

Narrow-Spectrum Cephalosporin Susceptibility Testing of *Escherichia coli* with the BD Phoenix Automated System: Questionable Utility of Cephalothin as a Predictor of Cephalexin Susceptibility[∇]

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The resistance of *Escherichia coli* to cephalothin was found to be overestimated when the Phoenix automated susceptibility system was used to determine resistance compared to reference broth microdilution, a finding that jeopardized the use of cephalexin for first-line treatment of urinary tract infections in children. In addition, using broth microdilution, we studied the accuracy of either cephalothin or cefazolin in predicting cephalexin susceptibility. In contrast to the recommendation of the Clinical Laboratory Standards Institute (CLSI), we found that cephalothin is not a reliable predictor of cephalexin susceptibility. Cefazolin performs no better in this role. We suggest that laboratories should consider testing and reporting cefazolin and cephalexin independently, according to clinical need.

Cephalexin is the empirical oral antibiotic of choice for the treatment of urinary tract infections (UTIs) in children at the Hospital for Sick Children. Cephalexin susceptibility is not routinely tested in the laboratory due to the absence of interpretive guidelines from the Clinical Laboratory Standards Institute (CLSI) (1). Its absence on most commercial panels reflects the CLSI recommendation that cephalothin should be used to predict cephalexin susceptibility (2). After implementing the Becton-Dickinson (BD) Phoenix Automated System method (PHX) in our laboratory, we noted an unusual susceptibility pattern in our urinary *Escherichia coli* isolates: ampicillin susceptible, cefazolin susceptible, and not susceptible to cephalothin (intermediate or resistant). Resistance to cephalothin rose from 5% to 86%, while resistance to ampicillin (52%) and cefazolin (4%) did not change. This alteration in the susceptibility profile resulted in the potential elimination of cephalexin as a first-line treatment for UTIs based on results using cephalothin to predict cephalexin susceptibility. Since the use of quinolones in children is not recommended, the default empirical oral choice of drug would be limited to the much more expensive and broader-spectrum drug, cefixime.

In this study, we attempted to compare the BD PHX to reference broth microdilution (BMD) for susceptibility testing of ampicillin and the narrow-spectrum cephalosporins cephalothin, cefazolin, and cephalexin. In addition, we also evaluated the validity of using either cephalothin or cefazolin as a predictor of cephalexin susceptibility.

This study examined 225 clinical isolates of *E. coli* (primarily

from urine cultures). The 225 isolates were categorized into four groups based on their susceptibility pattern to ampicillin, cephalothin, and cefazolin determined by BD PHX. Group 1 consisted of ampicillin-susceptible, cephalothin-susceptible, cefazolin-susceptible isolates. Group 2 consisted of ampicillin-susceptible, cephalothin-intermediate/resistant, cefazolin-susceptible isolates. Group 3 consisted of ampicillin-resistant, cephalothin-intermediate/resistant, cefazolin-susceptible isolates. Group 4 consisted of ampicillin-resistant, cephalothin-resistant, cefazolin-resistant isolates. Antimicrobial susceptibility testing (AST) was performed using PHX and BMD for four antibiotics, ampicillin, cephalothin, cefazolin, and cephalexin. PHX testing was performed according to the manufacturer's instructions, while BMD was performed according to CLSI guidelines (1).

Interpretive breakpoints for ampicillin, cephalothin, and cefazolin were determined according to CLSI guidelines (2). Specific breakpoints for cephalexin do not exist in the CLSI guidelines; the recommendation is to use cephalothin to predict cephalexin susceptibility. For the purpose of this study, cephalexin breakpoints were determined as for other narrow-spectrum cephalosporins according to CLSI guidelines, i.e., susceptible, ≤ 8 $\mu\text{g/ml}$; intermediate, 16 $\mu\text{g/ml}$; and resistant, ≥ 32 $\mu\text{g/ml}$. The rates of very major errors, major errors, and minor errors were calculated for ampicillin, cephalothin, cefazolin, and cephalexin (PHX versus BMD). The very major error rate should be $\leq 3\%$, while the rate for the combination of major and minor errors should be $\leq 7\%$ (4, 5). The evaluation of the utility of either cephalothin or cefazolin as a predictor of cephalexin susceptibility was based on reference BMD results.

On evaluation of *E. coli* susceptibility to ampicillin and narrow-spectrum cephalosporins, PHX results were 100% concordant with BMD results with respect to ampicillin susceptibility (Table 1). Although the error rate for very major errors was

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TABLE 1. Error rates for BD PHX AST compared to BMD MICs for ampicillin and narrow-spectrum cephalosporins in *E. coli*

| Antibiotic | Error rate (%) for: | | |
|-------------|--------------------------------|---------------------------|---------------------------|
| | Very major errors ^a | Major errors ^b | Minor errors ^c |
| Ampicillin | 0 (0/112) | 0 (0/112) | NA |
| Cefazolin | 3 (1/35) | 1 (2/183) | 6 (14/225) |
| Cephalothin | 9 (9/101) | 0 (0/36) | 42 (95/225) |
| Cephalexin | 0 (0/27) | 1 (2/159) | 15 (34/225) |

^a The very major error rate is the number of isolates that were susceptible by PHX/number of isolates that were resistant by BMD (the numbers used to calculate the error rate are shown in parentheses).

^b The major error rate is the number of isolates that were resistant by PHX/number of isolates that were susceptible by BMD (the numbers used to calculate the error rate are shown in parentheses).

^c The minor error rate is the number of isolates that were susceptible or resistant by either method (PHX or BMD)/total number of isolates (the numbers used to calculate the error rate are shown in parentheses). NA, not available for analysis.

3% and the error rate for major and minor errors combined was 7% when PHX was used to study cefazolin susceptibility (Table 1), these error rates were still within acceptable limits of variability. However, the error rate for very major errors was 9% and the error rate for major and minor errors combined was 42% when PHX was used to examine cephalothin susceptibility (Table 1). These error rates are considerably higher than the recommended acceptable upper limits (4, 5) and result in significant overestimates by PHX of resistance to cephalothin and therefore cephalexin. Sixty-one of 88 (69%) cephalothin-intermediate results by PHX were actually susceptible by BMD, and 40 of 101 (40%) resistant results by PHX were actually susceptible or intermediate by BMD. In a similar study, a 21% minor error rate in cephalothin susceptibility was observed when PHX results were compared to agar diffusion AST results (3).

When comparing PHX to BMD results for cephalexin, PHX resulted in an error rate of 16% for major and minor errors combined (Table 1), using CLSI-based interpretive breakpoints. Despite the absence of very major errors, this rate is higher than the acceptable limit (4, 5). Since there are no specific CLSI breakpoints for cephalexin, we evaluated the accuracy of cephalothin in predicting cephalexin susceptibility by BMD. In contrast to CLSI recommendations, we found that cephalothin is a poor predictor of cephalexin susceptibility. The error rate for major and minor errors combined was 40%, when the interpretive breakpoints for cephalexin that are recommended by CLSI for other narrow-spectrum cephalosporins were used (Table 2). In addition, cefazolin was proven to be a poor predictor, in that significantly elevated very major error rates (11%) were also observed (Table 2). These results suggested that neither cephalothin nor cefazolin can be used to predict cephalexin susceptibility; instead, cephalexin susceptibility should be tested independently.

It is noteworthy that 18 isolates were found to be susceptible to ampicillin, cefazolin, and cephalexin but intermediate to cephalothin by both methods. The reason for such a pattern is unclear; we have not been able to find evidence for a beta-lactamase enzyme that is active against narrow-spectrum cephalosporins (i.e., cephalothin) but inactive against ampicillin. One study has also shown that 72% of *E. coli* isolates resistant

TABLE 2. Comparison of cephalothin and cefazolin for predicting cephalexin susceptibility by using BMD MICs

| Predictor | Error rate (%) for: | | |
|-------------|--------------------------------|---------------------------|---------------------------|
| | Very major errors ^a | Major errors ^b | Minor errors ^c |
| Cephalothin | 0 (0/38) | 8 (13/172) | 32 (71/225) |
| Cefazolin | 11 (4/38) | <1 (1/172) | 7 (15/225) |

^a The error rate for very major errors is the number of isolates that were cephalothin susceptible/number of isolates that were cephalexin resistant or the number of isolates that were cefazolin susceptible/number of isolates that were cephalexin resistant (the numbers used to calculate the error rate are shown in parentheses).

^b The error rate for major errors is the number of isolates that were cephalothin resistant/number of isolates that were cephalexin susceptible or the number of isolates that were cefazolin resistant/number of isolates that were cephalexin susceptible (the numbers used to calculate the error rate are shown in parentheses).

^c The error rate for minor errors is the number of isolates that were susceptible or resistant to either drug (cephalothin or cefazolin)/total number of isolates (the numbers used to calculate the error rate are shown in parentheses).

to cephalothin were found to be susceptible to cefazolin (6). These data suggest that cephalothin is less stable to beta-lactamase than other narrow-spectrum cephalosporins in vitro. These findings may undermine its role as a predictor of susceptibility testing for other narrow-spectrum cephalosporins. While cephalothin may have been chosen for the role of “predictor” because it is known to overestimate resistance in other narrow-spectrum cephalosporins, the expediency afforded by this approach is unacceptable if it unnecessarily eliminates an antibiotic, such as cephalexin, from usage in appropriate clinical situations.

In summary, PHX overestimates cephalothin resistance compared to reference BMD. Susceptibility testing of cephalexin by PHX needs to be improved, since the error rates are significant, according to CLSI-based interpretive breakpoints. In addition, cephalothin was found to be a poor predictor of cephalexin susceptibility compared to BMD, in contrast to the current CLSI recommendation to use cephalothin to predict cephalexin susceptibility. Cefazolin is also not a reliable predictor of cephalexin susceptibility in vitro. Laboratories should test and report cefazolin and cephalexin susceptibility independently, since they are the only narrow-spectrum cephalosporins in common usage. Furthermore, CLSI should consider evaluating specific interpretive breakpoints for cephalexin, which could help retain its position as an effective antimicrobial and enhance the rational use of antibiotics.

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