AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Outbreak of OXA-48-Producing *Klebsiella pneumoniae* Involving a Sequence Type 101 Clone in Batna University Hospital, Algeria

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Seven nonredundant ertapenem-resistant *Klebsiella pneumoniae* isolates were collected between May 2014 and 19 January 2015 in the nephrology and hematology units of Batna University Hospital in Algeria. All strains coproduced the bla_{OXA-48}, $bla_{CTX-M-15}$, bla_{SHV-1}, and bla_{TEM-1D} genes. Six of these isolates belonged to the pandemic clone sequence type 101 (ST101). The bla_{OXA-48} gene was located on a conjugative IncL/M-type plasmid. This is the first known outbreak of OXA-48-producing *K. pneumoniae* isolates involving an ST101 clone in Batna University Hospital.

Carbapenems are a group of β-lactam drugs that constitute the last therapeutic option available for treating infections caused by multidrug-resistant *Enterobacteriaceae* (1). However, due to the emergence of carbapenem resistance, the antimicrobial activity of these drugs is no longer effective (2). Production of carbapenemases is one of the main mechanisms for carbapenem resistance (3). These carbapenemases belong to different Ambler classes (A, B, and D) and have been reported worldwide among *Enterobacteriaceae* isolates (4). The OXA-48 class D β-lactamase is called the "phantom menace" due to its weak but significant carbapenemase activity. This enzyme hydrolyzes penicillins at a high level and carbapenems at a low level but spares expanded-spectrum cephalosporins (5). OXA-48 was initially described in a clinical *Klebsiella pneumoniae* isolate from Istanbul, Turkey, in 2001 (6), and since then, several sporadic cases and outbreaks have been reported, especially in the Mediterranean countries (4).

Here, we describe a nosocomial outbreak in Batna University Hospital, Algeria, of ertapenem-resistant *K. pneumoniae* clinical isolates expressing OXA-48 associated with CTXM-15, SHV-1, and TEM-1D β-lactamases involving a sequence type 101 (ST101) clone.

Between May 2014 and 19 January 2015, seven patients hospitalized in the nephrology and hematology units at Batna University Hospital, Algeria, were infected with ertapenem-resistant *K. pneumoniae*. During the period of this study, the first OXA-48-positive *K. pneumoniae* strain was isolated in the hematology unit in May 2014 from a patient with myelosuppression and Fanconi anemia. This strain (Kp1) was assigned to sequence type 985 (ST985); however, it represented the only case until the outbreak. The index isolate Kp2 assigned to the pandemic ST101 was retrieved in August 2014 in the nephrology unit from pus on a catheter sample of a patient with chronic renal failure. During the outbreak, five patients in the hematology unit were infected with an ertapenem-resistant *K. pneumoniae* isolate of an ST101 clone, suggesting transmission via the hospital staff because the nephrology unit is in front of the hematology unit (Table 1).

Antimicrobial drug susceptibility was determined by the standard disc diffusion method recommended by the Antibiogram Committee of the French Society for Microbiology (http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM_EUCAST_V1_2015.pdf). In addition, the MICs of imipenem and colistin were determined using Etest strips (bioMérieux). All isolates were resistant to amoxicillin, cefoxitin, cefotaxime, ceftazidime, cefepime, aztreonam, amoxicillin-clavulanic acid, ertapenem, tobramycin, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole, but they were sensitive to colistin and displayed MIC values within the intermediate range for imipenem (3 to 4 μg/ml), except for imipenem-resistant strain Kp1 (MIC, >32 μg/ml). All the strains were positive for phenotypic carbapenemase production as tested by the modified Carba NP (MCNP) test (7). Real-time and standard PCR and sequencing for β-lactamase-encoding genes, which were performed using specific primers for the bla_{CTX-M}, bla_{SHV}, bla_{TEM}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{OXA-48} genes (8, 9), indicated the presence of the $bla_{CTX-M-15}$, bla_{SHV-1}, bla_{TEM-1D}, and bla_{OXA-48} genes in all isolates. Multilocus sequence typing (MLST) was performed according to the Institute Pasteur scheme (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html) and showed that all strains belonged to sequence type 101 (ST101) except for the Kp1 strain, which was assigned to ST985. An MLST concatenated gene sequence-based phylogenetic tree of our *K. pneumoniae* ST101 isolates with those responsible for most nosocomial OXA-48 outbreaks occurring in the Mediterranean countries was constructed to illustrate their phylogenetic position and clustering. MLST allele sequences of the strains not included in this work were retrieved from the *K. pneumoniae* MLST database through its web-

Received 9 March 2016 **Returned for modification** 6 July 2016
Accepted 6 September 2016
Accepted manuscript posted online 19 September 2016
Citation Loucif L, Kassah-Laouar A, Saidi M, Messala A, Chelaghma W, Rolain J-M. 2016. Outbreak of OXA-48-producing *Klebsiella pneumoniae* involving a sequence type 101 clone in Batna University Hospital, Algeria. Antimicrob Agents Chemother 60:7494–7497. doi:10.1128/AAC.00525-16.

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TABLE 1 Clinical features of the *K. pneumoniae* isolate outbreak

Patient no.	Isolate	Site of isolation	Date of positive isolate	Unit	Reason for hospitalization	Treatment(s)	Outcome
2	Kp2	Pus	August 2014	Nephrology	Chronic renal failure	Last step: colistin	Improved
3	Kp3	Blood	October 2014	Hematology	Acute myeloid leukemia, stage 2	1st step: cefotaxime, gentamicin, and metronidazole 2nd step: amikacin and piperacillin 3rd step: imipenem, vancomycin, and ofloxacin	Died
4	Kp4	Mouth ulcers	December 2014	Hematology	Acute myeloid leukemia, stage 5	1st step: cefotaxime, gentamicin, and metronidazole 2nd step: amikacin and piperacillin 3rd step: imipenem, vancomycin, and ofloxacin	Died
5	Kp5	Blood	December 2014	Hematology	Acute myeloid leukemia, stage 1	1st step: cefotaxime, gentamicin, and metronidazole 2nd step: amikacin and piperacillin 3rd step: imipenem, vancomycin, and ofloxacin	Died
6	Kp6	Blood	December 2014	Hematology	Acute myeloid leukemia, stage 4	1st step: cefotaxime, gentamicin, and metronidazole 2nd step: amikacin and piperacillin 3rd step: imipenem, vancomycin, and ofloxacin	Died
7	Kp7	Blood	January 2015	Hematology	Severe myelosuppression	1st step: cefotaxime, gentamicin, and metronidazole 2nd step: amikacin and piperacillin 3rd step: imipenem, vancomycin, and ofloxacin	Died

site (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). The phylogenetic tree was built using Mega 6 software (10–12) (Fig. 1). Transferability of the bla_{OXA-48} gene was tested using conjugation experiments among the *K. pneumoniae* isolates Kp2 and Kp4 and *Escherichia coli* strain J53, which is resistant to sodium azide. The obtained transconjugants were MCNP positive and resistant to only ertapenem and amoxicillin-clavulanic acid. PCR for β-lactamase determinants in transconjugants identified only the bla_{OXA-48} gene. The incompatibility group of the plasmid carrying the bla_{OXA-48} gene was determined by PCR using previously published primers (9) to be IncL/M.

With this study, we describe the first reported outbreak caused

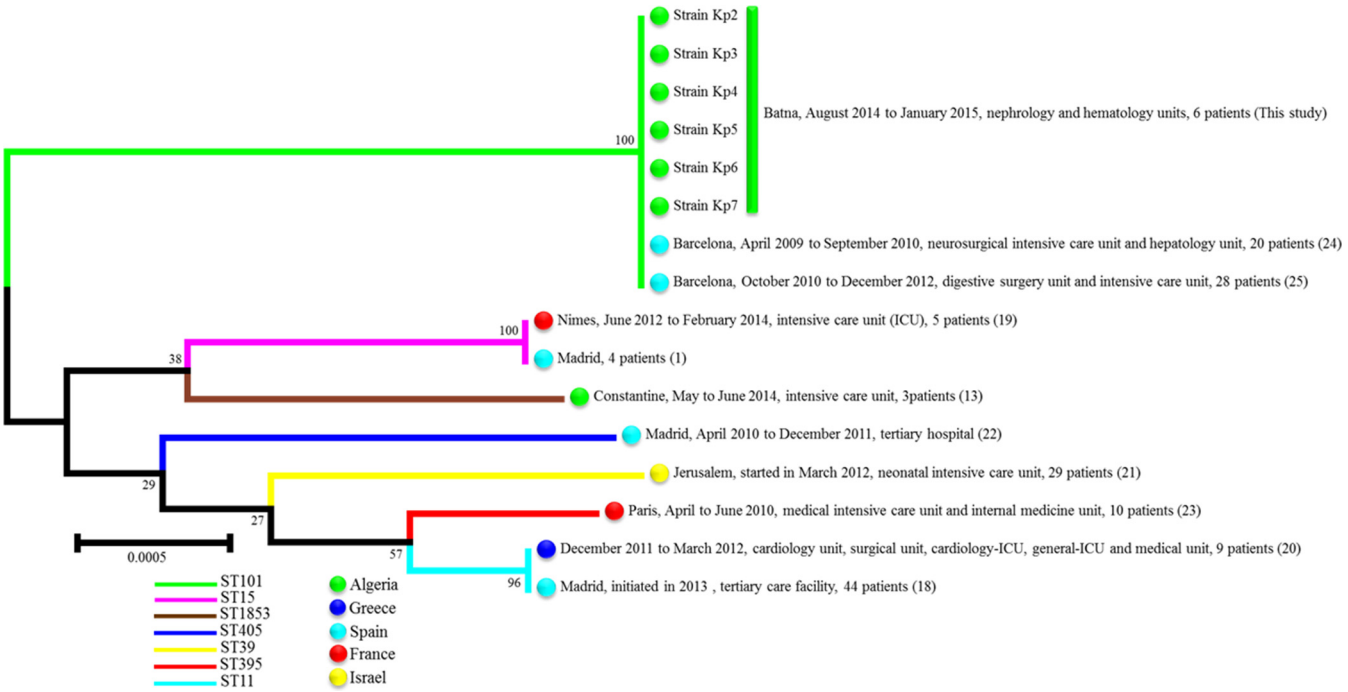

FIG 1 MLST-based phylogenetic tree of clinical *K. pneumoniae* isolates in this study and those responsible for most OXA-48 outbreaks in Mediterranean countries. The evolutionary history was inferred using the neighbor-joining method (11). The evolutionary distances were computed using the Kimura 2-parameter method (12). The numbers at the nodes are the levels of bootstraps from 1,000 replicates.

by OXA-48-producing *K. pneumoniae* isolates in Batna University Hospital in Algeria. This represents the second reported outbreak of OXA-48-producing *K. pneumoniae* in Algeria, after the recent study by Cuzon et al. (13), who characterized an outbreak of OXA-48-producing *K. pneumoniae* ST1853 in Constantine University Hospital in Algeria. In 2012, Poirel et al. (5) suggested that the OXA-48 enzyme may be endemic in Algeria, since its dissemination has been described worldwide (4), particularly in the Mediterranean area, including Egypt, Lebanon, Morocco, and Tunisia (14–17). This suggestion is supported by the isolation of OXA-48-producing *Enterobacteriaceae* strains from patients hospitalized in French hospitals who had recently travelled to or were hospitalized in Algeria (13). Previous studies reported the isolation of clinical *K. pneumoniae* strains coproducing extended-spectrum β-lactamase (ESBL) and the $bla_{\text{OXA-48}}$ gene (1, 13, 18). In this study, we identified the association of $bla_{\text{OXA-48}}$ with three different β-lactamase genes ($bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-1}}$, and $bla_{\text{TEM-1D}}$) that had not been previously described in Algeria; the same association was observed in Tunisia, except for the TEM variant (17). In the Mediterranean area, outbreaks involving OXA-48-producing isolates have implicated diverse sequence types, such as ST1853, ST15, ST11, ST39, ST405, ST395, and ST101 (Fig. 1) (13, 19–25). However, in this investigation, the outbreak was due to the pandemic ST101, which was detected for the first time in Algeria. The introduction of this ST101 clone in our hospital environment was attributed to index patient 2, who was probably infected during a prior hospitalization. OXA-48-producing *K. pneumoniae* isolates belonging to ST101 have already been implicated in two nosocomial outbreaks in Spain (Fig. 1) (24, 25). In addition to its involvement in the nosocomial outbreaks, there have been some sporadic cases reported in Tunisia, Switzerland, Bulgaria, and Germany (17, 26–28).

In conclusion, this study identified the first OXA-48-positive *K. pneumoniae* coproducing CTX-M-15, SHV-1, and TEM-1D β-lactamases in Batna University Hospital. These data, along with those of earlier studies and the more recent description of OXA-48-producing *Enterobacteriaceae* from companion animals and hospital cockroaches, confirm the widespread presence of OXA-48-producing *Enterobacteriaceae* in Algeria (29, 30).

ACKNOWLEDGMENTS

We are very grateful to Linda Hadjadj for technical assistance. We also thank TradOnline for English language corrections.

This work was partly funded by the Centre National de la Recherche Scientifique (CNRS7278) and the IHU Méditérranée Infection.

The authors report no conflicts of interest relevant to this article.

FUNDING INFORMATION

This work was funded by Centre National de la Recherche Scientifique (CNRS7278). This work was funded by IHU Méditérranée Infection.

REFERENCES

1. **Arana DM, Saez D, Garcia-Hierro P, Bautista V, Fernandez-Romero S, Angel de la Cal M, Alos JI, Oteo J.** 2015. Concurrent interspecies and clonal dissemination of OXA-48 carbapenemase. Clin Microbiol Infect **21:**148e1–148e4. http://dx.doi.org/10.1016/j.cmi.2014.07.008.
2. **Beyrouthy R, Robin F, Delmas J, Gibold L, Dalmasso G, Dabboussi F, Hamze M, Bonnet R.** 2014. IS1R-mediated plasticity of IncL/M plasmids leads to the insertion of bla OXA-48 into the *Escherichia coli* chromosome. Antimicrob Agents Chemother **58:**3785–3790. http://dx.doi.org/10.1128/AAC.02669-14.
3. **Nordmann P, Dortet L, Poirel L.** 2012. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med **18:**263–272. http://dx.doi.org/10.1016/j.molmed.2012.03.003.
4. **Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP.** 2014. Epidemiology of carbapenemase-producing *Enterobacteriaceae* and *Acinetobacter baumannii* in Mediterranean countries. Biomed Res Int **2014:**305784. http://dx.doi.org/10.1155/2014/305784.
5. **Poirel L, Potron A, Nordmann P.** 2012. OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother **67:**1597–1606. http://dx.doi.org/10.1093/jac/dks121.
6. **Poirel L, Heritier C, Tolun V, Nordmann P.** 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother **48:**15–22. http://dx.doi.org/10.1128/AAC.48.1.15-22.2004.
7. **Bakour S, Garcia V, Loucif L, Brunel JM, Gharout-Sait A, Touati A, Rolain JM.** 2015. Rapid identification of carbapenemase-producing *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* using a modified Carba NP test. New Microbes New Infect **7:**89–93. http://dx.doi.org/10.1016/j.nmni.2015.07.001.
8. **Mellouk FZ, Bakour S, Meradji S, Al-Bayssari C, Bentakouk MC, Zouyed F, Djahoudi A, Boutefnouchet N, Rolain JM.** 17 June 2016. First detection of VIM-4-producing *Pseudomonas aeruginosa* and OXA-48-producing *Klebsiella pneumoniae* in northeastern (Annaba, Skikda) Algeria. Microb Drug Resist http://dx.doi.org/10.1089/mdr.2016.0032.
9. **Poirel L, Bonnin RA, Nordmann P.** 2012. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob Agents Chemother **56:**559–562. http://dx.doi.org/10.1128/AAC.05289-11.
10. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol **30:**2725–2729. http://dx.doi.org/10.1093/molbev/mst197.
11. **Saitou N, Nei M.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol **4:**406–425.
12. **Kimura M.** 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol **16:**111–120. http://dx.doi.org/10.1007/BF01731581.
13. **Cuzon G, Bentchouala C, Vogel A, Hery M, Lezzar A, Smati F, Dortet L, Naas T.** 2015. First outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in Constantine, Algeria. Int J Antimicrob Agents **46:**725–727. http://dx.doi.org/10.1016/j.ijantimicag.2015.08.005.
14. **Poirel L, Abdelaziz MO, Bernabeu S, Nordmann P.** 2013. Occurrence of OXA-48 and VIM-1 carbapenemase-producing *Enterobacteriaceae* in Egypt. Int J Antimicrob Agents **41:**90–91. http://dx.doi.org/10.1016/j.ijantimicag.2012.08.015.
15. **Beyrouthy R, Robin F, Dabboussi F, Mallat H, Hamze M, Bonnet R.** 2014. Carbapenemase and virulence factors of *Enterobacteriaceae* in north Lebanon between 2008 and 2012: evolution via endemic spread of OXA-48. J Antimicrob Chemother **69:**2699–2705. http://dx.doi.org/10.1093/jac/dku181.
16. **Hays C, Benouda A, Poirel L, Elouennass M, Nordmann P.** 2012. Nosocomial occurrence of OXA-48-producing enterobacterial isolates in a Moroccan hospital. Int J Antimicrob Agents **39:**545–547. http://dx.doi.org/10.1016/j.ijantimicag.2012.03.002.
17. **Lahlaoui H, Poirel L, Barguellil F, Moussa MB, Nordmann P.** 2012. Carbapenem hydrolyzing class D beta-lactamase OXA-48 in *Klebsiella pneumoniae* isolates from Tunisia. Eur J Clin Microbiol Infect Dis **31:**937–939. http://dx.doi.org/10.1007/s10096-011-1389-5.
18. **Branas P, Villa J, Viedma E, Mingorance J, Orellana MA, Chaves F.** 2015. Molecular epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in a hospital in Madrid: successful establishment of an OXA-48 ST11 clone. Int J Antimicrob Agents **46:**111–116. http://dx.doi.org/10.1016/j.ijantimicag.2015.02.019.
19. **Pantel A, Richaud-Morel B, Cazaban M, Bouziges N, Sotto A, Lavigne JP.** 2016. Environmental persistence of OXA-48-producing *Klebsiella pneumoniae* in a French intensive care unit. Am J Infect Control **44:**366–368. http://dx.doi.org/10.1016/j.ajic.2015.09.021.
20. **Voulgari E, Zarkotou O, Ranellou K, Karageorgopoulos DE, Vrioni G, Mamali V, Themeli-Digalaki K, Tsakris A.** 2013. Outbreak of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* in Greece involving an ST11 clone. J Antimicrob Chemother **68:**84–88. http://dx.doi.org/10.1093/jac/dks356.
21. **Adler A, Solter E, Masarwa S, Miller-Roll T, Abu-Libdeh B, Khammash H, Najem K, Dekadek S, Stein-Zamir C, Nubani N, Kunbar A, Assous MV, Carmeli Y, Schwaber MJ.** 2013. Epidemiological and microbiolog-

ical characteristics of an outbreak caused by OXA-48-producing *Enterobacteriaceae* in a neonatal intensive care unit in Jerusalem, Israel. J Clin Microbiol **51:**2926–2930. http://dx.doi.org/10.1128/JCM.01049-13.

22. **Pano-Pardo JR, Ruiz-Carrascoso G, Navarro-San FC, Gomez-Gil R, Mora-Rillo M, Romero-Gomez MP, Fernandez-Romero N, Garcia-Rodriguez J, Perez-Blanco V, Moreno-Ramos F, Mingorance J.** 2013. Infections caused by OXA-48-producing *Klebsiella pneumoniae* in a tertiary hospital in Spain in the setting of a prolonged, hospital-wide outbreak. J Antimicrob Chemother **68:**89–96. http://dx.doi.org/10.1093/jac/dks364.
23. **Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P.** 2011. Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in France. Antimicrob Agents Chemother **55:**2420–2423. http://dx.doi.org/10.1128/AAC.01452-10.
24. **Pitart C, Sole M, Roca L, Fabrega A, Vila J, Marco F.** 2011. First outbreak of a plasmid-mediated carbapenem-hydrolyzing OXA-48 beta-lactamase in *Klebsiella pneumoniae* in Spain. Antimicrob Agents Chemother **55:**4398–4401. http://dx.doi.org/10.1128/AAC.00329-11.
25. **Cubero M, Cuervo G, Dominguez MA, Tubau F, Marti S, Sevillano E, Gallego L, Ayats J, Pena C, Pujol M, Linares J, Ardanuy C.** 2015. Carbapenem-resistant and carbapenem-susceptible isogenic isolates of *Klebsiella pneumoniae* ST101 causing infection in a tertiary hospital. BMC Microbiol **15:**177. http://dx.doi.org/10.1186/s12866-015-0510-9.
26. **Seiffert SN, Marschall J, Perreten V, Carattoli A, Furrer H, Endimiani A.** 2014. Emergence of *Klebsiella pneumoniae* co-producing NDM-1, OXA-48, CTX-M-15, CMY-16, QnrA and ArmA in Switzerland. Int J Antimicrob Agents **44:**260–262. http://dx.doi.org/10.1016/j.ijantimicag.2014.05.008.
27. **Markovska R, Stoeva T, Schneider L, Boyanova L, Popova V, Dacheva D, Kaneva R, Bauernfeind A, Mitev V, Mitov L.** 2015. Clonal dissemination of multilocus sequence type ST15 KPC-2-producing *Klebsiella pneumoniae* in Bulgaria. APMIS **123:**887–894. http://dx.doi.org/10.1111/apm.12433.
28. **Kaase M, Schimanski S, Schiller R, Beyreiss B, Thurmer A, Steinmann J, Kempf VA, Hess C, Sobottka L, Fenner L, Ziesing S, Burckhardt L, Von ML, Hamprecht A, Tammer I, Wantia N, Becker K, Holzmann T, Furitsch M, Volmer G, Gatermann SG.** 2016. Multicentre investigation of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in German hospitals. Int J Med Microbiol **306:**415–420. http://dx.doi.org/10.1016/j.ijmm.2016.05.009.
29. **Yousfi M, Touati A, Mairi A, Brasme L, Gharout-Sait A, Guillard T, De Champs C.** 2016. Emergence of carbapenemase-producing *Escherichia coli* isolated from companion animals in Algeria. Microb Drug Resist **22:**342–346. http://dx.doi.org/10.1089/mdr.2015.0196.
30. **Loucif L, Gacemi-Kirane D, Cherak Z, Chamlal N, Grainat N, Rolain JM.** 2016. First report of German cockroaches (*Blattella germanica*) as reservoirs of CTX-M-15 extended-spectrum-β-lactamase- and OXA-48 carbapenemase-producing *Enterobacteriaceae* in Batna University Hospital, Algeria. Antimicrob Agents Chemother **60:**6377–6380. http://dx.doi.org/10.1128/AAC.00871-16.