AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Carbapenem- and Colistin-Resistant *Enterobacter cloacae* from Delta, Colorado, in 2015

Andrew P. Norgan,[a] Jarred M. Freese,[b] Patricia M. Tuin,[b] Scott A. Cunningham,[c] Patricio R. Jeraldo,[d] Robin Patel[c,e]

Department of Laboratory Medicine and Pathology, Rochester, Minnesota, USA[a]; Delta County Memorial Hospital, Delta, Colorado, USA[b]; Division of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota, USA[c]; Department of Surgery, Mayo Clinic, Rochester, Minnesota, USA[d]; Division of Infectious Diseases, Mayo Clinic, Rochester, Minnesota, USA[e]

Resistance to carbapenems in *Enterobacteriaceae* is a clinical problem of growing significance. Difficulty in treating multidrug-resistant Gram-negative organisms with conventional antibiotics has led to a renewed and increasing use of polymyxin compounds, such as colistin. Here, we report the isolation of carbapenem- and colistin-resistant *Enterobacter cloacae* from a polymicrobial lower extremity wound in an ambulatory patient. Whole-genome sequencing demonstrated the presence of chromosomal bla_{IMI-1} and bla_{AmpC}, as well as numerous efflux pump genes.

Carbapenem resistance among *Enterobacteriaceae* is an increasing problem of great clinical concern (1). Imipenem-hydrolyzing β-lactamase (IMI) enzymes are Ambler class A serine β-lactamases with carbapenemase activity that are closely related to NMC-A-type β-lactamases (2–5). The chromosomal carbapenemase gene bla_{IMI-1} was first reported in 1984 from two *Enterobacter cloacae* isolates in California and subsequently characterized in 1996 (5). IMI-1 confers resistance to penicillins, first- and second-generation cephalosporins, carbapenems (in particular, imipenem), and aztreonam. IMI-1 poorly hydrolyzes aminothiazole-containing cephalosporins (e.g., ceftriaxone and cefepime) and is inhibited by clavulanic acid (5, 6).

IMI-producing clinical isolates are rare but have been reported in the last decade in France, Finland, Singapore, and China (7–10). Recently, IMI-1-producing *Enterobacter asburiae* and *E. cloacae* isolates with concomitant colistin resistance were described in Ireland and China (11, 12). Historically, polymyxin resistance in *Enterobacteriaceae* has either been intrinsic or acquired via chromosomal mutation (13). This paradigm was recently challenged by the report of a plasmid-borne colistin resistance gene, *mcr-1*, in *Escherichia coli* from China (14).

In February 2015 in Delta, CO, a carbapenemase-producing *E. cloacae* strain was isolated from an outpatient wound culture from a 72-year-old woman with a history of cutaneous scleroderma, medically complicated obesity, and venous insufficiency. Antimicrobial susceptibility testing by agar dilution revealed broad β-lactam resistance, including resistance to penicillins, cephalosporins (with the exception of cefepime), and carbapenems (imipenem, meropenem, and ertapenem), but susceptibility to other classes of antibiotics (Table 1). She responded to outpatient trimethoprim-sulfamethoxazole and wound care management, with negative follow-up repeat cultures.

Because of the detection of carbapenem resistance, additional characterization of the *Enterobacter* isolate was undertaken. Carbapenemase activity was confirmed by modified Hodge and Carba NP tests. PCR for bla_{KPC} and bla_{NDM}, performed as previously described, was negative (15). Purified nucleic acid from the isolate was assayed with the Check-MDR CT103XL microarray (CheckPoints BV, Wageningen, The Netherlands); none of the 27 assay targets were detected. Additional PCR targeting genes encoding GES-, IMP-, OXA-48 group-, SME-, VIM-, NMC-, IMP-, SPM-, GIM-, and SIM-type enzymes revealed an 1,800-bp product amplified using the NMC-type primer set (6, 16–19). Amplified DNA was sequenced bidirectionally and aligned using BLAST to a reference IMI-1 carbapenemase (GenBank accession no. JX090311.1) with almost complete identity. Due to carbapenem resistance, colistin and fosfomycin susceptibility testing was performed by agar dilution (Table 1); the colistin MIC was >4 μg/ml (resistant), and the fosfomycin MIC was >128 μg/ml (resistant per EUCAST version 5.0 guidelines) (20).

Given the concomitant carbapenem and colistin resistance, whole-genome sequencing (WGS) was performed to better characterize the resistance determinants. WGS was performed on TruSeq version 3 paired-end and Nextera mate-pair libraries (target mate-pair insert size, 8 kbp) using a MiSeq platform (Illumina, Inc., San Diego, CA) with a 2 × 300-cycle kit, resulting in an average genomic coverage of 83×. Sequencing reads were processed for library adapter removal and filtering using Trimmomatic 0.32 (21), assembled with SPAdes 3.1.1 (22), processed with HMMER 3.1b2 (23), and resistance genes identified using ResFams 1.2 (24) and ResFinder 2.1 (25).

WGS revealed only chromosomal DNA and no plasmids. Multiple efflux pumps, a fosfomycin resistance determinant (*fosA*), a carbapenemase (bla_{IMI-1}), and a β-lactamase (bla_{AmpC}) were identified, with no nearby mobile elements (Table 2). The gene encoding IMI-1 had 100% sequence identity to a reference bla_{IMI-1} gene (GenBank accession no. U50278) (5), while the bla_{AmpC} gene had 96% sequence identity (the highest level found) to an AmpC β-lactamase ($bla_{AZECL-27}$) from a Chinese urinary isolate of *E. cloacae* (GenBank accession no. KJ949108; UniProt: A0A0A0Q8L1) (26). *fosA* was 98% identical to a reference *E. cloacae* glutathione

Received 23 December 2015 **Returned for modification** 22 January 2016
Accepted 9 February 2016

Accepted manuscript posted online 16 February 2016

Citation Norgan AP, Freese JM, Tuin PM, Cunningham SA, Jeraldo PR, Patel R. 2016. Carbapenem- and colistin-resistant *Enterobacter cloacae* from Delta, Colorado, in 2015. Antimicrob Agents Chemother 60:3141–3144. doi:10.1128/AAC.03055-15.

Address correspondence to Robin Patel, patel.robin@mayo.edu.

TABLE 1 Antimicrobial susceptibility of the *E. cloacae* isolate from a wound swab, Delta, CO

Antibiotic(s)	MIC (μg/ml)[a]	Susceptibility[b]
Amoxicillin-clavulanate	>16/8	R
Ampicillin	>16	R
Ampicillin-sulbactam	>16/8	R
Piperacillin-tazobactam	>64/4	R
Ticarcillin-clavulanate	>64/2	R
Cefazolin	>16	R
Cefoxitin	>16	R
Cefuroxime	>16	R
Cefotetan	>32	R
Cefotaxime	>32	R
Ceftazidime	>16	R
Ceftriaxone	>32	R
Cefepime	≤8	S
Imipenem	>8	R
Meropenem	>8	R
Ertapenem	>4	R
Aztreonam	>16	R
Ciprofloxacin	≤1	S
Levofloxacin	≤2	S
Tetracycline	≤4	S
Gentamicin	≤4	S
Amikacin	≤16	S
Tobramycin	≤4	S
Trimethoprim-sulfamethoxazole	≤2/38	S
Fosfomycin	>128	R[c]
Colistin	>4	R

[a] Breakpoint testing only.
[b] R, resistant; S, susceptible.
[c] Susceptible interpretation based on EUCAST 5.0 guidelines for *Enterobacteriaceae*.

TABLE 2 Antimicrobial resistance genes identified by whole-genome sequencing in an *E. cloacae* isolate from a wound swab, Delta, CO

Resistance mechanism	Function	NCBI accession no.	Identity (%)[a]
bla_{IMI-1}	Class A carbapenem-hydrolyzing β-lactamase	U50278.1	100
bla_{AmpC}	Class A β-lactamase	KJ949108	96
ampR	Transcriptional regulator of bla_{AmpC}	CP009850	87
ampD	Enzymatic repressor of bla_{AmpC} induction	CP009850	93
fosA	Fosfomycin resistance	CP012167	98
cat	Chloramphenicol acetyltransferase	CP009850.1	86
emrB	Multidrug efflux	CP012162.1	96
macB	Macrolide transporter	CP007546.1	93
mexE	Multidrug efflux	CP012162.1	95
mexX	Multidrug efflux	CP012162.1	95
acrA	Multidrug efflux	DQ679966.1	95
acrB	Multidrug efflux	DQ679966.1	94
tolC	Multidrug efflux	CP003737.1	95
robA	Multidrug efflux	CP007546.1	94
msbA	Multidrug efflux	CP011591.1	92
ompL	Porin	EGK63765	99*
oprD	Porin	WP_028014341	99*
ompC	Porin	WP_028013256	99*
phoP	Transcriptional regulatory protein	CP003737	95
phoQ	Sensor histidine kinase	CP003737	91

[a] Identity is nucleotide identity, except for those with an asterisk, which represents predicted amino acid identity.

transferase (GenBank accession no. CP012167) (27). No sequences with significant identity to the newly described mobile polymyxin resistance gene *mcr-1* were identified, nor were other chromosomal determinants of colistin resistance.

To our knowledge, this is the first report in the United States of an IMI carbapenemase-producing *E. cloacae* strain that was also resistant to colistin. Recent surveillance for colistin resistance in the United Kingdom and Ireland revealed prevalences of 7% and 14% in blood and lower respiratory tract *Enterobacter* isolates, respectively (28). Similarly, 15% of the *Enterobacter* isolates from Canadian hospitals in 2008 were colistin resistant (29). The selection of carbapenem and colistin resistance in *Enterobacter* species undergoing antibiotic therapy has been reported (30), possibly due to the existence of environmental heteroresistant *Enterobacter* populations (31, 32). The mechanisms of colistin resistance in *Enterobacter* species are not well understood. Colistin resistance in Gram-negative bacilli is associated with mutations in the two-component regulatory proteins PhoPQ and PmrAB that result in alterations in lipopolysaccharide biosynthesis or overexpression of EptA-like phosphoethanolamine transferase proteins (13, 14, 33, 34). The *phoPQ* sequences from this isolate were similar to those of reference *Enterobacter* sequences, being without the loss-of-function mutations associated with colistin resistance (13). As with other recent reports, our patient was not documented to have ever received a carbapenem or polymyxin.

IMI-1 carbapenemases and AmpC β-lactamases are typically poor hydrolyzers of third- and fourth-generation cephalosporins, although resistance to third-generation cephalosporins via stable AmpC derepression is well known in *Enterobacter* species (35). Sequence analysis revealed no mutations in the IMI-1 regulatory (*imiR*) or enzymatic (*imiA*) genes. The chromosomal AmpC was relatively novel by sequence alignment (96% nucleotide identity to GenBank accession no. KJ949108 and 98% amino acid identity to *Enterobacter* AmpC GenBank accession no. KSX59524) and did not possess mutations in active-site residues (36). Derepression of *ampC* can result from mutations in *ampR* or *ampD*; dysfunction of AmpD has also been reported to result in the overexpression of *nmcA*-type carbapenemases (37). Analysis of the *ampR* nucleotide and predicted protein sequences did not reveal any mutations previously determined to result in increased *ampC* expression. Compared with a wild-type *ampD* reference sequence (GenBank accession no. U40785, *ampD* from *E. cloacae* 029), the *ampD* of this isolate contained several predicted amino acid substitutions, including Pro175Arg (previously described in temperature-sensitive or hyperinducible *ampD* variants) and substitutions Ala71Arg and Arg138His (38–41). Further investigation is required to determine the significance of these mutations. No mutations were identified in the detected porins (OprD, OmpL, and OmpC). Several efflux pumps were present by genomic analysis; expression-level analysis would be required to fully characterize their significance.

A majority of extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing isolates of *Enterobacteriaceae* are susceptible to fosfomycin (42), with susceptibility in 47 to 72% *E. cloacae* isolates (EUCAST criteria) (43, 44). This isolate was resistant to fosfomycin, consistent with the chromosomally encoded glutathione transferase (*fosA*). FosA family proteins are responsi-

ble for a majority of enzymatic fosfomycin resistance among *Enterobacteriaceae*, but the overall prevalence of *fosA* in *Enterobacter* species is not well defined. With increased clinical interest in the use of intravenous fosfomycin to treat multidrug- and carbapenem-resistant *Enterobacteriaceae*, the surveillance of fosfomycin susceptibility in carbapenem-resistant *Enterobacter* strains will be important (45).

Our report highlights the isolation of carbapenem- and colistin-resistant *E. cloacae* from a patient being treated in a rural health care setting with minimal or no exposure to those drugs. Health care providers should be aware of the potential for isolation of carbapenem- and/or colistin-resistant organisms in health care settings not typically associated with their emergence. We also highlight the value of phenotypic testing for carbapenemase production to detect unusual carbapenemases unlikely to be targeted by commonly used nucleic acid amplification tests.

Nucleotide sequence accession number. The sequencing reads and assembly were deposited in DDBJ/ENA/GenBank under BioProject accession no. PRJNA310238.

ACKNOWLEDGMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

REFERENCES

1. **Naas T, Nordmann P.** 1994. Analysis of a carbapenem-hydrolyzing class A β-lactamase from *Enterobacter cloacae* and of its LysR-type regulatory protein. Proc Natl Acad Sci U S A **91:**7693–7697. http://dx.doi.org/10.1073/pnas.91.16.7693.
2. **Ambler RP.** 1980. The structure of β-lactamases. Phil Trans R Soc Lond B Biol Sci **289:**321–331. http://dx.doi.org/10.1098/rstb.1980.0049.
3. **Pottumarthy S, Moland ES, Juretschko S, Swanzy SR, Thomson KS, Fritsche TR.** 2003. NmcA carbapenem-hydrolyzing enzyme in *Enterobacter cloacae* in North America. Emerg Infect Dis **9:**999–1002. http://dx.doi.org/10.3201/eid0908.030096.
4. **Walther-Rasmussen J, Høiby N.** 2007. Class A carbapenemases. J Antimicrob Chemother **60:**470–482. http://dx.doi.org/10.1093/jac/dkm226.
5. **Rasmussen BA, Bush K, Keeney D, Yang Y, Hare R, O'Gara C, Medeiros AA.** 1996. Characterization of IMI-1 β-lactamase, a class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. Antimicrob Agents Chemother **40:**2080–2086.
6. **Queenan AM, Bush K.** 2007. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev **20:**440–458. http://dx.doi.org/10.1128/CMR.00001-07.
7. **Yu Y-S, Du X-X, Zhou Z-H, Chen Y-G, Li L-J.** 2006. First isolation of bla_{IMI-2} in an *Enterobacter cloacae* clinical isolate from China. Antimicrob Agents Chemother **50:**1610–1611. http://dx.doi.org/10.1128/AAC.50.4.1610-1611.2006.
8. **Naas T, Cattoen C, Bernusset SEP, Cuzon G, Nordmann P.** 2012. First identification of bla_{IMI-1} in an *Enterobacter cloacae* clinical isolate from France. Antimicrob Agents Chemother **56:**1664–1665. http://dx.doi.org/10.1128/AAC.06328-11.
9. **Österblad M, Kirveskari J, Hakanen AJ, Tissari P, Vaara M, Jalava J.** 2012. Carbapenemase-producing *Enterobacteriaceae* in Finland: the first years (2008–11). J Antimicrob Chemother **67:**2860–2864. http://dx.doi.org/10.1093/jac/dks299.
10. **Teo JWP, La M-V, Krishnan P, Ang B, Jureen R, Lin RTP.** 2013. *Enterobacter cloacae* producing an uncommon class A carbapenemase, IMI-1, from Singapore. J Med Microbiol **62:**1086–1088. http://dx.doi.org/10.1099/jmm.0.053363-0.
11. **Boo TW, O'Connell N, Power L, O'Connor M, King J, McGrath E, Hill R, Hopkins KL, Woodford N.** 2013. First report of IMI-1-producing colistin-resistant *Enterobacter* clinical isolate in Ireland, March 2013. Euro Surveillance **18:**pii=20548. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20548.
12. **Huang L, Wang X, Feng Y, Xie Y, Xie L, Zong Z.** 2015. First identification of an IMI-1 carbapenemase-producing colistin-resistant *Enterobacter cloacae* in China. Ann Clin Microbiol Antimicrob **14:**51. http://dx.doi.org/10.1186/s12941-015-0112-2.
13. **Olaitan AO, Morand S, Rolain J-M.** 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol **5:**643. http://dx.doi.org/10.3389/fmicb.2014.00643.
14. **Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu L-F, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu J-H, Shen J.** 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis **16:**161–168. http://dx.doi.org/10.1016/S1473-3099(15)00424-7.
15. **Cunningham SA, Noorie T, Meunier D, Woodford N, Patel R.** 2013. Rapid and simultaneous detection of genes encoding *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) and New Delhi metallo-β-lactamase (bla_{NDM}) in Gram-negative bacilli. J Clin Microbiol **51:**1269–1271. http://dx.doi.org/10.1128/JCM.03062-12.
16. **Senda K, Arakawa Y, Ichiyama S, Nakashima K, Ito H, Ohsuka S, Shimokata K, Kato N, Ohta M.** 1996. PCR detection of metallo-β-lactamase gene (bla_{IMP}) in gram-negative rods resistant to broad-spectrum beta-lactams. J Clin Microbiol **34:**2909–2913.
17. **Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC.** 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother **45:**1151–1161. http://dx.doi.org/10.1128/AAC.45.4.1151-1161.2001.
18. **Queenan AM, Torres-Viera C, Gold HS, Carmeli Y, Eliopoulos GM, Moellering RC, Jr, Quinn JP, Hindler J, Medeiros AA, Bush K.** 2000. SME-type carbapenem-hydrolyzing class A β-lactamases from geographically diverse *Serratia marcescens* strains. Antimicrob Agents Chemother **44:**3035–3039. http://dx.doi.org/10.1128/AAC.44.11.3035-3039.2000.
19. **Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, Nordmann P.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother **44:**891–897. http://dx.doi.org/10.1128/AAC.44.4.891-897.2000.
20. **European Committee on Antimicrobial Susceptibility Testing.** 2015. Breakpoint tables for interpretation of MICs and zone diameters, version 5.0. European Committee on Antimicrobial Susceptibility Testing, Växjö, Sweden. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf.
21. **Bolger AM, Lohse M, Usadel B.** 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30:**2114–2120. http://dx.doi.org/10.1093/bioinformatics/btu170.
22. **Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Prjibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Clingenpeel SR, Woyke T, Mclean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA.** 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J Comput Biol **20:**714–737. http://dx.doi.org/10.1089/cmb.2013.0084.
23. **Eddy SR.** 2011. Accelerated profile HMM searches. PLoS Comput Biol **7:**e1002195. http://dx.doi.org/10.1371/journal.pcbi.1002195.
24. **Gibson MK, Forsberg KJ, Dantas G.** 2015. Improved annotation of antibiotic resistance determinants reveals microbial resistomes cluster by ecology. ISME J **9:**207–216. http://dx.doi.org/10.1038/ismej.2014.106.
25. **Zankari E, Hasman H, Kaas RS, Seyfarth AM, Agerso Y, Lund O, Larsen MV, Aarestrup FM.** 2013. Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. J Antimicrob Chemother **68:**771–777. http://dx.doi.org/10.1093/jac/dks496.
26. **Lahiri SD, Johnstone MR, Ross PL, McLaughlin RE, Olivier NB, Alm RA.** 2014. Avibactam and class C β-lactamases: mechanism of inhibition, conservation of the binding pocket, and implications for resistance. Antimicrob Agents Chemother **58:**5704–5713. http://dx.doi.org/10.1128/AAC.03057-14.
27. **Karageorgopoulos DE, Wang R, Yu X-H, Falagas ME.** 2012. Fosfomycin: evaluation of the published evidence on the emergence of antimicrobial resistance in Gram-negative pathogens. J Antimicrob Chemother **67:**255–268. http://dx.doi.org/10.1093/jac/dkr466.
28. **Reynolds R, Kidney A, Mushtaq S, BSAC Working Party on Resistance Surveillance.** 2013. Surprisingly high prevalence of colistin-resistance in *Enterobacter* spp. in the UK and Ireland, abstr P1311. 23rd Eur Cong Clin Microbiol Infect Dis (ECCMID), 27 to 30 April 2013, Berlin, Germany.
29. **Walkty A, DeCorby M, Nichol K, Karlowsky JA, Hoban DJ, Zhanel GG.**

2009. *In vitro* activity of colistin (polymyxin E) against 3,480 isolates of Gram-negative bacilli obtained from patients in Canadian hospitals in the CANWARD study, 2007–2008. Antimicrob Agents Chemother **53:**4924–4926. http://dx.doi.org/10.1128/AAC.00786-09.

30. **Thiolas A, Bollet C, La Scola B, Raoult D, Pagès J-M.** 2005. Successive emergence of *Enterobacter aerogenes* strains resistant to imipenem and colistin in a patient. Antimicrob Agents Chemother **49:**1354–1358. http://dx.doi.org/10.1128/AAC.49.4.1354-1358.2005.
31. **Aubron C, Poirel L, Ash RJ, Nordmann P.** 2005. Carbapenemase-producing *Enterobacteriaceae*, U.S. rivers. Emerg Infect Dis **11:**260–264. http://dx.doi.org/10.3201/eid1102.030684.
32. **Napier BA, Band V, Burd EM, Weiss DS.** 2014. Colistin heteroresistance in *Enterobacter cloacae* is associated with cross-resistance to the host antimicrobial lysozyme. Antimicrob Agents Chemother **58:**5594–5597. http://dx.doi.org/10.1128/AAC.02432-14.
33. **Lesho E, Yoon E-J, McGann P, Snesrud E, Kwak Y, Milillo M, Onmus-Leone F, Preston L, St Clair K, Nikolich M, Viscount H, Wortmann G, Zapor M, Grillot-Courvalin C, Courvalin P, Clifford R, Waterman PE.** 2013. Emergence of colistin-resistance in extremely drug-resistant *Acinetobacter baumannii* containing a novel *pmrCAB* operon during colistin therapy of wound infections. J Infect Dis **208:**1142–1151. http://dx.doi.org/10.1093/infdis/jit293.
34. **Telke AA, Rolain J-M.** 2015. Functional genomics to discover antibiotic resistance genes: the paradigm of resistance to colistin mediated by ethanolamine phosphotransferase in *Shewanella algae* MARS 14. Int J Antimicrobial Agents **46:**648–652. http://dx.doi.org/10.1016/j.ijantimicag.2015.09.001.
35. **Chow JW, Fine MJ, Shlaes DM, Quinn JP, Hooper DC, Johnson MP, Ramphal R, Wagener MM, Miyashiro DK, Yu VL.** 1991. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. Ann Intern Med **115:**585–590. http://dx.doi.org/10.7326/0003-4819-115-8-585.
36. **Dubus A, Ledent P, Lamotte-Brasseur J, Frère JM.** 1996. The roles of residues Tyr150, Glu272, and His314 in class C β-lactamases. Proteins **25:**473–485.
37. **Naas T, Massuard S, Garnier F, Nordmann P.** 2001. AmpD is required for regulation of expression of NmcA, a carbapenem-hydrolyzing β-lactamase of *Enterobacter cloacae*. Antimicrob Agents Chemother **45:**2908–2915. http://dx.doi.org/10.1128/AAC.45.10.2908-2915.2001.
38. **Ehrhardt AF, Sanders CC, Romero JR, Leser JS.** 1996. Sequencing and analysis of four new *Enterobacter ampD* alleles. Antimicrob Agents Chemother **40:**1953–1956.
39. **Kaneko K, Okamoto R, Nakano R, Kawakami S, Inoue M.** 2005. Gene mutations responsible for overexpression of AmpC β-lactamase in some clinical isolates of *Enterobacter cloacae*. J Clin Microbiol **43:**2955–2958. http://dx.doi.org/10.1128/JCM.43.6.2955-2958.2005.
40. **Stapleton P, Shannon K, Phillips I.** 1995. DNA sequence differences of *ampD* mutants of *Citrobacter freundii*. Antimicrob Agents Chemother **39:**2494–2498. http://dx.doi.org/10.1128/AAC.39.11.2494.
41. **Bagge N, Ciofu O, Hentzer M, Campbell JIA, Givskov M, Høiby N.** 2002. Constitutive high expression of chromosomal β-lactamase in *Pseudomonas aeruginosa* caused by a new insertion sequence (IS*1669*) located in *ampD*. Antimicrob Agents Chemother **46:**3406–3411. http://dx.doi.org/10.1128/AAC.46.11.3406-3411.2002.
42. **Kaase M, Szabados F, Anders A, Gatermann SG.** 2014. Fosfomycin susceptibility in carbapenem-resistant *Enterobacteriaceae* from Germany. J Clin Microbiol **52:**1893–1897. http://dx.doi.org/10.1128/JCM.03484-13.
43. **Lu CL, Liu CY, Huang YT, Liao CH, Teng LJ, Turnidge JD, Hsueh PR.** 2011. Antimicrobial susceptibilities of commonly encountered bacterial isolates to fosfomycin determined by agar dilution and disk diffusion methods. Antimicrob Agents Chemother **55:**4295–4301. http://dx.doi.org/10.1128/AAC.00349-11.
44. **Stock I, Grüger T, Wiedemann B.** 2001. Natural antibiotic susceptibility of strains of the *Enterobacter cloacae* complex. Int J Antimicrob Agents **18:**537–545. http://dx.doi.org/10.1016/S0924-8579(01)00463-0.
45. **Michalopoulos AS, Livaditis IG, Gougoutas V.** 2011. The revival of fosfomycin. Int J Infect Dis **15:**e732–e739. http://dx.doi.org/10.1016/j.ijid.2011.07.007.