.

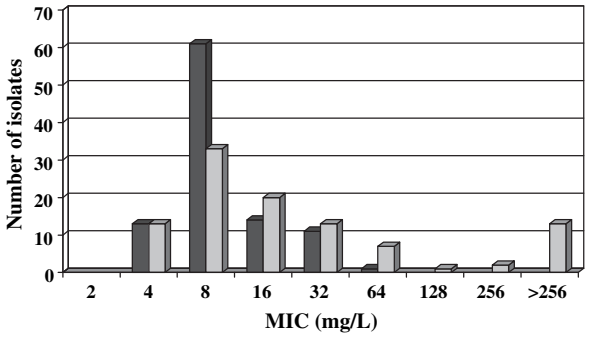

Fig. 1. Distribution of amoxycillin–clavulanate MICs determined by NCCLS (black bars) and Comité de l'Antibiogramme de la Société Française de Microbiologie (grey bars) methods for 100 consecutive *E. coli* urinary tract isolates that were non-susceptible to amoxycillin–clavulanate by disk diffusion.

≥32 mg/L) by the NCCLS method, compared to 35 isolates (MIC > 16 mg/L) by the CA-SFM method.

The differences in the breakpoints (Table 1) also had a marked effect on the amoxycillin–clavulanate susceptibility categories. If the NCCLS agar dilution method was taken as the reference method, the CA-SFM disk diffusion test gave the correct frequency of amoxycillin–clavulanate resistance, but considerably overestimated the frequency of intermediate isolates, while the CA-SFM agar dilution method overestimated the frequencies of both intermediate and resistant isolates. MICs were only measured for isolates that were classified as non-susceptible by the disk diffusion method, but by extrapolating the results to all *E. coli* isolates, it was estimated that 89.6% of isolates would be fully susceptible to amoxycillin–clavulanate according to NCCLS guidelines, compared to 64.4% according to CA-SFM guidelines. Similarly, only 4.8% of isolates were resistant by NCCLS criteria, compared to 14.4% by CA-SFM criteria. These results demonstrated that the large differences in the frequency of *E. coli* isolates susceptible to amoxycillin–clavulanate observed between France and other European countries [8,11–13] are probably attributable largely to technical differences in the guidelines used for antimicrobial susceptibility testing and in the MIC breakpoints used for the interpretation of susceptibility. These susceptibility differences reflect the amount of clavulanate available to inhibit β-lactamase.

No clinical studies have assessed which susceptibility-testing method is the best predictor of clinical efficacy. However, the NCCLS breakpoints were found to be fully predictive of in-vivo efficacy in a rat abscess model [14], in which isolates that would be defined as intermediate to amoxycillin–clavulanate *in vitro* according to CA-SFM methods were found to be susceptible *in vivo*. In addition, a study in a Yucatan miniature pig model showed that concentrations of amoxycillin–clavulanate similar to those seen in human plasma had bactericidal potential against *E. coli* strains [15]. The observed discordance between the MIC value and the efficacy of the antimicrobial combination was probably caused by an overestimation of the MIC value generated by the fixed concentration of clavulanic acid. Given these results, it is likely that all isolates intermediate by the CA-SFM method are susceptible to amoxycillin–clavulanate *in vivo*.

Considering the pharmacokinetic properties of amoxycillin–clavulanate, this combination is probably active against most urinary isolates of *E. coli* [11]. In France, expert opinion is that amoxycillin–clavulanate should not be used for treatment of acute uncomplicated cystitis because of the high rate of resistance in *E. coli*. This and other studies [8,16] demonstrate that this assertion is at least partially unfounded, even though β-lactam agents tend to be less effective than non-β-lactams in eradicating initial bacteriuria and in preventing reoccurrence [7].

In conclusion, the apparent high frequency of non-susceptibility of *E. coli* to amoxycillin–clavulanate in French hospitals is probably caused mostly by a methodological difference rather than a localised resistance problem. Considering the worrying increase in the frequency of fluoroquinolone-resistant *E. coli* isolates, it seems that amoxycillin–clavulanate could be used as an alternative to fluoroquinolones for the treatment of *E. coli* infections, particularly urinary tract infections. Susceptibility tests for amoxycillin–clavulanate should be standardised worldwide to avoid discrepancies resulting from technical differences.

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RESEARCH NOTE

Chlamydia trachomatis infections in heterosexuals attending sexually transmitted disease clinics in Slovenia

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ABSTRACT

This study assessed the age and gender distribution of *Chlamydia trachomatis* infections among patients attending two clinics for sexually transmitted diseases (STDs) in Slovenia. Between January 1999 and December 2003, 1714 heterosexual male and 892 heterosexual female patients were tested for *C. trachomatis*. The prevalence of *C. trachomatis* infection was 19.5% ($n = 334$) for male patients and 10.7% ($n = 96$) for female patients, with the highest prevalence in the group aged 15–30 years. The prevalence decreased between 2000 and 2003 among female patients. The results support the implementation of routine screening for *C. trachomatis* genital infection among male and female patients aged < 30 years attending STD clinics in Slovenia.

Keywords *Chlamydia trachomatis*, infection, prevalence, sexually transmitted disease, Slovenia, urethritis

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Chlamydia trachomatis has been recognised as a major bacterial sexually transmitted disease (STD) in north America and western Europe [1]. The prevalence of infection for men in STD settings is 15–20%, with a corresponding figure for women

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