

Characterization of an IncA/C Multidrug Resistance Plasmid in *Vibrio alginolyticus*

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Cephalosporin-resistant *Vibrio alginolyticus* was first isolated from food products, with β -lactamases encoded by *bla*_{PER-1}, *bla*_{VEB-1}, and *bla*_{CMY-2} being the major mechanisms mediating their cephalosporin resistance. The complete sequence of a multidrug resistance plasmid, pVAS3-1, harboring the *bla*_{CMY-2} and *qnrVC4* genes was decoded in this study. Its backbone exhibited genetic homology to known IncA/C plasmids recoverable from members of the family *Enterobacteriaceae*, suggesting its possible origin in *Enterobacteriaceae*.

Vibrio alginolyticus, like other *Vibrionaceae* species, such as *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, may cause a range of human infections, such as gastroenteritis, septicemia, and necrotizing fasciitis. Of the pathogenic *Vibrio* species, *V. alginolyticus* is one of the top three causes of infections in humans and is genetically homologous to *V. parahaemolyticus* (1, 2). Resistance to expanded-spectrum cephalosporins and fluoroquinolones has also been reported in *V. parahaemolyticus* and *V. cholerae* but not in other *Vibrio* species such as *V. vulnificus* and *V. alginolyticus* (3, 4). Here we report (i) the isolation from food products of *V. alginolyticus* strains that are resistant to expanded-spectrum cephalosporins and (ii) the resistance mechanisms involved.

V. alginolyticus strains were isolated from food samples, including shrimp, chicken, pork, and beef, in Shenzhen, China, from June 2014 to August 2015 as previously described (5). Suspicious colonies from thiosulfate-citrate-bile salt sucrose and CHROMagar plates were screened by PCR and DNA sequencing and confirmed with API 20E test strips (bioMérieux, Inc.). A total of 23 *V. alginolyticus* strains were isolated from 515 food samples (2 from 88 chicken samples, 1 from 258 pork samples, 1 from 121 beef samples, and 19 from 48 shrimp samples). These 23 *V. alginolyticus* strains were subjected to antimicrobial susceptibility testing according to the Clinical and Laboratory Standards Institute (6). These isolates exhibited a high rate of resistance to ampicillin (100%), followed by trimethoprim-sulfamethoxazole (48%); ceftriaxone and cefotaxime (26%); chloramphenicol (13%); cefoxitin and amoxicillin-clavulanic acid (8.7%); and nalidixic acid, ciprofloxacin, ofloxacin, and gentamicin (4.4%). All strains were susceptible to tetracycline, amikacin, and meropenem. Five of six cephalosporin-resistant strains were isolated from shrimp samples in the same market on different dates, whereas one strain, VAS3-1, was isolated from a chicken sample (Table 1). Characterization of these isolates by pulsed-field gel electrophoresis (PFGE) was performed as previously described (7) and revealed that these isolates had distinct PFGE profiles, suggesting a high level of genetic diversity of these isolates even though most of them were recovered from the same market at different times (see Fig. S1 in the supplemental material). The

prevalence of β -lactamase genes among the strains was determined by PCR assays as previously described (8) and showed that the *bla*_{PER-1} β -lactamase gene is present in strains VA1 and VA6 and that the *bla*_{CMY-2} gene is present in VAS3-1. The *bla*_{VEB-1} element was detected in strains V2 and V5, but no known β -lactamase gene was found in strain V4 (Table 1). It should be noted that this is the first time these cephalosporinases have been described in *V. alginolyticus* and that this is the first time the *bla*_{VEB-1} element has been recovered from a *Vibrio* species. Conjugation experiments performed with these strains showed that three strains, namely, VA1, VAS3-1, and VA5, could successfully transfer their cephalosporin resistance phenotypes to a recipient strain, *Escherichia coli* J53. Plasmid typing results showed that the conjugative plasmid recovered from VAS3-1 belonged to the IncA/C type, whereas conjugative plasmids from VA1 and VA5 were untypeable. Our laboratory has recently recovered an IncA/C-type conjugative plasmid from a *V. parahaemolyticus* strain (3). We therefore further characterized the IncA/C conjugative plasmid recovered from strain VAS3-1 in this study and investigated whether it is genetically related to the one obtained from *V. parahaemolyticus*. S1 PFGE and hybridization with a *bla*_{CMY-2} probe performed with VAS3-1 showed that the strain contains two *bla*_{CMY-2}-positive plasmids of ~180 and 300 kb, respectively, but only the ~180-kb plasmid was transferrable to the *E. coli* J53 recipient strain (see Fig. S2 in the supplemental mate-

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TABLE 1 Antibiotic susceptibility profiles of cephalosporin-resistant *V. alginolyticus* strains and corresponding recipient transconjugant *E. coli* strain J53

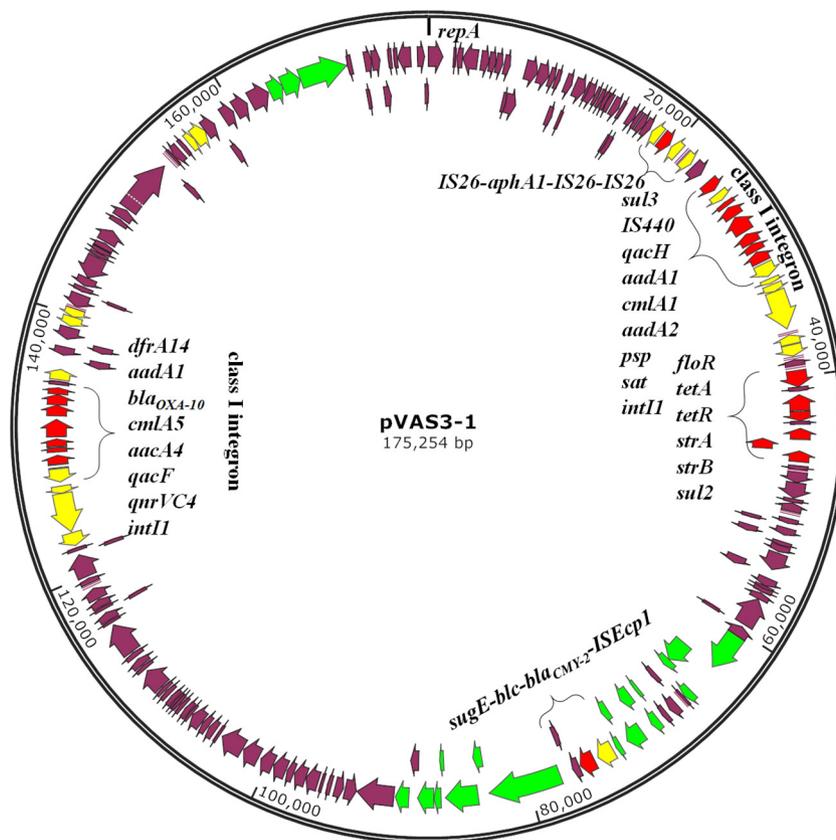
| Strain | 2015 isolation date | Source | β-Lactamase gene product | MIC (mg/liter) ^b of: | | | | | | | | | | |
|----------|---------------------|---------|--------------------------|---------------------------------|-------|------|-------|-----|-------|-----|-----|------|------|-----------|
| | | | | AMP | AMC | CTX | CRO | NAL | CIP | AMK | TET | CHL | GEN | SXT |
| J53 | | | | 1 | 4/2 | 0.03 | 0.015 | 1 | 0.03 | 0.5 | 0.5 | 2 | 0.25 | 0.25/4.75 |
| VA1 | 1/14 | Shrimp | PER-1 | >64 | 16/8 | >16 | >16 | 0.5 | 0.06 | 4 | 2 | 0.25 | 32 | >16/304 |
| T-VA1 | | | PER-1 | >64 | 8/4 | >16 | >16 | 4 | 0.015 | 0.5 | 8 | 2 | 32 | 16/304 |
| VA2 | 1/20 | Shrimp | VEB-1 | >64 | 4/2 | >16 | >16 | 32 | >16 | 1 | 8 | 32 | 4 | >16/304 |
| VAS3-1 | 3/14 | Chicken | CMY-2 | >64 | 64/32 | 8 | 16 | 16 | 0.5 | 0.5 | 16 | 32 | 1 | >16/304 |
| T-VAS3-1 | | | CMY-2 | >64 | 64/32 | 8 | >16 | 8 | 0.5 | 0.5 | 8 | 16 | 1 | 8/152 |
| VA4 | 3/4 | Shrimp | ND ^a | >64 | 4/2 | >16 | >16 | 0.5 | 0.5 | 2 | 4 | >32 | 4 | 8/152 |
| VA-5 | 3/14 | Shrimp | VEB-1 | >64 | 8/4 | >16 | >16 | 2 | 1 | 4 | 0.5 | 1 | 8 | 0.25/4.75 |
| T-VA5 | | | VEB-1 | >64 | 4/2 | 2 | 2 | 8 | 0.03 | 0.5 | 0.5 | 2 | 8 | 0.25/4.75 |
| VA6 | 3/30 | Shrimp | PER-1 | >64 | 4/2 | 16 | 8 | 1 | 0.25 | 4 | 2 | 2 | 4 | 8/152 |

^a ND, not detected.^b AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CRO, ceftriaxone; CTX, cefotaxime; NAL, nalidixic acid; CIP, ciprofloxacin; AMK, amikacin; TET, tetracycline; CHL, chloramphenicol; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole.

rial). This 180-kb conjugative plasmid is larger than the ~150-kb *V. parahaemolyticus* plasmid reported in our previous study (3).

The plasmid from a transconjugant, T-VAS3-1, was sequenced with the Illumina NextSeq 500 platform and the PacBio RSII single-molecule real-time sequencing platform. The completed plasmid sequence was confirmed by PCR and then annotated with the RAST tool and the NCBI Prokaryotic Genome Annotation Pipeline. The plasmid was designated pVAS3-1 and was found to be a

circular IncA/C plasmid of 175,254 bp with a G+C content of 52.3%. It contained 216 predicted open reading frames, among which three core regions were disrupted by three resistance-encoding fragments (Fig. 1). The backbone exhibited a high degree of genetic similarity to six other known IncA/C plasmids (99% identity with 82 to 91% coverage) and was found to harbor genes responsible for plasmid replication, horizontal transfer, and maintenance of genetic stability (9, 10) (Fig. 2). All seven plasmids were bla_{CMY}-harboring IncA/C conjugative plasmids, and each

**FIG 1** Gene map of conjugative IncA/C plasmid pVAS3-1. Yellow arrows represent mobile elements. Red arrows represent resistance genes. Blue arrows represent genes related to conjugal transfer functions.

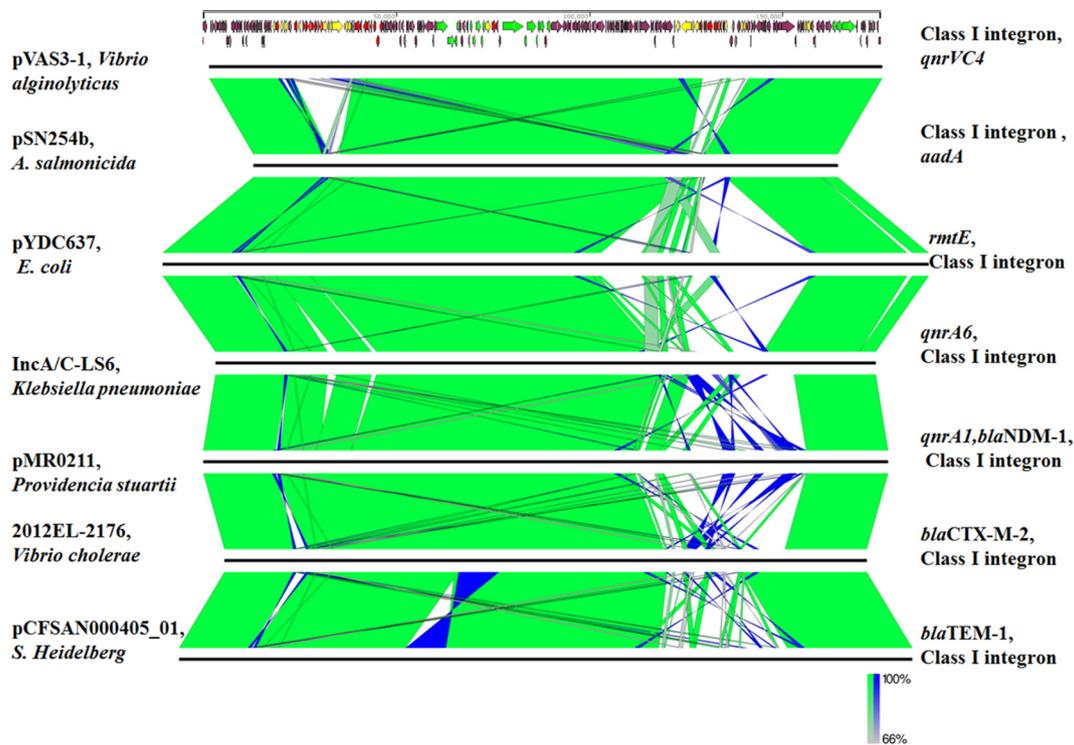


FIG 2 Whole-plasmid alignment of highly homologous *bla*_{CMY-2} IncA/C plasmids recovered from various bacterial species. Plasmid names and sources are on the left; elements located in variable mosaic regions are on the right. GenBank accession numbers: [KJ909290](https://www.ncbi.nlm.nih.gov/nuccore/KJ909290), [KP056256](https://www.ncbi.nlm.nih.gov/nuccore/KP056256), [JX442976](https://www.ncbi.nlm.nih.gov/nuccore/JX442976), [JN687470](https://www.ncbi.nlm.nih.gov/nuccore/JN687470), [CP007636](https://www.ncbi.nlm.nih.gov/nuccore/CP007636), and [KR091911](https://www.ncbi.nlm.nih.gov/nuccore/KR091911).

contained a mobile mosaic multidrug resistance (MDR) region located between conserved backbone genes *ybaA* and *rhsD*. Presumably, with the aid of transposons and other mobile elements, different resistance determinants (*rmtE*, *bla*_{NDM-1}, the *mer* operon, etc.) were found incorporated into the plasmid backbone (9, 11). Resembling other *bla*_{CMY-2} positive plasmids, the *ISEcp1-bla*_{CMY-2} region was also found to be located between the *traA* and *traC* genes in pVAS3-1 (Fig. 1) (12). The *bla*_{CMY-2} gene was contained in a mobile element, *ISEcp1-bla*_{CMY-2}-*blc-sugE*, that has previously been found mainly in members of the family *Enterobacteriaceae*, may originate from the *Citrobacter freundii* chromosome, and is transferrable to other bacterial species through *ISEcp1*-mediated mobilization and conjugative plasmids (12, 13). It has also recently been recovered in IncA/C plasmids harbored by *Aeromonas salmonicida* and *Vibrio parahaemolyticus* (3, 14). In the region upstream of the conserved *bla*_{CMY-2} fragment, another conserved MDR region common to the seven plasmids that contained the *floR*, *tet(A/R)*, *strA*, *strB*, and *sul2* elements was also detected. In addition, an IS26-*aphA1*-IS26 fragment and a non-classic class I integron without the 3' coding sequence region were also found in the upstream region of pVAS3-1 (Fig. 1). This integron, which harbored six gene cassettes, was interrupted by the IS440 and *sul3* elements. Such an integron structure was found in an *Enterobacteriaceae* species only once before (15). The presence of this unique integron in pVAS3-1 and not in the other six IncA/C plasmids suggests that pVAS3-1 may have originated from one of these six IncA/C plasmids (Fig. 2). Although some researchers have speculated that an aquatic environment may be the source of IncA/C plasmids (16), in view of the above data and the increasing reports of *bla*_{CMY-2} positive plasmids in aquatic bacte-

ria such as *Aeromonas* and *Vibrio* species (GenBank accession no. [KJ909290](https://www.ncbi.nlm.nih.gov/nuccore/KJ909290) and [CP007636](https://www.ncbi.nlm.nih.gov/nuccore/CP007636)) in recent years, we believe that dissemination of *bla*_{CMY-2} plasmids from *Enterobacteriaceae* to aquatic pathogens may be the most likely event.

The hypervariable region of pVAS3-1 was found to contain a ca. 20-kb MDR-encoding fragment, including the novel class I integron In1222 (*qnrVC4*, *qacF*, *aacA4*, *cmlA5*, *bla*_{OXA-10}, *aadA1*, and *dfrA14*), a macrolide resistance determinant (*mphA-mrx-mphR*), and a mercury resistance operon. The *qnrVC4* gene was first identified in *Aeromonas punctata* in China in a classic class I integron (17). The class I integron found in pVAS3-1 is partially homologous to the one reported in *Salmonella enterica* serovar Rissen (18) in Thailand and different from the one reported in *A. punctata* (see Fig. S3 in the supplemental material). *qnrVC* alleles that encode quinolone resistance determinants that mediate decreased susceptibility to fluoroquinolones have emerged globally (17, 18). To date, seven different *qnrVC* alleles have been identified, mainly in the *Vibrionaceae* family; however, these resistance elements have also been recovered in other pathogens, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (7, 18, 19), highlighting the possibility that *qnrVC* alleles can be readily transmitted from aquatic-environment organisms to clinical pathogens.

In conclusion, comparative analysis of the genetic features of pVAS3-1 and plasmids recoverable from other bacterial species indicates that such elements exhibit a high degree of genetic plasticity and that the increasing reports of IncA/C plasmids in aquatic bacteria may be due to the transmission of these plasmids from *Enterobacteriaceae*.

Nucleotide sequence accession number. The sequence of plasmid pVAS3-1 has been deposited in GenBank under accession no. KU160531.

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We have no conflicts of interest to declare.

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REFERENCES

- Xie ZY, Hu CQ, Chen C, Zhang LP, Ren CH. 2005. Investigation of seven *Vibrio* virulence genes among *Vibrio alginolyticus* and *Vibrio parahaemolyticus* strains from the coastal mariculture systems in Guangdong, China. *Lett Appl Microbiol* 41:202–207. <http://dx.doi.org/10.1111/j.1472-765X.2005.01688.x>.
- Newton A, Kendall M, Vugia DJ, Henao OL, Mahon BE. 2012. Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. *Clin Infect Dis* 54(Suppl 5):S391–S395. <http://dx.doi.org/10.1093/cid/cis243>.
- Li R, Lin D, Chen K, Wong MH, Chen S. 2015. First detection of AmpC β -lactamase bla(CMY-2) on a conjugative IncA/C plasmid in *Vibrio parahaemolyticus* of food origin. *Antimicrob Agents Chemother* 59:4106–4111. <http://dx.doi.org/10.1128/AAC.05008-14>.
- Wu J, Xie L, Zhang F, Ni Y, Sun J. 2015. Molecular characterization of ISCR1-mediated bla_{PER-1} in a non-O1, non-O139 *Vibrio cholerae* strain from China. *Antimicrob Agents Chemother* 59:4293–4295. <http://dx.doi.org/10.1128/AAC.00166-15>.
- Di Pinto A, Terio V, Novello L, Tantillo G. 2011. Comparison between thiosulphate-citrate-bile salt sucrose (TCBS) agar and CHROMagar *Vibrio* for isolating *Vibrio parahaemolyticus*. *Food Control* 22:124–127. <http://dx.doi.org/10.1016/j.foodcont.2010.06.013>.
- CLSI. 2005. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; proposed guideline. CLSI document M45-P. Clinical and Laboratory Standards Institute, Wayne, PA.
- Belotti PT, Thabet L, Laffargue A, Andre C, Coulange-Mayonnove L, Arpin C, Messadi A, M'Zali F, Quentin C, Dubois V. 2015. Description of an original integron encompassing blaVIM-2, qnrVC1 and genes encoding bacterial group II intron proteins in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 70:2237–2340. <http://dx.doi.org/10.1093/jac/dkv103>.
- Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *J Antimicrob Chemother* 65:490–495. <http://dx.doi.org/10.1093/jac/dkp498>.
- Lee CS, Li JJ, Doi Y. 2015. Complete sequence of conjugative IncA/C plasmid encoding CMY-2 β -lactamase and RmtE 16S rRNA methyltransferase. *Antimicrob Agents Chemother* 59:4360–4361. <http://dx.doi.org/10.1128/AAC.00852-15>.
- Call DR, Singer RS, Meng D, Broschat SL, Orfe LH, Anderson JM, Herndon DR, Kappmeyer LS, Daniels JB, Besser TE. 2010. bla_{CMY-2}-positive IncA/C plasmids from *Escherichia coli* and *Salmonella enterica* are a distinct component of a larger lineage of plasmids. *Antimicrob Agents Chemother* 54:590–596. <http://dx.doi.org/10.1128/AAC.00055-09>.
- Villa L, Guerra B, Schmogger S, Fischer J, Helmuth R, Zong Z, Garcia-Fernandez A, Carattoli A. 2015. IncA/C plasmid carrying bla_{NDM-1}, bla_{CMY-16}, and fosA3 in a *Salmonella enterica* serovar Corvallis strain isolated from a migratory wild bird in Germany. *Antimicrob Agents Chemother* 59:6597–6600. <http://dx.doi.org/10.1128/AAC.00944-15>.
- Verdet C, Gautier V, Chachaty E, Ronco E, Hidri N, Decre D, Arlet G. 2009. Genetic context of plasmid-carried bla_{CMY-2}-like genes in Enterobacteriaceae. *Antimicrob Agents Chemother* 53:4002–4006. <http://dx.doi.org/10.1128/AAC.00753-08>.
- Barlow M, Hall BG. 2002. Origin and evolution of the AmpC beta-lactamases of *Citrobacter freundii*. *Antimicrob Agents Chemother* 46:1190–1198. <http://dx.doi.org/10.1128/AAC.46.5.1190-1198.2002>.
- Vincent AT, Trudel MV, Paquet VE, Boyle B, Tanaka KH, Dallaire-Dufresne S, Daher RK, Frenette M, Derome N, Charette SJ. 2014. Detection of variants of the pRAS3, pAB5S9, and pSN254 plasmids in *Aeromonas salmonicida* subsp. *salmonicida*: multidrug resistance, interspecies exchanges, and plasmid reshaping. *Antimicrob Agents Chemother* 58:7367–7374. <http://dx.doi.org/10.1128/AAC.03730-14>.
- Vinué L, Saenz Y, Rojo-Bezares B, Olarte I, Undabeitia E, Somalo S, Zarazaga M, Torres C. 2010. Genetic environment of sul genes and characterisation of integrons in *Escherichia coli* isolates of blood origin in a Spanish hospital. *Int J Antimicrob Agents* 35:492–496. <http://dx.doi.org/10.1016/j.ijantimicag.2010.01.012>.
- Fricke WF, Welch TJ, McDermott PF, Mammel MK, LeClerc JE, White DG, Cebula TA, Ravel J. 2009. Comparative genomics of the IncA/C multidrug resistance plasmid family. *J Bacteriol* 191:4750–4757. <http://dx.doi.org/10.1128/JB.00189-09>.
- Xia R, Guo X, Zhang Y, Xu H. 2010. qnrVC-like gene located in a novel complex class 1 integron harboring the ISCR1 element in an *Aeromonas punctata* strain from an aquatic environment in Shandong Province, China. *Antimicrob Agents Chemother* 54:3471–3474. <http://dx.doi.org/10.1128/AAC.01668-09>.
- Fonseca EL, Vicente AC. 2013. Epidemiology of qnrVC alleles and emergence out of the Vibrionaceae family. *J Med Microbiol* 62:1628–1630. <http://dx.doi.org/10.1099/jmm.0.062661-0>.
- Po KHL, Wong MHY, Chen S. 2015. Identification and characterisation of a novel plasmid-mediated quinolone resistance gene, qnrVC7, in *Vibrio cholerae* of seafood origin. *Int J Antimicrob Agents* 45:667–668. <http://dx.doi.org/10.1016/j.ijantimicag.2015.02.002>.