Journal of Antimicrobial Chemotherapy (2003) **52**, 2–4
DOI: 10.1093/jac/dkg284
Advance Access publication 29 May 2003

JAC

AmpC β-lactamases: what do we need to know for the future?

Nancy D. Hanson*

Department of Medical Microbiology and Immunology, Center for Research in Anti-Infectives and Biotechnology, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178, USA

Keywords: AmpC, β-lactamase

AmpC β-lactamases have been a target of study since the late 1970s. Most of these enzymes are cephalosporinases but are capable of hydrolysing all β-lactams to some extent.[1,2] Researchers have examined characteristics of both inducible and non-inducible AmpC β-lactamases such as physical properties, hydrolytic activity, the molecular mechanisms involved in chromosomal expression, and comparative studies between genera on the induction potential of the enzyme.[1,3] In the late 1980s, these inducible chromosomal genes were detected on plasmids (most without induction capabilities) and were transferred to organisms, which typically do not express these types of β-lactamase such as *Klebsiella* spp., *Escherichia coli*, or *Salmonella* spp. The plasmid-encoded or 'imported' *ampC* β-lactamase complicates the job of clinical microbiologists working in hospital laboratories. No longer can a Gram-negative organism be considered a potential AmpC-producing organism based on identification. In addition, many clinical microbiologists are unaware of plasmid-encoded AmpC β-lactamases because phenotypic detection is difficult at best and these β-lactamases can be misidentified as extended spectrum β-lactamases (ESBLs). This article serves to point out new developments and/or gaps in the basic knowledge of our understanding of AmpC β-lactamases.

Molecular aspects

The hydrolytic properties of AmpC β-lactamases are similar regardless of their genetic origin. Generally speaking, these enzymes have low V_{max} values and high K_m values for the third generation cephalosporins.[1] There are notable exceptions, however, including the cefotaxime V_{max} values reported for the plasmid-encoded AmpC β-lactamases, MIR-1 and MOX-1, and the kinetic values of the *Serratia marcescens* AmpC β-lactamase reported for ceftazidime.[4–7] These types of data are of limited value due to the lack of standardization of AmpC enzymic analyses between laboratories. Therefore, comparisons of data generated on specific enzymic activities cannot be made between laboratories and the specific roles these enzymic activities play in the overall resistance pattern of organisms will remain obscure.

Regardless of the subtle differences in the hydrolytic properties of different AmpC β-lactamases, organisms expressing these enzymes are not resistant to the third generation cephalosporins unless the AmpC β-lactamase is expressed at high-levels.[1] It has been clearly established that chromosomal *ampC* gene expression in organisms such as *Citrobacter freundii*, *Enterobacter cloacae*, *Morganella morganii*, *Hafnia alvei* and *Serratia marcescens* is inducible by β-lactam antibiotics such as cefoxitin and imipenem but poorly induced (if at all) by the third- or fourth-generation cephalosporins.[8–10] Induction requires the DNA-binding protein AmpR, and is a reversible process once the inducing agent is removed.[1,3] Mechanisms of *ampC* gene expression have been analysed using two model organisms, *E. cloacae* and *C. freundii*.[3,11–14] However, a recent publication describing *ampC* expression in *S. marcescens* suggests that genus-specific variation will play a role in the overall regulation of *ampC* gene expression.[15] Elucidating these genus-specific variations will provide insight for understanding differences observed between genera regarding responses to different β-lactam antibiotics.

Variations in β-lactam MICs have been noted for organisms expressing different plasmid-encoded AmpC β-lactamases.[5] Little if anything is understood about the mechanisms controlling plasmid-encoded *ampC* expression. Two assumptions have been made in the literature in an attempt to explain the high-level expression of plasmid-encoded *ampC* genes. These assumptions include: (i) high-level expression is due to high gene copy number associated with the plasmid;[16,17]and (ii) the absence of *ampR* for many of these plasmid-encoded *ampC* genes would increase expression levels by two- to six-fold because of the release of *ampC* repression by AmpR and muropeptide cofactor binding.[14,16,18]

A recent publication addressed the contributions of gene copy number and promoter strength to overall *ampC* gene expression.[19] By using a new methodology, the relative copy number of several plasmid-encoded *ampC* genes has been determined. Plasmid-encoded *ampC* genes such as bla_{ACT-1}, bla_{CMY-2}, bla_{FOX-5} and bla_{CMY-7} have been found in low copy number (2–4), whereas only the bla_{MIR-1} β-lactamase gene copy number has been demonstrated as moderate (12 copies)[19] (M. Reisbig, V. Herrera, A. Hossain & N. Hanson, unpublished results). Therefore, the accepted assumption of high-level expression of plasmid-encoded *ampC* genes being mediated by high-copy plasmids was not substantiated in these studies. Evaluation of gene expression after normalization for copy number indicated that expression of plasmid-encoded *ampC* genes in the absence of AmpR resulted in much more expression than the two- to six-fold

*Tel: +1-402-280-5837; Fax: +1-402-280-1875; E-mail: ndhanson@creighton.edu

increase predicted in the literature. It is more likely that promoter modifications made during the recombination event, which created the plasmid-encoded *ampC* gene, are responsible for high-level expression of the gene and copy number in most cases contributes only minimally to overall *ampC* gene expression. Because the driving force for AmpC-mediated resistance seems to be high-level expression mediated by promoter mutations, questions regarding how expression levels will alter resistance patterns of organisms still remain. These questions include: (i) Will plasmid-encoded *ampC* gene expression fluctuate depending upon the genetic background from which it is expressed; and (ii) Could variation in *ampC* gene expression play a role in the variability observed for β-lactam MIC values for organisms expressing plasmid-encoded *ampC* genes of different or similar origins? A recent publication has examined β-lactam MICs for *E. coli* transformants expressing different plasmid-encoded *ampC* genes derived from *C. freundii*.[20] These data indicate that the variation reported in the literature between clinical strains expressing similar AmpC β-lactamases could be due to variable *ampC* expression.

Clinical implications of plasmid-encoded AmpC-mediated resistance

For clinical microbiologists, the most immediate problem is detection of plasmid-encoded AmpC-mediated resistance in Gram-negative organisms. There are no guidelines in place for detection of this resistance mechanism and yet there is as much need for clinical laboratories to address this issue as there is for the detection of ESBLs. A recent publication by Coudron *et al.*[21] argues the need to distinguish cefoxitin-resistant AmpC producers from cefoxitin-resistant non-AmpC producers. Distinguishing between these two types of organisms could impact treatment options, using extended-spectrum cephalosporins for cefoxitin-resistant non-AmpC, non-ESBL producers and carbapenems for the cefoxitin-resistant AmpC producers. Discrimination between these types of organisms would influence the usage of cephalosporins and carbapenems and therefore impact the selective pressure driving ESBL, AmpC, or plasmid-encoded class A carbapenem resistance gene propagation.

An added caveat for problematic detection is the appearance of inducible plasmid-encoded AmpC β-lactamases. It is well known that mutations in AmpD are implicated in the derepressed phenotype of organisms, which encode an inducible chromosomal *ampC*.[3,22] What is not well known is that the majority of Gram-negative organisms encode *ampD*.[19,23] Spontaneous *ampD* mutations which should occur in clinical isolates of *E. coli*, *K. pneumoniae* and *Salmonella* spp. have not been described because there is no detectable phenotype in the absence of an inducible chromosomal *ampC*. Noticeable increases in ESBL MICs are predicted for clinical isolates of *E. coli* and *K. pneumoniae* when plasmid-encoded inducible *ampC* genes are expressed in the presence of *ampD* mutations.[19] Will these increases in MIC values contribute to the confusion in the identification of plasmid-encoded AmpC producers and ESBL producers in clinical microbiology laboratories? Time will tell, but do we have that luxury?

In addition to isolates from humans, plasmid-encoded AmpC β-lactamases have been found in isolates from livestock such as swine and cattle, and from companion animals such as dogs.[24,25] These additional sources of AmpC-producing isolates add another level of urgency for accurately detecting this resistance mechanism. A community-based source for AmpC-mediated resistance suggests that hospital-based clinical laboratories should be screening isolates from community-based patients before hospitalization to prevent the spread of community-acquired plasmid-encoded AmpC-mediated resistance within the hospital. Surveillance studies of community-acquired plasmid-encoded AmpC β-lactamase genes are warranted. But, what approach can be used for screening these isolates?

Phenotypic susceptibility testing to distinguish the difference between organisms producing ESBLs or plasmid-encoded AmpC β-lactamases is difficult. Resistance to cefoxitin can indicate the possibility of AmpC-mediated resistance but can also indicate reduced outer membrane permeability.[26] Some phenotypic tests are available to help distinguish the difference between cefoxitin resistant non-AmpC producers and cefoxitin resistant AmpC producers. These include the three-dimensional test and a new AmpC disc test.[27,28] In addition, the use of β-lactamase inhibitors can help identify possible AmpC producing organisms.[26] None of these tests are standardized and can be time consuming when screening large numbers of isolates. A recently developed multiplex PCR for the detection of plasmid-encoded *ampC* genes has proved useful as a rapid screening tool to distinguish cefoxitin resistant non-AmpC producers from cefoxitin resistant AmpC producers.[29] In addition to *ampC* gene detection, the data generated from the multiplex PCR method can distinguish which family of *ampC* gene is present in the resistant organism thereby distinguishing possible inducible AmpC producers from non-inducible producers of AmpC. Furthermore, this PCR-based method can distinguish hyper-producing chromosomal AmpC *E. coli* isolates from *E. coli* isolates encoding an 'imported' *ampC* gene. Type identification of AmpC or ESBLs may aid in hospital infection control and the ability of the physician to prescribe the most appropriate antibiotic, thus decreasing the selective pressure, which generates antibiotic resistance.[21,30] If we fail to distinguish between ESBL and plasmid-encoded AmpC β-lactamase producers do we run the risk of the emergence of extended-spectrum AmpC β-lactamases (ESACs)?[31] With that horrifying possibility in mind proper surveillance becomes a priority. Proper surveillance will require the implementation of molecular testing in the clinical laboratory to help distinguish between organisms producing plasmid-encoded AmpC β-lactamases, ESBLs, or production of both enzymes in a single organism. Surveillance is key in controlling the Gram-negative β-lactamase resistance mechanisms we face today and for the first time help stop the emergence of a new type of β-lactamase, the ESACs.

Indeed, we have gained much knowledge in the past 25 years on the topic of AmpC β-lactamases. Yet, reality indicates that, because of our lack of knowledge, we have not made any progress in controlling the spread of this resistance mechanism. More effort needs to be directed towards understanding *ampC* expression, detection of resistance mechanisms in the clinical setting for both outpatients and inpatients, and the clinical implications of patients infected with organisms producing plasmid-encoded AmpC β-lactamases.

Acknowledgements

I, too, would like to express my deepest sympathy to the family of Dr Martin Wood and to the British Society for Antimicrobial Chemotherapy. This article is dedicated in remembrance of Dr Martin Wood.

References

1. Sanders, C. C. (1987). Chromosomal cephalosporinases responsible for multiple resistance to newer β-lactam antibiotics. *Annual Review of Microbiology* **41**, 573–93.

2. Bush, K., Jacoby, G. A. & Medeiros, A. A. (1995). A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy* **39**, 1211–33.

3. Hanson, N. D. & Sanders, C. C. (1999). Regulation of inducible AmpC β-lactamase expression among Enterobacteriaceae. *Current Pharmaceutical Design* **5**, 881–94.

4. Raimondi, A., Sisto, F. & Nikaido, H. (2001). Mutation in *Serratia marcescens* AmpC β-lactamase producing high-level resistance to ceftazidime and cefpirome. *Antimicrobial Agents and Chemotherapy* **45**, 2331–9.

5. Philippon, A., Arlet, G. & Jacoby, G. A. (2002). Plasmid-determined AmpC-type β-lactamases. *Antimicrobial Agents and Chemotherapy* **46**, 1–11.

6. Papanicolaou, G. A., Medeiros, A. A. & Jacoby, G. A. (1990). Novel plasmid-mediated β-lactamase (MIR-1) conferring resistance to oxyimino- and α-methoxy β-lactams in clinical isolates of *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy* **34**, 2200–9.

7. Horii, T., Arakawa, Y., Ohta, M. *et al.* (1994). Characterization of a plasmid-borne and constitutively expressed bla_{MOX-1} gene encoding AmpC-type β-lactamase. *Gene* **139**, 93–8.

8. Jones, R. N. (1998). Important and emerging β-lactamase-mediated resistances in hospital-based pathogens: the AmpC enzymes. *Diagnostic Microbiology and Infectious Disease* **31**, 461–6.

9. Minami, S., Yotsuji, A., Inoue, M. *et al.* (1980). Induction of β-lactamase by various β-lactam antibiotics in *Enterobacter cloacae*. *Antimicrobial Agents and Chemotherapy* **18**, 382–5.

10. Sanders, C. C. & Sanders, W. E. (1986). Type I β-lactamases of Gram-negative bacteria: interactions with β-lactam antibiotics. *Journal of Infectious Diseases* **154**, 792–800.

11. Lindquist, S., Lindberg, F. & Normark, S. (1989). Binding of the *Citrobacter freundii* AmpR regulator to a single DNA site provides both autoregulation and activation of the inducible *ampC* β-lactamase gene. *Journal of Bacteriology* **171**, 3746–53.

12. Lindberg, F. & Normark, S. (1987). Common mechanism of AmpC β-lactamase induction in Enterobacteria: regulation of the cloned *Enterobacter cloacae* P99 β-lactamase gene. *Journal of Bacteriology* **169**, 758–63.

13. Honore, N., Nicolas, M. H. & Cole, S. T. (1986). Inducible cephalosporinase production in clinical isolates of *Enterobacter cloacae* is controlled by a regulatory gene that has been deleted from *Escherichia coli*. *EMBO Journal* **5**, 3709–14.

14. Lindberg, F., Westman, L. & Normark, S. (1985). Regulatory components in *Citrobacter freundii* AmpC β-lactamase induction. *Proceedings of the National Academy of Sciences, USA* **82**, 4620–4.

15. Mahlen, S. D., Morrow, S. S., Abdalhamid, B. *et al.* (2003). Analyses of *ampC* gene expression in *Serratia marcescens* reveal new regulatory properties. *Journal of Antimicrobial Chemotherapy* **51**, 791–802.

16. Bush, K. (2001). New β-lactamases in Gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clinical Infectious Diseases* **32**, 1085–9.

17. Gazouli, M., Tzouvelekis, L. S., Prinarakis, E. *et al.* (1996). Transferable cefoxitin resistance in Enterobacteria from Greek hospitals and characterization of a plasmid-mediated group 1 β-lactamase (LAT-2). *Antimicrobial Agents and Chemotherapy* **40**, 1736–40.

18. Poirel, L., Guibert, M., Girlich, D. *et al.* (1999). Cloning, sequence analyses, expression, and distribution of *ampC-ampR* from *Morganella morganii* clinical isolates. *Antimicrobial Agents and Chemotherapy* **43**, 769–76.

19. Reisbig, M. D., Hossain, A. & Hanson, N. D. (2003). Factors influencing gene expression and resistance for Gram-negative organisms expressing plasmid-encoded *ampC* genes of Enterobacter origin. *Journal of Antimicrobial Chemotherapy* **51**, 1141–51.

20. Barlow, M. & Hall, B. G. (2002). Origin and evolution of the AmpC β-lactamases of *Citrobacter freundii*. *Antimicrobial Agents and Chemotherapy* **46**, 1190–8.

21. Coudron, P., Hanson, N. D. & Climo, M. W. (2003). Occurrence of extended-spectrum and AmpC β-lactamases in bloodstream isolates of *Klebsiella pneumoniae*: isolates harbor plasmid-mediated FOX-5 and ACT-1 AmpC β-lactamases. *Journal of Clinical Microbiology* **41**, 772–7.

22. Medeiros, A. A. (1997). Relapsing infection due to Enterobacter species: lessons of heterogeneity. *Clinical Infectious Diseases* **25**, 341–2.

23. McClelland, M., Sanderson, K. E., Spieth, J. *et al.* (2001). Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. *Nature* **413**, 852–6.

24. Sanchez, S., McCrackin S. M. A., Hudson, C. R. *et al.* (2002). Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *Journal of Clinical Microbiology* **40**, 3586–95.

25. Winokur, P. L., Brueggemann, A., Desalvo, D. L. *et al.* (2000). Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC β-lactamase. *Antimicrobial Agents and Chemotherapy* **44**, 2777–83.

26. Thomson, K. S. (2001). Controversies about extended-spectrum and AmpC β-lactamases. *Emerging Infectious Diseases* **7**, 333–6.

27. Thomson, K. S. & Sanders, C. C. (1992). Detection of extended-spectrum β-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests. *Antimicrobial Agents and Chemotherapy* **36**, 1877–82.

28. Black, J., Moland, E. S. & Thomson, K. S. (2002). A simple disk test for detection of plasmid-mediated AmpC production in *Klebsiella*. In *Program and Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, 2002*. Abstract D-534, p. 140. American Society for Microbiology, Washington, DC, USA.

29. Perez-Perez, F. J. & Hanson, N. D. (2002). Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. *Journal of Clinical Microbiology* **40**, 2153–62.

30. Paterson, D. L., Ko, W. C., Von Gottberg, A. *et al.* (2001). Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. *Journal of Clinical Microbiology* **39**, 2206–12.

31. Barnaud, G., Labia, R., Raskine, L. *et al.* (2001). Extension of resistance to cefepime and cefpirome associated to a six amino acid deletion in the H-10 helix of the cephalosporinase of an *Enterobacter cloacae* clinical isolate. *FEMS Microbiology Letters* **195**, 185–90.