2014

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C. S. McWilliams
Northwell Health

S. Condon
Northwell Health

R. M. Schwartz
Hofstra Northwell School of Medicine

C. C. Ginocchio
Hofstra Northwell School of Medicine

JCM
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Incidence of Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates That Test Susceptible to Cephalosporins and Aztreonam by the Revised CLSI Breakpoints

Carla S. McWilliams,[a]* Susan Condon,[b] Rebecca M. Schwartz,[c,d] Christine C. Ginocchio[b,d]

North Shore-LIJ Health System, Division of Infectious Diseases, Department of Medicine, Manhasset, New York, USA[a]; North Shore-LIJ Health System Laboratories, Department of Pathology and Laboratory Medicine, Lake Success, New York, USA[b]; North Shore-LIJ Health System, Department of Population Health, Great Neck, New York, USA[c]; Hofstra North Shore-LIJ School of Medicine, Hempstead, New York, USA[d]

The incidence of aztreonam and cephalosporin susceptibility, determined using the revised CLSI breakpoints, for extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates was evaluated. Our analysis showed that results for aztreonam and/or ≥1 cephalosporin were reported as susceptible or intermediate for 89.2% of ESBL-producing *E coli* isolates (569/638 isolates) and 67.7% of ESBL-producing *K. pneumoniae* isolates (155/229 isolates).

Extended-spectrum-β-lactamase (ESBL)-producing *Enterobacteriaceae* strains represent a challenging problem for health care providers, particularly in acute-care and long-term-care facilities and more recently in community-acquired infections (1–5). ESBL enzymes are capable of inactivating penicillins, aztreonam, cephalosporins, and β-lactamase inhibitors, which limits the number of effective antibiotics for treatment (1, 2, 4, 5).

The presence of an ESBL is suspected in *Escherichia coli* and *Klebsiella pneumoniae* infections when resistance to one or more of the extended-spectrum cephalosporins (ESCs) (cefotaxime, ceftazidime, ceftriaxone, or cefepime) is detected (1, 2, 4). Based on pre-2010 guidelines from the Clinical and Laboratory Standards Institute (CLSI) (Wayne, PA), laboratories then confirmed the presence of an ESBL using labor-intensive manual methods. This supplemental testing often delayed ESBL identification by 24 to 48 h. Confirmatory testing for ESBLs has also been incorporated into automated susceptibility test systems. Since resistance to some ESCs and aztreonam may not always be detected by *in vitro* methods, strains were reported as resistant to all penicillins, cephalosporins (excluding the cephamycins), and aztreonam based on positive confirmatory test results, independent of the initial susceptibility test results. These guidelines were followed to prevent strains being reported inadvertently as being susceptible to ESCs and aztreonam, leading to potentially inappropriate treatment.

In 2010, the CLSI Antibiotic Subcommittee lowered the MIC breakpoints and increased the disk diffusion size criteria for reporting of aztreonam, cefazolin, cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime results. Interpretive criteria for cefuroxime and cefepime were not changed, because the committee determined that the available data did not support any changes in the breakpoints for these two drugs (6). In 2014, the CLSI recommended changing the MIC breakpoints for cefepime to <2 μg/ml for sensitive, 4 to 8 μg/ml for sensitive dose dependent (SDD), and >16 μg/ml for resistant (7). The CLSI advises that treatment of ESBL-producing strains can be predicted solely on the basis of MIC values, regardless of the underlying resistance mechanisms. More-stringent interpretive criteria would eliminate the need for confirmatory testing for ESBL, and results could be reported as tested. In theory, this would decrease the time needed to identify ESBL-producing *Enterobacteriaceae* and the costs associated with additional laboratory work.

There were significant concerns that the 2010 interpretive criteria might not detect all resistance, based on studies that demonstrated that MICs do not always predict clinical responses, inoculum effects may decrease the potential to detect subpopulations with different susceptibility profiles, different test methods can yield different results, and MIC results are not always reproducible and can vary by >3 dilutions (8). Therefore, we evaluated the rates of cephalosporin and aztreonam susceptibility that would be reported when using the lower breakpoints for ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae*.

A total of 638 unique ESBL-producing *E. coli* and 229 unique ESBL-producing *K. pneumoniae* clinical isolates collected between October 2012 and December 2012 were selected. Isolate specimen sources are listed in Tables 2 and 4. Microscan Gram-negative MIC panel 61 (Siemens, Tarrytown, NY) was used to determine antimicrobial susceptibility profiles. The panel contains confirmatory wells for ESBL (cefotaxime and clavulanate at 0.25 and 4.0 μg/ml or 2.0 and 4.0 μg/ml, respectively). MIC values were interpreted as sensitive, intermediate, or resistant results based on 2010 CLSI breakpoints for aztreonam, cefotaxime, ceftazidime, and ceftriaxone. Cefepime interpretations were evaluated using 2010 and 2014 guidelines. Descriptive analyses of the numbers and percentages of categorical interpretations incorrectly reported as sensitive or intermediate were determined using SPSS version 21 statistical software.

Based on 2010 breakpoints, 89.2% of ESBL-producing *E. coli* strains (569/638 strains) would have been reported as sensitive or

Received 31 December 2013 **Returned for modification** 27 January 2014
Accepted 18 April 2014
Published ahead of print 30 April 2014
Editor: R. Patel
Address correspondence to Carla S. McWilliams, carla.s.mcwilliams@gmail.com.
* Present address: Carla S. McWilliams, Lutheran Medical Center, Department of Medicine, Brooklyn, New York, USA.

doi:10.1128/JCM.03613-13

TABLE 1 Susceptibility profiles of 638 ESBL-producing *E. coli* isolates, interpreted using 2010 and 2014 CLSI breakpoints

Drug	No. (%) with CLSI interpretation of:		
	Sensitive	Intermediate	Resistant
Aztreonam	69 (10.8)	53 (8.3)	516 (80.9)
Cefotaxime	0 (0)	14 (2.2)	624 (97.8)
Ceftazidime	141 (22.1)	62 (9.7)	435 (68.2)
Ceftriaxone	18 (2.8)	4 (0.6)	616 (96.6)
Cefuroxime	23 (3.6)	7 (1.1)	608 (95.3)
Cefepime			
2010 breakpoints	126 (19.7)	51 (8.0)	461 (72.3)
2014 breakpoints	126 (19.7)[a]		512 (80.3)

[a] Sensitive or sensitive dose dependent.

TABLE 3 Susceptibility profiles of ESBL-producing *K. pneumoniae* isolates, interpreted using 2010 and 2014 CLSI breakpoints

Drug	No. (%) with CLSI interpretation of:		
	Sensitive	Intermediate	Resistant
Aztreonam	11 (4.8)	5 (2.2)	213 (93.0)
Cefotaxime	0 (0)	10 (4.4)	219 (95.6)
Ceftazidime	13 (5.7)	8 (3.5)	208 (90.8)
Ceftriaxone	7 (3.1)	2 (0.9)	220 (96.1)
Cefuroxime	8 (3.5)	4 (1.7)	217 (94.8)
Cefepime			
2010 breakpoints	67 (29.3)	20 (8.7)	142 (62.0)
2014 breakpoints	67 (29.3)[a]		162 (70.7)

[a] Sensitive or sensitive dose dependent.

intermediate for aztreonam (19.1%) and/or ≥1 cephalosporin, i.e., ceftazidime (31.8%), cefepime (27.7%), cefuroxime (4.7%), ceftriaxone (3.4%), or cefotaxime (2.2%) (Table 1). A total of 170 *E. coli* isolates (26.6%) were reported as sensitive or intermediate for multiple (2 to 6) drugs. Categorical interpretations according to specimen sources are listed in Table 2. Evaluation of ESBL-producing *K. pneumoniae* isolates demonstrated that 67.7% (155/229 isolates) would have been reported as sensitive or intermediate for aztreonam (7.0%) and/or one or more cephalosporins, i.e., cefepime (38.0%), ceftazidime (9.2%), cefuroxime (5.2%), cefotaxime (4.4%), or ceftriaxone (4.0%) (Table 3). Twenty-six isolates (11.4%) were incorrectly reported as sensitive or intermediate for multiple (2 to 6) drugs. Categorical interpretations according to specimen sources are listed in Table 4. The 2014 cefepime breakpoints increased the reporting of the resistant category from 72.3% to 80.3% for ESBL-producing *E. coli* isolates and from 62% to 70.7% for ESBL-producing *K. pneumoniae* isolates.

Our data are similar to those of Kristo et al., who found that 6.4% of the ESBL-producing strains were susceptible to cefotaxime, 44.6% to ceftazidime, and 55.4% to cefepime; as many as 71.8% were susceptible to at least one ESC (9). Among the *E. coli* isolates examined, 8.0%, 58.0%, and 52.7% were called susceptible to cefotaxime, ceftazidime, and cefepime, respectively; among the *K. pneumoniae* isolates, 2.3%, 7.0%, and 58.1% were called susceptible to the aforementioned ESCs. Wang et al. found that, with the new breakpoints, 41.8 to 45.6% of ESBL-producing *E. coli* strains appeared to be susceptible to ceftazidime and cefepime and 20.1% of ESBL-producing *K. pneumoniae* strains were susceptible to cefepime (10).

These data show that, by eliminating confirmatory testing for ESBL, a laboratory would report a significant number of ESBL-producing *E. coli* and *K. pneumoniae* strains as susceptible or intermediate for aztreonam and one or more ESCs, including approximately 20% of the isolates for cefepime. Pharmacokinetic (PK)-pharmacodynamic (PD) models based primarily on Monte Carlo simulations have demonstrated that the use of higher doses of cefepime in the presence of an ESBL-producing strain may achieve time above the MIC (free fraction of the dosing interval) of at least 50% (11, 12). Published data are limited by retrospective study designs, smaller sample sizes, and lack of prospective randomization (13). No head-to-head trials of cefepime versus a carbapenem for treatment of ESBL-producing *E. coli* or *K. pneumoniae* isolates have been published to date.

The 2010 and 2014 CLSI breakpoints were instituted to reflect more accurately the clinical efficacy of these drugs with contemporary isolates, recommended antibiotic dosage regimens, and a better understanding of PK-PD data. Not all types of ESBLs confer resistance to aztreonam and/or ESCs, which could be therapeutically effective. However, susceptibility test results can be inaccurate due to the type of ESBL present, the resistance mechanism, inoculum effects, or testing variability. Inoculum effects in the

TABLE 2 Susceptibility profiles of ESBL-producing *E. coli* isolates according to specimen source, interpreted using 2010 CLSI breakpoints

Isolate source[a]	No. (%)[b]			
	Total	Susceptible result for 1 drug	Susceptible result for ≥2 drugs	Resistant
Blood	34	2 (5.9)	9 (26.5)	23 (67.6)
Bronchial secretion or sputum	13	2 (15.4)	2 (15.4)	9 (69.2)
Surgical or tissue specimen	15	3 (20.0)	4 (26.7)	8 (53.3)
Urine	537	86 (16.0)	140 (26.1)	311 (57.9)
Unknown	30	2 (6.7)	12 (40.0)	16 (53.3)
Total	638	95 (14.9)	170 (26.7)	373 (58.5)

[a] Throat, skin, rectal, and genital swabs ($n = 9$) are not listed.
[b] The drugs evaluated were aztreonam, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, and cefepime.

TABLE 4 Susceptibility profiles of ESBL-producing *K. pneumoniae* isolates according to specimen source, interpreted using 2010 CLSI breakpoints

Isolate source	No. (%)[a]			
	Total	Susceptible result for 1 drug	Susceptible result for ≥2 drugs	Resistant
Blood	26	7 (26.9)	3 (11.5)	16 (61.5)
Bronchial secretion or sputum	17	6 (35.3)	2 (11.8)	9 (52.9)
Surgical or tissue specimen	7	0 (0)	0 (0)	7 (100)
Urine	166	52 (31.3)	19 (11.4)	95 (57.2)
Unknown	13	2 (15.4)	2 (15.4)	9 (69.2)
Total	229	67 (29.3)	26 (11.4)	136 (59.4)

[a] The drugs evaluated were aztreonam, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, and cefepime.

host are complex and involve multiple factors (e.g., patient weight, drug metabolism, renal function, and site of infection) that can be only partially accounted for in laboratory models. Again, most available data are from *in vitro* studies, and infectious disease physicians are wary of how such data apply *in vivo*, particularly for patients with more-serious infections such as bacteremia (14–18). Therefore, the standard of care for managing infections due to ESBL-producing organisms is treatment with a carbapenem. Clinical outcome studies must sufficiently assess the true clinical responses and determine the appropriate use of aztreonam and ESCs for the treatment of infections due to ESBL-producing *Enterobacteriaceae* strains. Physicians, pharmacists, and microbiologists should be aware of the frequency of reports of susceptible/intermediate results for aztreonam and/or ESCs for ESBL-producing *Enterobacteriaceae* strains when the 2010/2014 interpretive guidelines are used. This information should facilitate the development of institutional policies for both treatment and the reporting of susceptibilities for aztreonam and ESCs.

The major limitation of the study was that it was retrospective and therefore no genetic analysis was performed to determine specific resistance mechanisms. Nonetheless, based on these data, our institution determined that, until more clinical outcome data are available, susceptibility reporting using a combination of the lowered breakpoints and confirmatory ESBL testing, with adjustment of reporting for aztreonam and ESCs, will be continued.

ACKNOWLEDGMENTS

We thank the North Shore-LIJ Microbiology Laboratory for performing organism identification and susceptibility testing.

We declare no conflicts of interest.

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