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Cefditoren versus ceftazidime in inducer-substrate combinations for the evaluation of AmpC production in a disc approximation test

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ABSTRACT

Objective: To evaluate cefditoren in inducer-substrate combinations to screen for AmpC induction.

Methods: 100 clinical isolates (25 *P. aeruginosa*, 25 *E. cloacae*, 14 *M. morganii*, 13 *S. marcescens*, 12 *C. freundii*, 7 *P. rettgeri*, and 4 *E. aerogenes*) were tested by the Kirby-Bauer disc approximation method using cefditoren and ceftazidime discs as substrates, and cefditoren and imipenem discs as inducers.

Results: None of the strains showed induction of AmpC with cefditoren-ceftazidime as inducer-substrate combination. Imipenem-cefditoren as inducer-substrate combination was not useful for evaluating strains of *P. aeruginosa* since no inhibition zones surrounding the cefditoren disc were found. Among evaluable enterobacteria (those showing substrate inhibition zone), inducible Amp C was detected in 48 out of 63 (76.2%) with cefditoren, and in 33 out of 68 (48.5%) isolates with ceftazidime as substrate. Significantly (p= 0.013) higher number of AmpC producers were detected with cefditoren versus ceftazidime (76.2% vs. 48.5%), due to the differences found for *E. cloacae* (72.8% vs. 21.7%; p= 0.0009) and *S. marcescens* (100% vs. 54.5%; p= 0.03). Higher mean reductions of diameters around substrate discs were found for cefditoren (4.17 mm) vs. ceftazidime (3.79 mm), reaching statistical significance (p<0.05) for indol-positive proteae: *M. morganii* (5.32 mm vs. 3.92 mm) and *P. rettgeri* (3.47 mm vs. 2.64 mm).

Conclusion: Cefditoren showed no induction capability, and when used as substrate (with imipenem as inducer) it offered detection rates of AmpC inducible enterobacteria higher than the imipenem-ceftazidime combination, mainly for *Enterobacter spp.* and *Serratia spp.*, with higher diameter reductions for indol-positive proteae.

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Key words: Inducible ß-lactamase; cefditoren; ceftazidime; imipenem; ß-lactamase detection

Cefditoren versus ceftazidima en combinaciones inductor-substrato para detectar la producción de AmpC mediante el método de disco

RESUMEN

Objetivo: Evaluar el cefditoren en combinaciones inductor-substrato para la detección de inducción de AmpC.

Métodos: 100 aislados clínicos (25 *P. aeruginosa*, 25 *E. cloacae*, 14 *M. morganii*, 13 *S. marcescens*, 12 *C. freundii*, 7 *P. rettgeri*, and 4 *E. aerogenes*) se ensayaron por el método de disco Kirby-Bauer utilizando discos de cefditoren y ceftazidima como substratos y de cefditoren e imipenem como inductores.

Resultados: Ninguna cepa mostró inducción de AmpC con la combinación cefditoren-ceftazidima como inductor-substrato. La combinación de imipenem-cefditoren como inductor-substrato no fue adecuada para evaluar las cepas de *P. aeruginosa* ya que no hubo halo de inhibición alrededor del disco de cefditoren. En las enterobacterias que se pudieron evaluar (por presentar halo de inhibición alrededor del substrato), se detectó AmpC inducible en 48 de 63 (76.2%) con cefditoren, y en 33 de 68 (48.5%) de los aislados con ceftazidima como substrato. Se detectó un número significantivamente (p= 0.013) mayor de productores de AmpC con cefditoren que con ceftazidima (76.2% vs. 48.5%), debido a las diferencias encontradas en *E. cloacae* (72.8% vs. 21.7%; p= 0.0009) y *S. marcescens* (100% vs. 54.5%; p= 0.03). Las reducciones medias del diámetro alrededor de los discos del substrato fueron mayores para cefditoren (4.17 mm) que para ceftazidima (3.79 mm), alcanzando significación estadística (p<0.05) en proteáceas indol-positivo: *M. morganii* (5.32 mm vs. 3.92 mm) y *P. rettgeri* (3.47 mm vs. 2.64 mm).

Conclusión: Cefditoren no presentó capacidad de in-

ducción, y utilizado como substrato (con imipenem como inductor) ofreció una tasa de detección de AmpC inducible en enterobacterias superior de la de la combinación imipenem-ceftazidima, principalmente en *Enterobacter* spp. y *Serratia* spp., con mayores reducciones del diámetro en proteáceas indol-positivo.

Palabras clave: ß-lactamasa inducible; cefditoren; ceftazidima; imipenem; detección de ß-lactamasas

INTRODUCTION

Upon exposure to inducing agents (aminopenicillins, first-generation cephalosporins, cephamycins and carbapenems as strong inducers, and second- or third-generation cephalosporins, acylureidopenicillins or monobactams as weakly inducers), isolates of *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Serratia marcescens*, *Morganella morganii* and *Providencia rettgeri*, among enteric bacteria, and *Pseudomonas aeruginosa*, among human colonisers, have potential to produce AmpC inducible chromosomal ß-lactamases[1,2]. This production returns to basal levels hardly detectable when the inducer is removed, thus when isolated from patients, bacteria are found to be susceptible to third-generation cephalosporins, but when these drugs are clinically used selection of derepressed mutants (constitutively producing ß-lactamase) occurred with contingent clinical failure[2,3]. Association between use of third-generation cephalosporins and resistance emergence among organisms with inducible chromosomally encoded AmpC ß-lactamases has been established[3].

Cefditoren is a third-generation cephalosporin licensed for the treatment of upper and lower respiratory tract infections. Cefditoren and cefotaxime exhibit similar MIC_{50}/MIC_{90} values for *P. aeruginosa* (≥16/≥16 µg/ml), *Citrobacter spp.* (≤1/≥16 µg/ml) and *Enterobacter spp.* (≤1/≥16 µg/ml), but not for indol-positive proteae (0.12/≥16 µg/ml for cefditoren vs. ≤0.06/2 µg/ml for cefotaxime)[4,5]. Third-generation cephalosporins as cefotaxime or ceftazidime have weak induction potential for AmpC but are labile to it[2], so they can serve as substrates to screen AmpC induction[6].

The aim of this study was to evaluate cefditoren in inducer-substrate combinations to screen for AmpC induction.

MATERIAL AND METHODS

One hundred clinical isolates (25 *P. aeruginosa*, 25 *E. cloacae*, 14 *M. morganii*, 13 *S. marcescens*, 12 *C. freundii*, 7 *P. rettgeri*, and 4 *E. aerogenes*) were tested by the Kirby-Bauer disc approximation method7 using cefditoren 5 and 10 µg discs and ceftazidime 30 µg discs as substrates, and cefditoren 5 and 10 µg and imipenem 10 µg discs as inducers. Discs were located as shown in Figure 1 with a 25 mm distance between them. A positive induction was considered when the inhibition zone of the substrate disc was reduced by ≥2 mm[1].

In addition production of extended-spectrum ß-lactamase (ESBL) was investigated by including in the template ceftazidime (30 µg) and ceftazidime/clavulanate (30 + 10 µg) disc located as shown in Figure 1. ESBL production was inferred when there was a reduction ≥5 mm in inhibition zones[8] in the ceftazidime versus the ceftazidime/clavulanate disc. A cefotaxime disc (30 µg) was also included in the template. Those strains showing absence of inhibition for ceftazidime (due to possible derepressed AmpC and/or ESBL) and ceftazidime/clavulanate (due to the possible induction of AmpC by clavulanate[2]), were plated on a second template containing cefepime discs (30 µg) to differentiate both types of enzymes[9]. Photographs of the incubated plates were taken using visualization *Gel Doc* (Bio Rad, Madrid, Spain) and inhibition zones were measured using the ImageJ program (http://rsbweb.nih.gov/ij/). Comparisons of percentages of AmpC isolates detected with the different substrates were performed with the Fisher's exact test, and reductions in diameters were compared by the Student's t-test.

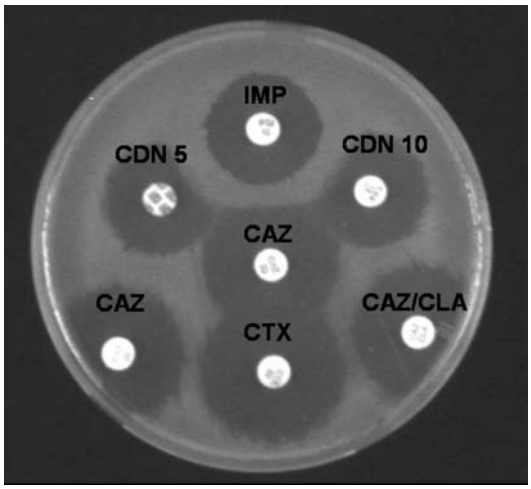

Figure 1 — **Photograph (*C. freundii*) showing the placement of antibiotic discs: IMP (imipenem 10 µg), CDN 5 (cefditoren 5 µg), CDN 10 (cefditoren 10 µg), CAZ (ceftazidime 30 µg), CAZ/CLA (ceftazidime/clavulanate 30 µg + 10 µg), and CTX (cefotaxime 30 µg).**

RESULTS AND DISCUSSION

When using cefditoren-ceftazidime as inducer-substrate combination (whether with 5 µg or 10 µg cefditoren discs), none of the strains showed AmpC induction, thus discarding cefditoren as inducer.

With regard to cefditoren as substrate, the combination imipenem-cefditoren as inducer-substrate was not useful for evaluating *P. aeruginosa* since zones of inhibition surrounding the cefditoren discs were not present. This only occurred in one

Table 1 **Number (n) of valuable strains (those showing inhibitory zone) among the *Enterobacteriaceae* tested; strains (n; %) showing ≥2 mm reduction when using cefditoren and ceftazidime as substrate , and mean ± SD reduction in the inhibitory zone.**

	Cefditoren 10 µg			Ceftazidime 30 µg		
	n	No. (%) strains with reduction in inhibition zone	Reduction (mean ± SD; mm)	n	No. (%) strains with reduction in inhibition zone	Reduction (mean ± SD; mm)
C. freundii	7	6 (85.7%)	5.16 ± 2.37	10	7 (70.0%)	4.94 ± 2.98
M. morganii	13	10 (76.9%)	5.32 ± 1.11*	13	11 (84.6 %)	3.92 ± 1.59
P. rettgeri	7	3 (42.9%)	3.47 ± 0.25*	7	2 (28.6%)	2.64 ± 0.02
S. marcescens	11	11 (100.0%)**	3.50 ± 0.93	11	6 (54.5%)	2.78 ± 0.95
E. cloacae	22	16 (72.8%)**	4.19 ± 1.00	23	5 (21.7%)	3.88 ± 2.39
E. aerogenes	3	2 (66.6%)	3.45 ± 0.97	4	2 (50.0%)	2.76 ± 0.03
Total	**63**	**48 (76.2%)****	**4.17 ± 1.41**	**68**	**33 (48.5%)**	**3.79 ± 2.02**

*Significantly ($p<0.05$) higher reduction than ceftazidime.

**Significantly ($p<0.05$) higher percentage of AmpC strains detected with cefditoren versus ceftazidime

strain with ceftazidime (that showed inhibition zone around the cefepime disc). Of the remaining 24 strains tested with the imipenem-ceftazidime combination, 18 strains (75%) presented inducible AmpC, three of them with concomitant ESBL production.

Among the 75 *Enterobacteriaceae* tested, there was absence of inhibition zone in 14, 12 and 7 isolates with cefditoren 5 µg, cefditoren 10 µg and ceftazidime 30 µg, respectively. Among evaluable strains, inducible Amp C was detected in 48 out of 61 (78.7%), 48 out of 63 (76.2%), and 33 out of 68 (48.5%) isolates with cefditoren 5 µg, cefditoren 10 µg and ceftazidime 30 µg as substrates, respectively. Table 1 shows number and percentage of valuable isolates showing inducible AmpC using cefditoren 10 µg and ceftazidime 30 µg as substrates by species. Significantly (p= 0.013) higher number of AmpC producers were detected with cefditoren 10 µg versus ceftazidime 30 µg (76.2% vs. 48.5%). This difference was due to the significant difference found for *E. cloacae* isolates (72.8% vs. 21.7%; p= 0.0009) and *S. marcescens* (100% vs. 54.5%; p= 0.03) since no differences were found for other species. With respect to reductions of diameters around substrate discs, higher reductions were found for cefditoren 10 µg vs. ceftazidime 30 µg (mean reductions of 4.17 mm and 3.79 mm, respectively), reaching statistical significance for indol-positive proteae: mean reductions of 5.32 mm vs. 3.92 mm (p= 0.03) for *M. morganii* and 3.47 mm vs. 2.64 mm (p= 0.02) for *P. rettgeri*. No differences were found between cefditoren 5 µg and 10 µg with respect to AmpC detection or mean reductions in positive isolates.

When defining as inducible strains those positive with any inducer-substrate combination, 53 out of 75 (70.7%) *Enterobacteriaceae* complied with this criterion. Of these 53 strains, cefditoren detected 48 (90.57% sensitivity; 95%CI 82.7-98.4) and ceftazidime 33 (62.26% sensitivity; 95% CI 49.2-75.3).

With respect to the seven *Enterobacteriaceae* showing absence of inhibition zone around ceftazidime and ceftazidime/clavulanate discs (derepressed AmpC with or without ESBL), only one *E. cloacae* showed a resistance pattern to cefepime suggesting ESBL production in addition to derepressed AmpC.

In conclusion although the combination imipenem-cefditoren as inducer-substrate was not useful for AmpC detection in non-fermenters as *P. aeruginosa*, it offered detection rates of AmpC inducible *Enterobacteriaceae* higher than the imipenem-ceftazidime combination, mainly for *Enterobacter spp.* and *Serratia spp.* isolates, with higher mean diameter reductions for indol-positive proteae. These findings can be relevant for microbiologists for investigational or epidemiological purposes to better discriminate isolates of these *Enterobacteriaceae* species without inducible AmpC in bacterial collections, but logically have limited relevance from the clinical perspective since inducible AmpC should be suspected, and subsequently empirically covered by antibiotic therapy, in isolates of these *Enterobacteriaceae* species.

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