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Meire Maria da Silva

Bactérias Gram-Negativas com Resistência Adquirida a Antibióticos de Importância Médica Veterinária na região do Sertão do Estado da Paraíba, Brasil

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Tese submetida ao Programa de Pós-Graduação em Medicina Veterinária da Universidade Federal de Campina Grande, como requisito parcial para obtenção do grau de Doutora em Medicina Veterinária.

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MEIRE MARIA DA SILVA

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A Deus, por guiar os meus passos que levaram-me a chegar até aqui.

Dedico

Aos meus pais, Antônio Gustavo e Maria Silva, por esta sempre ao meu lado nos momentos mais difíceis e nos momentos alegres da minha vida.

Dedico

“Atropelei e cancelei sonhos, venci as mágoas, convivi com os fracos, suportei os falsos, e hoje sei a força que tenho para enfrentar qualquer obstáculo. Força essa que vem de Deus, que mim fez enxergar no reflexo de mim mesma, o maior sentimento que o filho de Deus deixou na terra, o amor”.

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RESUMO

Com o objetivo de caracterizar a prevalência de bactérias Gram-negativas com resistência adquirida a antibióticos de importancia na medicina veterinária, essa tese é composta por três capítulos. No capitulo I foi detectado a presença do gene CTX-M-15 na cepa *Klebsiella pneumoniae-KP76* (ST231) em um cão sem raça definida (SRD), onde foi realizado o sequênciamento genômico depositado no Banco de dados (GenBank) com número de acesso NBMT00000000, esse gene é muito comum em humanos no Brasil e pouco registrado em cães. No capitulo II **f**oi realizado o sequênciamento genômico e plasmidial da cepa identificada como *Eschechia coli*-ECPB39 (ST711) reportada tanto em humanos como em animais, depositado no Banco de dados (GenBank) com número de acesso PDMU01000000. No capitulo III foi realizado o sequênciamento genômico e plasmidial de um isolado da bacteria *Eschechia coli*-ECPB17 (ST224) recuperado de uma infecção pulmonar em gato doméstico, que morreu devido a pneumonia, onde detectou-se o gene CTX-M-8, depositado no Banco de dados (GenBank) com número de acesso PNFE00000000. Todas as cepas estão disponivel em: www.ncbi.nlm.nih.gov/genbank/. Os dados genômicos dos tês artigos foram analisados usando ferramentas de Bioinformática online, utilizando uma plataforma de NextSeq500 de ilumina e montado pelo CIC genomic Workbench.

PALAVRAS-CHAVE: *Klebsiella pneumoniae, Eschechia coli,* resistência adquirida, sequênciamento genómico, antibióticos.

ABTRACT

In order to characterize the prevalence of Gram-negative bacteria with antibiotic-acquired resistance of importance in veterinary medicine, this thesis is composed of three chapters. In chapter I was detected the presence of the CTX-M-15 gene in the strain Klebsiella pneumoniae-KP76 (ST231) in a defined breedless dog (SRD), where the genomic sequence was made deposited in the database (GenBank) with NBMT00000000 access number, that gene is Very common in humans in Brazil and little recorded in dogs. In chapter II, the genomic and plasmidial sequence of the strain identified as Eschechia coli-ECPB39 (ST711) was reported in both humans and animals, deposited in the database (GenBank) with PDMU01000000 access number. In chapter III, the genomic and plasmidial sequence of an isolate of the bacteria Eschechia coli-ECPB17 (ST224) recovered from a pulmonary infection in domestic cat, which died due to pneumonia, where the CTX-M-8 gene was detected, deposited in the bank Data (GenBank) with PNFE00000000 access number. All strains are available at: www.ncbi.nlm.nih.gov/genbank/. The genomic data of the T articles were analyzed using online bioinformatics tools, using a NextSeq500 platform of illumination and mounted by the CIC genomic Workbench.

KEY-WORDS: *Klebsiella pneumoniae, Eschechia coli,* acquired resistance, genomic sequencing, antibiotics.

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CAPÍTULO II

INTRODUÇÃO GERAL

As β-lactamases de espectro ampliado (ESBL) foram reportadas como um dos principais mecanismos de resistência em bactérias Gram-negativas aos betalactamicos, principalmente na família Enterobacteriaceae, e com maior prevalência em *E. coli* e *Klebisiella pneumoniae* (Kotapati, 2005). Desde sua descoberta na década de 1980, estas enzimas vem mostrando grande capacidade de disseminação entre essas bactérias (BUSH & FISCHER, 2011). O monitoramento da resistência bacteriana e a pesquisa de cepas produtoras de ESBL em bactérias Gram-negativas de interesse clínico contribuem para delinear a amplitude do problema e para definir opções de tratamento e medidas adequadas para prevenir essas resistências (LUZZARO, 2006).

A disseminação da resistência bacteriana entre diferentes ecossistemas vem sendo estudada como um conceito amplo de saúde pública (*one health*), que retrata a interação entre humanos, animais e meio ambiente. Esta estratégia tem se tornado fundamental para garantir a aplicação de práticas corretas relacionadas à prevenção, vigilância e detecção de zoonoses. O intuito proposto é a conscientização sobre os riscos associados ao uso incorreto de antibióticos tanto na criação de animais de companhia quanto de produção, o que consequentemente reflete no homem e no meio ambiente (CONRAD et al., 2013). Segundo Silva & Lincopan (2012), as ESBL em Enterobacteriaceae é um problema de saúde pública mundial, pois apresenta importância tanto na medicina veterinária quanto na medicina humana. Os mesmos autores afirmam que mesmo estando disseminadas no território brasileiro, não há a notificação de ESBL em todas as unidades federativas. Some-se a isso o fato de que o país ainda não possui um programa nacional de vigilância da resistência microbiana.

Dentre as ESBL, as enzimas CTX-M tem sido amplamente disseminadas no mundo inteiro em diferentes ecosistemas. Essas enzimas sao divididas em seis grupos: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25 e KLUC. No Brasil, a produção de enzimas CTX-M tornou-se o mecanismo mais comum de resistência adquirida às cefalosporinas de amplo espectro em bactérias Gram-negativas, a partir de amostras clínicas humanas e animais, bem como em ambientes aquáticos (SILVA et al., 2013; AIZAWA et al., 2014; ANDRADE et al., 2014; CARVALHO-ASSEF et al., 2014; CASELLA et al., 2015; DROPA et al., 2015; LEIGUE et al., 2015; NOGUEIRA et al., 2015). Concomitantemente, a identificação de cepas de *E. coli* produtoras de CTX-M-2 e CTX-M-8 tem sido reportada em carne de frango comercializado em

mercados brasileiros (CASELLA et al., 2015); complementarmente relatos prévios documentaram a presença de ambos os genes em cepas de *E. coli* isoladas a partir de amostras de carne de frango oriundas da América do Sul exportadas para países europeus (DHANJI et al., 2010, EGERVÄRN et al., 2014; WARREN et al., 2008).

No cenário atual, as variantes CTX-M-2 e CTX-M-15, tem sido amplamente identificadas no Brasil, como as variantes mais prevalentes, seguidas pela identificação de CTX-M-8 e CTX-M-9 (ROCHA et al., 2017). Durante muitos anos as variantes de CTX-M mais identificadas em território brasileiro eram apenas as CTX-M-2, CTX-M-8 e CTX-M-9 (ZAVASCKI et al., 2012; SILVA & LINCOPAN, 2012; CABRAL et al., 2012; QUEIROZ et al., 2012; SEKI et al., 2013; BUENO et al., 2013; LAHLAOUI et al., 2014; QUERESHI & DOI, 2014;). Neste contexto, estudos epidemiológicos moleculares têm relatado uma ligação estreita e bem significativa de genes blaCTX-M em plasmídeos, pertencentes aos grupos IncF, IncI, IncN, IncHI2, IncL/M e IncK. Em *E. coli*, IncF e IncN, onde estão sendo associados com a disseminação de diversas variantes de ESBL do tipo CTX-M (*bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-2}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-32}}$, *bla*$_{\text{CTX-M-40}}$ e *bla*$_{\text{CTX-M-65}}$) (NOVAIS et al., 2006; GONULLU et al., 2008; MSHANA et al., 2009; VILLA et al., 2010; ZHAO; HU, 2013; ZURFLUH et al., 2015).

Blair et al. (2015) afirmaram que, dentro do contexto genético, as bactérias são adaptadas para sobreviver a condições adversas, como a presença de um antimicrobiano, sendo realizada por meio da aquisição de mutações ou incorporação de elementos móveis que carregam genes de resistência. A aquisição de plasmídeos que carregam esses genes, tem sido um dos maiores desafios para controle da disseminação da resistência bacteriana. Os plasmídeos são moléculas de DNA dupla-fita extra cromossômais, circulares ou lineares e que possuem a capacidade de replicação independente (CARATTOLI, 2013). Esses plasmídeos e a disseminação de genes de resistência são sequências de DNA que podem ser encontrados no cromossomo bacteriano ou em elementos genéticos móveis como os integrons e transposons, conferindo resistência aos antimicrobianos de diversas classes. Os plasmídeos apresentam um importante papel na adaptação e na evolução bacteriana, e também possuem a capacidade de transferência de genes entre bactérias (PARTRIDGE et al., 2009; HALL, 2012).

A transferência horizontal de genes de resistência acontece de forma dinâmica entre membros de uma mesma espécie ou entre espécies de diferentes gêneros

bacterianos que compõem os diversos ecossistemas, transferência esta, mediada por conjugação, transformação e/ou transdução. Essa transferencia plasmídial que carregam genes de resistência ou virulencia, contribui com a evolução dos procariontes e a sua adaptabiliodade às mudanças dentro de um determinado ambiente (SLATER et al., 2008; FONDI & FANI, 2010). A classificação dos plasmídeos pode ser realizada baseando-se em diferentes critérios: (i) número de cópias na célula, (ii) tamanho, (iii) capacidade de transferência e (iv) grupo de incompatibilidade (SHINTANI et al., 2015). Os plasmídeos conjugativos (apresentam pilus F, plasmídeos F+) em geral são plasmídeos grandes (>20kb), que possuem poucas cópias dentro da célula bacteriana, e carregam os genes necessários à transferência (genes tra). Entretanto, os plasmídeos não conjugativos são menores (<10kb).

O uso excessivo de cefalosporinas tanto na medicina veterinária quanto na humana, contribuiu com a pandemia das enzimas do tipo CTX-M, resultando na utilização dos carbapenêmicos, antibióticos de "última linha", no tratamento de infecções ocasionadas por bactérias produtoras de ESBL (BUSH; JACOBY, 2010; CARATTOLI, 2013; HARRIS et al., 2015). Entretanto, com a utilização exacerbada desses antibióticos na medicina humana, surgiram as carbapenemases mediadas por plasmídeos, principalmente bla_{KPC} e bla_{NDM}, como mecanismo principal de resistência aos β-lactâmicos. Atualmente, *Klebsiella pneumoniae* produtora da enzima KPC, está disseminada em vários continentes, ocasionando diversos surtos e endemias (MATHERS et al., 2015). Com o surgimento de Enterobacteriaceae produtora de carbapenemases - New Delhi Metalobetalactamase (NDM) também foi restringido o uso dos carbapenêmicos, tornando as opções para tratamento de bactérias produtoras de carbapenemases limitadas à utilização apenas de polimixinas e tigecilina (NORDMANN et al., 2011).

Em 1970 o uso clínico das polimixinas foi suspenso devido a sua alta nefrotoxicidade nos pacientes, sendo substituído pelo uso de aminoglicosideos, quinolonas e β-lactâmicos. Entretanto, em 2000 com a alta incidência de casos de infecções causadas por bactérias resistentes NDR, as polimixinas foram reintroduzidas como recurso de última escolha terapêutica no tratamento de infecções causadas por bactérias produtoras de carbapenemases (RHOUMA & LETELLIER, 2017). Infelizmente, em novembro de 2015 foi reportado pela primeira vez, em isolados de *E. coli* provenientes da China, a resistência transferível à colistina codificada pelo gene

mcr-1, o qual foi localizado em um plasmídeo conjugativo do tipo IncI2, destacando-se como uma nova ameaça para a saúde pública (LIU et al., 2016).

Os plasmídeos que carregam o gene *mcr-1* vêm sendo encontrados em diferentes espécies de Enterobacteriaceae, tais como *K. pneumoniae*, *Enterobacter* spp., *Salmonella enterica*, *Shigella sonnei, Citrobacter freundii* e *Kluvyera* spp. (OLAITAN et al., 2014; SCHWARZ & JOHNSON, 2016; QUESADA et al., 2016; ZHAO et al., 2016). No entanto, a ocorrência destes plasmídeos tem sido mais predominante nos isolados de *E. coli*, principalmente devido à sua grande versatilidade genética (PERRETEN et al., 2016; POIREL et al., 2016; QUESADA et al., 2016; SELLERA et al., 2017). É importante resaltar que, a ubiquidade desses plasmídeos vem sendo observada em diferentes hospedeiros, como, humanos (infectados e saudáveis), animais de companhia, aves migratórias, répteis exóticos; bem como, nos alimentos de origem animal e vegetal e também em ambientes aquáticos. Essa variação, reforça a estabilidade desses plasmídeos (SKOV & MONNET, 2016). Em um panorama geral vem sendo observado que, diversas atividades antropogênicas contaminam o meio ambiente, como o descarte impróprio de esgoto industrial e/ou hospitalar, que podem carrear resíduos de antibióticos, bem como, a descarga de bactérias resistentes, criando grandes reservas ambientais de resistência e contribuindo com a modificação de diversos ecossistemas (ROCA et al., 2015).

O gene *mcr-1* em plasmídeos Incx4 tem sido identificado no Brasil em isolados da bacteria *E. coli* em animais de produção (FERNANDES et al., 2016a), infeções em humanos (FERNANDES et al., 2016b, ROCHA et al., 2017), aves migratórias (SELLERA et al., 2017), carne de frango (MONTE et al., 2017) e ambiente marinho (FERNANDES et al., 2017). Com os avanços na genômica, a epidemiologia molecular de plasmídeos que carregam os genes $bla_{\text{CTX-M}}$ e *mcr-1* tem sido explanada por pesquisadores do mundo inteiro (ZURFLUH et al., 2015; FERNANDES et al., 2016b; XAVIER et al., 2016; WANG et al., 2016). Entretanto, todos esses estudos que visam investigar aspectos relacionados à relação ancestral e pan-resistoma plasmidial (conjunto de genes de resistência em plasmídeo) podem fornecer uma base científica para monitorar e elucidar a rota epidemiológica de possíveis fontes de aquisição desses genes de resistência. Portanto, as aplicações de ferramentas de bioinformática e moleculares, podem ter uma contribuição prática, baseada na identificação da origem e dos veículos de disseminação desses genes de resistência, podendo ser aplicado para ter

um controle efetivo e estabelecer políticas de vigilância epidemiológica em relação a essas disseminações.

Esta Tese de Doutorado é composta por três capítulos constituídos por artigos científicos originais. O Capítulo I é referente a um artigo científico publicado na revista Comparative Immunology, Microbiology & Infectious Diseases, ISSN: 0147-9571 (Fator de impacto 1.875 - Qualis B1), no qual foi isolado uma cepa de *K. pneumoniae* ST231 em um cão sem raça definida (SRD) com presença do gene CTX-M-15. O Capítulo II é composto por uma carta informativa submetida à revista Jornal of Antimicrobial Chemotherapy, ISSN: 1460-2091/03057453 (Fator de impacto: 5.071 - Qualis A1, no qual foi idendificado uma variante do gene CTX-M-8 (*mcr-5.3)* de uma *E. coli* ST711 isolada de uma infecção em um cavalo que venho a óbito. O Capítulo III compreende uma comunicação científica submetida à revista Journal of Feline Medicine and Surgery, ISSN Online: 1532-2750 (Fator de impacto: 1.131 – Qualis B1, onde foi identificada uma *E. coli* ST224 com a presença do gene CTX-M-8 ceftiofur-resistente realizado sequênciamento genômico e plasmidial, proviniente de uma amostra de pulmão de um gato doméstico que foi a óbito por Pneumonia.

REFERÊNCIAS

AIZAWA, J.; NEUWIRT, N.; BARBATO, L., et al. Identification of fluoroquinolone-resistant extended-spectrum β-lactamase (CTX-M-8)-producing Escherichia coli ST224, ST2179 and ST2308 in buffalo (Bubalus bubalis). **J. Antimicrob. Chemother**., 69:2866-2869, 2014.

ANDRADE, L.N., VITALI, L., GASPAR, G.G., et al. Expansion and evolution of a virulent, extensively drugresistant (polymyxin B-resistant), QnrS1-, CTX-M-2-, and KPC-2-producing Klebsiella pneumoniae ST11 international high-risk clone. **J. Clin. Microbiol.**, 52: 2530-2535, 2014.

BLAIR, J.M, WEBBER, M.A, BAYLAY, A.J., et al. Molecular mechanisms of antibiotic resistance. **Nat. Rev. Microbiol.,** 13:42-51, 2015.

BUENO, M.F., et al. Coproduction of 16S rRNA methyltransferase RmtD or RmtG with KPC-2 and CTX-M group extended-spectrum β-lactamases in Klebsiella pneumoniae. **Antimicrob. Agents Chemother**., 57: 2397-2400, 2013.

BUSH, K.; JACOBY, G. A. Updated functional classification of beta-lactamases. **Antimicrob. Agents Chemother**., 54:969-976, 2010.

BUSH, K.; FISHER, J. F. Epidemiological expansion, strutural studies, and clinical challenges of new β-lactamases from Gram-negative bactéria. **Annu. Ver. Microbiol**., 65: 455-478, 2011.

CABRAL, A.B., et al. Multidrug resistance genes, including bla(KPC) and bla(CTX)-M-2, among Klebsiella pneumoniae isolated in Recife, Brazil. **Rev. Soc. Bras. Med. Trop**., v. 45, p. 572-578, 2012.

CARATTOLI, A. Plasmids and the spread of resistance. Inter. **J. Medical Microbiol**., 303: 298– 304, 2013.

CARVALHO-ASSEF, A.P., et al. Detection of NDM-1-, CTX-M-15-, and qnrB4-producing Enterobacter hormaechei isolates in Brazil. **Antimicrob. Agents Chemother**., 58:2475-2476, 2014.

CONRAD, P.A.; MEEK, L.A.; DUMIT, J. Operationalizing a One Health approach to global health challenges. **Comp. Immunol. Microbiol. Infect. Dis.,** 36:211-6, 2013.

CASELLA, T., et al. Detection of blaCTX-M-type genes in complex class 1 integrons carried by Enterobacteriaceae isolated from retail chicken meat in Brazil. Int. **J. Food Microbiol.**, 197:88- 91, 2015.

DHANJI, H.; MURPH,Y. N.M.; DOUMITH, M., et al. Cephalosporin resistance mechanisms in Escherichia coli isolated from raw chicken imported into the UK. **J. Antimicrob. Chemother.**, 65:2534- 2537, 2010.

DROPA, M., GHIGLIONE, B., MATTÉ, M.H., et al. Molecular and Biochemical Characterization of CTX-M-131, a Natural Asp240Gly Variant Derived from CTX-M-2, Produced by a Providencia rettgeri Clinical Strain in São Paulo, Brazil. **Antimicrob. Agents Chemother**., 59: 1815-1817, 2015.

EGERVÄRN, M., et al. *Escherichia coli* with extended-spectrum beta-lactamases or transferable AmpC beta-lactamases and *Salmonella* on meat imported into Sweden. Int. **J. Food. Microbiol.**, 171: 8-14, 2014.

FERNANDES, M.R.; MOURA, Q.; SARTORI, L. et al. Silent dissemination of colistin-resistant Escherichia coli in South America could contribute to the global spread of the mcr-1 gene. **Euro Surveill**, 28; 21, 2016a.

FERNANDES, M.R.; MCCULLOCH, J.A.; VIANELLO, M.A., et al. First Report of the Globally Disseminated IncX4 Plasmid Carrying the mcr-1 Gene in a Colistin-Resistant Escherichia coli Sequence Type 101 Isolate from a Human Infection in Brazil. **Antimicrob. Agents. Chemother.**, 23:6415-7, 2016b.

FERNANDES, M.R.; SELLERA, F.P.; ESPOSITO, F., et al. Colistin-Resistant mcr-1-Positive Escherichia coli on Public Beaches, an Infectious Threat Emerging in Recreational Waters. **Antimicrob. Agents. Chemother.**, 61:pii: e00234-17, 2017.

FONDI, M.; FANI, R. The horizontal flow of the plasmid resistome: clues from inter-generic similarity networks. **Environ. Microbiol**., v. 12, p. 3228-3242, 2010.

GONULLU, N., et al. Dissemination of CTX-M-15 beta-lactamase genes carried on Inc FI and FII plasmids among clinical isolates of *Escherichia coli* in a university hospital in Istanbul, Turkey. **J. Clinical Microbiol**., 46, 1110–1112, 2008.

HALL, A. R. Integrons and gene cassettes: hotspots of diversity in bacterial genomes. **Ann. N Y. Acad. Sci**., v.1267, p. 71-78, 2012.

KOTAPATI, S.; KUTI, J. L.; NIGHTINGALE, C. H., et al. Clinical implications of extended spectrum b-lactamase (ESBL) producing Klebsiella species and Escherichia coli on cefepime effectiveness. **Journal of Infection**, 51, 211–217, 2005.

LAHLAOUI, H., et al. Epidemiology of Enterobacteriaceae producing CTX-M type extended spectrum β-lactamase (ESBL). **Med. Mal. Infect.,** 44: 400-404, 2014.

LEIGUE, L.; WARTH J.F.; MELO, L.C., et al. MDR ST2179-CTX-M-15 *Escherichia coli* coproducing RmtD and AAC(6')-Ib-cr in a horse with extraintestinal infection, Brazil. **J. Antimicrob. Chemother**., 70(4):1263-5, 2015.

LUZZARO, F.; MEZZATESTA, M.; MUGNAIOLI, C., et al. Trends in Production of Extended-Spectrum-Lactamases among Enterobacteria of Medical Interest: Report of the Second Italian Nationwide Survey. **J. Clin. Microbiol**., 44:1659-1664, 2006.

MONTE, D.F., MEM, A.; FERNANDES, M.R., et al. Chicken Meat as a Reservoir of ColistinResistant *Escherichia coli* Strains Carrying mcr-1 Genes in South America. **Antimicrob Agents Chemother**., 61: pii: e02718-16, 2017.

MSHANA, S.E., et al. Conjugative IncFI plasmids carrying CTX-M-15 among Escherichia coli ESBL producing isolates at a University hospital in Germany. **BMC Infect. Dis**., 9:97, 2009.

NOGUEIRA, K.S.; NOGUEIRA, K.D.A.S.; CONTE, D., et al. Distribution of extended-spectrum β-lactamase types in a Brazilian tertiary hospital. **Rev. Soc. Bras. Med. Trop.**, v. 48, p. 162-169, 2015.

NOVAIS, A., et al. Dissemination and persistence of blaCTX-M-9 are linked to class 1 integrons containing CR1 associated with defective transposon derivatives from Tn402 located in early antibiotic resistance plasmids of IncHI2, IncP1-alpha, and IncFI groups. **Antimicrob. Agents. Chemother**., 50 :2741–2750, 2006.

OLAITAN, A.O.; MORAND, S.; ROLAIN, J.M. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. **Front. Microbiol**., 5:643, 2014.

PARTRIDGE, S.R.; TSAFNAT, G.; COIERA, E.; IREDELL, J.R. Gene cassettes and cassette arrays inmobile resistance integrons. **FEMS Microbiol. Rev**., 33 :757-784, 2009.

QUEIROZ, M.L., et al. Characterization of extended-spectrum beta-lactamases, antimicrobial resistance genes, and plasmid content in *Escherichia coli* isolates from different sources in Rio de Janeiro, Brazil. Diagn. **Microbiol. Infect. Dis.,** 74:91-64, 2012.

QUERESHI, Z.A; DOI, Y. *Escherichia coli* sequence type 131: epidemiology and challenges in treatment. **Expert. Ver. Anti-Infect. Ther.,** 12: 597-609, 2014.

QUESADA, A.; UGARTE-RUIZ, M.; IGLESIAS, M.R., et al. Detection of plasmid mediated colistin resistance (MCR-1) in *Escherichia coli* and *Salmonella enterica* isolated from poultry and swine in Spain. **Res. Vet. Sci.,** 105:134-5, 2016.

ROCA, I.; AKOVA, M.; BAQUERO, F., et al. The global threat of antimicrobial resistance: science for intervention. **New Microbes New Infect.,** 6:22-9, 2015.

ROCHA. I.V.; ANDRADE, C.A.D.N.; CAMPOS, T.L., et al. Ciprofloxacin-resistant and extendedspectrum β-lactamase-producing Escherichia coli ST410 strain carrying the mcr-1 gene associated with bloodstream infection. **Int. J. Antimicrob. Agents**., 49:655-656, 2017.

SCHWARZ, S.; JOHNSON, A.P. Transferable resistance to colistin: a new but old threat. J. **Antimicrob. Chemother.,** 71:2066-70, 2016.

SELLERA, F.P.; FERNANDES, M.R.; SARTORI, L., et al. *Escherichia coli* carrying IncX4 plasmid-mediated mcr-1 and blaCTX-M genes in infected migratory Magellanic penguins (Spheniscus magellanicus). **J. Antimicrob. Chemother**., 72:1255-1256, 2017.

SEKI, L.M. et al. Molecular epidemiology of CTX-M producing Enterobacteriaceae isolated from bloodstream infections in Rio de Janeiro, Brazil: emergence of CTX-M-15. **Braz. J. Infect. Dis**., v. 17, p. 640-646, 2013.

SILVA, K.C; LINCOPAN, N. Epidemiologia das beta-lactamases de espectro estendido no Brasil: impacto clínico e implicações no agronegócio. **J. Bras. Patol. Med. Laboratorial**, 2: 91- 99, 2012.

SHINTANI, M.; SANCHEZ, Z.K.; KIMBARA, K. Genomics of microbial plasmids: classification and identification based on replication and transfer systems and host taxonomy. **Front . Microbiol**., v. 6, p. 242, 2015.

SILVA, K.C., et al. Emergence of Extended-Spectrum-_-Lactamase CTX-M-2-Producing Salmonella enterica Serovars Schwarzengrund and Agona in Poultry Farms. Antimicrob. **Agents Chemeoth**., 57 : 3458-3459, 2013.

SKOV, R.L.; MONNET, D.L. Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds. **Euro Surveill**, 21:30155, 2016.

SLATER, F.R.; BAILEY, M.J.; TETT, A.J.; TURNER, S.L. Progress towards understanding the fate of plasmids in bacterial communities. **FEMS Microbiol. Ecol**., 66:3–13, 2008.

VILLA, L.; GARCIA-FERNANDEZ, A.; FORTINI, D.; CARATTOLI, A. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. **J. Antimicrob. Chemother.**, 65:2518-2529, 2010.

WANG, J.; STEPHAN, R.; POWER, K., et al. Nucleotide sequences of 16 transmissible plasmids identified in nine multidrug-resistant Escherichia coli isolates expressing an ESBL phenotype isolated from food-producing animals and healthy humans. **J. Antimicrob. Chemother**., 69:2658-2668, 2016.

WARREN, R.E. et al. 2008. Imported chicken meat as a potential source of quinolone-resistant Esche-richia coli producing extended-spectrum beta-lactamases in the UK. **J.Antimicrob. Chemother.,** 61:504-508, 2008.

XAVIER, B.B.; LAMMENS, C.; RUHAL, R., et al. Identification of a novel plasmid-mediated colistin- resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. **Euro Surveill**, 21(27), 2016.

ZHAO, W.H.; HU, Z.Q. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. **Crit. Ver. Microbiol.,** 39: 79-101, 2013.

ZHAO, F.; ZONG, Z. Kluyvera ascorbata Strain from Hospital Sewage Carrying the mcr-1 Colistin Resistance Gene. **Antimicrob. Agents. Chemother**., 60:7498-7501, 2016.

ZAVASCKI, A.P., et al. Outbreak of carbapenem-resistant Providencia stuartii in an intensive care unit. **Infect. Control. Hosp. Epidemiol.,** 33: 627-630, 2012.

ZURFLUH, K., et al. Replicon typing of plasmids carrying blaCTX-M-15 among Enterobacteriaceae isolated at the environment, livestock and human interface. **Sci Total Environ.**, 521-522:75-78, 2015.

CAPITULO I

TITULO: Multidrug-resistant CTX-M-15-producing Klebsiella pneumoniae ST231 associated with infection and persistent colonization of dog

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Multidrug-resistant CTX-M-15-producing Klebsiella pneumoniae ST231 associated with infection and persistent colonization of dog

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ABSTRACT

Extended spectrum β-lactamase (ESBL)-producing bacterial infections in veterinary medicine are a clinical and epidemiological challenge. We report a case of CTX-M-15-producing *Klebsiella pneumoniae* infection followed by persistent colonization, in a dog presenting with bilateral purulent nasal discharge and dyspnea. In this regard, 5 broad-spectrum cephalosporin-resistant *K. pneumoniae* isolates were recovered from infection and surveillance cultures, collected during 1 year and eight months study. Genomic analysis of a representative clone of K. pneumoniae (*Kp*PB76) revealed the presence of the human-associated lineage ST231, whereas resistome data confirmed the presence of genes conferring resistance to aminoglycosides, β-lactams, fluoroquinolones, fosfomycin, phenicols, sulfonamides, tetracyclines and trimethoprim. In the absence of therapeutic options, meropenem therapy was used, contributing to the control of infection during persistent carriage of *K. pneumoniae* CTX-M-15/ST231. Persistent colonization of companion animals with ESBL-producing bacteria could be result from a variety of situations, including multi introduction from the owner or household family members to pets, or from environmental exposure; whereas colonized animals may serve as an important source for the spread of ESBL-producing strains in the human-animal interface.

Keywords: Carbapenems, Companion animal, Pets ESBL.

The emergence and rapid dissemination of CTX-M extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in companion animals is a critical issue that have compromised the treatment and clinical outcome of bacterial infections, representing a clinical challenge for small animal veterinarians (Ewers et al., 2014; So et al., 2012). In this regard, while in human beings carbapenems (doripenem, ertapenem, imipenem, and meropenem) have been used as the lastresort drugs for the treatment of infections caused by multidrug-resistant (MDR) bacteria, including ESBL producers (Liu et al., 2017), in domestic animals carbapenems have been only used empirically, since there are no reports of

pharmacokinetic and pharmacodynamics validating its use for veterinary practice (Byun et al., 2016).

In July 2015, a 5-year-old female mixed-breed dog was admitted to a veterinary teaching hospital, presenting with bilateral purulent nasal discharge and dyspnea. A detailed clinical examination revealed chronic sinusitis and pneumonia. Nasal discharge sample was collected and cultivated on MacConkey agar, and a ceftiofur-resistant *K. pneumoniae* isolate (*Kp*PB7) was identified by VITEK 2 system (bioMérieux). Empirical treatment with amoxicillin/potassium clavulanate (20 mg/kg twice a day for 10 days) was started, but not clinical improvement was observed. After a month, frontal sinus trephination was performed and a tracheal aspirate sample was collected and cultured, revealing the persistent of the MDR ceftiofur-resistant *K. pneumoniae* isolate (*Kp*PB34). Antibiotic treatment with marbofloxacin (4 mg/kg once a day) and ciprofloxacin (10 mg/kg twice a day) was performed for 12 days. Subsequently to these interventions, the dog presented a slight improvement, however purulent nasal discharge was still observed. Therefore, since MDR profile of *K. pneumoniae* restricted the use of antibiotics, a new treatment with intravenous infusion of meropenem (10 mg/kg twice a day) for 7 days was started, resulting in the clinical improvement and absence of nasal discharge.

In the course of the following 6 months, the dog returned to the veterinary hospital presenting closed pyometra. During hysterectomy, intrauterine content was collected by direct puncture and, surprisingly, a ceftiofur-resistant *K. pneumoniae* isolate (*Kp*PB76) was detected again, after culture. One year after, in the absence of clinical infections, nasal and rectal samples were collected for surveillance cultures, where 2 ceftiofur-resistant *K. pneumoniae* strains (*Kp*PB101 and *Kp*PB102) were identified.

Bacterial isolates (*Kp*PB7, KpPB34, *Kp*PB76, *Kp*PB101 and *Kp*PB102) were identified by matrix-assisted laser desportion/ionization time-of-flight mass spectrometry (MALDI-TOF). Antibiotic susceptibility profiles were determined by Kirby-Bauer method (CLSI, 2017). Additionally, cefotaxime/cefotaxime plus clavulanic acid (CT/CTL), ertapenem, imipenem, and meropenem MICs were determined by E-test method, whereas colistin MIC was determined by microdilution broth (EUCAST, 2016). DNA samples were extracted and subject to PCR, in order to identify genes encoding resistance to broad-spectrum cephalosporins (Nascimento et al., 2017). The clonal relatedness of the strains was evaluated by enterobacterial repetitive intergenic consensus (ERIC) PCR (Aydin et al., 2007), and ERIC patterns were analyzed using Bionumerics software (Applied Maths).

All 5 K. pneumoniae strains were clonally related, as determined by ERIC-PCR analysis, being recovered from infection and surveillance cultures collected during 1 year and 8 months study. The strains displayed an identical MDR profile (Magiorakos et al., 2012), to amoxicillin/clavulanic acid, cefepime, ceftiofur, amikacin, gentamicin, ciprofloxacin, enrofloxacin, nalidixic acid, chloramphenicol, tetracycline and trimethoprim/sulfamethoxazole remaining susceptible to ertapenem, imipenem, meropenem (0.032 μg/mL), and colistin (1 μg/mL). In all K. pneumoniae strains, ESBL phenotype was confirmed by cefotaxime/cefotaxime plus clavulanic acid (MIC, 32/0.125 μg/mL) ESBL strips. Moreover, PCR analysis identified the presence of the $bla_{\text{CTX-M-15}}$ gene.

Plasmid DNA was extract and conjugation and transformation of ceftiofur-resistant K. pneumoniae isolates were performed by broth mating method and electroporation, respectively, using streptomycin-resistant Escherichia coli C600 and *E. coli* TOP10 as recipient lineages. While transconjugant cells were selected by using MacConkey agar plates supplemented with 4 μg/mL cefotaxime and 200 μg/mL streptomycin, transformed cells were selected in LB agar containing cefotaxime 4 μg/mL. PCR and sequencing were carried out to confirm the presence of $bla_{\text{CTX-M-15}}$ gene in *E. coli* transformants.

Genomic DNA of representative *Kp*PB76 strain, selected as being recovered from a rare surgical-site infection (i.e., closed pyometra), was extracted (PureLinkTM Invitrogen) and used to construct a paired-end library (150 bp), being sequenced by the NextSeq platform (Illumina). Genome data were analyzed using bioinformatic tools available in the Center for Genomic Epidemiology (www.genomicepidemiology.org).

WGS analysis of KpPB76 (GenBank accession number: NBMT00000000) revealed that this strain belonged to ST231 and capsular serotype K51 (Brisse et al., 2013). CTX-M-15/K51/ST231 *Kp*PB76 strain harbored kfuA3 and kfuC6 (iron uptake marker), mrkH1 (coding biofilm formation) and ybtP122 (yersiniabactin) virulence genes. On the other hand, resistome analysis revealed the presence of genes conferring resistance to aminoglycosides [*aadB, aph(3')-Ia,aac(6')Ib-cr, aac(3')IIa, strA, strB, aadA1 and aadA2*], β-lactams ($bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV-1}}$ and $bla_{\text{TEM-1}}$), fluoroquinolones (oqxA, oqxB and qnrB66), fosfomycin (fosA), macrolides [mph(A)], phenicols (catB3), sulfonamides (sul1 and sul2), tetracyclines [tet(A)], and trimethoprim (drfA12, drfA14 and drfA21). Furthermore, the GyrA mutation (Ser83Ile) associated with ciprofloxacin resistance was identified in KpPB76. Additionally; this strain carried the heavy metal gene silR8, which confers resistance to silver, and efflux pump genes *acrR2, envR2, fis1, marA5, marR2, oqxA9, oqxR4, rarA2, rob3, soxR2* and *soxS2*.

Finally, incompatibility plasmid groups FIB and FII were detected, whereas transformation assays revealed that blaCTX-M-15 was carried by a ~90-kb IncF plasmid, as determined by PCR-replicon type, and by comparing plasmid band patterns obtained in agarose gel electrophoresis.

In this study, infection accompanied by persistent colonization of the pet was associated to the presence of clonally related K. pneumoniae isolates belonging to the ST231. This lineage has only been reported in human infections, so far; being related to MDR and carbapenemase-producing phenotypes identified in Asian and European countries (Abdul Momin et al., 2017; Giske et al., 2012; Mancini et al., 2017).

Thus, we report the first description (to our knowledge) of this human-associated K. pneumoniae lineage in animal infection.

In the absence of effective antibiotics, we decided to use meropenem therapy (Byun et al., 2016). However, while in human medicine carbapenems are considered the antibiotics of choice for treatment of ESBL-producing Enterobacteriaceae (Kim et al., 2018), in veterinary medicine carbapenems have no legal indication (Melo et al., 2017). Although, the pet achieved a clinical significant improvement, surveillance cultures revealed the presence of the CTX-M-15-producing K. pneumonia ST231 in nasal and rectal cavities, supporting a persistent colonization for this lineage. In this regard, a number of human studies have shown that intestinal colonization with ESBL-producing Enterobacteriaceae is common among inpatients and outpatients after carbapenem treat-ment, persisting for at least 3 months (Haverkate et al., 2017). So, persistent colonization of companion animals with ESBL-producing bacteria could be result from a variety of situations, including multi introduction from the owner or household family members to pets, or from environmental exposure (Fernandes et al., 2018). Therefore, persistent colonization of companion animals with ESBL-producing bacteria must be considered a critical issue, since these carriers may serve as an important source for the spread of ESBL producers in the human-animal interface.

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Competing Interest

None declared.

Ethical Approval

Not required.

References

Abdul Momin MHF, Liakopoulos A, Phee LM, Wareham DW. Emergence and nosocomial spread of carbapenem-resistant OXA-232-producing Klebsiella pneumoniae in Brunei Darussalam. J Glob Antimicrob Resist 2017;9:96–9.

Aydin F, Gümüşsoy KS, Atabay HI, Iça T, Abay S. Prevalence and distribution of Arcobacter species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR. J Appl Microbiol 2007;103:27–35.

Brisse S, Passet V, Haugaard AB, Babosan A, Kassis-Chikhani N, Struve C, et al. Wzi gene sequencing, a rapid method for determination of capsular type for Klebsiella strains. J Clin Microbiol 2013;51:4073–8.

Byun SY, Jeong JW, Choi JH, Lee KP, Youn HY, Maeng HJ, et al. Pharmacokinetic study of meropenem in healthy beagle dogs receiving intermittent hemodialysis. J Vet Pharmacol Ther 2016;39:560–5.

Clinical and laboratory standards institute (CLSI). Performance Standards for Antimicro-bial Disk Susceptibility Tests. Approved Standard M100. 27th ed. Wayne, PA, USA: CLSI; 2017.

EUCAST. Breakpoint tables for interpretation of MICs and zone diameters, v 6. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf, 2016.

Ewers C, Stamm I, Pfeifer Y, Wieler LH, Kopp PA, Schønning K, et al. Clonal spread of highly successful ST15-CTX- M-15 Klebsiella pneumoniae in companion animals and horses. J Antimicrob Chemother 2014;69:2676–80.

Fernandes MR, Sellera FP, Moura Q, Carvalho MPN, Rosato PN, Cerdeira L, et al. Zooanthroponotic transmission of drug-resistant Pseudomonas aeruginosa, Brazil. Emerg Infect Dis 2018;24:1160–2.

Giske CG, Fröding I, Hasan CM, Turlej-Rogacka A, Toleman M, Livermore D, et al. Diverse sequence types of Klebsiella pneumoniae contribute to the dissemination of blaNDM-1 in India, Sweden, and the United Kingdom. Antimicrob Agents Chemother 2012;56: 2735–8.

Haverkate MR, Platteel TN, Fluit AC, Cohen Stuart JW, Leverstein-van Hall MA, Thijsen SFT, et al. Quantifying within-household transmission of extended-spectrum β-lactamase producing bacteria. Clin Microbiol Infect 2017;23:46.e1–7.

Kim SA, Altshuler J, Paris D, Fedorenko M. Cefepime versus carbapenems for the treatment of urinary tract infections caused by extended-spectrum beta-lactamase producing Enterobacteriaceae. Int J Antimicrob Agents 2018;51:155–8.

Liu Y, Yang Y, Chen Y, Xia Z. Antimicrobial resistance profiles and genotypes of extended-spectrum β-lactamase- and AmpC β-lactamase-producing Klebsiella pneumonia isolated from dogs in Beijing, China. J Glob Antimicrob Resist 2017;10:219–22.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.

Mancini S, Poirel L, Tritten ML, Lienhard R, Bassi C, Nordmann P. Emergence of an MDR Klebsiella pneumoniae ST231 producing OXA-232 and RmtF in Switzerland. J Antimicrob Chemother 2017;73:821–3.

Melo LC, Boisson MN, Saras E, Médaille C, Boulouis HJ, Madec JY, et al. OXA-48-producing ST372 Escherichia coli in a French dog. J Antimicrob Chemother 2017; 72:1256–8.

Nascimento T, Cantamessa R, Melo L, Fernandes MR, Fraga E, Dropa M, et al. International high-risk clones of Klebsiella pneumoniae KPC-2/CC258 and Escherichia coli CTX-M 15/CC10 in urban lake waters. Sci Total Environ 2017;598:910–5.

So JH, Kim J, Bae IK, Jeong SH, Kim SH, Lim SK, et al. Dissemination of multidrug resistant Escherichia coli in Korean veterinary hospitals. Diagn Microbiol Infect Dis 2012;73:195-9.

CAPITULO II

TITULO: Novel *mcr-5.3* variant in a CTX-M-8-producing *Escherichia coli* ST711 isolated from an infected horse

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Research Letter

Novel *mcr-5.3* variant in a CTX-M-8-producing *Escherichia coli* ST711 isolated from an infected horse

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Sir,

Following the first description of the mobile phosphoethanolamine transferase gene *mcr-1*, causing colistin resistance in Enterobacteriaceae of human and animal origin, a rapid dissemination and emergence of novel *mcr* variants have been globally described.[1-4] In this regard, two recent reports published in *JAC* have documented the identification of novel *mcr-5* and *mcr-5.2* gene variants in *d*-tartrate-fermenting *Salmonella enterica* serovar Paratyphi B and *Escherichia coli*, respectively, from food and food-producing animals in Germany.[3,4] Interestingly, *mcr-5* has been further identified in *E. coli* isolates from diseased pigs in Japan,[5] and from human vaginal microbiome in China.[6] In this study, we report the occurrence of a novel variant of *mcr-5* (named *mcr-5.3*) in South America.

A retrospective genomic study conducted to evaluate the evolution of clinically significant antibiotic resistant Gram-negative bacteria in the human-animal interface, in Brazil, led to the identification of a CTX-M-8-producing *E. coli* strain (ECPB39) carrying a *mcr-5*-type gene, which was isolated from a diseased horse, who died due to pneumonia. Veterinary medical records revealed that E. coli ECPB39 was isolated in 2012, from a lung tissue culture obtained at necropsy, in a veterinary hospital in north-eastern Brazil. This strain exhibited an MDR profile including ampicillin, amoxicillin/clavulanic acid, ceftiofur, ceftriaxone, cefotaxime (>32mg/L), cefepime, amikacin, gentamicin, trimethoprim/sulfamethoxazole and tetracycline, but remained susceptible to cefoxitin, ciprofloxacin, enrofloxacin, ertapenem, imipenem and meropenem (http://www.eucast.org/). ESBL production was confirmed by using a double-disc synergy test, whereas ESBL genes were screened for by PCR and Sanger sequencing, revealing the presence of blaCTX-M-8.

Even though ECPB39 exhibited a colistin MIC of 2 mg/L (http://www.eucast.org/), it showed a positive result in the rapid polymyxin NP test,[9] which was reverted in the presence of EDTA.[10]

Whole genomic DNA was extracted (PureLink™; Invitrogen) and used to prepare a library that was sequenced using the NextSeq550 platform (2x 75 bp paired-end) (Illumina). De novo assemblies were accomplished by using the CLC Genomic Workbench 10.0. Multilocus STs, serotypes, virulomes, resistomes and incompatibility plasmid groups were screened for using bio-informatics tools available from the Center for Genomic Epidemiology (http://genomicepidemiology.org/).

WGS analysis revealed that ECPB39 belonged to serotype O45:H20 and ST711, the latter described in the E. coli MLST database as a human, animal, food and environmental pathogen in countries throughout Europe and North America (http://enterobase.warwick.ac.uk/). Regarding virulence, the presence of gad (glutamate decarboxylase), lpfA (long polar fimbriae) and iss (increased serum survival) genes was found. The resistome analysis identified the presence of genes conferring resistance to b-lactams (blaCTX-M-8 and blaTEM-1B), aminoglycosides [aac(3)-IId, aadA2, strA and strB], sulphonamides (sul2) trimethoprim (dfrA12) and polymyxins (mcr-5-type).

E. coli ECPB39 harboured IncI1, IncHI1A, IncQ1, IncFIB, IncFIA and IncFII plasmids. For plasmid assembly, a de novo strategy was used in combination with manual curation. In this regard, a novel 5361 bp plasmid (pECPB39, belonging to an unknown replicon type) carrying the mcr-5-type gene could be identified. It incorporated the complete backbone of the pKP13a plasmid (2459 bp, GenBank accession number CP003996), as previously observed,[4] with a mobilization gene and two hypothetical proteins (Figure 1a). The mcr-type gene (1644 bp), termed as mcr-5.3-type,[2] showed 99.9% sequence identity (G1240T) to mcr-5, resulting in 7479% mutation coverage (i.e. number of reads mapping at that position). Therefore, the MCR-5.3 variant differed by one amino acid (Ala414Ser) from MCR-5 and MCR-5.2 (Figure 1b). The mutation was confirmed by Sanger sequencing, using primers mcr-5.3 F (50-CGATAACCAGTCGGGCTGTA-30) and mcr-5.3 R (50-CCAGAAGGTCCAACTCTGGC-30) (55°C annealing).

Based on a previous analysis of the mcr-5.2 variant,[4] the genetic context of mcr-5.3 revealed that pSE13-SA01718 (KY807921), pEC1066 (MG587003) and pEC2380 (MG587004) carried the same transposon (Tn3-type), differing only by the ProP protein present in Cupriavidus gilardii CR3 (CP010516) (Figure S1, available as Supplementary data at JAC Online).[3,4] On the other hand, pECPB39 (MG886287) and pEC0674 (MF684783) plasmids lack tnpA and tnpR of the mcr-5 transposon. While the plasmid carrying the mcr-5.3 gene could not be transferred by conjugation (using MacConkey plates supplemented with 200 mg/L streptomycin and 0.5 or 1 mg/L colistin), an IncI1/ST113 plasmid (90 kb) carrying $bla_{\text{CTX-M-8}}$ was transferred to the recipient streptomycin-resistant E. coli C600S^{TR}.

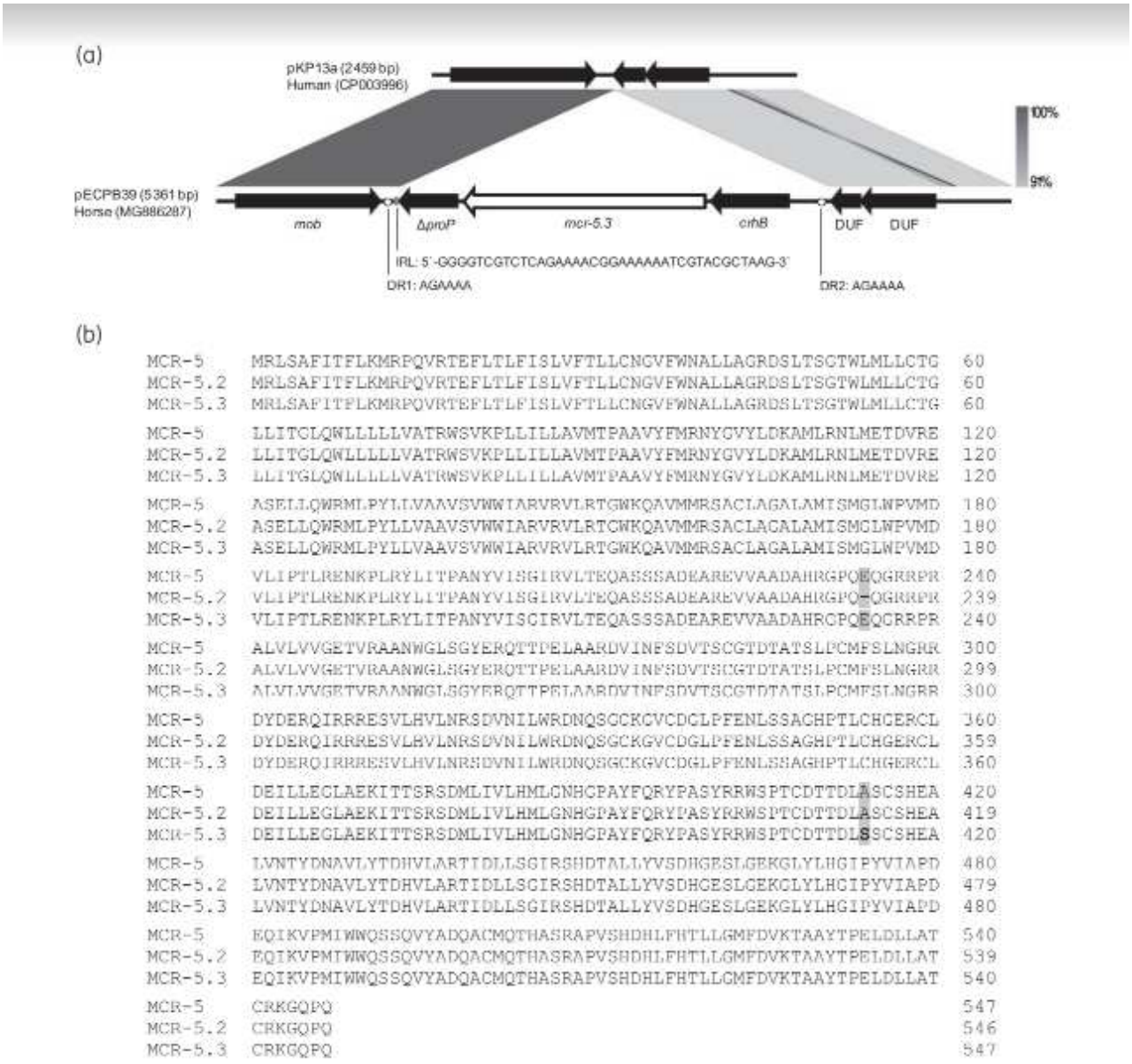

Figure 1. (a) Genetic backbone of pECPB39 (MG886287) and pKP13a (CP003996) plasmids. In pECPB39, the DproP-mcr-5.3-chrB array has been incorporated into the pKP13a backbone. In fact, the mcr-5.3 gene is embedded within a Tn3-family transposon with a 38 bp IRL, as previously reported.[3] DUF, domain of unknown function proteins. (b) Comparison of MCR-5-type phosphoethanolamine transferase proteins of S. enterica subsp. Enterica serovar Paratyphi B and E. coli strains identified in Germany[3,4] and Brazil (this study). The position of the amino acid deletion in MCR-5.2 (Glu233) is indicated by a dash (highlighted in grey), whereas the amino acid substitution in MCR-5.3 (Ala414Ser) is indicated in bold (highlighted in grey).

Although, E. coli ECPB39 was susceptible to colistin (MIC 2 mg/L, just below the EUCAST breakpoint), in silico structural analysis of MCR-5.3 revealed that the Ala414Ser mutation would not have a significant impact on the overall architecture of the catalytic domain, where zinc ions and residues seem to remain conserved and properly coordinated in comparison to MCR-1, despite the low amino acid identity (Figure S2). Thus, the

identification of an mcr-5-positive isolate showing a colistin MIC of 2 mg/L, in the present study, indicates that the silent spread of this gene might happen. Indeed, some E. coli isolates carrying the mcr-1, mcr-3 or mcr-5 gene have showed lower colistin MICs.[6,11–13]

In conclusion, we report the identification of a novel variant of mcr-5 in a CTX-M-8-producing E. coli from an infected horse, which had not received colistin previously. These results suggest that mcr-5 variant genes have been present in the South American veterinary scenario for at least 6 years, highlighting an urgent need to monitor plasmid-mediated mcr-type genes in human and veterinary medicine.

Nucleotide sequence accession number

The nucleotide sequence of pECPB39 has been deposited at GenBank under the accession number MG886287.

Acknowledgements

FAPESP (2016/08593-9) and CNPq (462042/2014-6) research grants are gratefully acknowledged. We thank Cefar Diagnóstica Ltda (Brazil) for kindly supplying antibiotic discs for susceptibility testing.

Funding

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Transparency declarations

None to declare.

Supplementary data

Figures S1, S2 and S3 are available as Supplementary data at JAC Online.

References

1 Liu YY, Wang Y, Walsh TR et al. 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 2016; 16: 161–8.

2 Partridge SR, Di Pilato V, Doi Y et al. Proposal for assignment of allele numbers for mobile colistin resistance (mcr) genes. J Antimicrob Chemother 2018; **73**: 2625–30.

3 Borowiak M, Fischer J, Hammerl JA et al. Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting Salmonella enterica subsp. enterica serovar Paratyphi B. J Antimicrob Chemother 2017; **72**: 3317–24.

4 Hammerl JA, Borowiak M, Schmoger S et al. mcr-5 and a novel mcr-5.2 variant in Escherichia coli isolates from food and food-producing animals, Germany, 2010 to 2017. J Antimicrob Chemother 2018; **73**: 1433–5.

5 Yang YQ, Li YX, Lei CW et al. Novel plasmid-mediated colistin resistance gene mcr-7.1 in *Klebsiella pneumoniae*. J Antimicrob Chemother 2018; **73**: 1791–5.

6 Fukuda A, Sato T, Shinagawa M et al. High prevalence of mcr-1, mcr-3 and mcr-5 in Escherichia coli derived from diseased pigs in Japan. Int J Antimicrob Agents 2018; **51**: 163-4.

7 Chen L, Zhang J, Wang J et al. Newly identified colistin resistance genes, mcr-4 and mcr-5, from upper and lower alimentary tract of pigs and poultry in China. PLoS One 2018; **13**: e0193957.
8 Zhang J, Chen L, Wang J et al. Molecular detection of colistin resistance genes (mcr-1 to mcr-5) in human vaginal swabs. BMC Res Notes 2018; **11**:143.
9 Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in Enterobacteriaceae. Emerg Infect Dis 2016; **22**: 1038–43.
10 Esposito F, Fernandes MR, Lopes R et al. Detection of colistin-resistant MCR-1-positive *Escherichia coli* by use of assays based on inhibition by EDTA and zeta potential. J Clin Microbiol 2017; **55**: 3454–65.
11 Feng Y. Transferability of MCR-1/2 polymyxin resistance: complex dissemination and genetic mechanism. ACS Infect Dis 2018; **4**: 291–300.
12 Liassine N, Assouvie L, Descombes MC et al. Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract Enterobacteriaceae in Switzerland.Int J Infect Dis 2016; **51**: 4–5.
13 Matuschek E, A ̈hman J, Webster C et al. Antimicrobial susceptibility testing of colistin-evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter* spp. Clin Microbiol Infect 2018; **24**: 865–70.

Figures S1, S2 and S3.

Supplementary data

Figures S1

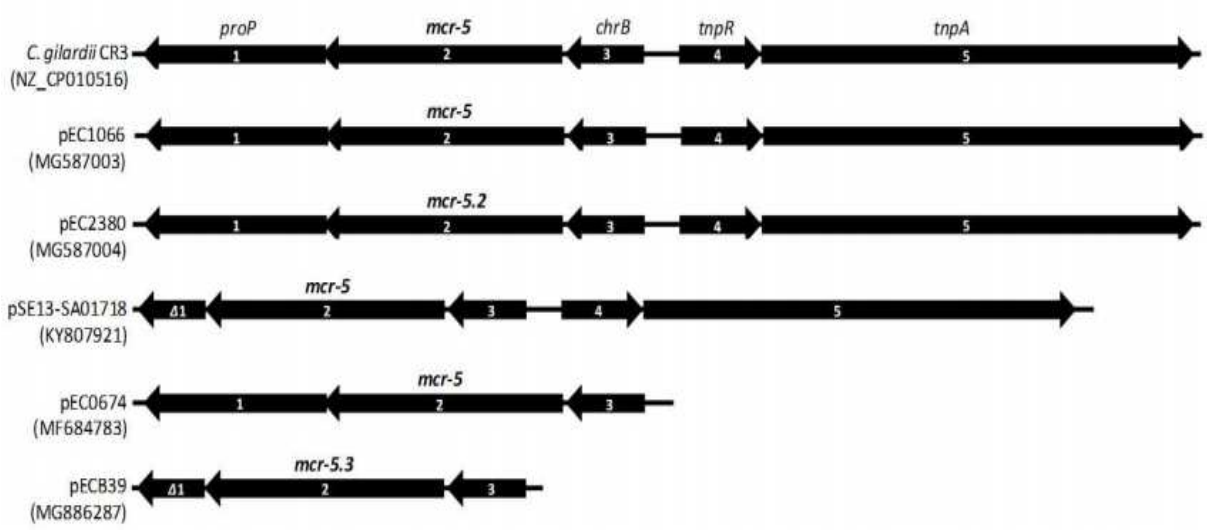

Figura S1. Comparative analyses of Tn6452 transposon carried by Cupriavidus gilardi CR3 (NZ_CP010516), Pec1066 (MG587003), Pec2380 (MG587004), Pse13-SA01718 (KY807921), Pec0674 (MF684783), and (MG886287).

Figure S2

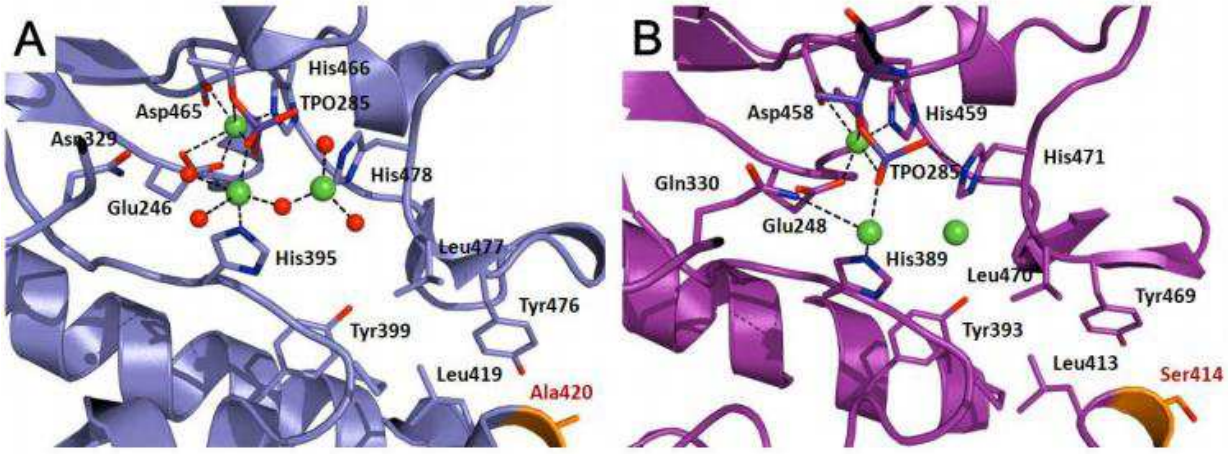

Figura S2. Models of MCR-1 (A) and MCR-5.3 (B) phosphoethanolamine transferase proteins. Orange, single mutation Ala420Ser. Green spheres, Zn(II) ions. Red spheres, water moleculares. Black dotted, main hydrogen bonds coordinating the active site.

Figures S3

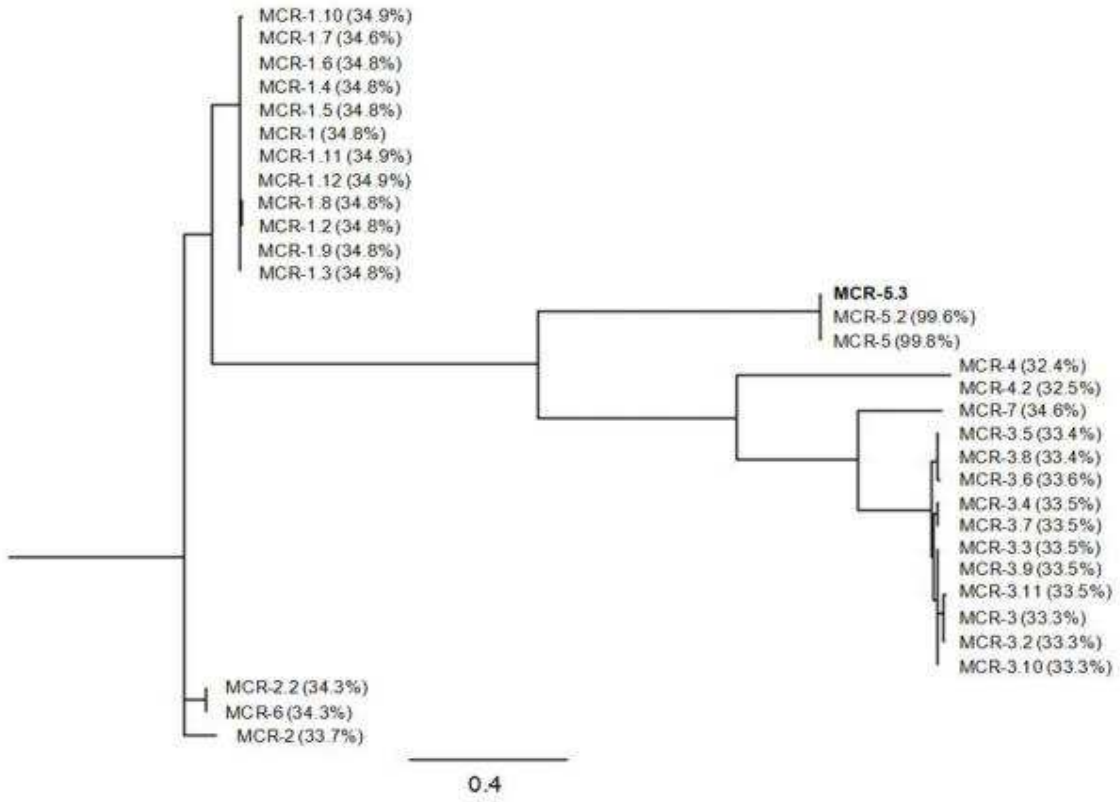

Figura S3. Phygelogenetic tree obtained for all the identified MCR-like enzymes including all MCR-5 variantes by distance method using Neighbor-Joining algorythm (RaxML 8.2.11). Branch lengths are drawn to scale and are proportional to the number of amino acids substitutions with 100 bootstrap replications. The distance along the vertical axis has no significance.

CAPÍTULO III

TITULO: Genomic features of a highly virulent ceftiofur-resistant CTX-M-8-producing *Escherichia coli* ST224 causing fatal infection in a domestic cat

(Artigo publicado na revista Journal of Global Antimicrobial Resistance, ISSN 2213-7165
(Fator de impacto: 2.022 - Qualis B1).

Genome note

Genomic features of a highly virulent ceftiofur-resistant CTX-M-8- producing *Escherichia coli* ST224 causing fatal infection in a domestic cat

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ABSTRACT

Objectives: *Escherichia coli* strains producing extended-spectrum β-lactamases (ESBL), especially of CTX-M type, have been largely described in companion animals; howevergenomic data are lacking to clarify the clinical impact of ESBL producers in these hosts. The aim of this study was to present genomic features of a highly virulent ceftiofurresistant CTX-M-8-producing *E. coli* lineage from a case of pneumonia in cat with fatal outcome. **Methods:** Genomic DNA was sequenced using an Illumina NextSeq500 platform and assembled by CLC Genomic Workbench. Genomic data was analyzed using bioinformatics online tools. **Results:** Genome size was evaluated at 5,1 Mb, with 5,334 protein-coding sequences. The strain was assigned to ST224 and presented genes conferring resistance to β-lactams (*bla*$_{CTX-M-8}$), sulphonamides (*sul2*), tetracycline (*tetA*) and trimethoprim (*dfrA14*), as well as chromosomal point mutations in ParC (S80I), GyrA (S83L),

and GyrB (D87N). Additionally, the presence of *cba*, *gad*, *ipfA*, *iroN*, *iss*, *mchF* and *tsh* virulence genes was detected. **Conclusion:** This draft genome sequence might provide important data for a better comprehension about genomic aspects regarding the dissemination of CTX-M-8-producing *E. coli* in a human-animal-environment interface.

Keywords: companion animals; ESBL; MDR bacteria; pets; whole-genome sequencing.

TEXT

Escherichia coli strains producing extended-spectrum β-lactamases (ESBL), especially of CTX-M-type, have been recognized as a major threat to human and animal health. Although colonization in companion animals has been widely reported, genomic data are lacking to clarify the clinical impact of ESBL producers in these hosts. [1]. In this study, we present the genetic features of a highly virulent ceftiofur-resistant *E. coli* lineage collected post mortem from a case of pneumonia in a domestic cat.

In April 2015, an 8-year-old female Siamese cat presenting dyspnea for 6 months and progressive weight loss was admitted to a small-animal hospital in Northeast Brazil. Airways obstructions and frequent cough were observed at clinical examination. The animal had no outdoor access and was fed with non-raw pet food products. An emergency treatment was performed with oxygen supplementation, bronchodilators drugs and fluid therapy; however, the animal died 6 hours after starting the support treatment. Indeed, fulminant course of the infection made it impossible to establish antibiotic therapy. Pneumonia was diagnosed based on development of respiratory symptoms, radiological aspects and microbiological findings (positive culture for *Escherichia coli*).

The *E. coli* isolate (ECPB17) was recovered from a lung tissue sample obtained

at necropsy and was identified by matrix-assisted laser desorption/ionization time-offlight mass spectrometry (MALDI-TOF). *E. coli* ECPB17 exhibited a multidrug-resistant profile, including resistance to amoxicillin-clavulanic acid, ceftiofur, ceftriaxone, cefotaxime (MIC>32 mg/L), cefepime, ciprofloxacin, enrofloxacin, nalidixic acid, trimethoprim/sulfamethoxazole and tetracycline, but remained susceptible to cefoxitin, amikacin, gentamicin, ertapenem, imipenem and meropenem, according to the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org).

Genomic DNA was extracted using the PureLink™ Quick Gel Extraction kit (Life Technologies, Carlsbad, CA), according to the manufacturer's guidelines. DNA quality and quantity were assessed by spectrometry (NanoDrop spectrophotometer; Thermo Scientific) and fluorometry (Qubit 2.0 fluorometer; Life Technologies, Carlsbad, CA) methods. Genomic library was prepared using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA) and sequenced using an Illumina NextSeq500 platform, with 2 x 75-bp read lengths (paired-end). Read sequences were trimmed and *de novo* assembled using CLC Genomics Workbench 10 (CLC Bio, Aarhus, Denmark). Theresulting contigs were submitted to automatic annotation by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

The assembled sequences were analyzed to identify antimicrobial resistance genes and chromosomal point mutations in the quinolone resistance-determining region (QRDR), plasmid replicons and plasmid sequence type (ST), serotype and *E. coli* virulence genes, using multiple databases, available from the Center for Genomic Epidemiology (http://genomicepidemiology.org/).

Genome size of *E. coli* ECPB17 strain was calculated at 5,135,031 bp, with 190x coverage, N_{50} value of 95,340 and GC content of 50.7%. A total of 315 contigs were produced, with 5,381 genes, 5,334 protein-coding sequences, 3 rRNAs, 39 tRNAs, 5 ncRNAs,

312 pseudogenes, and 1 CRISPR array. This strain was assigned to the serotype O66:H23-*fimH*61, whereas virulome analysis detected the presence of *cba* (colicin B), *gad* (glutamate decarboxylase), *ipfA* (long polar fimbriae) *iroN* (enterobactin siderophore receptor protein), *iss* (increased serum survival), *mchF* (ABC transporter protein MchF) and *tsh* (temperature-sensitive hemagglutinin) virulence genes.

Multilocus sequence typing (MLST) analysis assigned *E. coli* ECPB17 to the global multidrug-resistant *E. coli* clone ST224. In fact, this ST appears to be well adapted to the human-animal interface, being reported in Asia, Europe and South America, mostly in association with plasmid-mediated *bla*$_{\text{CTX-M}}$-type genes (https://enterobase.warwick.ac.uk/species/index/ecoli). In Brazil, the occurrence of ST224 has so far been restricted to swine and buffalo from the food-producing industry, linked to the production of CTX-M-8 and CTX-M-15 enzymes, respectively [2,3].

Resistome analysis revealed four genes that confer resistance to β-lactams (*bla*$_{\text{CTXM-8}}$), sulphonamides (*sul2*), tetracycline (*tetA*) and trimethoprim (*dfrA14*). Additionally, the presence of chromosomal point mutations in ParC (S80I), GyrA (S83L), and GyrB (D87N) were identified, which may explain the fluoroquinolone resistance profile. IncI1, IncFIA, IncFIB and IncY replicon types were identified; however, since we used shortread sequencing technology, we could not assemble the plasmids sequences and, therefore, it was not possible to confirm the exact location of the *bla*$_{\text{CTX-M-8}}$ gene.

Although the origin of CTX-M-8-positive *E. coli* could not be elucidated, some studies performed by our research group have documented that humans can transmit highrisk resistant pathogens to pets in a reverse zoonotic event, called zooanthroponosis [4]. On the other hand, raw pet food products have been shown to be an important risk factor for colonization and infection by ESBL-producing bacteria in household cats [1], and can also represent a risk for pet owners when handling raw pet food products.

In summary, the emergence and dissemination of CTX-M-producing pathogens in companion animals creates important therapeutic limitations in veterinary medicine. Finally, considering that the One Health approach has been encouraged to unify human-animal-environment health in a single standard, the rapid spread of clinically relevant antibiotic resistance genes in companion animals is worrisome within a broader public health perspective, since companion animals carrying ESBL bacteria may contribute to the spread of these pathogens at the human-animal interface.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession PNFE00000000. The version described in this paper is version PNFE00000000.1.

Declarations

Funding: This work was funded by research grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2016/08593-9; FAPESP 2015/13527-2), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 462042/2014-6) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Finance Code 001).

Ethical Approval: Not required.

Competing Interests: None declared.

Acknowledgments

We thank Cefar Diagnóstica Ltda. (São Paulo, Brazil) for kindly supplying antibiotic discs for susceptibility testing.

References

1. Baede VO, Broens EM, Spaninks MP, Timmerman AJ, Graveland H, Wagenaar JA, *et al*. Raw pet food as a risk factor for shedding of extended-spectrum betalactamase- producing Enterobacteriaceae in household cats. PLoS One 2017;12:e0187239.

2. Silva KC, Moreno M, Cabrera C, Spira B, Cerdeira L, Lincopan N, *et al*. First Characterization of CTX-M-15-producing *Escherichia coli* Strains Belonging to Sequence Type (ST) 410, ST224, and ST1284 from Commercial Swine in South America. Antimicrob Agents Chemother 2016;60:2505-8.

3. Aizawa J, Neuwirt N, Barbato L, Neves PR, Leigue L, Padilha J, *et al*. Identification of fluoroquinolone-resistant extended-spectrum β-lactamase (CTX-M-8)-producing *Escherichia coli* ST224, ST2179 and ST2308 in buffalo (*Bubalus bubalis*). J Antimicrob Chemother 2014;69:2866-9.

4. Fernandes MR, Sellera FP, Moura Q, Carvalho MPN, Rosato PN, Cerdeira L, *et al*. Zooanthroponotic Transmission of Drug-Resistant *Pseudomonas aeruginosa*, Brazil. Emerg Infect Dis 2018;24:1160-1162.

CONCLUSÕES

Resumidamente, os três trabalhos que compõem a presente tese chegaram às seguintes conclusões:

- Detecção da presença do gene CTX-M-15 numa cepa de *Klebsiella pneumoniae* ST231 em um cão sem raça definida (SRD), gene este muito comum em humanos no Brasil e pouco registrado em cães, (*Klebsiella pneumoniae*-KPPB76) com número de acesso no GenBank NBMT00000000. Disponivel em: www.ncbi.nlm.nih.gov/genbank/. Uma colonização persistente por que desenvolveu infecções nasais e uterinas, onde a análise genômica revelou que os isolados de *K. pneumoniae* foram clonalmente relacionados pertencentes à linhagem ST231 associada a humanos, com resistência a aminoglicosídeos, β-lactamas, fluoroquinolonas, fosfomicina, phenicols, sulfonamidas, tetraciclinas e trimetoprima.
- Foi realizado o sequênciamento genômico e plasmidial da cepa identificada como *Eschechia coli* ST711 reportada tanto em humanos como em animais, (*Eschechia coli*-ECPB39) com número de acesso no GenBank PDMU01000000). Disponivel em: www.ncbi.nlm.nih.gov/genbank/. Onde foi relatado a identificação de uma nova variante do MCR-5 do gene CTX-M-8 em uma cepa de *E. coli* isolada de um cavalo infectado, no Brasil. Estes resultados sugerem que os genes da variante MCR-5 estão presentes na América do Sul durante pelo menos 6 anos, destacando uma necessidade urgente de monitorar genes de tipo MCR mediados por plasmídeos em medicina humana e veterinária.
- Foram determinados o sequênciamento genômico e plasmidial da cepa identificada como *Eschechia coli* ST225, com a presença do gene CTXM-8, infecção pulmonar fatal (pneumonia fatal) em felino, (*Eschechia coli*-ECPB17) com número de acesso no GenBank: PNFE00000000). Disponivel em: www.ncbi.nlm.nih.gov/genbank/. Sendo o primeiro sequênciamente de genoma de um $bla_{\text{CTX-M-8}}$-abrigando em *E. coli* isolado de uma amostra de fragmento pulmonar de um gato infectado que veio a óbito.
- A rápida propagação de genes de resistência aos antibióticos clinicamente relevantes em animais domésticos é preocupante dentro de uma perspectiva de saúde única mais ampla, uma vez que esses animais podem contribuir para a disseminação desses organismos. Portanto, este trabalho de genômica pode ser útil para elucidar características genéticas de CTX-M-8 em *E. coli* e CTX-M-15 em *K. pneumoniae* em uma interface humano-animal-ambiente. Entretanto, novas pesquisas deverão ser realizadas para continuar caracterizando esses genes

com o objetivo de previnir novos casos de infecções causadas por bacterias que apresentam resistencias aos antibióticos, considerado mundialmente no momento um quadro preocupante.

ANEXO 1: Instruções aos autores para publicações na revista Diagnostic Microbiology and Infectious Disease.

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ANEXO 2: Instruções aos autores para publicações na revista Journal of Antimicrobial Chemotherapy.

Impact Factor
5.217
5 year Impact Factor
5.173

Editor-in-Chief
Dr Peter Donnelly
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Instructions for Authors

PROCESSING OF PAPERS

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Article types and format

All documents should be double spaced, and the margins should not be excessively wide. A clear, legible single font (which is readily available internationally) and point size should be employed throughout. For symbols, please use the 'insert symbol' function and ONLY select characters from the 'normal text' subset. **All submitted articles should be line numbered (using continuous line numbers). To do this in Word, use File, Page Setup, Layout, Line Numbers and select continuous line numbering. Please DO NOT insert page numbers (as the pdf proof created by the online submission system will automatically be page numbered).**

All articles should include a title page comprising: article title; author names and their affiliations (each affiliation address must be given separately and in full); telephone, fax and e-mail contact details for the corresponding author; and a short running title. In addition, all articles must include a Funding section (if reporting original research) and a Transparency declarations section.

Article titles. All articles reporting the results of original research must have a descriptive title. For example ‘Effect of streptomycin in tuberculosis’ is acceptable; ‘Streptomycin cures tuberculosis’ is not acceptable. Leading articles, which are expressions of opinion, are permitted to have declarative titles. Please note that claims of priority are not permitted in article titles as such claims are impossible to verify; only history will reveal the first example. For instance ‘First NDM-1 *Escherichia coli* isolated in Andorra’ would not be permitted. Authors are permitted to indicate in the article that, to the best of their knowledge, a finding is the first of its kind.

Original articles and Brief reports must have a structured synopsis. The headings for the structured synopsis are as follows: Background (optional), Objectives, Patients and methods (or Methods), Results, and Conclusions.

Original articles . There is a limit of 3500 words in the main text of the article (everything from the Introduction to the end of the Discussion). Papers must be written as concisely as possible. Original articles are divided into the following sections: Synopsis (250 words maximum), Introduction, Materials (or Patients) and methods, Results, Discussion, Acknowledgements, Funding, Transparency declarations and References. Repetition of

content between sections must be avoided. A combined Results and Discussion section is acceptable.

Brief reports . These should have the same format as Original articles, but should have no more than two figures/tables, should have a maximum of 20 references and should not exceed 1500 words of main text.

Antimicrobial practice . Articles on topics related to the use of antimicrobials, format as for Original articles/Brief reports.

Correspondence . Letters on topics of concern or interest in the field of antimicrobial chemotherapy, particularly arising from papers or letters already published in the Journal. These should be addressed to the Editor-in-Chief and must not exceed 800 words, one figure or table and 10 references.

Case reports. JAC will publish Case reports that are of sufficient calibre and potential importance, and they should be submitted in the form of Correspondence (see above). Please note that patient anonymity MUST be preserved in Case reports (see the later section on Ethics approval and patient consent/privacy).

Systematic review articles. There is no length limit for this format. A systematic review, as defined by the Cochrane Handbook, is 'A review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyse data from the studies that are included in the review. Statistical methods (meta-analysis) may or may not be used to analyse and summarize the results of the included studies.' They should include a structured synopsis (with appropriate headings; these may differ from the headings used for Original articles etc.).

Review articles . There is no length limit for this format. These generally aim to give an overview of a field suitable for a wide audience, and they should include a synopsis (250 words maximum). Most reviews are invited. We are pleased to consider unsolicited reviews, but authors are encouraged to consult the Editor-in-Chief in advance of writing to avoid duplicating commissioned material.

Leading articles . These articles are usually in the region of 800-1000 words and may contain the expression of opinion as well as fact. They should address a topical subject, perhaps taking a particular viewpoint and throwing new light on a current debate. A leading article should include a short synopsis (150 words maximum) that should convey the topics and ideas the article covers. Those wishing to contribute a Leading article are encouraged to

contact the Editor-in-Chief to discuss their ideas before writing to prevent clashes with any articles already in the pipeline.

For debate. These articles should air contentious issues or discuss controversies so as to stimulate discussion in the Journal on any given topic on antimicrobial chemotherapy. Articles should be as clear and concise as possible, consist of 800-2500 words and must be accompanied by an unstructured synopsis of up to 150 words.

The Editor-in-Chief particularly welcomes pairs of For debate articles offering two opposing viewpoints that aim to persuade readers of their cases. The two resultant articles will be published side by side in the same issue.

Those wishing to contribute a For debate article should first contact the Editor-in-Chief to discuss their ideas and secure a clear agreement before submission. Unsolicited For debate articles will not be considered.

Please note that on publication all Original articles and Brief reports, as well as Antimicrobial practice papers, will be published under the heading of Original research so that articles on similar topics can be grouped together when assigned to an issue. In addition each piece of Correspondence will be published as either a Research letter or a Letter to the Editor.

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After preliminary examination of the submission by Editorial Office staff to check that all the necessary elements are present, the paper is passed to the Editor-in-Chief. The Editor-in-Chief then assigns the paper to an appropriate Senior Editor. The Senior Editor is then responsible for selecting an Editor to handle the article. Articles can be rejected immediately by the Editor-in-Chief, a Senior Editor or an Editor without further peer review. The assigned Editor is responsible for selecting referees and obtaining referee reports.

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Senior Editors, Editors and referees are asked to consider whether they have any conflicts of interest when they are assigned a paper, and if necessary to decline to handle the paper. See the section 'Conflicts of interest' for more information on this subject.

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The Editor-in-Chief, Senior Editors and Editors reserve the right to request more rounds of revision and resubmission/refereeing, or reject a paper outright, if they judge that any revised version does not adequately address the concerns raised by the referees and the Editor. Once the Editor is satisfied that a revised version has adequately dealt with any points raised they may accept the paper.

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After acceptance the paper is sent for copy-editing and typesetting prior to production of proofs for author correction.

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JAC does not bar Editors (including Senior Editors and the Editor-in-Chief) from submitting articles to the Journal. Articles submitted by Editors are handled in the same fashion as other articles subject to the following considerations: these articles are never assigned to the submitting Editor, or an Editor from the same institution; the submitting Editor is unable to access details of their article through the online submission system; and, like other authors, the submitting Editor will not know the identity of the handling Editor (in cases of rejection) or referees.

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Late corrections, Advance Access and Errata

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In-press and submitted papers that are important for the review of a paper MUST be uploaded when the paper is submitted and referred to in the covering letter that accompanies the submission. Authors should be aware of the issues of redundant/duplicate publication. For further information, please see the following Editorial:

Reeves DS, Wise R, Drummond CWE. Duplicate publication: a cautionary tale. *J Antimicrob Chemother* 2004; **53** : 411-2.

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The authorship of the paper should be confined to those who have made a significant contribution to the design and execution of the work described. In the case of clinical trials/randomized control trials it is compulsory for the contribution of each author to be clearly stated in the Transparency declarations section, after the information on conflicts of interest. Authors of other types of article may indicate the contribution made by each author if they wish.

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Many large collaborative studies are organized under a group name that represents all of the participants. *JAC* will not accept a group name as an 'author' of an article. All articles must have at least one named individual as author. Authors of large collaborative studies should list the author(s) of the article and follow this with 'on behalf of the [GROUP NAME]'. The names of all of the participants should then be listed in the Acknowledgements section.

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JAC supports the use of the ARRIVE Guidelines

(https://www.nc3rs.org.uk/sites/default/files/documents/Guidelines/NC3Rs%20ARRIVE%20Guidelines%20Checklist%20(fillable).pdf) and articles reporting research in animals must include a completed ARRIVE checklist (https://www.nc3rs.org.uk/sites/default/files/documents/Guidelines/NC3Rs%20ARRIVE%20Guidelines%20Checklist%20(fillable).pdf) which must be uploaded with the article so it is available for the scrutiny of the Editor and referees.

Funding

ALL papers submitted to *JAC* reporting original research MUST include a 'Funding' section. This section should appear after the 'Acknowledgements' section. Details of all funding sources for the work in question must be given. Authors must list any internal funding. If no specific funding has been received then this should be clearly stated; equally if data have been generated as part of the routine work of an organization, this too should be stated. Ongoing financial support for any of the authors should also be included under the Funding heading.

If a professional medical writer or similar service was involved in the origin or preparation of a manuscript and this support was funded, the source must be declared in the Funding section.

Sources of funding may of course still be thanked in the Acknowledgements section, but should not be listed again in the Transparency declarations (see below), unless there is an important reason for doing so. For example if the funder played any decision-making role in the research this must be stated.

The following rules should be followed:

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- Grant numbers should be complete and accurate and provided in brackets as follows: '(grant number ABX CDXXXXXX)'

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An example is given here: ‘This work was supported by the National Institutes of Health (P50 CA098252 and CA118790 to R. B. S. R.) and the Alcohol & Education Research Council (HFY GR667789).

Crossref Funding Data Registry

In order to meet your funding requirements authors are required to name their funding sources, or state if there are none, during the submission process. For further information on this process or to find out more about the CHORUS initiative please click here.

Conflicts of interest

Conflicts of interest have the potential to affect authors, referees and Editors (including Senior Editors and the Editor-in-Chief). *JAC* has the following systems in place to deal with conflicts of interest:

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Authors should either include appropriate declarations or state ‘None to declare’. Importantly, the declarations should be kept as concise as possible, should avoid giving financial details (e.g. sums received, numbers of shares owned etc.), and should be restricted to declarations that are specific to the paper in question. Authors will of course need to consider whether or not the transparency declarations need to be amended when revisions are submitted.

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All papers submitted to *JAC* must include a transparency declarations section; papers that do not include such a section will not enter the review process; they will be returned to the corresponding author so that the appropriate section can be added. Following resubmission the paper will then be progressed to peer review.

In the case of clinical trials/randomized control trials it is compulsory for the contribution of each author to be clearly stated in the Transparency declarations section, after the information on conflicts of interest. Authors of other types of article may indicate the contribution made by each author if they wish.

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In some instances (often when the authors themselves have no interests to declare) it may be helpful to readers as background information to give brief details of organizations that do have an interest but do not appear elsewhere in the article, for example 'Fantastazole is owned by Wonder Pharmaceuticals'.

Misconduct

We will energetically pursue accusations of misconduct directed at authors, Editors or referees and have a number of sanctions at our disposal including the option to inform employers about accusations and ask them to mount their own internal investigations. Accusations should not be made lightly or in the absence of the likelihood of supporting evidence being obtainable. The Journal may take the view that accusations are malicious if supporting evidence cannot be found and may direct sanctions against accusers in such cases. Any accusation of misconduct should be addressed to the Editor-in-Chief (unless it involves the Editor-in-Chief, in which case it should be directed to the President of BSAC).*JAC* is a member of COPE and will follow its guidelines on the handling of investigations into research misconduct.

Clinical trials/Randomized controlled trials

Registration and data publication

must register their trials in one of the databases dedicated to registration of trials. In addition, authors must state the database and provide the unique registration number – both in the abstract and in the main body of the paper.

JAC will consider for publication clinical trials for which there has been prior publication of trial data in results databases (such as http://www.clinicalstudyresults.org/about/or others), however, authors MUST declare in the covering letter and the Acknowledgements section of the article that they have previously published data in a results database.

Contributions

The contribution of each author must be clearly stated in the Transparency declarations section, after the information on conflicts of interest.

Reporting standards

All involved in the publication of health intervention research have a duty to patients and society at large to ensure that this research is reported in a complete, accurate and transparent fashion. This includes authors, referees, Editors and Journals. *JAC* takes this responsibility seriously and endorses the work of organizations such as the EQUATOR network (http://www.equator-network.org/), an international initiative that seeks to improve the reliability and value of the medical research literature.

There is a wide range of reporting guidelines, each specific for different types of study. Some of those for study types that are frequent in *JAC* are mentioned specifically below. Authors should consult the EQUATOR network website (http://www.equator-network.org/) for links to the latest versions of guidelines, which are organized by the study type.

Randomized controlled trials

Authors should comply with the Consolidated Standards of Reporting Trials (CONSORT) statement (www.consort-statement.org/) and use the resources within it (for example the checklist and flow diagram) to ensure they have addressed potential criticisms and provided all necessary information. Authors should include a CONSORT flow diagram in their article, and provide a copy of the completed checklist.

Systematic reviews and meta-analyses

For systematic reviews and meta-analyses of randomized controlled trials authors should comply with the PRISMA statement (which replaces the QUORUM statement), which consists of a checklist and flow diagram (http://www.prisma-statement.org/index.htm). Authors should include a PRISMA flow diagram in their article, and provide a copy of the completed checklist.

Outbreaks and intervention studies in nosocomial infection

Authors should comply with the ORION statement (www.idrn.org/orion.php), which is the CONSORT equivalent for infection control studies. Its purpose is to increase the quality of research and reporting in the area of nosocomial infection.

Economic evaluations

Authors of articles describing economic evaluations of antimicrobial interventions are encouraged to make use of the following resources, where applicable, in order to ensure that their work is both optimal and adequately described.

International Society of Pharmacoeconomics and Outcomes Research (ISPOR) Checklist for retrospective database studies, which can be accessed at:http://www.ispor.org/workpaper/healthscience/ret_dbTFR0203.asp

Quality of Health Economic Studies (QHES) Instrument. See Table 1 in:http://www.amcp.org/data/jmcp/Formulary Management-53-61.pdf

Observational epidemiology studies

Authors of articles reporting observational epidemiology studies should follow the STROBE guidelines (https://www.strobe-statement.org/index.php?id=strobe-home) and complete the relevant checklist for the type of study they have conducted. The completed checklist should be supplied as part of the article submission process.

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JOURNAL STYLE

General

In addition to reading the information provided here, authors should consult a recent issue of the Journal for the layout and conventions used.

The past tense should be used throughout for description of the results of the paper, the present tense should be used when referring to previously established and generally accepted results.

Where possible SI units should be used.

Please ensure that characters with a similar appearance are consistent throughout the document and not from different Unicode sub ranges as with the Greek Delta.

Language editing

Particularly if English is not your first language, before submitting your manuscript you may wish to have it edited for correct usage of English. This is not a mandatory step, but may help to ensure that the academic content of your paper is fully understood by journal editors and reviewers. Language editing does not guarantee that your manuscript will be accepted for publication. If you would like information about such services, please click https://academic.oup.com/journals/pages/authors/language_services. There are other specialist language editing companies that offer similar services and you can also use any of these. Authors are liable for all costs associated with such services.

Spelling

British spelling should be used. Spelling should follow that of the *Oxford Dictionary for Scientific Writers and Editors* and where this gives no guidance the *Concise Oxford Dictionary* . Spelling of drug names should conform with that given in the latest edition of the *British National Formulary* (published by the British Medical Association and the Royal Pharmaceutical Society of Great Britain and available online at http://www.bnf.org/bnf), but please note that *JAC* will continue to use methicillin (not meticillin).

Abbreviations

Non-standard abbreviations should be defined at the first occurrence and introduced only where multiple use is made. See here for abbreviations that may be used without definition, as well as antimicrobial abbreviations (which may be used in Tables and Figures).

Dosage frequencies and routes of administration

Latin dosage frequency abbreviations are not permitted (qd, bd, bid, tds etc.), however, constructions q12h, q8h and so on are permitted as there is less likelihood of confusion. . Routes of administration other than intramuscular (im) and intravenous (iv), which may be abbreviated after definition, should be given in full in English.

MICs

Please note that all MIC data in *JAC* must be expressed in terms of mg/L (not μg/mL).

Nomenclature

Authors are required to check and ensure that in all instances the most up to date nomenclature is being used.

Bacterial nomenclature

When genus and species are given together use a capital letter for the genus and a lowercase letter for the species and italicize both e.g. *Staphylococcus aureus* . After the initial use in the text of the full name of an organism the generic name should then be abbreviated to the initial letter, e.g. *E. coli.*

When the genus is used as a noun or adjective use lowercase roman unless the genus is specifically referred to e.g. 'staphylococci and streptococci' but 'organisms of the genera *Staphylococcus* and *Streptococcus* '.

The name of an order has an initial capital but is not italicized, e.g. Enterobacteriaceae. For genera in the plural, use lowercase roman, e.g. salmonellae.

When the species is used alone use lowercase e.g. viridans streptococci. For trivial names, use lowercase roman e.g. meningococcus.

Authors should use bacterial names present in the *Approved List of Bacterial Names, Amended Edition* (1989), Skermanm, V.B.D., McGowan, V. & Sneath, P.H.A., Eds, ASM Press, Washington, DC, USA (ISBN 1-55581-014-4), with subsequent alterations validly published by announcement in Validation Lists of the *International Journal of Systematic and Environmental Microbiology* (formally the *International Journal of Systematic Bacteriology*). A full list of validly published bacterial names is given athttp://www.bacterio.cict.fr/allnames.html

Genetic and amino acid nomenclature

Bacterial genetics . Genotype designations are indicated with italic lowercase three-letter locus codes (e.g. *par* , *his* , *ara*). If several loci are involved in a related function the individual loci are designated by the addition of an uppercase italic letter to the locus code (*parC* , *ompF*).

Phenotype designations (for example the protein product of a bacterial gene) are given in roman type with an initial capital letter (OmpF, LacZ).

Erythromycin gene nomenclature should follow that described in: Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J & Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999; **43**: 2823-30.

Yeast genetics . Wild-type alleles are all uppercase and italicized (*LEU2*), mutant alleles are all lowercase and italicized (*leu2*), and gene products are capitalized on the first letter and are not italicized (Leu2).

General . Authors should ensure that they confine discussion of changes in amino acid sequence to the context of the protein (e.g. OmpF) and nucleotide changes to the context of the gene (e.g. *ompF*). Please also be aware of the difference between a mutant (a strain with one or more mutations) and a mutation (a change in the sequence of the genetic material).

Amino acids . The full residue names or three-letter abbreviations are preferred in the text (e.g. a methionine residue at position 184 should be symbolized Met-184). The single

letter codes may be used in figures. Amino acid changes should be designated Met-184→Val or M184V.

When comparing nucleotide or amino acid sequences authors should exercise care in the use of the term homology. Homology should only be used when a common evolutionary origin is being implied; it is incorrect to give a percentage homology between two sequences. The wing of a bird and the human arm are homologous structures (they are believed to have a common evolutionary origin), homology cannot be quantified. For sequence comparison authors should use the terms identity and similarity. Sometimes 'equivalent' or 'counterpart' is more appropriate than 'homologue'.

Beta-lactamase nomenclature

Authors submitting articles reporting the identification of new beta-lactamases must provide evidence that they have contacted the relevant clearinghouse (http://www.lahey.org/Studies/) to deposit the new sequence data and receive a unique designation for the new enzyme.

Macrolide-lincosamide-streptogramin resistance determinant nomenclature

Nomenclature for macrolide-lincosamide-streptogramin resistance determinants should follow the structure suggested by: Roberts MC, Sutcliffe J, Courvalin P *et al* . Nomenclature for macrolide and macrolide-lincosamide-streptogramin B antibiotic resistance determinants. *Antimicrob Agents Chemother* 1999; **43** : 2823-30. A new gene must have ≤79% amino acid identity with all previously characterized MLS genes before receiving a new unique name. Adding subscripts or superscripts to established genes is not acceptable. See:http://faculty.washington.edu/marilynr/ . Before submitting a sequence to GenBank or submitting a manuscript for publication, please contact Professor Marilyn Roberts (marilynr@u.washington.edu). Once a new name has been assigned you must indicate in your article that you have received approval by the nomenclature centre for the new gene name.

Tetracycline resistance determinant nomenclature

Nomenclature for tetracycline resistance determinants should follow that suggested by: Levy SB, McMurry LM, Barbosa TM *et al* . Nomenclature for new tetracycline resistance determinants. *Antimicrob Agents Chemother* 1999; **43** : 1523-4. A new gene must have ≤79%

amino acid identity with all previously characterized *tet* genes before receiving a new unique name. Adding subscripts or superscripts to established genes is not acceptable. See:http://faculty.washington.edu/marilynr/ . The Levy Group is responsible for coordinating the naming of new *tet* genes and before submitting a sequence to GenBank or submitting a manuscript for publication, please contact Laura McMurry (laura.mcmurry@tufts.edu). Once a new name has been assigned you must indicate in your article that you have received approval by the nomenclature centre for the new gene name.

***qnr* gene/allele nomenclature**

Authors submitting articles reporting the identification of new *qnr* genes or alleles must provide evidence that they have contacted the relevant clearinghouse (http://www.lahey.org/qnrStudies/) to deposit the new sequence data and receive a unique designation. Authors should consult Jacoby G, Cattoir V, Hooper D *et al* . *qnr* gene nomenclature. *Antimicrob Agents Chemother* 2008; **52** : 2297-9.

FICI data

Fractional inhibitory concentration index (FICI) experiments are performed in order to study drug interactions and they must be interpreted in the following way:

FICI<=0.5 = synergy

FICI>4.0 = antagonism

FICI>0.5-4 = no interaction

For further information please see the following Editorial:

Odds FC. Synergy, antagonism, and what the chequerboard puts between them. *J Antimicrob Chemother* 2003; **52** : 1.

Microarray data

Authors of articles containing microarray data must ensure that the full datasets are lodged with an appropriate publicly available online database (the data must not be supplied for publication as Supplementary data alongside the article). The data should be supplied with the submitted article if they are not already publicly available. The name of the database and the accession numbers should be provided in the article. Authors must ensure that their data are available for public scrutiny from the online publication date of their article at the latest.

Chemistry

General nomenclature . The IUPAC recommendations on chemical nomenclature should be followed (*IUPAC Compendium of Chemical Terminology* (1987, ISBN 0 632 01767 8, Blackwell Scientific Publications, Oxford). All chemical names are run together except those of acids, acetals, esters, ethers, glycosides, ketones and salts, which are printed as separate words; hyphens are used to separate numbers, Greek letters and some configurational prefixes, e.g.*p* -nitrophenol. Italics are used for certain prefixes, e.g. *cis* - , *trans* - and *N* . Small capitals are used for dextro- and laevo- prefixes, e.g. L -glutamine.

Drugs . Spelling of drug names should conform with that given in the latest edition of the British National Formulary. Chemical or generic names of drugs should be used; trade names may be referred to once only upon first use of the generic or chemical name. The content of proprietary formulations should be given if relevant. Generic names should not be abbreviated in the text; abbreviations may be used in Tables if there is limited space. If compounds are referred to by code name or company number either the structure or a reference to a paper illustrating the structure must be given, any previous code names or designations should be given on first use.

Supplier locations are required for all smaller/local suppliers.

References

Authors are responsible for the accuracy of all references, which must be checked against the original material. Reference citations should be restricted to those that are essential for introducing the purpose and context of the paper, describing methods that are not given in detail, and for discussing the results and any relevant issues raised by them. Authors are responsible for ensuring that references are quoted accurately and not taken out of context. References must not be cited in the synopsis.

Where possible authors should avoid citing conference abstracts or posters (partly because they are not peer reviewed and also because they often report interim findings and the final published studies can often come to substantially different conclusions) and authors MUST NOT cite abstracts that are more than 2 years old without excellent justification for doing so. In addition, abstracts must only be cited if they appear in published abstract books, journal supplements or in a permanent online archive.

References should be cited in the text using sequential numbers. Superscript numbers should be used and should be placed after any punctuation. When referring to several references, separate individual numerals by a comma or a hyphen for a range greater than two

references. For instance: This was first discovered by Jones, [1] and later confirmed by several other groups of investigators. [2,3,5-7]

Papers accepted for publication, but not yet published, may be included in the reference list; they should be listed as 'in press', with the name of the journal and the likely year of publication. Submitted work should be quoted as 'unpublished results'. Personal communications and unpublished results, which are permitted in the text only, must include the initials and surnames of all the workers involved; for the former citation, the person's affiliation must be stated, e.g. '(J. Bloggs, NIH, personal communication)', and documentary evidence (an e-mail will suffice) from the person quoted, showing their agreement to be so quoted, must be provided (the agreement must include the exact wording that appears in the paper).

All references should be listed numerically at the end of the text. Each reference should be preceded by a bold number (not superscript). Please see the following examples. Failure to conform to Journal style will result in the manuscript being returned to authors.

Examples

Journal reference (<= three authors)

Sanschagrin F, Levesque RC. A specific peptide inhibitor of the class B metallo-B-lactamase L-1 from *Stenotrophomonas maltophilia* identified using phage display. *J Antimicrob Chemother*2005; **55** : 252-5.

Journal reference (> three authors)

Williams I, Gabriel G, Cohen H *et al* . Zidovudine-the first year of experience. *J Infect* 1989; **18**Suppl 1: 23-31.

Journal reference (online journal)

Bell A, Lewandowski K, Myers R *et al* . Genome sequence analysis of Ebola virus in clinical samples from three British healthcare workers, August 2014 to March 2015. *Euro Surveill*2015; **20** : pii=21131.

Whole book

Long HC, Blatt MA, Higgins MC *et al* . *Medical Decision Making* . Boston: Butterworth-Heinemann, 1997.

Book chapter

Manners T, Jones R, Riley M. Relationship of overweight to haitus hernia and reflux oesophagitis. In: Newman W, ed. *The Obesity Conundrum* . Amsterdam: Elsevier Science, 1997; 352-74.

NCCLS/CLSI methods

National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Sixth Edition: Approved Standard M7-A6* . NCCLS, Wayne, PA, USA, 2003.

Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Fifteenth Informational Supplement M100-S15* . CLSI, Wayne, PA, USA, 2005.

Meeting abstract

Hou Y, Qiu Y, Vo NH *et al* . 23-O derivatives of OMT: highly active against *H. influenzae* . In:*Abstracts of the Forty-third Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2003* . Abstract F-1187, p. 242. American Society for Microbiology, Washington, DC, USA.

Online material

References to online material should be given in the reference list. Please note that URLs for the suppliers of materials must not be given in either the text or the references. The Journal does not accept any responsibility for the content of web pages cited. NB – it is no longer necessary to provide the 'date last accessed' for URLs. Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents* . Department of Health and Human Services. http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf.

Tables

These should be employed sparingly and should be generally comprehensible without reference to the text. Each table should be supplied on a separate sheet and numbered consecutively using Arabic numerals in the order they are referred to in the text. Each must have a brief descriptive heading. Column headings must clearly explain the content of the column and indicate any units used. Footnotes should be kept to a minimum.

Tables must be created using the Table function in Word; they must not be inserted as images. Each data item should occupy a single cell and return characters should not be used within any Table. *JAC* reserves the right to move complicated Tables to online-only Supplementary data.

Figures

These must be employed sparingly to demonstrate important specific points. Figures should be numbered using Arabic numerals in the order in which they are referred to in the text. **In figure LEGENDS, symbols should be described in words (e.g. filled circles, open squares etc.)** .

Wherever possible, figures should be two-dimensional. Authors should NOT supply 'three-dimensional' figures unless this is actually necessary to represent the data.

The quality of reproduction in *JAC* is limited by the quality of the submitted material. All figures must be of high quality - they should be sharply focused, have good contrast and any lettering must be clear and legible. Colour illustrations can be reproduced if there is sufficient scientific merit in doing so. Authors will be expected to pay for the cost of colour origination in the print version of the Journal (£350/US$600/€525.00 per figure). Alternatively, black and white figures can appear in the printed version of an article with colour versions appearing online (for which there is no charge) – figure legends will need to be suitably worded, e.g. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC* . Please state your preferred option (i.e. agreement to pay £350/US$600/€525.00 per figure for print and online colour or preference for online-only colour with no charge) in your covering letter.

Guidance for preparation of Figures

Figures should be sized to fit a single column of the Journal where possible (88 mm) or a double column if necessary (180 mm). The preferred font for lettering is Times; lettering should have an upper case height of 2 mm and a lower case height of 1 mm at publication size (corresponding to point size 8). Line thickness should be set at 0.5 points. Shading used on line drawings should be clear and distinctive; shades of grey and heavy stippling do not reproduce well. Lines and symbols should be drawn boldly enough to withstand reduction. The preferred symbols are filled circles, open circles, filled squares, open squares, filled triangles and open triangles, and should be no smaller than 1 mm (height/diameter) at

publication size. Part labels should be lower case letters within parentheses, e.g. (a), (b), (c) etc.

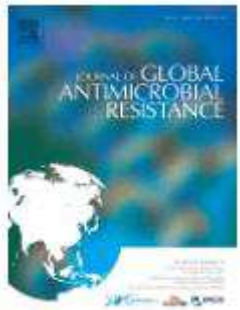
Authors must be ready to supply original gel pictures if requested to do so.

ANEXO 3: Instruções aos autores para publicações na revista Journal of Global Antimicrobial Resistance.

JOURNAL OF GLOBAL ANTIMICROBIAL RESISTANCE

ELSEVIER

AUTHOR INFORMATION PACK



ISSN: 2213-7165

GUIDE FOR AUTHORS

These guidelines generally follow the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals". The complete document appears at http://www.icmje.org.

Types of paper
The following types of manuscripts are routinely accepted (please note that word count is from abstract to references but excluding references):

Original Articles: The form of these articles is discussed fully below; an abstract is required. They should be no longer than 4000 words and 40 references (as above, please note that word count also excludes tables, figures and legends). JGAR will be happy to consider papers of veterinary origin as long as there is some linkage of the scientific work back to human antibiotic use.

Letters: Headings should not be used in a letter; no abstract or keywords are required. The text should be no more than 800 words; there should be a maximum of 5 references and one table or figure may be included.

Reviews: An abstract and keywords are required. The text should be divided into sections by suitable headings. Tables and figures may be used as appropriate for the text. They should be no longer than 5000 words.

Short Communications: These should be no more than 2,500 words, with up to 20 references and a maximum of 3 figures or tables.

Hot topics: These tend to be invited papers but unsolicited Hot topics are welcome. There are no abstract, keywords or section headings.

Genome note: Should be no more than 800 words (excluding the abstract); there should be a maximum of 5 references and one table or figure may be included. An abstract and keywords are required. A Genome note must state why the genome is of general interest and report on important or novel resistance or virulence mechanisms and if possible present the genome's MLST or a similar molecular unambiguous typing method.

Contact details for submission
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Submission checklist
You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:
One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
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• Relevant declarations of interest have been made
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

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BEFORE YOU BEGIN
Ethics in publishing Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.