Evaluation of Ceftazidime contents in antibiotic discs by capillary electrophoresis

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SUMMARY

Good quality of antibiotic discs is a fundamental prerequisite to accurate antibiotic susceptibility tests. Capillary electrophoresis (CE) is a widely used method for quantitative analysis. Here, using ceftazidime as an example, we report an easy-to-perform strategy to determine ceftazidime content in discs. First, a serial of ceftazidime standard solutions was prepared to determine the detection linearity. Utilizing the background buffer containing 50 mmol/L Na_2HPO_4, 0.045 mmol/L β-cyclodextrin and 3.15 mmol/L Tris (hydroxymethyl) aminomethane, ceftazidime of concentrations ranging from 1.875 to 60 μg/ml showed a linear response to detected peak areas. Subsequently, four kinds of ceftazidime discs were selected from different manufacturers. The discs were homogenized using 1 ml deionized water, and then detected by CE after filtration. The results showed that one kind of discs were of poor quality as further confirmed by disc diffusion tests using standard strains. This study proved the potential of CE as an easy choice to perform disc quality control under appropriate conditions.

KEY WORDS: Ceftazidime, Capillary electrophoresis, Quality control

Received December 24, 2007 *Accepted* February 19, 2008

INTRODUCTION

A correct and timely antibiotic susceptibility test report plays a key role in selecting proper treatment for curing infections. The worldwide increase in antibiotic resistance is a public health concern. The fact that the choice of dose and treatment duration can affect the selection of antibiotic-resistant mutants is becoming more evident (Olofsson and Cars, 2007). Thus, the antibiotic reports are not only the basis for physicians to prescribe suitable antibiotics, but also a good measure to prevent new resistance's arising. Many methods can be adopted to perform antibiotic susceptibility tests, e.g., disc diffusion, E-test and broth dilution (Toraman *et al.*, 2004; Prashanth and Badrinath 2004; Sieradzki and Tomasz 2006; Sieradzki *et al.*, 1999).

Traditionally, the disc diffusion method, deemed the K-B method, has been more popular in clinical laboratories because of its lower cost, easy performance and good reproducibility (Lopez-Oviedo *et al.*, 2006). Because the antibiotic susceptibilities of microbes are evaluated by the zones of inhibition, which are sensitive to the antibiotic content in the discs, the exact antimicrobial agent quantity in the discs is a core factor for a reliable report.

The quality of antibiotic discs, from every aspect, has been described in documents provided by Clinical Laboratory Standards Institute (CLSI). To ascertain the quality of discs, CLSI also des-

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ignates many standard strains to be used to perform quality control (Brown and Traczewski 2007). The culture-based evaluation method is time-consuming and it cannot give a definite content of certain agent in the disc.

Ceftazidime (CAZ) is a representative 3rd generation cephalosporin with a broad spectrum of antimicrobial activity against gram-positive and gram negative microorganims including *Pseudomonas aeruginosa*, and thus has gained widely application in anti-inflammatory treatment clinically (Baraniak *et al*., 2002). Its high degree of activity and tolerability profile makes it a useful option for the treatment of severe infections especially in intensive care units (Traunmuller *et al*., 2002). It is also recommended as an indicator agent to screen some extended-spectrum beta-lactamases (ESBLs) producing enterobacteria routinely by CLSI (Nogueira *et al*., 2006). In order to determine the exact contents of CAZ in commercial discs, we employed a capillary electrophoresis (CE) method to rapidly and quantitatively detect CAZ. This CE-based method was also applied to real evaluation of four kinds of CAZ discs from different manufacturers. The results were also compared with traditional culture method results using standard strains.

MATERIALS AND METHODS

Equipment and regents

All quantitative experiments were performed using a Beckman P/ACE System MDQ capillary electrophoresis system (Palo Alto, CA, USA) equipped with a DAD detector. Analyte separation was carried out in the 75 cm long fused-silica capillaries (70 cm effective length) with the inner diameter of 50 μm provided by Yongnian Optical Fiber Company (Hebei, China). Detection wavelength was set at 254 nm and the capillary column temperature was maintained at 20°C. In order to minimize sample evaporation, the sample compartment was thermostated at 10°C. Samples were injected by pressure at 0.5psi (34.47 kPa) in 5 s, and separated by a constant voltage of 15 kV. Prior to first use, new capillary columns were rinsed with 1 M and 0.1 M NaOH for 2 min respectively, and then treated with background buffer solution for 2 min. The electrode buffer was replaced after every third run.

Standard ceftazidime pentahydrate (84.7% anhydrous ceftazidime) and Δ^2-isomer powder was kindly donated by the Pharmaceutical Department of Dalian Municipal Central Hospital. All the products were used without further purification and stored at -40°C. Water used in the experiments was purified with Mili-Q system (Millipore, Bedford, MA, USA).

CAZ discs

4 kinds of CAZ discs (in brief A, B, C and D) were commercially available products from different manufacturers. Their labeled contents were all 30 μg/disc for each. The discs were stored at -40 with dessicant.

K-B disc diffusion tests

Three American Type Culture Collection strains-*E. coli* (ATCC25922), *S. aureus* (ATCC25923) and *P. aeruginosa* (ATCC27853) were utilized as standard control strains. Fresh cultures were acquired using MH broth. Newly prepared suspensions of 0.5 Mcf, determined by a bioMerieux (France) DENSIMAT transmissometer, were utilized to perform K-B disc diffusion antibiotic susceptibility tests according to the procedures well established by CLSI.

Statistical analysis

Statistical analysis was performed using Minitab v.14 (Minitab, USA).

RESULTS

Optimization of analytical conditions

Optimization of the analytical conditions was focused on the modification of ratios among Na_2HPO_4, β-cyclodextrin and Tris (hydroxymethyl) aminomethane in the background buffer.

At last background buffer containing 50mmol/L Na_2HPO_4, 0.045 mmol/L β-cyclodextrin and 3.15 mmol/L Tris (hydroxymethyl) aminomethane was thought to be suitable because of the relatively short analytical time and good resolution between CAZ and its isomer. Under the optimized conditions, the electrophoregram was shown in Figure 1. The migration time of CAZ was 3.1 min (RSD=3%, n=5), and its isomer was 2.2 min (RSD=4%, n=5).

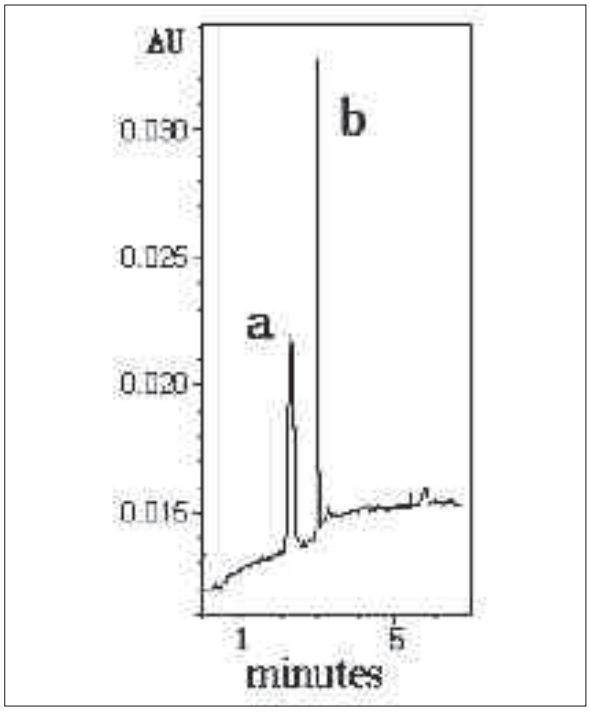

FIGURE 1 - *Electrophoregram of CAZ and its Δ^2-isomer. Peak with migration time of 3.1 min was CAZ (b). Its isomer was 2.2 min(a). Detection wavelength was 254 nm.*

Detection linearity of the method

A series of CAZ solutions were prepared with the concentrations of 60.000, 30.000, 15.000, 7.500, 3.750 and 1.875 µg/ml respectively. Each solution was then subjected to detection by CE in triplicate. The concentrations of CAZ (y) showed a linear response to peak areas (x) in the CE electrophoregrams with regression equation of $y = 0.0005x - 0.6053$ ($R^2 = 0.997$).

Evaluation of CAZ contents in discs

Every 10 discs were randomly selected from the four manufacturers' products. Each disc was put into an aseptic ampoule containing 1 ml aseptic deionized water, and homogenized by sucking and releasing using aseptic pipette tips until the fiber was evenly suspended. After filtration by a 0.22 µm membrane, the solution was detected by CE. The results were given in Table 1 and Figure 2.

Evaluating the quality of the discs by standard strains

K-B disc diffusion tests proposed by CLSI were performed using 10 randomly selected discs from each manufacturer. For the three recommended ATCC strains, their corresponding zones of inhibition (diameter in mm) were shown in Table 2.

DISCUSSION

The quality of antibiotic discs is the key issue in the performance of susceptibility tests. CAZ contains two kinds of isomers. Its Δ^2-isomer deriving from an isomerization process is usually regarded as an impurity with no antimicrobial effects; we select an additive - beta-cyclodextrin, to discriminate the Δ^2 -isomer from its counterpart (Hammitzsch-Wiedemann and Scriba 2007) in case of an occasionally transformed form from the active component of CAZ. We did not find the Δ^2 - isomer component in any of the electrophoregrams. This result meant that the purity of CAZ was ensured properly by all the selected manufacturers, and the storage was also satisfied because of no isomer transformation taking place.

From the CE detection results in Figure 2 we could conclude that the discs from manufacturers C and D were of relatively good quality, because their 95% confidence intervals of means

TABLE 1 - *Comparison of CAZ contents in the discs from different manufacturers.*

Disc manufactures	*CAZ contents (µg) (mean ± 2SD)*	*P values*		
		B	*C*	*D*
A	27.28±2.17	0.046	0.036	0.064
B	26.02±2.96		0.002	0.002
C	28.14±0.62			0.340
D	28.02±0.49			

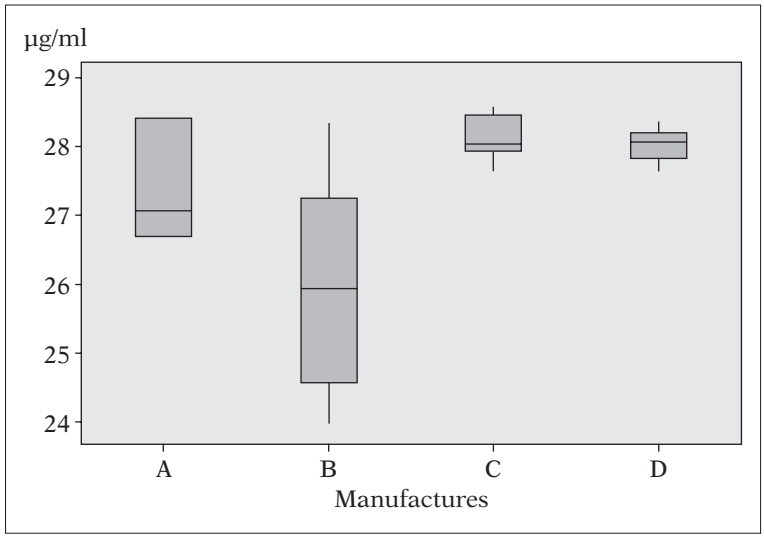

FIGURE 2 - *Box plots of CAZ contents in discs from 4 different manufacturers with 95% confidence intervals of corresponding means.*

TABLE 2 - *Results of zones of inhibition detected by K-B method (mean ±2SD).*

Test strains	*Disc manufacturers*			
	A	*B*	*C*	*D*
ATCC25922 (25-32)*	26.8±3.5	26.4±6.3	29.0±1.6	29.1±1.5
ATCC25923 (16-20)	17.9±1.9	17.8±2.1	18.1±1.5	18.2±1.3
ATCC27853 (22-29)	26.2±2.9	26.2±3.5	27.2±1.89	27.3±2.1

*Reference zones of inhibition (mm) from CLSI

were in narrow ranges (near to 30 μg). Although CAZ contents in discs from A and B distributed in relatively broad scales, quality of discs from manufacturer A was acceptable to some extent. This conclusion was based on the fact that the zones of inhibition, determined by discs from manufacturer A using three standard strains, were all in the permitted ranges. One case failed when using 10 discs from manufacturer B to perform K-B antibiotic susceptibility tests. The failure took place in one performance using ATCC 25922, which zone of inhibition was 24 mm. From Table 2, we could also discern the great variance in the zones of inhibition results of disc B for every strain. In view of CAZ contents, the inferior quality of discs of manufacturer B could also be confirmed in that this kind of discs had the lowest content of CAZ (Figure 2 and Table 1).

From the results listed in Table 1, the content of CAZ in every disc was not 30 μg as labeled. This was thought to be the result of sample preparation. CAZ was not very stable in aqueous solution, and was sensitive to temperature when dissolved. In order to ensure the accuracy of detection, avoiding daylight overexposure, storage at low temperature and rapid processing was very important (Wade *et al*., 1991; Zhou and Notari 1995). In reality, from the sample preparation to finishing the detection by CE, The whole performance could be accomplished in 30 min.

Due to the lower sample volume required, higher separation efficiency and relatively simple operation, CE has gained wide application in many aspects. In microbiology, CE had been employed to aid bacteriology researches in many aspects (Gao *et al*., 2007; Gao *et al*., 2006). For appraisal of antibiotic disc quality, we seldom found the application of CE. Not only to that, although many methods had been proposed to aid the quality control of antimicrobial disc susceptibil-

ity testing, the applicable measures aiming at detecting the exact content of a certain agent in antibiotic discs were also scarce (Barry *et al*., 1972). In this study, the developed CE method could finish single detection in 30 min including the several minutes of CE detection. The exact content of CAZ in the disc was easily deduced. Compared to the traditional K-B disc diffusion strategy, our CE approach was time-saving and easy to perform. No culture or special equipment was needed. With rational selection of the components in the background buffer, this CE-based tactic might be developed to be a useful complement to using the reference strains method for the evaluation of antibiotic disc quality in clinical laboratory.

ACKNOWLEDGEMENT

The research was partially sponsored by Dalian Center of Clinical Laboratory. We would like to thank the technologists in the Clinical Laboratory of Dalian Municipal Central hospital for their cooperation.

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