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ENTEROBACTERIACEAE ENDOFÍTICAS PRODUTORAS DE β-LACTAMASES DE ESPECTRO ESTENDIDO EM HORTALIÇAS COMERCIAIS

Tese apresentada ao Programa de Pós-Graduação em Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Doutor em Ciências.

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RESUMO

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A disseminação de bactérias produtoras de β-lactamases de espectro estendido (ESBL) é um desafio que não está mais restrito aos ambientes hospitalares, mas também representa um problema crescente que envolve segurança alimentar e integridade ambiental. Neste estudo, foi realizada a investigação de bactérias endofíticas produtoras de ESBL isoladas de hortaliças comercializadas no Brasil e em Portugal. A caracterização genômica dos isolados foi realizada por WGS e o estilo de vida endofítico foi avaliado utilizando o feijoeiro comum (*Phaseolus vulgaris*) como modelo para o estudo. Linhagens endofíticas de *Escherichia coli* (ST38, ST648, ST4012), *Klebsiella pneumoniae* (ST198, ST2739) e *Enterobacter cloacae* (ST927) produtoras de CTX-M-15 foram isoladas de espinafre, repolho, rúcula e alface do Brasil. *E. coli* (ST10 e ST novo) produtoras de CTX-M-14 foram isoladas de alface e agrião de Portugal. As linhagens avaliadas colonizaram eficientemente o interior da raiz e da parte aérea das plantas do feijoeiro, confirmando a capacidade de estilo de vida endofítico. A análise dos genomas revelou genes de resistência aos beta-lactâmicos ($bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{SHV-11}}$, $bla_{\text{SHV-28}}$, $bla_{\text{OXA-1}}$, bla_{ACT}), aminoglicosídeos (*strA*, *strB*, *aac(3)−II*, *aac(6')Ib−cr*, *aadA1*, *aadA2*, *aadA5*, *aadA12*, *aph(3'')-Ib*, *aph(6)-Id*), (fluoro)quinolonas (*aac(6')Ib−cr*, *qnrB*, *oqxA*, *oqxB*), fenicóis (*catA1*, *catB3*, *cmlA1*, *floR*), tetraciclinas (*tetA*, *tetB*, *tetX*), macrolídeos (*ermB*, *mphA*, *mdfA*), trimetoprima (*dfrA14*, *dfrA15*, *dfrA17*), sulfonamidas (*sul1*, *sul2*) e fosfomicina (*fosA*), bem como mutações em regiões determinantes de resistência às (fluoro)quinolonas. Múltiplos genes associados à virulência para animais e seres humanos foram encontrados, sendo *E. coli* ST38, ST648 e ST novo pertencentes ao altamente virulento filogrupo D, enquanto *E. coli* ST10 e ST4012 foram atribuídas ao filogrupo A de baixa virulência. Genes que favorecem a associação bacteriana com plantas também foram identificados nas linhagens endofíticas. Para todas as linhagens isoladas de amostras brasileiras, plasmídeos carreadores de $bla_{\text{CTX-M-15}}$ pertenceram ao IncF ou IncHI2A e foram transferíveis horizontalmente. Para as linhagens de *E. coli* isoladas de amostras portuguesas, plasmídeos carreadores de $bla_{\text{CTX-M-14}}$ pertenceram ao IncI1 e foram transferíveis em duas das três linhagens. Em *K. pneumoniae* ST198, o plasmídeo IncF, denominado pKP301cro (147,4 kb), também carreou os *clusters* gênicos *cusSRCFBA*, *copABCDRSE* e *arsRDABC* codificadores de resistência à prata/cobre, cobre e arsênio, respectivamente. Finalmente, além do contexto genético internacional IS*Ecp1*-$bla_{\text{CTX-M-15}}$-*orf477*, dois novos contextos de $bla_{\text{CTX-M-15}}$ foram encontrados em *E. coli* ST38 e ST4012. Em conclusão, hortaliças podem representar uma possível rota para a disseminação de bactérias, incluindo clones internacionais, carreadoras de genes de resistência clinicamente significantes para seres humanos e animais, representando uma séria ameaça à saúde pública.

Palavras-chave: *Enterobacteriaceae*, ESBL, CTX-M, WGS, hortaliças, segurança alimentar.

ABSTRACT

LOPES, R. B. M. **Extended-spectrum β-lactamase-producing endophytic *Enterobacteriaceae* in commercial vegetables.** 2019. 90 p. Ph.D. thesis (Microbiology) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2019.

The dissemination of extended-spectrum β-lactamase (ESBL)-producing bacteria is a challenge that is no longer restricted to hospital settings but also represents a growing problem involving environmental and food safety. In this study, the investigation of ESBL-producing endophytic bacteria isolated from vegetables marketed in Brazil and Portugal was carried out. Genomic characterization of the isolates was performed by WGS and the endophytic lifestyle was evaluated using common bean (*Phaseolus vulgaris*) as the model for the study. *Escherichia coli* (ST38, ST648, ST4012), *Klebsiella pneumoniae* (ST198, ST2739), and *Enterobacter cloacae* (ST927) endophytic strains producing CTX-M-15 were isolated from spinach, cabbage, arugula, and lettuce from Brazil. CTX-M-14-producing *E. coli* (ST10 and new ST) strains were isolated from lettuce and watercress from Portugal. Evaluated strains efficiently colonised the interior of the common bean roots and shoots, confirming their endophytic lifestyle. Genome analysis revealed resistance genes to beta-lactams ($bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{SHV-11}}$, $bla_{\text{SHV-28}}$, $bla_{\text{OXA-1}}$, bla_{ACT}), aminoglycosides (*strA*, *strB*, *aac(3)−II*, *aac(6')Ib−cr*, *aadA1*, *aadA2*, *aadA5*, *aadA12*, *aph(3'')-Ib*, *aph(6)-Id*), (fluoro)quinolones (*aac(6')Ib−cr*, *qnrB*, *oqxA*, *oqxB*), phenicols (*catA1*, *catB3*, *cmlA1*, *floR*), tetracyclines (*tetA*, *tetB*, *tetX*), macrolides (*ermB*, *mphA*, *mdfA*), trimethoprim (*dfrA14*, *dfrA15*, *dfrA17*), sulfonamides (*sul1*, *sul2*), and fosfomycin (*fosA*), as well as mutations in quinolone resistance-determining regions. Multiple virulence-associated genes for animals and humans were found, with *E. coli* ST38, ST648 and new ST belonging to the highly virulent phylogroup D, while *E. coli* ST10 and ST4012 were attributed to low virulence phylogroup A. Genes that favor bacterial association with plants were also identified in endophytic strains. For all strains isolated from Brazilian samples, plasmids carrying $bla_{\text{CTX-M-15}}$ belonged to IncF or IncHI2A and were horizontally transferable. For *E. coli* strains isolated from Portuguese samples, plasmids carrying $bla_{\text{CTX-M-14}}$ belonged to IncI1 and were transferable from two of three strains. In *K. pneumoniae* ST198, IncF plasmid, designated pKP301cro (147.4 kb), also harbored *cusSRCFBA*, *copABCDRSE*, and *arsRDABC* gene clusters encoding resistance to silver/copper, copper, and arsenic, respectively. Finally, in addition to the international IS*Ecp1*-$bla_{\text{CTX-M-15}}$-*orf477* genetic context, two novel contexts of $bla_{\text{CTX-M-15}}$ were identified in endophytic *E. coli* ST38 and ST4012 strains. In conclusion, vegetables can represent a possible route for the dissemination of bacteria, including international clones, carrying clinically significant resistance genes for humans and animals, posing a serious threat to public health.

Keywords: *Enterobacteriaceae*, ESBL, CTX-M, WGS, vegetables, food safety.

INTRODUÇÃO

Um dos mecanismos mais importantes de resistência aos antimicrobianos em bactérias pertencentes à família *Enterobacteriaceae* é a produção de β-lactamase de espectro estendido (ESBL) mediada por plasmídeos. As enterobactérias multirresistentes produtoras de ESBL têm se tornado um desafio frente ao tratamento de infecções e constituem um dos maiores problemas de saúde pública em todo o mundo. As principais ESBLs são dos tipos CTX-M, TEM e SHV (Bush e Jacoby, 2010; Hemlata et al., 2016), das quais as mais prevalentes são as enzimas CTX-M, codificadas pelas variantes do gene $bla_{\text{CTX-M}}$ (Cantón et al., 2012). Nos dias atuais, CTX-M-15 é a ESBL de maior importância clínica e tem substituído outras variantes em muitas partes do mundo (Bevan et al., 2017).

Interessantemente, *Kluyvera ascorbata*, uma bactéria comumente encontrada na rizosfera, foi proposta como a fonte original de genes $bla_{\text{CTX-M}}$, mobilizados por sequências de inserção tais como IS*26* e IS*Ecp1* (Humeniuk et al., 2002). Após a mobilização inicial de $bla_{\text{CTX-M}}$ para plasmídeos, esses genes então se disseminaram entre bactérias gram-negativas, em que a ubiquidade das enterobactérias contribuiu grandemente para a sua rápida disseminação global (Cantón et al., 2006; Lu et al., 2010; Carattoli, 2013)

De fato, estudos epidemiológicos têm mostrado nos últimos anos que a disseminação de bactérias produtoras de ESBL não é um problema restrito aos hospitais, mas também se estende à segurança alimentar e à integridade ambiental (Cantas et al., 2013; Ur Rahman et al., 2018). Assim, desde ao início da década de 2000, as taxas de detecção de produtores de ESBL, principalmente do tipo CTX-M, na comunidade têm aumentado. Além disso, vários fatores, como fontes ambientais, alimentos de origem animal e migração humana, aceleram a disseminação global de bactérias produtoras de ESBL (Chong et al., 2018).

As culturas agrícolas também podem ser contaminadas por fontes ambientais, animais ou humanas (Olaimat e Holley, 2012). Ambientes aquáticos, por exemplo, que podem constituir uma importante fonte de contaminação, têm sido reportados como reservatórios de linhagens bacterianas de alto risco produtoras de CTX-M (Nascimento et al., 2017). Dessa maneira, bactérias multirresistentes têm sido cada vez mais relatadas em vegetais comerciais nos últimos anos (Reuland et al., 2014; Zurfluh et al., 2015; Hölzel et al., 2018). Da mesma forma, surtos de doenças causadas por linhagens altamente virulentas associadas ao consumo de vegetais crus também têm sido descritos com maior frequência. Um exemplo é o recente surto causado por *Escherichia coli* O157:H7 relacionado ao

consumo de alface romana, envolvendo 210 casos de doença e cinco mortes na América do Norte em 2018 (https://www.cdc.gov/ecoli/2018/o157h7-04-18/index.html). Nesse contexto, a veiculação de patógenos multirresistentes por vegetais frescos é uma questão preocupante de segurança alimentar, uma vez que a demanda global por vegetais utilizados na alimentação é crescente, principalmente devido à maior preocupação dos consumidores em se manterem saudáveis e com uma dieta equilibrada.

Embora enterobactérias, como *E. coli*, *Enterobacter cloacae* e *Klebsiella* spp., estejam frequentemente associados com plantas (Shankar et al., 2011; Dublan et al., 2014; Reyna-Flores et al., 2018), pouco se conhece sobre o risco real de bactérias produtoras de ESBL em vegetais frescos e muito menos sobre bactérias endofíticas com essa característica. As bactérias endofíticas colonizam os tecidos internos de plantas sem causar danos aparentes ao vegetal (Hallmann et al., 1997; Lopes et al., 2015) e podem representar um modo silencioso de disseminação de bactérias multirresistentes e determinantes de resistência para a microbiota humana e animal. Neste estudo, foi realizada uma investigação genômica de bactérias endofíticas produtoras de ESBL isoladas de vegetais comercializados no Brasil e em Portugal, dois países com alta prevalência de ESBL, estreitas relações culturais e frequente fluxo migratório entre ambos.

CONCLUSÕES

A emergência de enterobactérias endofíticas produtoras de ESBL (CTX-M) em vegetais é um relato importante para a saúde pública, pois os vegetais são frequentemente consumidos crus e as bactérias endofíticas resistem aos métodos convencionais de desinfecção dos mesmos. Nesse sentido, vegetais comercializados para o consumo alimentar podem ter uma relevância maior do que se pensava até então na transmissão de bactérias carreadoras de genes de resistência clinicamente significantes para humanos e animais, representando assim uma ameaça à saúde pública. Medidas apropriadas, como a melhoria das práticas agrícolas e da qualidade da água de irrigação, além de regulamentações mais rigorosas, contribuirão para aumentar a segurança alimentar. Considerando ainda o papel crescente do Brasil para o abastecimento do mercado global, é necessária a instauração de programas de vigilância que investiguem bactérias emergentes de relevância clínica em culturas agrícolas com o intuito de controlar a sua disseminação e de seus genes de resistência na comunidade, uma vez que a dose infecciosa pode ser muito baixa.

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