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Cephalothin is not a reliable surrogate marker for oral cephalosporins in susceptibility testing of Enterobacteriaceae causing urinary tract infection☆,☆☆

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ABSTRACT

Vitek® 2 (bioMérieux) is a widely used commercial antimicrobial susceptibility test (AST) system. AST-N244 card includes cephalothin as first-generation cephalosporin. We compared the cephalothin susceptibility results obtained with Vitek® 2 AST-N244 to those obtained by broth microdilution (BMD) and disk diffusion (DD) for 212 urinary Enterobacteriaceae. We also evaluated the differences between cefazolin and cephalothin susceptibility results. The overall performance of Vitek® 2 for cephalothin testing was 74.5% and 76.4% category agreement compared to BMD and DD, respectively; 84.4% essential agreement; very major errors 15.2% and 11.1% compared to BMD and DD; major errors 0% compared to both methods; and minor errors 22.2% and 21.7% compared to BMD and DD. Regarding correlation between cephalothin and cefazolin, the differences observed were statistically significant ($P < 0.0001$) for the 167 *Escherichia coli* included (39.5% cephalothin susceptible versus 92.2% cefazolin susceptible by BMD; 41.9% cephalothin susceptible versus 93.4% cefazolin susceptible by DD). Vitek® 2 should provide cefazolin instead of cephalothin as a surrogate marker for oral cephalosporins on the urinary AST-244 cards in order to follow the CLSI (2016) recommendations.

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1. Introduction

Cephalothin has been recommended in the past by the Clinical and Laboratory Standards Institute (CLSI) to predict susceptibility to oral agents such as cefadroxil, cefpodoxime, cephalexin, and loracarbef. The predictability of cephalothin as a class marker was based on old data (Barry et al., 1978; Preston et al., 1983). Former CLSI guidelines (2014 and 2015) indicated that testing cefazolin was preferred to testing cephalothin to predict results for oral cephalosporins when used for therapy of uncomplicated urinary tract infections (uUTIs) (CLSI, 2014, 2015a). This indefinite statement might suggest that both antimicrobials could be indistinctly used as surrogate markers in Enterobacteriaceae causing uUTIs. However, several authors have suggested that CLSI should reconsider its recommendation to use cephalothin susceptibility as the predictor of susceptibility to cefadroxil, cefpodoxime, cephalexin, and loracarbef, especially among Enterobacteriaceae uUTI isolates (Bookstaver et al., 2014; Bookstaver et al., 2015; Nguyen and Graber, 2013; Schuetz et al., 2013; Zhang et al., 2007). Actually, the latest guidelines from the CLSI (2016) have deleted cephalothin interpretive breakpoints (CLSI, 2016).

The MicroScan Walk-Away® (Siemens, Dade Behring Inc., West Sacramento, CA, USA) was the automated susceptibility testing system routinely used in our clinical laboratory until 2011, which included cefazolin as first-generation cephalosporin agent in Combo Urines 37 panel. The average resistance rate of Enterobacteriaceae lacking inducible chromosomal AmpC isolated from urine cultures to cefazolin was 14% in 2009 and 13% in 2010, applying the CLSI 2009–based criteria (susceptible, ≤8 μg/mL; resistant, ≥32 μg/mL) (CLSI, 2009). After we implemented the Vitek® 2 (bioMérieux, Marcy l'Étoile, France) system (using the AST-N244 card, which does not contain cefazolin but does include cephalothin), a remarkable high resistance rate of Enterobacteriaceae isolated from urine cultures to cephalothin (22% in 2011, 24% in 2012) was noticed.

The aim of this study was to compare the results of cephalothin testing against Enterobacteriaceae lacking inducible chromosomal AmpC isolated from urine cultures obtained with Vitek® 2 AST-N244 card with those obtained with the reference methods broth microdilution (BMD) and disk diffusion (DD). Selection of this group of Enterobacteriaceae (including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter koseri*, and *Klebsiella oxytoca*) was made

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because first-generation cephalosporins remain active against them (Leclercq et al., 2013). This is due to the fact that chromosomal ampC genes are expressed constitutively at a low level in this group, unlike other Enterobacteriaceae such as *Enterobacter* spp., *Citrobacter* spp., and *Serratia* spp., which carry an inducible ampC gene. In these cases, the gene is strongly induced by β-lactams, such as cefoxitin and imipenem, with expression mediated by the regulator AmpR, conferring resistance to first-generation cephalosporins. The regulation of chromosomal ampC expression in *E. coli* differs considerably from that in other Enterobacteriaceae. *E. coli* lacks ampR, and thus, ampC expression is not inducible (Jacoby, 2009).

Additionally, we also evaluated the differences between *in vitro* susceptibility to cephalothin and cefazolin in the same set of organisms.

2. Materials and methods

2.1. Bacterial isolates

A total of 212 Enterobacteriaceae obtained from urine samples submitted to our service between March 2014 and April 2014 were studied. Production of inducible chromosomal AmpC was excluded phenotypically by antibiogram interpretative reading, based on EUCAST Expert rules in antimicrobial susceptibility testing (AST) (Leclercq et al., 2013). The Enterobacteriaceae included were *E. coli* (n = 167; 78.8%), *K. pneumoniae* (n = 23; 10.8%), *P. mirabilis* (n = 11; 5.19%), *C. koseri* (n = 6; 2.8%), and *K. oxytoca* (n = 5; 2.4%).

2.2. Automated identification and susceptibility testing

Each isolate was tested with the Vitek® 2 system, according to the manufacturer's instructions, using a gram-negative identification card and an antimicrobial susceptibility testing card for urinary Enterobacteriaceae (AST-N244). The MIC calling range for cephalothin on the Vitek® 2 AST-N244 card was ≤2 μg/mL to ≥64 μg/mL in doubling dilutions. MIC results were classified as susceptible, intermediate, or resistant based on the 2014 CLSI breakpoints. The quality control strains tested with each run included *E. coli* ATCC 25922 and *K. pneumoniae* 700603.

2.3. Susceptibility testing of cephalothin and cefazolin by reference methods

Susceptibility to cephalothin and cefazolin was determined by both BMD and DD, following the CLSI guidelines (CLSI, 2015b, 2015c). The MICs were assessed in microdilution plates containing cation-adjusted Mueller–Hinton broth (Oxoid LTD, Basingstoke, Hants, UK). The wells contained serial 2-fold dilutions of cephalothin or cefazolin ranging from 0.125 to 128 μg/mL. DD was performed using Mueller–Hinton agar plates (Oxoid) and disks (Oxoid) containing 30 μg of cephalothin and cefazolin, respectively. *E. coli* ATCC 25922 was included as quality control strain.

Categorical interpretation (susceptible, intermediate, or resistant) of antimicrobial susceptibility test results from Vitek® 2, BMD, and DD was defined according to the CLSI (2015a) interpretive criteria for Enterobacteriaceae in the case of cephalothin and CLSI (2016) for uUTIs in the case of cefazolin (Table 1).

2.4. Data analysis

Categorical agreement, very major, major, and minor errors between the automated system Vitek® 2 and the 2 reference methods were calculated following previous recommendations by the US Department of Health and Human Services Food and Drug Administration (US Department of Health and Human Services, 2009). The comparative analysis of cephalothin and cefazolin susceptibility results was determined using Fisher's exact test (statistical significance was tested at $\alpha \leq 0.05$).

3. Results

The cephalothin and cefazolin susceptibility testing results for the 212 urinary tract isolates evaluated by the 2 reference methods and by the Vitek® 2 system are summarized in Table 2.

On evaluation of Enterobacteriaceae susceptibility to cephalothin, the Vitek® 2 system displayed a 74.5% category agreement compared to BMD and 76.4% compared to DD. The essential agreement between the automated system and BMD accounted for 84.4%. Error rates are provided in Table 3 for comparison.

Remarkable discrepancies between reference methods, BMD and DD, were found for testing cephalothin (Table 4). In fact, 18.6% (11/59) and 26.1% (12/46) of the isolates were defined as intermediate or resistant to cephalothin by microdilution but were considered susceptible and intermediate, respectively, by DD. Additionally, there were 2 *E. coli* strains for which the 2 reference methods had a category disagreement in cefazolin susceptibility results. One isolate showed a 15-mm zone of inhibition (susceptible), while the MIC was 64 μg/mL (resistant), and the second one presented an 18-mm zone (susceptible) and an MIC of 32 μg/mL (resistant).

Overall, a higher percentage of isolates were susceptible to cefazolin (93.4% by BMD and 94.3% by DD) than to cephalothin (50.5% by BMD and 52.8% by DD). The difference observed between cephalothin and cefazolin susceptibility rate was statistically significant ($P < 0.0001$) for the 167 *E. coli* strains studied (39.5% susceptible to cephalothin versus 92.2% susceptible to cefazolin by BMD; 41.9% susceptible to cephalothin versus 93.4% susceptible to cefazolin by DD).

The MIC distributions of cephalothin and cefazolin for the *E. coli* strains we have studied are shown in Fig. 1. MIC_{50} for cephalothin was 16 μg/mL, whereas MIC_{50} for cefazolin was 2 μg/mL. In addition, MIC_{90} for cephalothin was 128 μg/mL, while MIC_{90} for cefazolin was 16 μg/mL. Using the current breakpoints, 79.7% versus 39.5% of the isolates had cefazolin MIC and cephalothin MIC in the susceptible range, respectively. A total of 88 (88/101; 87.1%) *E. coli* strains classified as intermediate or resistant to cephalothin were susceptible to cefazolin by BMD method.

Fig. 2 shows the concordance between disk zone diameter (millimeters) for cefazolin versus cephalothin. By DD method, 86 *E. coli* strains (86/156; 55.1%) susceptible to cefazolin showed resistance or reduced susceptibility (intermediate category) to cephalothin.

4. Discussion

Oral first-generation cephalosporins (cephalexin and cefadroxil) are one of the alternatives for empirical therapy of uUTI in children, as indicated by several clinical guidelines, both in Spain (Biblioteca de Guías de Práctica Clínica del Sistema Nacional de Salud, Programa de Guías de Práctica Clínica en el Sistema Nacional de Salud (España),

Table 1
CLSI breakpoints for cephalothin and cefazolin in Enterobacteriaceae causing uUTIs.

	Antimicrobial agent	Disk content	Zone diameter (mm)			MIC (μg/mL)		
			S	I	R	S	I	R
CLSI 2014/2015	Cephalothin (parenteral, uUTI)	30 μg	≥18	15–17	≤14	≤8	16	≥32
CLSI 2016	Cefazolin (parenteral and oral, uUTI)	30 μg	≥15		≤14	≤16		≥32

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