The in vitro transfer of plasmids containing the bla_{NDM-1} gene in our study confirms that this carbapenemase gene can be readily mobilized among different species of *Enterobacteriaceae*. Moreover, *E. coli* TOP10 transformants containing the bla_{NDM-1} gene presented similar characteristics of the original clinical isolate, with increased MIC to β-lactams and positive results of the combined-disc assay with EDTA. Although a plasmid of the same molecular weight (~110 bp) was observed in 6 of 9 transformants, the identification of other plasmids (~52 bp and ~154 bp) suggests that the bla_{NDM-1} gene is located in different mobile genetic elements.

Molecular investigations involving both the characterization of isolates of NDM-positive bacteria and the characterization of the plasmids containing bla_{NDM-1} genes reveal a highly complex picture. The plasmids encoding NDM also appear highly heterogeneous based on molecular size, incompatibility type, and linked antibiotic-resistance genes.[2] Moreover, our data support the findings from Brazil in which a variety of plasmids were found. The gene bla_{NDM-1} was identified on plasmid with an estimated size of 420–490 kb in *Enterobacter hormaechei*.[8] In *Enterobacter cloacae*, *Providencia rettgeri*, and *Klebsiella pneumoniae*, the plasmid was reported to be ~230 kb.[9] *Escherichia coli* and *Enterobacter hormaechei* had plasmid sizes of 70 kb and 90 kb, respectively.[10] The plasmid size in *Acinetobacter baumannii* was 100 kb.[7]

In summary, the results of this study demonstrate the variety of plasmids observed in the transformants and suggests that strains producing bla_{NDM-1} harbor plasmids of different sizes, demonstrating the plasticity of these mobile genetic elements. These findings highlight the need for continuous monitoring of the presence of carbapenemases. Our results contribute to the understanding of carbapenem resistance in *Enterobacteriaceae* and to the molecular characterization of NDM-1–producing isolates in Brazil.

ACKNOWLEDGMENTS

Financial support: CAPES Foundation, Ministry of Education of Brazil, Brasília, Brazil. FIPE/HCPA (Research and Events Support Fund at Hospital de Clínicas de Porto Alegre). A.L.B. is a research fellow from the CNPq, Ministry of Science and Technology, Brazil (grant no. 458489/2014-0).

Potential conflicts of interest: All authors report no conflicts of interest relevant to this article.

Franciéli P. Rozales, MsC;[1,2]
Cibele M. Magagnin, MsC;[1]
Juliana C. Campos, MsC;[3]
Mariana Pagano, PhD;[1]
Luciana S. Nunes, PhD;[1,4]
Lisiane R. Pancotto;[1]
Jorge L. M. Sampaio, PhD;[3]
Alexandre P. Zavascki, PhD;[1,5]
Afonso L. Barth, PhD[1,2]

Affiliations: 1. Laboratório de Pesquisa em Resistência Bacteriana (LABRESIS), Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil; 2. Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; 3. Universidade de São Paulo (USP), São Paulo, Brazil; 4. Curso de Medicina, Universidade Federal do Pampa, Uruguaiana, Brazil; 5. Infectious Diseases Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil.

Address correspondence to Franciéli Pedrotti Rozales, MsC, Laboratório de Pesquisa em Resistência Bacteriana (LABRESIS), Hospital de Clínicas de Porto Alegre, Ramiro Barcelos 2350, Porto Alegre, Brazil (frozales@hotmail.com).
Infect Control Hosp Epidemiol 2017;38:634–636
 0899-823X/2017/3805-0029. DOI: 10.1017/ice.2017.28

REFERENCES

1. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis* 2009;9:228–236.
2. Johnson AP, Woodford N. Global spread of antibiotic resistance: the example of New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance. *J Med Microbiol* 2013;62:499–513.
3. Carvalho-Assef AP, Pereira PS, Albano RM, et al. Isolation of NDM-producing *Providencia rettgeri* in Brazil. *J Antimicrob Chemother* 2013;68:2956–2957.
4. Rozales FP, Ribeiro VB, Magagnin CM, et al. Emergence of NDM-1-producing Enterobacteriaceae in Porto Alegre, Brazil. *Int J Infect Dis* 2014;25:79–81.
5. do Carmo Junior NV, Filho HF, Gomes E, Costa DA, et al. First report of a NDM-producing *Providencia rettgeri* strain in the state of São Paulo. *Braz J Infect Dis* 2015;19:675–676.
6. Pagano M, Poirel L, Martins AF, et al. Emergence of NDM-1-producing *Acinetobacter pittii* in Brazil. *Int J Antimicrob Agents* 2015;45:444–445.
7. Pillonetto M, Arend L, Vespero EC, et al. First report of NDM-1-producing *Acinetobacter baumannii* sequence type 25 in Brazil. *Antimicrob Agents Chemother* 2014;58:7592–7594.
8. Carvalho-Assef AP, Pereira PS, Albano RM, et al. Detection of NDM-1-, CTX-M-15-, and *qnrB4*-producing *Enterobacter hormaechei* isolates in Brazil. *Antimicrob Agents Chemother* 2014;58:2475–2476.
9. Quiles MG, Rocchetti TT, Fehlberg LC, et al. Unusual association of NDM-1 with KPC-2 and *armA* among Brazilian *Enterobacteriaceae* isolates. *Braz J Med Biol Res* 2015;48:174–177.
10. Campos JC, da Silva MJ, Dos Santos PR, et al. Characterization of Tn3000, a transposon responsible for bla_{NDM-1} dissemination among Enterobacteriaceae in Brazil, Nepal, Morocco, and India. *Antimicrob Agents Chemother* 2015;59:7387–7395.

Is AGREE II a counsel of perfection? A letter commenting on Lytvyn et al[1]

To the Editor—We read the systematic survey (review) of *Clostridium difficile* (CD) guidelines (August 2016) with interest. We suggest that Lytvyn et al are proposing a counsel of perfection, ignoring the realities of producing practical guidelines to address rising infection levels. In particular, we question their data extraction from the UK guidelines and

their views that (1) a systematic review is a pre-requisite of guideline writing; (2) relatively weak evidence should not result in strong recommendations; (3) ecological studies are grade 5 evidence; and (4) probiotics have the highest level of evidence for any CD prevention intervention.

Guidelines are produced in response to emergence of new diseases, new evidence on management, or rising levels of existing disease. The latter circumstance, plus a high-profile political drive to reduce CD, prompted revision of the UK guidelines. As we explained "A formal systematic review with grading of the level of evidence (in) each study was not done … (we) did not consider that evidence had changed sufficiently to … warrant extra time and resources." We would like to ask Lytvyn et al which studies, published up to the end of 2007, were excluded from our review that would have materially affected the final recommendations? We suggest that AGREE II amend their standard to allow the guideline authors to justify why systematic reviews were not done.

Lytvyn et al state that the UK guidelines did not update prevention-related information. However, our introduction explicitly stated, "This guidance updates and replaces the 1994 report … outlines newer evidence and approaches to good infection control and environmental hygiene … taking into account national clinical governance frameworks which did not exist in 1994." The example of antibiotic stewardship illustrates the difference in prevention-related information between 1994 and 2008. The former simply recommend, "adoption of an antibiotic policy" and "use of narrow spectrum antibiotics whenever the causative pathogen is known." The 2008 guidelines recommend "restrictive antibiotic guidelines using narrow-spectrum agents … for empirical and definitive treatment … ." They specify which antibiotics to avoid, and they recommend the formation of antimicrobial management teams including antimicrobial pharmacists and information technology specialists to facilitate feedback of antibiotic and CD data. In 1994, neither antimicrobial teams, antimicrobial pharmacists, information technologists, nor data feedback existed.

Lytvyn et al are concerned that guidelines fail to explain how low-quality evidence leads to strong recommendations. The UK guidelines were explicit that lower-quality evidence could result in strong recommendations if "supported by non-RCT studies and/or by clinical governance reports and/or the Code." Maybe they overlooked this statement or did not appreciate that the Hygiene Code has legal status, with hospitals and individuals facing legal sanctions for noncompliance. Governance reports based on public inquiries or investigations by regulatory authorities cannot be ignored and must be considered alongside the scientific evidence. Do Lytvyn et al suggest that a lack of RCTs or systematic reviews of RCTs of the effects of restrictive antibiotic policies on CD levels means that antibiotic restriction should not be strongly recommended? How well do they think clinicians would follow a "moderate" recommendation? The proof of the pudding is in the eating. Since the United Kingdom adopted these guidelines, fluoroquinolone, cephalosporin, and other broad-spectrum antibiotic prescriptions have declined markedly, with the levels of CDI falling by 70%–80%. No other country has achieved this result.[2,3]

Why do Lytvyn et al regard ecological studies as "grade 5 evidence"? Such studies recognize the environmental determinants of disease, are a well-accepted pragmatic design for evaluating public health interventions, use widely available data, and provide a wider range of exposures than a trial, thus increasing the generalizability of findings. Potential limitations are minimized if measurement, analysis, and interpretation are performed at group level, if data are available and reliable, and if inference from group to individual is avoided.[4] One such study, using 4 years of data from all English acute-care hospitals, showed a very strong independent association between soap use and CD rates.[5] Would Lytvyn et al really not consider such evidence that supports a strong recommendation for hand-hygiene with soap to prevent CD?

Lytvyn et al took all guidelines to task for not recommending probiotics, which they regard as "the prevention strategy with highest-level evidence." All but 1 of these guidelines, however, was prepared or published before the Cochrane review of probiotics was published. The single guideline published afterward rightly regards the case for probiotics as unproven, due to few large robustly designed studies, pooling of data on different probiotics, and large amounts of missing CD data. The Cochrane review's own reanalysis using complete data sets revealed no effect of probiotics.

We do not agree that clinical guidelines should address resource implications. Their development may require substantial extra work and research, delaying guidance needed to address rising infection levels. For example, calculating the additional isolation capacity required for CD, its cost, opportunity costs, and their potential offset by reduced CD may be less important than getting the guidelines published. Insisting that both systematic reviews and cost estimations are done, although ideal, may risk accusations of "fiddling while Rome burns." As authors of guidelines ourselves,[6,7] we consider them to be guidance from which authors may depart when there are good grounds or circumstances to do so.[8] Clinical guideline writing is neither an isolated academic exercise nor so difficult that it requires a "methodologist" lead. It requires experienced clinicians and researchers to apply its principles wisely in the clinical and national contexts where the guideline will be applied. The United Kingdom was experiencing an epidemic of CDI. The publication of the 2008 guidelines was part of a concerted ultimately successful public health campaign to reduce the CDI epidemic.

ACKNOWLEDGMENTS

Financial support: No financial support was provided relevant to this article.

Potential conflicts of interest: M.W. has done research work for and received consultancy and/or lecture fees from Actelion, Cubist, Astellas, Optimer, Sanofi-Pasteur, Summit, Biomerieux, Da Volterra and Qiagen; consultancy fees from Seres, Merck, Valneva; and a consultancy fee and a departmental grant from ALera. P.H. has received personal fees from Merck, Optimer, Novolytics, Novasta, Biselea, Wyeth, MagisCommunications, Pfizer, and

Novartis as well as grants and personal fees from Eumedica and Pfizer. S.P.S. has no conflict of interest relevant to this article.

Sheldon P. Stone, BSc, MD, FRCP;[1]
Mark Wilcox, MD, FRCPath, BMedSci, BM, BS;[2]
Peter Hawkey, BSc, DSc, MBBS, MD, FRCPath, FFPath, FRCP[3]

Affiliations: 1. Royal Free Campus, Royal Free and University College Medical School, University College London, London, United Kingdom; 2. Department of Microbiology, Leeds Teaching Hospitals and University of Leeds, Old Medical School, Leeds General Infirmary, Leeds, United Kingdom; 3. National Infection Service, Public Health England, Birmingham Heartlands Hospital, Birmingham, United Kingdom.

Address correspondence to Sheldon P. Stone, BSc, MD, FRCP, Senior Lecturer, Stroke Physician and Consultant Physician for Older People, Royal Free Campus, Royal Free and University College Medical School, University College London, Rowland Hill Street, Hampstead, London NW3 2PF United Kingdom (sheldon.stone@ucl.ac.uk).

Infect Control Hosp Epidemiol 2017;38:636–638
 0899-823X/2017/3805-0030. DOI: 10.1017/ice.2017.5

REFERENCES

1. Lytvyn L, Mertz D, Sadeghirad B, et al. Prevention of *Clostridium difficile* infection: a systematic survey of clinical practice guidelines. *Infect Control Hosp Epidemiol* 2016;37:901–908.
2. The National Archives. Public Health England. Mandatory surveillance of Clostridium difficile. Previous C. difficile monthly tables. http://webarchive.nationalarchives.gov.uk/20140714084352/http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1259151898525. Accessed March 13, 2017.
3. Dingle KE, Didelot X, Phuong Quan T, Eyre DW, Stoesser N, Golubchik T, et al. Effects of control interventions on Clostridium difficile infection in England: an observational study. *Lancet Infect Dis* 2017; doi: http://dx.doi.org/10.1016/S1473-3099(16)30514-X.
4. Stevenson M, McClure R. Use of ecological study designs for injury prevention. *Inj Prev* 2005;11:2–4.
5. Stone S, Fuller C, Savage J, et al. Evaluation of the national Cleanyourhands campaign to reduce Staphylococcus aureus bacteraemia and *Clostridium difficile* infection in hospitals in England and Wales by improved hand hygiene: four year, prospective, ecological, interrupted time series study. *BMJ* 2012;344:e3005.
6. Pratt R. The EPIC project: developing national evidence-based guidelines for preventing healthcare associated infections. *J Hosp Infect* 2001;47:S1–S82.
7. Stone SP, Cooper BS, Kibbler CC, et al. The ORION STATEMENT guidelines for transparent reporting of outbreak reports and intervention studies of nosocomial infection. *Lancet Infect Dis* 2007;7:282–288.
8. Stone SP, Cookson BD. Commentary: Endorsing reporting guidelines: infection control literature gets ahead of the game. *Am J Infect Control* 2016;44:1446–1448.