cause of the illness. The PCR for amplification of a 470-bp fragment of citrate synthase gene was performed according to a previously published protocol (*5*). DNA was extracted with QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) from whole blood and the crust of the eschar collected on day 5 of illness. In addition, serum samples were tested by indirect immunofluorescent assay for specific IgG and IgM against *Francisella tularensis* and *Rickettsia* spp. (spotted fever and typhus group) 5 days and 10 weeks after onset of fever.

Diagnosis of ATBF was affirmed by positive PCR result from the crust of the eschar; further sequence analysis revealed the infection with *R. africae*. Serologic testing demonstrated seroconversion of IgG to *R. conorii* and *R. rickettsii*, which cross-reacts with *R. africae* (negative immunofluorescent assay IgG titer at initial testing and 1:1,024 for *R. conorii* and *R. rickettsii* 10 weeks later) (*6*). Thick and thin blood smears were negative for malaria.

ATBF is the second most well-established cause of febrile illness among travelers to sub-Saharan Africa, after malaria. Usually it manifests by fever (59%–100% of cases), headache (62%–83%), eschar (53%–100%), lymphadenopathy (43%–100%), and rash (15%–46%). The clinical and laboratory findings in the patient reported here were similar to those previously reported among *R. africae*–infected patients (*1*). In the acute phase of illness, a biopsy and culture from an eschar, as well as PCR, are the most suitable methods to confirm the ATBF diagnosis. In this case, ATBF was proven by PCR and subsequent sequencing from a crust sample but not from whole blood and seroconversion of IgG.

The first information about *R. africae* in ticks in Uganda was published in 2013 by Lorusso et al. (*7*), but previously *R. conorii* also was found (*8*). The prevalence rate of *R. africae* infection among *Amblyomma variegatum* ticks in Uganda was 97.1% (*9*). Recently, Proboste et al. established the presence of previously undetected tickborne pathogens in rural dogs and associated ticks in Uganda. Tick species *Haemaphysalis leachi*, *Rhipicephalus* spp., and *A. variegatum* were infected by *Rickettsia* spp. (18.9%), including *R. conorii* and *R. massiliae*; by *Ehrlichia* spp. (18.9%), including *E. chaffeensis*; and by *Anaplasma platys* (*10*).

Our MEDLINE literature search found no previous descriptions of human *R. africae* infection in Uganda. This case indicates that ATBF should be included as a possible diagnosis in persons with febrile illness who have traveled to Uganda, a well-known tourist destination.

References

1. Jensenius M, Fournier PE, Kelly P, Myrvang B, Raoult D. African tick bite fever. Lancet Infect Dis. 2003;3:557–64. http://dx.doi.org/10.1016/S1473-3099(03)00739-4
2. Kelly PJ, Beati L, Mason PR, Matthewman LA, Roux V, Raoult D. *Rickettsia africae* sp. nov., the etiological agent of African tick bite fever. Int J Syst Bacteriol. 1996;46:611–4. http://dx.doi.org/10.1099/00207713-46-2-611
3. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev. 2013;26:657–702. http://dx.doi.org/10.1128/CMR.00032-13
4. Jensenius M, Fournier PE, Vene S, Hoel T, Hasle G, Henriksen AZ, et al.; Norwegian African Tick Bite Fever Study Group. African tick bite fever in travelers to rural sub-Equatorial Africa. Clin Infect Dis. 2003;36:1411–7. http://dx.doi.org/10.1086/375083
5. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int J Syst Bacteriol. 1997;47:252–61. http://dx.doi.org/10.1099/00207713-47-2-252
6. Fournier PE, El Karkouri K, Leroy Q, Robert C, Giumelli B, Renesto P, et al. Analysis of the *Rickettsia africae* genome reveals that virulence acquisition in *Rickettsia* species may be explained by genome reduction. BMC Genomics. 2009;10:166. http://dx.doi.org/10.1186/1471-2164-10-166
7. Lorusso V, Gruszka KA, Majekodunmi A, Igweh A, Welburn SC, Picozzi K. *Rickettsia africae* in *Amblyomma variegatum* ticks, Uganda and Nigeria. Emerg Infect Dis. 2013;19:1705–7. http://dx.doi.org/10.3201/eid1910.130389
8. Socolovschi C, Matsumoto K, Marie JL, Davoust B, Raoult D, Parola P. Identification of rickettsiae, Uganda and Djibouti. Emerg Infect Dis. 2007;13:1508–10. http://dx.doi.org/10.3201/eid1310.070078
9. Nakao R, Qiu Y, Igarashi M, Magona JW, Zhou L, Ito K, et al. High prevalence of spotted fever group rickettsiae in *Amblyomma variegatum* from Uganda and their identification using sizes of intergenic spacers. Ticks Tick Borne Dis. 2013;4:506–12. http://dx.doi.org/10.1016/j.ttbdis.2013.07.001
10. Proboste T, Kalema-Zikusoka G, Altet L, Solano-Gallego L, Fernández de Mera IG, Chirife AD, et al. Infection and exposure to vector-borne pathogens in rural dogs and their ticks, Uganda. Parasit Vectors. 2015;8:306. http://dx.doi.org/10.1186/s13071-015-0919-x

Address for correspondence: Petra Bogovic, Department of Infectious Diseases, University Medical Center Ljubljana, Japljeva 2, 1525 Ljubljana, Slovenia; email: petra.bogovic@kclj.si

Polymyxin B Resistance in Carbapenem-Resistant *Klebsiella pneumoniae*, São Paulo, Brazil

Flávia Bartolleti, Bruna Mara Silva Seco, Carla Capuzzo dos Santos, Carolina Bragança Felipe, Mara Elisa Borsato Lemo, Tatiane da Silva Alves, Lilian F. Passadore, Marcelo J. Mimica, Suely Carlos Ferreira Sampaio, Alexandre Prehn Zavascki, Jorge Luiz Mello Sampaio

Author affiliations: University of São Paulo School of Pharmaceutical Sciences, São Paulo, Brazil (F. Bartolleti,

B.M.S. Seco, J.L. Mello Sampaio); Santa Casa de São Paulo School of Medical Sciences, São Paulo (F. Bartolleti, M.J. Mimica, S.C.F. Sampaio); Fleury Medicine and Health, São Paulo (C.C. dos Santos, C.B. Felipe, M.E.B. Lemo, T.S. Alves, J.L.M. Sampaio); University of São Paulo, São Paulo (L.F. Passadore); Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil (A.P. Zavascki)

To the Editor: Infections caused by carbapenem-resistant *Enterobacteriaceae* have been associated with higher death rates than infections caused by carbapenem-susceptible strains, and resistant infections are mostly treated with polymyxins (*1*). Several outbreaks caused by carbapenem- and polymyxin-resistant *Klebsiella pneumoniae* (CPRKp) have been reported, mainly from Europe, and represent an emerging threat.

Carbapenem-resistant *K. pneumoniae* (CRKp) are endemic to Brazil, where polymyxin B (PMB) has been largely used against infections caused by these microorganisms. We evaluated PMB resistance rates and clonal diversity among CRKp isolates from patients in São Paulo, Brazil. The study was approved by the Research Review Board of Fleury Institute in São Paulo.

All *K. pneumoniae* isolates, except those from urine and active surveillance samples, recovered from inpatients during January 1, 2011–December 31, 2015, at 10 private tertiary-care hospitals in São Paulo were included in the study. *K. pneumoniae* isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; we analyzed only the first isolate from each patient, unless other isolates were recovered after a 90-day interval. To determine antimicrobial drug MICs, we used nonautomated broth microdilution (*2*) polystyrene plates with cation-adjusted Mueller–Hinton broth (Becton Dickinson, Franklin Lakes, NJ, USA) for PMB and tigecycline; the Etest (bioMérieux, Marcy l'Etoile, France) for fosfomycin; and the disk-diffusion method (*3*) for all other antimicrobial drugs. Isolates with a MIC of ≤2 mg/L for PMB were considered susceptible; this value is the EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoint for colistin in *Enterobacteriaceae* (*2*). Tigecycline and fosfomycin MICs were interpreted according to EUCAST guidelines (*2*). Imipenem and meropenem MICs were determined using the Etest for all isolates that were nonsusceptible to at least 1 carbapenem (ertapenem, meropenem, or imipenem) by disk-diffusion (*3*). We phenotypically detected class A carbapenemases as previously described (*4*).

We used convenience sampling to select 62 CPRKp isolates that were detected during 2014–2015 and used pulsed-field gel electrophoresis (PFGE) to evaluate their genomic DNA macrorestriction profiles after *Xba*I

digestion. Dice similarity indexes were calculated using the UPGMA method with 1.25% tolerance and optimization (*5*). The minimal Dice index for a clonal group was defined as 80%.

We performed multilocus sequence typing as described (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html) for 11 isolates that represented the 2 major PFGE clonal groups, CPRKp1 and CPRKp2. The full bla_{KPC} nucleotide sequence was determined for these isolates (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/11/16-0695-Techapp1.pdf).

We included a total of 3,085 *K. pneumoniae* isolates in the analysis (online Technical Appendix Table 2). A significant increase in carbapenem resistance (p<0.001) was seen from 2011 (6.8%) to 2015 (35.5%) (Figure, panel A). During the last year of analysis, we detected *K. pneumoniae* carbapenemase (KPC) in 96.2% of CRKp isolates.

PMB MICs showed a bimodal distribution that was clearly differentiated by a 2 mg/L MIC (Figure, panel B). When we stratified MICs by year and carbapenem resistance, a significantly increasing trend of resistance was seen among CRKp isolates from 2011 (0%) to 2014 (24.8%) to 2015 (27.1%) (p<0.001) (Figure, panel C). Resistance among carbapenem-susceptible *K. pneumoniae* varied from 0.7% in 2011 to 3.9% in 2014 (p = 0.002).

We did not evaluate the mechanism of PMB resistance. However, this resistance in KPC-producing *K. pneumoniae* is probably caused by the loss of *mgrB* function or the presence of nonsynonymous substitutions in *pmrB* that upregulate the *pmrCAB* and *arnBCADTEF-pmrE* operons, resulting in modification of lipid A. All these genes are located on the bacterial chromosome. Susceptibility testing showed that amikacin and tigecycline were the most active non–β-lactam antimicrobial agents against CPRKp isolates (amikacin 73.8%, tigecycline 69.4%) and CRKp isolates (amikacin 79.9%, tigecycline 72.2%) (online Technical Appendix Table 3).

PFGE identified 2 major clonal groups. The largest group, CPRKp1 (n = 30), belonged to sequence type (ST) 11, and the other group, CPRKp2 (n = 17), belonged to ST437. Both STs belonged to clonal complex (CC) 258. Interhospital and intrahospital dissemination among private and public hospitals was observed. All isolates tested had the bla_{KPC-2} gene (online Technical Appendix Figure).

In a previous study, the PMB resistance rate was 27% among 22 CRKp isolates from patients at a tertiary hospital in São Paulo during 2008–2010 (*6*). This rate is much higher than the rate we obtained for 2010, possibly because the previous study had a small number of isolates. CPRKp has been reported in various European countries at rates similar to those we report (*7*).

The predominance of CC258 among KPC-2-producing *K. pneumoniae*, but not among CPRKp, was reported
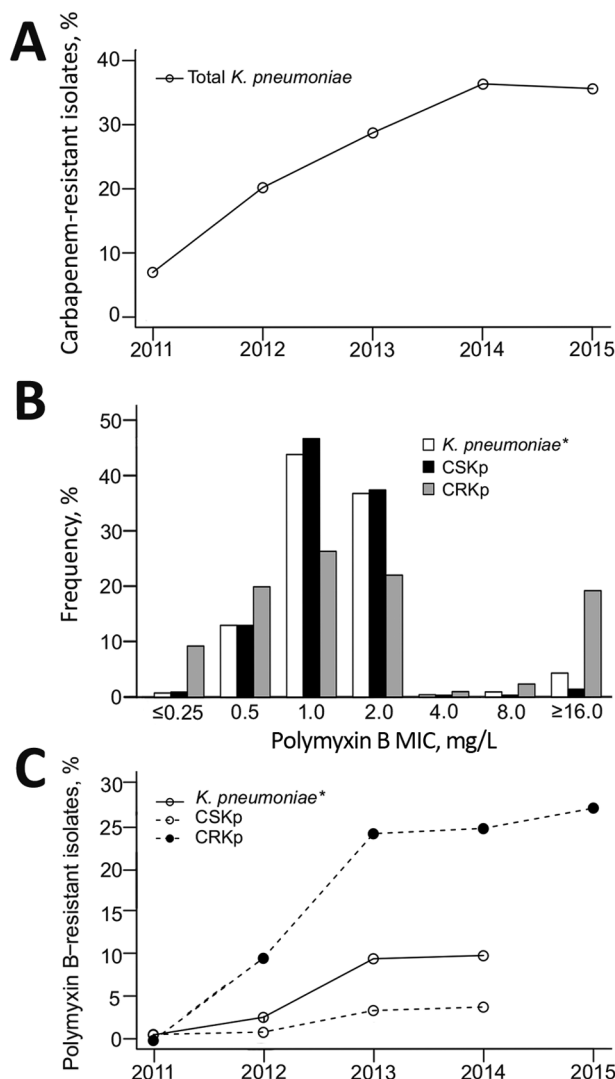
in Brazil (*8*), and ST11, a variant of ST258, has occasionally been detected in colistin-resistant KPC-producing isolates in Spain (*9*). The ST437 clone has been reported in KPC-2 producers in China (*10*), but we found no reports of CPRKp among this clonal group. Our findings show an alarming yearly increase in the rate of PMB resistance among CRKp isolates, mostly KPC-2 producing, and the occurrence of interhospital and intrahospital dissemination of CPRKp from CC258 in São Paulo.

Figure. Antimicrobial resistance profile of *Klebsiella pneumoniae* isolated from hospital inpatients in São Paulo, Brazil. A) Carbapenem resistance trend among all *K. pneumoniae* isolates cultured during January 1, 2011–December 31, 2015 (n = 3,085; p<0.001). B) Polymyxin B MIC distribution stratified by carbapenem susceptibility. C) Polymyxin B resistance trend stratified by carbapenem susceptibility, 2011–2015. B, C) Carbapenem-susceptible *K. pneumoniae* (CSKp) isolated during January 1, 2011–June 30, 2014 (n = 1,511) and carbapenem-resistant *K. pneumoniae* (CRKp) isolated during January 1, 2011–December 31, 2014 (n = 436); *during July 1, 2015–December 31, 2015, only CRKp were tested for polymyxin B susceptibility (n = 377). All statistical analyses were conducted using SAS Studio 3.4 (SAS Institute, Inc., Cary, NC, USA). The statistical significance of a trend in resistance rates was evaluated using the χ^2 test, in which p values <0.05 were considered significant: *K. pneumoniae*, p<0.001; CSKp, p = 0.004; CRKp, p = 0.003.

References

1. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant *Enterobacteriaceae* infections. Emerg Infect Dis. 2014;20:1170–5. http://dx.doi.org/10.3201/eid2007.121004
2. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 5.0 [cited 2016 Aug 12]. http://www.eucast.org/fileadmin/ src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Break-point_Table_01.pdf
3. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement (M100–S25). Wayne (PA): The Institute; 2015.
4. Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A, Petropoulou D, et al. Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumoniae* isolates in the clinical laboratory. J Clin Microbiol. 2009;47:362–7. http://dx.doi.org/10.1128/JCM.01922-08
5. Heras J, Dominguez C, Mata E, Pascual V, Lozano C, Torres C, et al. GelJ—a tool for analyzing DNA fingerprint gel images. BMC Bioinformatics. 2015;16:270. http://dx.doi.org/10.1186/ s12859-015-0703-0
6. Pereira GH, Garcia DO, Mostardeiro M, Ogassavara CT, Levin AS. Spread of carbapenem-resistant *Klebsiella pneumoniae* in a tertiary hospital in Sao Paulo, Brazil. J Hosp Infect. 2011;79:182–3. http://dx.doi.org/10.1016/j.jhin.2011.05.023
7. Giske CG. Contemporary resistance trends and mechanisms for the old antibiotics colistin, temocillin, fosfomycin, mecillinam and nitrofurantoin. Clin Microbiol Infect. 2015;21:899–905. http://dx.doi.org/10.1016/j.cmi.2015.05.022
8. Andrade LN, Curiao T, Ferreira JC, Longo JM, Climaco EC, Martinez R, et al. Dissemination of *bla*$_{KPC-2}$ by the spread of *Klebsiella pneumoniae* clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among *Enterobacteriaceae* species in Brazil. Antimicrob Agents Chemother. 2011;55:3579–83. http://dx.doi.org/10.1128/ AAC.01783-10
9. Ruiz-Garbajosa P, Curiao T, Tato M, Gijon D, Pintado V, Valverde A, et al. Multiclonal dispersal of KPC genes following the emergence of non-ST258 KPC-producing *Klebsiella pneumoniae* clones in Madrid, Spain. J Antimicrob Chemother. 2013;68:2487–92. http://dx.doi.org/10.1093/jac/dkt237
10. Wang LH, Wei DD, Wan LG, Yu Y, Deng Q, Liu Y. Diversity of the genetic environment of the *bla*$_{KPC-2}$ gene among *Klebsiella pneumoniae* clinical isolates in a Chinese hospital. Microb Drug Resist. 2016;22:15–21. http://dx.doi.org/10.1089/ mdr.2014.0281

Address for correspondence: Jorge Luiz Mello Sampaio, Faculdade de Ciências Farmacêuticas, Avenida Professor Lineu Prestes, 580, Butantã, São Paulo, SP, Brazil, 05508-900; email: sampaio@usp.br