AmpC Disk Test for Detection of Plasmid-Mediated AmpC β -Lactamases in *Enterobacteriaceae* Lacking Chromosomal AmpC β -Lactamases

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Although plasmid-mediated AmpC β -lactamases were first reported in the late 1980s, many infectious disease personnel remain unaware of their clinical importance. These enzymes are typically produced by isolates of *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, and *Salmonella* spp. and are associated with multiple antibiotic resistance that leaves few therapeutic options. Plasmid-mediated AmpC β -lactamases have been associated with false in vitro susceptibility to cephalosporins. Many laboratories do not test for this resistance mechanism because current tests are inconvenient, subjective, lack sensitivity and/or specificity, or require reagents that are not readily available. In this study a new test, the AmpC disk test, based on filter paper disks impregnated with EDTA, was found to be a highly sensitive, specific, and convenient means of detection of plasmid-mediated AmpC β -lactamases in organisms lacking a chromosomally mediated AmpC β -lactamase. Using cefoxitin insusceptibility as a screen, the test accurately distinguished AmpC and extended-spectrum β -lactamase production and differentiated AmpCs from non- β -lactamase mechanisms of cefoxitin insusceptibility, such as reduced outer membrane permeability. The test is a potentially useful diagnostic tool. It can provide important infection control information and help to ensure that infected patients receive appropriate antibiotic therapy.

β -Lactamase production is the most common mechanism of β -lactam drug resistance in gram-negative bacteria. Newer β -lactamases that hydrolyze cephamycins, oxymino and zwitterionic cephalosporins, monobactams, or carbapenems are of increasing concern because they restrict therapeutic options, cause treatment failures, and are increasing in occurrence (5, 9, 14, 15). Many clinical laboratories currently test *Escherichia coli* and *Klebsiella* spp. for production of extended-spectrum β -lactamases (ESBLs) but do not attempt to detect plasmid-mediated AmpC β -lactamases (also known as imported, transmissible, foreign, or mobile AmpC β -lactamases). Like ESBLs, plasmid-mediated AmpC β -lactamases have a broad substrate profile that includes penicillins, cephalosporins (apart from zwitterionic cephalosporins), and monobactams. In contrast to ESBLs, they hydrolyze cephamycins and are not inhibited by commercially available β -lactamase inhibitors. These enzymes are typically associated with multiple antibiotic resistance, leaving few therapeutic options (7, 24, 26). Bacteria, mostly *Klebsiella pneumoniae* and *E. coli*, producing plasmid-mediated AmpC β -lactamases have been responsible for nosocomial outbreaks of infection and colonization (16, 17, 20, 21).

According to the report of Pai et al. (20), plasmid-mediated AmpCs are associated with potentially fatal errors of false susceptibility in routine susceptibility tests. These authors reported therapeutic failures with cefotaxime and ceftazidime among 28 bacteremia cases caused by AmpC-producing *K.*

pneumoniae, some of which involved isolates that did not respond to “definitive treatment,” i.e., were susceptible to these agents in vitro. The failures included deaths and failures until therapy was switched to a carbapenem. This report raised concerns similar to those associated with ESBLs (8, 22). It also indicated that some plasmid-mediated AmpC β -lactamases may be clinically more harmful than others. In this study the inducible DHA-1 enzyme was associated with a crude mortality rate of 46%, whereas the constitutively produced CMY-1-like enzyme was associated with only a 14.3% mortality rate. Given the relatively small number of cases, it is unclear how much significance should be attributed to the findings, but it is clear that they should be taken seriously and that further investigations are warranted. For this to happen, it is essential that clinical laboratories accurately detect isolates producing a plasmid-mediated AmpC β -lactamase.

Testing for plasmid-mediated AmpC β -lactamases is not widely attempted by clinical laboratories because the available phenotypic tests are either inconvenient, subjective, lack sensitivity and/or specificity, or require reagents that are not readily available (2, 4, 10, 12, 29, 31, 18). For this reason, a study was designed to evaluate the potential of a novel disk test to detect production of plasmid-mediated AmpC β -lactamases in isolates that lack a chromosomally encoded AmpC β -lactamase.

MATERIALS AND METHODS

Bacterial strains. (i) Clinical isolates: 140 nonduplicate recent U.S. hospital isolates of *Klebsiella* species, *Proteus mirabilis*, and *Salmonella* species with cefoxitin MICs of ≥ 16 $\mu\text{g/ml}$ were used. The isolates were obtained from a 2000–2001 study involving 63 United States clinical sites. The sites providing the isolates are listed in Acknowledgments.

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FIG. 1. Representative results obtained with the AmpC disk test with six isolates being tested on a 90-mm plate, with one isolate inoculated onto disks placed on either side of three cefoxitin (FOX) disks.

(ii) **Control strains.** Plasmid-mediated AmpC-producing strains, *K. pneumoniae* HVAMC 39 (high level ACT-1) and *K. pneumoniae* UMJM14 (low level DHA-1); three South African strains of *K. pneumoniae* with reduced outer membrane permeability (one of which produced SHV-2); and phenotypically β -lactamase-negative *E. coli* ATCC 25922 (actually possesses *ampC* gene but only produces an insignificant amount of AmpC β -lactamase) were used.

β -Lactamase investigations. The types of β -lactamase production of the clinical isolates were investigated by phenotypic inhibitor-based microdilution tests using cefpodoxime, cefepime, cefotaxime, or ceftazidime tested alone and in combination with clavulanate; tests for inducibility with cefoxitin and/or imipenem by the disk approximation method (27) and/or by a broth induction method in which spectrophotometric assays using crude cell sonicates were used to compare the level of β -lactamase production that had occurred in log-phase growth in the presence and absence of 1/4 the MIC of the inducing agent (28); previously described isoelectric focusing overlay procedures to determine the pIs of β -lactamases, the capabilities of clavulanate and cloxacillin to inhibit the β -lactamases, and the capabilities of the β -lactamases to hydrolyze cefotaxime (15); microbiological and spectrophotometric assays to investigate carbapenem hydrolysis (13, 28); molecular identification tests utilizing multiplex PCR for plasmid-mediated AmpC β -lactamases (23); PCR primers specific for TEM-, SHV-, OXA-, CTX-M-, PSE-, and OXY-derived β -lactamases; and DNA sequencing (some enzymes only) (15) were used.

AmpC disk test. The test is based on use of Tris-EDTA to permeabilize a bacterial cell and release β -lactamases into the external environment. AmpC disks (i.e., filter paper disks containing Tris-EDTA, available from BD Diagnostic Systems, Sparks, MD) were prepared in-house by applying 20 μ l of a 1:1 mixture of saline and 100 $\times$ Tris-EDTA (catalog code T-9285; Sigma-Aldrich Corp., St. Louis, MO) to sterile filter paper disks, allowing the disks to dry, and storing them at 2 to 8°C. The surface of a Mueller-Hinton agar plate (Oxoid Ltd., Basingstoke, Hampshire, England) was inoculated with a lawn of cefoxitin-susceptible *E. coli* ATCC 25922 according to the standard disk diffusion method (19). Immediately prior to use, AmpC disks were rehydrated with 20 μ l of saline and several colonies of each test organism were applied to a disk. A 30- μ g cefoxitin disk (Becton Dickinson, Sparks, MD) was placed on the inoculated surface of the Mueller-Hinton agar. The inoculated AmpC disk was then placed almost touching the antibiotic disk with the inoculated disk face in contact with the agar surface. The plate was then inverted and incubated overnight at 35°C in ambient air. After incubation, plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result) (Fig. 1).

RESULTS

Forty-four of the 140 cefoxitin-insusceptible clinical isolates yielded positive AmpC disk tests (31%), and the remaining 96 were negative (69%) (Table 1). The negative isolates were confirmed as negative for AmpC production with the three-dimensional test (29). Forty-two of the 44 positive isolates were confirmed as being plasmid-mediated AmpC β -lactamase producers by the isoelectric focusing overlay technique and multiplex PCR. Most β -lactamases were identified only to the

TABLE 1. AmpC disk test results with clinical strains

Outcome or organism	No. of isolates	β -Lactamase
Positive results		
<i>K. pneumoniae</i>	1	ACT-like (inducible)
	4	FOX-5
	2	FOX-5 plus SHV ESBL
	1	FOX-5 plus SHV ESBL and TEM ESBL
	6	FOX-like
	15	FOX-like plus SHV ESBL
	1	DHA-like (inducible)
	1	KPC-2 plus SHV ESBL
	1	Unidentified β -lactamase, pI 8.0
<i>K. oxytoca</i>	4	FOX-like
	1	DHA-like (inducible) plus SHV ESBL
<i>P. mirabilis</i>	1	FOX-5
	4	AmpC of <i>Citrobacter</i> origin
<i>Salmonella</i> spp.	2	AmpC of <i>Citrobacter</i> origin
Negative results		
<i>K. pneumoniae</i>	38	SHV ESBL
	44	SHV-1
<i>K. oxytoca</i>	4	SHV ESBL
	3	High-level K1
	3	Low-level K1
<i>P. mirabilis</i>	4	None

family level (e.g., FOX-like), but a few enzymes were identified definitively by sequencing. The two test-positive isolates that were not confirmed as producing a plasmid-mediated AmpC β -lactamase were an imipenem-resistant isolate of *K. pneumoniae* (strain 01BH79; MIC, 64 μ g/ml), which produced the class A carbapenem-hydrolyzing β -lactamase, KPC-2, and also an SHV-derived ESBL; and *K. pneumoniae* 01JMH4, which produced an as-yet-unidentified, clavulanate-sensitive, cloxacillin-resistant β -lactamase with a pI of 8.0. Based on these findings, the disk test exhibited 100% sensitivity and 98% specificity for detection of plasmid-mediated AmpC β -lactamases in these isolates. The two AmpC-producing control strains yielded positive test results, and the negative controls strains, including the three porin mutants, yielded negative test results.

DISCUSSION

There is a paucity of data about the prevalence and clinical significance of plasmid-mediated AmpC β -lactamases. It is now well over a decade since the first confirmed reports of these enzymes (3, 21). Where accurate testing has been done, isolates producing plasmid-mediated AmpC β -lactamases have been shown to be now widespread (24). For example, during the period 1992 to 2000, Alvarez et al. reported plasmid-mediated AmpC-producing *K. pneumoniae* isolates in the United States to have a prevalence of 8.5% among clinical isolates of this species and to occur at 20 of 70 centers (29%) (1). Plasmid-mediated AmpC-producing *Salmonella* and *E. coli* isolates are also a veterinary problem, affecting livestock, pets, and their human contacts (6, 11, 25, 30). In view of the apparently uncontained spread and the concern of false-susceptible in vitro test results with cephalosporins (20), there is good justification for clinical and veterinary microbiology laboratories to test for plasmid-mediated AmpC β -lactamases.

In this study the AmpC disk test provided a simple, convenient, and accurate means of detection of plasmid-mediated AmpC β -lactamases in organisms lacking a chromosomally mediated AmpC β -lactamase, i.e., *K. pneumoniae*, *Klebsiella oxytoca*, *Salmonella* spp., and *P. mirabilis*. These organisms were chosen for the study because they are convenient indicator organisms for this resistance mechanism in that a positive test can unequivocally indicate the presence of a foreign, or plasmid-mediated, AmpC β -lactamase. The test accurately distinguished between cefoxitin insusceptibility caused by AmpC production and non- β -lactamase mechanisms, such as reduced outer membrane permeability (porin mutations). Distinguishing between these types of mechanisms is a current diagnostic problem for laboratories wanting to detect AmpC β -lactamases. Coproduction of ESBLs did not interfere with the detection of the AmpC β -lactamases.

Care is required in interpreting the test with isolates exhibiting reduced carbapenem susceptibility, since this may be due to other, currently rare β -lactamases capable of hydrolyzing cefoxitin, e.g., carbapenemases.

E. coli isolates that produce plasmid-mediated AmpC β -lactamases were not included in this study, because the test does not discriminate between positive results due to upregulated chromosomally mediated AmpC β -lactamases and those due to plasmid-mediated AmpC β -lactamases. Additional studies showed that the AmpC disk test also reliably detected plasmid-

mediated AmpC β -lactamases in *E. coli* and also high-level production of chromosomally mediated AmpC β -lactamases in *E. coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. (unpublished results). The follow-up of positive disk tests with *E. coli* isolates should, ideally, be done by genetic testing, e.g., AmpC multiplex PCR testing (23). However, if this is not feasible, an experienced microbiologist may sometimes be able to presumptively distinguish between chromosomal and plasmid-mediated AmpC β -lactamases by comparing the *E. coli* susceptibility pattern with that of a *Klebsiella* sp. (or other organism) from the same patient population that is already known to produce a plasmid-mediated AmpC β -lactamase.

Optimal screening agents for plasmid-mediated AmpC β -lactamases have yet to be defined. Currently used screens include that for cefoxitin insusceptibility in *Klebsiella*, *E. coli*, *P. mirabilis*, or *Salmonella* and positive ESBL screens that are not confirmed by ESBL confirmatory tests.

Given the need for a test for AmpC β -lactamases and the fact that many clinical laboratories are often short staffed and overworked, the AmpC disk test could fill a current gap in diagnostic microbiology. Adoption of this test would make it possible to learn more about the clinical implications of plasmid-mediated AmpC β -lactamases and to contain the spread of organisms possessing this resistance mechanism. The potential benefits would include better patient outcomes in terms of avoiding inappropriate therapy and a reduction in the escalation of antibiotic resistance through better infection control.

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REFERENCES

1. Alvarez, M., J. H. Tran, N. Chow, and G. A. Jacoby. 2004. Epidemiology of conjugative plasmid-mediated AmpC beta-lactamases in the United States. *Antimicrob. Agents Chemother.* **48**:533-537.
2. Barnaud, G., G. Arlet, C. Verdet, O. Gailliot, P. H. Lagrange, and A. Philippon. 1998. *Salmonella enteritidis*: AmpC plasmid-mediated inducible β -lactamase (DHA-1) with an *ampR* gene from *Morganella morganii*. *Antimicrob. Agents Chemother.* **42**:2352-2358.
3. Bauernfeind, A., Y. Chong, and S. Schweighart. 1989. Extended broad-spectrum β -lactamase in *Klebsiella pneumoniae* including resistance to cephamycins. *Infection* **17**:316-321.

4. Black, J. A., K. S. Thomson, and J. D. Pitout. 2004. Use of beta-lactamase inhibitors in disk tests to detect plasmid-mediated AmpC beta-lactamases. *J. Clin. Microbiol.* **42**:2203–2206.
5. Bradford, P. A. 2001. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol. Rev.* **14**:933–951.
6. Bradford, P. A., P. J. Petersen, I. M. Fingerman, and D. G. White. 1999. Characterization of expanded-spectrum cephalosporin resistance in *E. coli* isolates associated with bovine calf diarrhoeal disease. *J. Antimicrob. Chemother.* **44**:607–610.
7. Bradford, P. A., C. Urban, N. Mariano, S. J. Projan, J. J. Rahal, and K. Bush. 1997. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC β -lactamase, and the loss of an outer membrane protein. *Antimicrob. Agents Chemother.* **41**:563–569.
8. Brun-Buisson, C., P. Legrand, A. Philippon, F. Montravers, M. Ansquer, and J. Duval. 1987. Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. *Lancet* **ii**:302–306.
9. Bush, K. 2001. New beta-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin. Infect. Dis.* **32**:1085–1089.
10. Coudron, P. E., N. D. Hanson, and M. W. Climo. 2003. Occurrence of extended-spectrum and AmpC beta-lactamases in bloodstream isolates of *Klebsiella pneumoniae*: isolates harbor plasmid-mediated FOX-5 and ACT-1 AmpC beta-lactamases. *J. Clin. Microbiol.* **41**:772–777.
11. Fey, P. D., T. J. Safranek, M. E. Rupp, E. F. Dunne, E. Ribot, P. C. Iwen, P. A. Bradford, F. J. Angulo, and S. H. Hinrichs. 2000. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. *N. Engl. J. Med.* **342**:1242–1249.
12. Manchanda, V., and N. P. Singh. 2003. Occurrence and detection of AmpC beta-lactamases among Gram-negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. *J. Antimicrob. Chemother.* **51**:415–418.
13. Masuda, G., S. Tomioka, and M. Hasegawa. 1976. Detection of beta-lactamase production by gram-negative bacteria. *J. Antibiot. (Tokyo)* **29**:662–664.
14. Moland, E. S., J. A. Black, A. Hossain, N. D. Hanson, K. S. Thomson, and S. Pottumarthy. 2003. Discovery of CTX-M-like extended-spectrum beta-lactamases in *Escherichia coli* isolates from five U.S. states. *Antimicrob. Agents Chemother.* **47**:2382–2383.
15. Moland, E. S., J. A. Black, J. Ourada, M. D. Reisbig, N. D. Hanson, and K. S. Thomson. 2002. Occurrence of newer beta-lactamases in *Klebsiella pneumoniae* isolates from 24 U.S. hospitals. *Antimicrob. Agents Chemother.* **46**:3837–3842.
16. M'Zali, F. H., J. Heritage, D. M. Gascoyne-Binzi, M. Denton, N. J. Todd, and P. M. Hawkey. 1997. Transcontinental importation into the UK of *Escherichia coli* expressing a plasmid-mediated AmpC-type beta-lactamase exposed during an outbreak of SHV-5 extended-spectrum beta-lactamase in a Leeds hospital. *J. Antimicrob. Chemother.* **40**:823–831.
17. Nadjar, D., M. Rouveau, C. Verdet, L. Donay, J. Herrmann, P. H. Lagrange, A. Philippon, and G. Arlet. 2000. Outbreak of *Klebsiella pneumoniae* producing transferable AmpC-type beta-lactamase (ACC-1) originating from *Hafnia alvei*. *FEMS Microbiol. Lett.* **187**:35–40.
18. Nasim, K., S. Elsayed, J. D. Pitout, J. Conly, D. L. Church, and D. B. Gregson. 2004. New method for laboratory detection of AmpC beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*. *J. Clin. Microbiol.* **42**:4799–4802.
19. National Committee for Clinical Laboratory Standards. 2003. Performance standards for antimicrobial disk susceptibility testing; eighth informational supplement. Approved standard M2–A7. National Committee for Clinical Laboratory Standards, Wayne, Pa.
20. Pai, H., C. I. Kang, J. H. Byeon, K. D. Lee, W. B. Park, H. B. Kim, E. C. Kim, M. D. Oh, and K. W. Choe. 2004. Epidemiology and clinical features of bloodstream infections caused by AmpC-type-beta-lactamase-producing *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* **48**:3720–3728.
21. Papanicolaou, G. A., A. A. Medeiros, and G. A. Jacoby. 1990. Novel plasmid-mediated beta-lactamase (MIR-1) conferring resistance to oxyimino- and alpha-methoxy beta-lactams in clinical isolates of *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* **34**:2200–2209.
22. Paterson, D. L., W.-C. Ko, A. Von Gottberg, J. M. Casellas, L. Mulazimoglu, K. P. Klugman, R. A. Bonomo, L. B. Rice, J. G. McCormack, and V. L. Yu. 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical laboratory. *J. Clin. Microbiol.* **39**:2206–2212.
23. Perez-Perez, F. J., and N. D. Hanson. 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J. Clin. Microbiol.* **40**:2153–2162.
24. Philippon, A., G. Arlet, and G. A. Jacoby. 2002. Plasmid-determined AmpC-type beta-lactamases. *Antimicrob. Agents Chemother.* **46**:1–11.
25. Pitout, J. D., M. D. Reisbig, M. Mulvey, L. Chui, M. Louie, L. Crowe, D. L. Church, S. Elsayed, D. Gregson, R. Ahmed, P. Tilley, and N. D. Hanson. 2003. Association between handling of pet treats and infection with *Salmonella enterica* serotype Newport expressing the AmpC beta-lactamase CMY-2. *J. Clin. Microbiol.* **41**:4578–4582.
26. Rodriguez-Martinez, J. M., A. Pascual, I. Garcia, and L. Martinez-Martinez. 2003. Detection of the plasmid-mediated quinolone resistance determinant *qnr* among clinical isolates of *Klebsiella pneumoniae* producing AmpC-type β -lactamase. *J. Antimicrob. Chemother.* **52**:703–706.
27. Sanders, C. C., and W. E. Sanders, Jr. 1979. Emergence of resistance to cefamandole: possible role of cefoxitin-inducible β -lactamases. *Antimicrob. Agents Chemother.* **15**:792–797.
28. Sanders, C. C., W. E. Sanders, Jr., K. S. Thomson, and J. P. Iaconis. 1989. Meropenem: activity against resistant gram-negative bacteria and interactions with beta-lactamases. *J. Antimicrob. Chemother.* **24**(Suppl. A):187–196.
29. Thomson, K. S., and C. C. Sanders. 1992. Detection of extended-spectrum β -lactamases in members of the family *Enterobacteriaceae*: comparison of the double-disk and three-dimensional tests. *Antimicrob. Agents Chemother.* **36**:1877–1882.
30. Winokur, P. L., A. Brueggemann, D. L. DeSalvo, L. Hoffmann, M. D. Apley, E. K. Uhlenhopp, M. A. Pfaller, and G. V. Doern. 2000. Animal and human multidrug-resistant, cephalosporin-resistant salmonella isolates expressing a plasmid-mediated CMY-2 AmpC beta-lactamase. *Antimicrob. Agents Chemother.* **44**:2777–2783.
31. Yong, D., R. Park, J. Yum, K. Lee, E. Choi, and Y. Chong. 2002. Further modification of the Hodge test to screen AmpC beta-lactamase (CMY-1)-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*. *J. Microbiol. Methods* **51**:407.