BRUNA TORRES FURTADO MARTINS

CLONALIDADE E PERFIS DE RESISTÊNCIA A ANTIBIÓTICOS EM *Yersinia enterocolitica* DE SUÍNOS E HUMANOS NO BRASIL

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Medicina Veterinária, para obtenção do título de *Doctor Scientiae*.

Orientador: Luís Augusto Nero

VIÇOSA - MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
M386c
2021

Martins, Bruna Torres Furtado, 1992-
Clonalidade e perfis de resistência a antibióticos em *Yersinia enterocolitica* de suínos e humanos no Brasil [recurso eletrônico] / Bruna Torres Furtado Martins. – Viçosa, MG, 2021.
1 tese eletrônica (70 f.): il. (algumas color.).

Inclui anexo.
Orientador: Luís Augusto Nero.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2021.032
Modo de acesso: Word Wide Web.

1. Suínos - Doenças - Aspectos genéticos. 2. *Yersinia enterocolitica* . 3. Persistência. 4. Resistência à drogas. 5. Micro- organismos - Efeito dos antibióticos. 6. Genoma. I. Universidade Federal de Viçosa. Departamento de Veterinária. Programa de Pós-Graduação em Medicina Veterinária. II. Título.

CDD 22. ed. 636.4089692

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB6 2523

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APROVADA: 31 de agosto de 2021

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AGRADECIMENTOS

A Deus, por iluminar minha jornada e me conduzir, com sabedoria, saúde e perseverança.

Ao meu orientador, professor Dr. Luís Augusto Nero, pelo apoio oferecido, ensinamentos, tempo desprendido, exemplo e parceria de trabalho, sou muito grata.

Ao professor Dr. Ricardo Seiti Yamatogi, que contribuiu na condução da pesquisa e no meu aprendizado.

A todos os demais professores, que colaboraram na minha trajetória de ensino dentro e fora da UFV.

Aos funcionários do Departamento de Veterinária, especialmente aos técnicos do setor de Medicina Veterinária Preventiva e a secretária do Programa de Pós-graduação, Rosi, pelo apoio e disponibilidade em ajudar.

Aos colegas de trabalho e estagiários, que por muitas vezes foram fundamentais para execução das atividades, meus sinceros agradecimentos. A ajuda oferecida foi crucial para a realização das atividades ou até mesmo solução de algum problema.

A todos do laboratório de Inspeção de Produtos de Origem Animal, que sempre se mostraram solícitos, dispostos a ouvir e contribuir comigo.

À todas as equipes de trabalho externas: WSU – Washigton State University, UFPR - Campus Palotina, Unesp – Campus Botucatu, FIOCRUZ-RJ, especialmente Laboratório de Zoonoses e Listeria (LABZOO) pela parceria firmada para o desenvolvimento desse projeto.

À UFV, por ser minha segunda casa, um lugar mais que especial que tem um lugar reservado no meu coração.

Viçosa por ser minha segunda cidade, durante esses 10 anos vividos foi capaz de me proporcionar muitas conquistas e aprendizagem.

Aos colegas de convivência diária do Setor de Medicina Veterinária Preventiva e Laboratório de Biologia Molecular.

Aos técnicos do Setor de Medicina Veterinária Preventiva e Laboratório de Biologia Molecular pela ajuda que sempre me ofereceram.

Ao meu filho José Vitor, que está comigo nessa reta final, me trouxe ainda mais esperança e força para vencer todos os desafios.

Ao Victor, por dividir comigo sua vida e ser tão especial.

A minha família, em especial meus pais e minha irmã, eles sempre foram meu alicerce e entenderam minhas escolhas com apoio e amor incondicional.

Aos (as) amigos (as) da pós-graduação, sei que nossa história e amizade ficarão marcadas para sempre em nossas vidas.

Aos (as) amigos (as) de sempre que ainda se fazem presentes apesar da distância.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de estudos.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pela concessão da bolsa de estudos.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de estudos.

“A persistência é o caminho do êxito.”

Charles Chaplin

RESUMO

MARTINS, Bruna Torres Furtado, D.Sc., Universidade Federal de Viçosa, agosto de 2021. **Clonalidade e perfis de resistência a antibióticos em *Yersinia enterocolitica* de suínos e humanos no Brasil**. Orientador: Luís Augusto Nero.

Yersinia enterocolitica é um patógeno associado usualmente a produtos derivados de suínos, seu principal reservatório. Esse patógeno é frequentemente isolado em tecidos linfáticos de suínos, em especial tonsilas palatinas e linfonodos mesentéricos. Por estar intimamente associada a cadeia produtiva de suínos, *Y. enterocolitica* está sujeita a todos os efeitos derivados dos procedimentos de manejo usualmente aplicados aos animais, como o desenvolvimento de resistência devido a aplicação preventiva ou terapêutica de antibióticos. Em um estudo prévio de nosso grupo, *Y. enterocolitica* foi identificada em uma cadeia produtiva de suínos e os isolados apresentaram alta similaridade de seus perfis de macro-restrição por XbaI. Assim, esse estudo teve como objetivo avaliar a persistência de *Y. enterocolitica* nessa cadeia produtiva de suínos, além de verificar a similaridade e potencial clonalidade dos isolados do bio-sorotipo 4/O:3 com isolados obtidos de casos de yersiniose humana, além de seus perfis de resistência a antibióticos. Amostras de tonsilas palatinas (n = 100), palatos (n = 30) e carne de cabeça (n = 17) foram obtidas durante o abate de suínos provenientes da mesma cadeia produtiva de suínos em que o estudo prévio de nosso grupo foi conduzido; as amostras foram submetidas a pesquisa de *Y. enterocolitica*, e os isolados obtidos submetidos a identificação de seus bio-sorotipos, pesquisa de genes de virulência (*ail*, *ystB*, *virF*, *myfA*, *ystA*, *ystC*, *fepA*, *fepD*, *fes*, *tccC*, *ymoA*, *hreP* e *sat*) e resistência múltipla a antibióticos (*emrD*, *yfhD* e *marC*). Parte desses isolados (n = 24) e isolados obtidos no estudo anterior de nosso grupo (n = 13) foram selecionados e caracterizados quanto aos seus perfis de macro-restrição com XbaI e NotI. Entre as amostras analisadas, 14 (9,5%) foram positivas para *Y. enterocolitica*, e os isolados obtidos (n = 24) foram identificados como pertencentes ao bio-sorotipo 4/O:3. Todos os isolados apresentaram resultados positivos para os genes de virulência e resistência múltipla a antibióticos pesquisados. Os isolados selecionados apresentaram alta similaridade após análise de seus perfis de macro-restrição, sendo agrupados em dois clusters com 33 (similaridade entre 82,4 e 100,0%) e 4 (similaridade entre 83,3 e 100,0%) isolados. Em seguida, parte desses isolados (n = 24) e isolados de *Y. enterocolitica* 4/O:3 obtidos de casos de yersiniose humana (n = 3) foram submetidos a sequenciamento completo de seus genomas e análises de similaridade, além de serem caracterizados quanto aos seus perfis de resistência a antibióticos pelo método de difusão

em disco (14 antibióticos). Pela análise de polimorfismo de nucleotídeo único (SNP), os isolados foram agrupados em dois grandes clados, sendo apenas um isolado (R31) no Clado A e os demais no Clado B, e um total de sete grupos clonais. As diferenças marcantes observadas no genoma do isolado R31 foram determinadas pela presença de vários genes associados a fagos, o que determinou a sua identificação como pertencente ao sorotipo O:5. Treze isolados apresentaram resistência a antibióticos de três ou mais classes, sendo caracterizados como multidroga resistentes. A análise dos genomas dos isolados permitiu a identificação de 17 genes associados a resistência a antibióticos. A maioria dos isolados apresentou resistência a cefalosporinas, porém sem a presença de genes correspondentes a esse grupo de antibiótico. O estudo permitiu identificar a persistência de *Y. enterocolitica* do bio-sorotipo 4/O:3 na cadeia produtiva de suínos analisada, além de uma alta similaridade genética entre isolados obtidos nessa cadeia produtiva com isolados obtidos de casos de yersiniose humana, confirmando a relevância de suínos na manutenção e transmissão desse patógeno.

Palavras-chave: *Yersinia enterocolitica*. Suíno. Persistência. Resistência. Antibióticos. Genoma.

ABSTRACT

MARTINS, Bruna Torres Furtado, D.Sc., Universidade Federal de Viçosa, August, 2021. **Clonality and antibiotic resistance profiles in pig and human *Yersinia enterocolitica* in Brazil.** Advisor: Luís Augusto Nero.

Yersinia enterocolitica is a foodborne pathogen usually associated to pork products, once pigs are their main reservoirs. This pathogen is often isolated from swine lymphatic tissues, especially tonsils and mesenteric lymph-nodes. Once it is close related to swine production, *Y. enterocolitica* is subjected to all effects resulted from the usual handling of pigs, such as the developing of antibiotic resistance as a consequence of preventive or therapeutic use of antimicrobials. *Y. enterocolitica* was previously identified by our group in a swine production chain and the obtained isolates presented high matching of band profiles after macro-restriction with XbaI. Thus, this study we aimed to evaluate the persistence of *Y. enterocolitica* in this swine production chain, the genetic similarity and potential clonality of the bio-serotype 4/O:3 isolates from this production chain with human yersiniosis isolates and their antibiotic resistance profiles. Samples of palatine tonsils (n = 100), palates (n = 30) and head meat (n = 17) were obtained from slaughtered pigs from the same production chain included in our previous study; samples were subjected to isolation of *Y. enterocolitica* and the isolates subjected to identification of their bio-serotypes, research of virulence genes (*ail*, *ystB*, *virF*, *myfA*, *ystA*, *ystC*, *fepA*, *fepD*, *fes*, *tccC*, *ymoA*, *hreP* and *sat*) and multidrug resistance related genes (*emrD*, *yfhD* and *marC*). A subset of these isolates (n = 24) and isolates obtained in our previous study (n = 13) were selected and characterized based on their band profiles after macro-restriction with XbaI and NotI. *Y. enterocolitica* was isolated in 14 (9.5%) of the analyzed samples, and the obtained isolates (n = 24) were identified as bio-serotype 4/O:3. All isolates presented the researched virulence and multidrug resistance related genes. The selected isolates presented high band matching after macro-restriction analysis, being grouped in two clusters with 33 (82.4 to 100.0% of band matching) and 4 (83.3 to 100.0% band matching) isolates. Then, a subset of these isolates (n = 24) and *Y. enterocolitica* 4/O:3 isolates obtained from human yersiniosis (n = 3) were subjected to whole genome sequencing, checked for genetic similarity and antibiotic resistance through the disk diffusion assay (14 antibiotics). Based on Single Nucleotide Polymorphism (SNP) analysis, isolates were grouped in two major clades: a single isolate (R31) was included in Clade A and other in Clade B, with a total of seven clonal groups. R31 presented several phage related genes, explaining its genetic

differences when compared to other isolates. Thirteen isolates were characterized as multidrug resistant, once they presented resistance to three or more antibiotic classes. Genome analysis revealed the presence of 17 antibiotic resistance related genes among *Y. enterocolitica* isolates, and most of the isolates presented resistance to cephalosporins, despite the absence of the related genes. We were able to demonstrate the persistence of *Y. enterocolitica* 4/O:3 in the studied swine production chain, despite a high genetic similarity among the obtained isolates and human yersiniosis isolates, confirming the relevance of the swine in the maintenance and transmission of this pathogen.

Keywords: *Yersinia enterocolitica*. Swine. Persistence. Resistance. Antibiotics. Genome.

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INTRODUÇÃO

Yersinia enterocolitica é uma bactéria Gram-negativa que está associada a casos de doenças em humanos, principalmente pela possibilidade de ser veiculada por alimentos. Yersiniose é a doença determinada por esse patógeno, sendo identificadas em 2015 como a terceira zoonose mais reportada na União Europeia (ECDC, 2018) e a quinta mais comum nos Estado Unidos (TACK et al., 2019). Os sintomas mais comuns da doença são diarreia e dor abdominal em crianças e jovens, com possíveis complicações imunológicas e até morte em alguns indivíduos. Os biotipos 1B, 2, 3, 4 e 5 são os apontados como patogênicos para humanos, e cepas do biotipo 1A são consideradas geralmente apatogênicas (PETSIOS et al., 2016; BOTTONE, 1999). No entanto, pesquisas mais recentes já investigam a relação do biotipo 1A com casos de doenças em humanos (ZADERNOWSKA et al., 2014; LUPI et al., 2013; BATZILLA et al., 2011).

Os isolados pertencentes aos sorogrupos O:3, O:5,27, O:8 e O:9 são os mais frequentemente isolados de amostras de alimentos e de casos clínicos e surtos em todo o mundo (VAN DAMME et al., 2015; FONDREVEZ et al., 2014; FÀBREGA; VILA, 2012). No Brasil, o sorotipo O:3 é o predominantemente encontrado (MARTINS et al., 2018; PAIXÃO et al., 2013; FALCÃO; FALCÃO, 2006). Esse sorotipo geralmente está associado ao biotipo 4, sendo o bio-sorotipo 4/O:3 relatado entre os mais isolados no mundo e sua maior prevalência geralmente está associada a tonsilas palatinas de suínos (MARTINS et al., 2018; IBAÑEZ et al., 2016; LIANG et al., 2015; PAIXÃO et al., 2013; RAHMAN et al., 2011; BOTTONE, 1997).

Muitos estudos revelaram a importância dos suínos como reservatórios dessa bactéria; nesses animais é possível isolar *Y. enterocolitica* com maior frequência na cavidade oral quando vivos e também em suas carcaças (VILAR et al., 2015; BOLTON et al., 2013; BONARDI et al., 2013). *Y. enterocolitica* é capaz de se disseminar e atingir o tecido linfático dos animais sem lhes causar nenhum prejuízo (ANDERSEN, 1988). Dessa forma, órgãos como linfonodos mesentéricos, linfonodos submandibulares e tonsilas palatinas são apontados como fontes importantes de contaminação e isolamento de *Y. enterocolitica* (VILAR et al., 2015; NESBAKKEN et al., 2003). Os intestinos e fezes também são locais em que o patógeno pode estar presente (LIANG et al., 2015; BHADURI; WESLEY, 2006). A presença inicial de *Y. enterocolitica* nas tonsilas e/ou fezes é associada à contaminação da carcaça nas áreas amostradas nos frigoríficos, inclusive quando é feita a divisão da cabeça junto com a carcaça e

incisão das tonsilas durante a retirada da do conjunto cabeça-língua (KICH et al., 2020; VAN DAMME et al., 2015; VILAR et al., 2015).

A contaminação por cepas patogênicas de *Y. enterocolitica* em órgãos dos animais abatidos tem relação direta com aumento das chances de contaminação da carcaça, o que pode ocasionar a veiculação do patógeno até o alimento na mesa do consumidor final (IBAÑEZ et al., 2016; VAN DAMME; HABIB; DE ZUTTER, 2010). Uma vez que os suínos são considerados os mais importantes reservatórios de cepas patogênicas de *Y. enterocolitica* para humanos (KICH, et al., 2020; FREDRIKSSON-AHOMAA, M. et al.; 2007; ESPENHAIN et al., 2019; BOTTONE, 1999), é de se esperar a presença dessa bactéria em abatedouros de suínos e a associação importante da carne suína com casos de yersiniose. Por esses motivos, os estudos para detecção de *Y. enterocolitica* patogênica se concentram basicamente nos suínos e seus produtos. Mais precisamente, a associação de cepas isoladas nas tonsilas e fezes dos animais com características semelhantes àquelas isoladas de casos clínicos em humanos foi descrita previamente (FREDRIKSSON-AHOMAA; KORKEALA, 2003).

LAUKKANEN-NINIOS; FREDRIKSSON-AHOMAA; KORKEALA, (2014) discutiram em uma revisão de literatura a prevalência de *Y. enterocolitica* na cadeia produtiva de carne suína. A avaliação de fezes de animais prontos para o abate para a pesquisa de *Y. enterocolitica* demonstrou resultados variados, o que pode estar associado principalmente às práticas de criação que são adotadas na granja (VIRTANEN et al., 2011). A prevalência de *Y. enterocolitica* entre esses grupos de animais varia entre 0 e 65,4% (LAUKKANEN-NINIOS; FREDRIKSSON-AHOMAA; KORKEALA, 2014). A recuperação de *Y. enterocolitica* no abatedouro foi possível em 38,4% das amostras de tonsilas, 3,8% das amostras de linfonodos íleocecais e 0,3% nas superfícies das carcaças antes da refrigeração (LAUKKANEN-NINIOS; FREDRIKSSON-AHOMAA; KORKEALA, 2014; GÜRTLER et al., 2005). No entanto, a dinâmica de infecção dos suínos na granja ainda é muito pouco conhecida e não há dados suficientes para propor medidas comprovadamente eficientes de redução da prevalência de *Y. enterocolitica* nos rebanhos (LAUKKANEN-NINIOS; FREDRIKSSON-AHOMAA; KORKEALA, 2014). A saúde dos animais não parece ter efeito direto sobre a prevalência de *Y. enterocolitica* no rebanho, mas sim fatores como contato com outras espécies (cães, roedores, aves), uso de antibióticos, manejo e reposição de plantel podem estar diretamente relacionados a contaminação e persistência do patógeno no plantel (LAUKKANEN-NINIOS; FREDRIKSSON-AHOMAA; KORKEALA, 2003; VIRTANEN et al., 2011).

Informações relacionadas a patógenos em alimentos no Brasil ainda são escassas e relativamente superficiais, inclusive em relação a *Y. enterocolitica*. Apesar da criação do Sistema Nacional de Vigilância Epidemiológica das Doenças Transmitidas por Alimentos em 2007, os dados são minimamente coletados quando comparados a realidade estimada de casos desse tipo de doença no país. Além disso, esses dados carecem de integralidade, mesmo os casos mais comuns não são adequadamente investigados e registrados (DRAEGER et al., 2019). Pouco se sabe sobre a prevalência de *Y. enterocolitica* no país, suas características, associação com a espécie suína e capacidade de resistência a antibióticos. Além disso, os estudos que buscam avaliar a similaridade de cepas de *Y. enterocolitica* no Brasil são muito escassos.

O uso de antibióticos é difundido na criação de animais desde 1950 (WIELINGA; SCHLUNDT, 2013). Estudos apontam que o consumo mundial de antibióticos na pecuária tende a aumentar 67% entre os anos de 2010 e 2030 (VAN BOECKEL et al., 2017). Os suínos se destacam na média de consumo de antibióticos por quilo de carne produzida (UPC), com valores em torno de 172 mg/UPC, ficando à frente da cadeia produtiva de carne de frango (148 mg/UPC) e de bovinos (45 mg/UPC) (VAN BOECKEL et al., 2015). Os cinco países que mais utilizaram antibióticos na produção animal em 2010 foram China (23%), Estados Unidos (13%), Brasil (9%), Índia (3%) e Alemanha (3%) (VAN BOECKEL et al., 2015).

Em 2018, a Organização Mundial da Saúde Animal relatou que 155 países submeteram relatórios sobre o uso de antibióticos na produção animal, dentre esses 45 relataram ter o uso de antibióticos como promotores de crescimento autorizado, inclusive o Brasil (OIE, 2018). Os antibióticos são utilizados na produção animal com três objetivos principais: a) promotor de crescimento (doses baixas, continuamente administradas através de ração); b) preventivo (tratamento em grupo com doses intermediárias de antibiótico antes ou durante transições críticas no processo de produção e c) terapêutico (altas doses para tratamento de doenças) (POSTMA; BACKHANS; COLLINEAU; LOESKEN et al., 2016; CHANTZIARAS; BOYEN; CALLENS; DEWULF, 2014). No Brasil ainda não há obrigatoriedade do registro do uso de antibióticos na pecuária, e as informações sobre bases e quantidades utilizadas são restritas as integradoras, fábricas de medicamentos e produtores. A proposta de controle de resíduos de antibióticos em produtos de origem animal foi iniciada entre 1980-2000, com a determinação pelo Ministério da Agricultura, Pecuária e Abastecimento (MAPA) de limites para o uso e resíduos de antibióticos nos produtos finais, buscando atender a melhoria da produtividade e da

qualidade dos alimentos disponíveis para consumo, além de adequação a normas e parâmetros internacionais de qualidade, inocuidade e comércio.

Quando são usados em doses sub-terapêuticas para incrementação do ganho de peso e produtividade, os antibióticos são capazes de exercer pressão seletiva sobre patógenos, bem como em micro-organismos comensais, o que favorece o desenvolvimento de populações resistentes (JAMALI; RADMEHR; ISMAIL, 2014; LAMMIE; HUGHES, 2016; MARSHALL; OCHIENG; LEVY, 2016; VAN BOECKEL et al., 2015). Muitas classes de agentes antibióticos que são utilizadas na criação de animais também são utilizadas em tratamentos médicos em humanos. MARSHALL & LEVY (2011) comprovaram que em criações pecuárias onde se fazia o uso de antibióticos como promotores de crescimento, as bactérias da microbiota intestinal dos trabalhadores e animais eram mais resistentes a antibióticos. Essa íntima relação entre humanos e animais propicia a colonização do indivíduo com micro-organismos semelhantes, o que está envolvido com a disseminação de cepas resistentes entre as espécies. O uso concomitante dessas bases entre seres humanos e animais potencializa o desenvolvimento de resistência cruzada aos medicamentos pelos micro-organismos, muitas vezes são usados antibióticos de classes semelhantes às usadas em humanos, o questionamento em torno do uso desses princípios ativos em doses sub-terapêuticas e sua contribuição para a seleção de bactérias resistentes a antibióticos, com consequentemente transferência de genes de resistência entre as bactérias presentes na microbiota dos animais, como por exemplo *Y. enterocolitica* (HOLMES et al., 2016). BARTON, 2014; VAN DER FELS-KLERX et al., 2011). Ainda, elementos genéticos associados a resistência a antibióticos podem ser transferidos entre bactérias por contato direto ou indireto; através de alimentos, água e resíduos dos animais.

A disseminação de cepas resistentes a antibióticos e a transferência horizontal de genes associados a resistência ocorre em escala exponencial no meio ambiente e nos locais de produção de alimentos (AARESTRUP, 2015; CHANTZIARAS; BOYEN; CALLENS; DEWULF, 2014; POSTMA; BACKHANS; COLLINEAU; LOESKEN et al., 2016). De uma maneira mais efetiva, a transferência horizontal de genes, como ocorre através de plasmídeos via conjugação, tem contribuído enormemente nessa propagação. Essa transferência intensa de genes associados a resistência a antibióticos entre bactérias patogênicas e comensais no organismo dos animais e nos alimentos pode colocar em risco a saúde humana, além da sanidade animal (MARSHALL; OCHIENG; LEVY, 2016; LANDERS et al., 2012; MARSHALL; LEVY, 2011; ZHOU; CALL; BROSCHAT, 2012). A resistência a antibióticos

geralmente está associada com a transferência horizontal de genes localizados em elementos genéticos móveis, como plasmídeos, integrons, transposons, sequências de inserção e fagos (SOUCY; HUANG; GOGARTEN, 2015; LEVERSTEIN-VAN HALL, 2002). A resistência a vários antibióticos em bactérias Gram-negativas é potencialmente relacionada a aquisição de genes por transferência horizontal, o que é comprovado através de experimentos de conjugação com espécies resistentes e não-resistentes, uma vez que foram observados altos valores de transferência completa de integrons (LEVERSTEIN-VAN HALL et al., 2002).

A emergência de resistência a antibióticos é considerada um problema de saúde global e o seu controle é um dos maiores desafios da sociedade moderna. Dessa forma, o controle da disseminação de bactérias resistentes pelo mundo vem sendo pauta de inúmeras discussões em caráter internacional (FERRI; RANUCCI; ROMAGNOLI; GIACCONE, 2017). No Brasil, este assunto também vem ganhando relevância, o que determinou a criação de um programa nacional pelo MAPA em 2017 que visa a identificação e vigilância da resistência a antibióticos na agricultura e pecuária. Esse programa tem vigência inicial entre 2018 e 2022, e prevê ações em uma abordagem ampla, voltada para o conceito de Saúde Única e contemplando ações integradas entre saúde humana, animal e ambiental, com estudos epidemiológicos e ferramentas de análise que visam o monitoramento sistemático de resistência a antibióticos em bactérias de sistemas agropecuários, alternativas para controle de infecções, uso racional de antibióticos e consequente combate a emergência de resistência (SHRESTHA; ACHARYA; SHRESTHA, 2018; BRASIL, 2017).

Nesse sentindo, pesquisas multidisciplinares são necessárias para entender a ligação desse fenômeno entre os micro-organismos, antibióticos, hospedeiros e descobertas de novos compostos (HOLMES et al., 2016). Análises aprofundadas em busca do entendimento sobre a dinâmica quando incluímos humanos-animais-ambiente são complexas. O desenvolvimento de ferramentas de genotipagem para a caracterização de micro-organismos vem sendo um elemento essencial para auxiliar no entendimento dessas questões (DENG; BAKKER; HENDRIKSEN, 2016; TYSON et al., 2015; GORDON et al., 2014; STRUELENS; BRISSE, 2013). O sequenciamento completo do genoma (whole genome sequencing, WGS) é capaz de fornecer informações, principalmente relacionadas a epidemiologia e transmissão de características entre os indivíduos. Atualmente, o WGS é considerado como a ferramenta mais moderna para ser usada na caracterização do genoma nesse tipo de abordagem (GORDON et al., 2014; TYSON et al., 2015; STRUELENS; BRISSE, 2013). Essa análise é capaz de contribuir efetivamente no mapeamento de surtos de doenças associadas ao consumo de

alimentos contaminados e delineamento dos pontos críticos, oferecendo um melhor entendimento sobre a influência do ambiente, pessoas e fluxo de cepas de microrganismos (HOLMES et al., 2016; STRUELENS; BRISSE, 2013).

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OBJETIVOS

Objetivo geral

Esse estudo teve como objetivo avaliar a persistência de *Y. enterocolitica* em uma cadeia produtiva de suínos.

Objetivos específicos

Avaliar similaridade e potencial clonalidade de *Y. enterocolitica* entre isolados obtidos na cadeia produtiva de carne suína e isolados obtidos em amostras de humanos no Brasil.

Caracterizar os seus perfis de resistência a antibióticos.

CAPÍTULO 1. Persistence of *Yersinia enterocolitica* bio-serotype 4/O:3 in a pork production chain in Minas Gerais, Brazil.

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**Manuscript published in Food Microbiology, 94:103660, 2021
ISSN: 0740-0020
DOI: 10.1016/j.fm.2020.103660

Abstract

Yersinia enterocolitica bio-serotype 4/O:3 was previously identified in a pork production chain in Brazil and the obtained isolates presented high identity by pulsed- field gel electrophoresis (PFGE, XbaI). For the current study, an additional 147 porcine samples (tonsils = 100, palate = 30, head meat = 17) were collected from the same pork production chain 2-years later and 14 (9.5%) tested positive for *Y. enterocolitica*. Isolates (n = 24, 1 to 2 per positive sample) were bio-serotype 4/O:3 and harbored virulence genes *ail*, *inv*, *wbbU*, *virF*, *myfA*, *ystA*, *ymoA*, *hreP* and *sat*, and the multidrug resistance related genes *emrD*, *marC* and *yfhD*. PFGE (XbaI) demonstrated no differences among isolates (100% similarity) and were identical to some *Y. enterocolitica* isolates (n = 13) obtained previously from the same pork chain. A second PFGE analysis (NotI) confirmed the high degree of similarity among isolates obtained over time, demonstrating the persistence of an apparent clonal *Y. enterocolitica* bio-serotype 4/O:3 in this particular pork production chain in Brazil.

Keywords: *Yersinia enterocolitica*, pork, PFGE, persistence

1. Introduction

Yersinia enterocolitica is a foodborne pathogen and the causative agent of yersiniosis, the fourth most reported zoonotic disease in the European Union (EFSA, 2019) and the fifth most common in the Unites States (TACK et al., 2019). Despite this relevance in EU and US, the status of yersiniosis in Brazil is unknown due to limited foodborne disease surveillance (DRAEGER et al., 2018). Yersiniosis is characterized by diarrhea and abdominal pain, and it can lead to immune complications and more serious problems in some individuals (BOTTONE, 2015). *Y. enterocolitica* pathogenicity is usually associated with its bio-serotype: biotypes 1B, 2, 3, 4 and 5 and serogroups O:3, O:5,27, O:8 and O:9 are considered pathogenic to people (FÀBREGA; VILA, 2012; FONDREVEZ et al., 2014; VAN DAMME et al., 2015). Based on this characterization scheme, Y. enterocolitica bio-serotype 4/O:3 is most commonly reported worldwide from yersiniosis cases and disease outbreaks (BOTTONE, 2015).

Swine are the main reservoir of *Y. enterocolitica* and this bacterium can survive successfully in all ages groups (VILAR et al., 2015). Swine tonsils are considered the best source of *Y. enterocolitica*, even when compared to feces and intestinal contents (FONDREVEZ et al., 2014). *Y. enterocolitica* contamination of pig carcasses and pork products can occur during slaughtering and processing due to poor handling of potentially contaminated sites, including entrails tonsils and mesenteric lymph-nodes (FOSSE; SEEGERS; MAGRAS, 2008; VAN DAMME et al., 2015). (FOSSE; SEEGERS; MAGRAS, 2008) estimated that 77% of yersiniosis cases are associated to pork consumption, and that cold storage might protect *Y. enterocolitica* due to its psychrotrophic characteristic (BOTTONE, 2015; VAN DAMME et al., 2015). Despite the limitations of epidemiological data of foodborne diseases in Brazil, FALCÃO et al., (2006). identified the swine as the main source of *Y. enterocolitica* 4/O:3 for humans based on isolates obtained from pork, swine and clinical samples collected from 1968 to 2000.

Y. enterocolitica bio-serotype 4/O:3 is predominant among human isolates, as well as in pork samples (FÀBREGA; VILA, 2012; FALCÃO et al., 2006a). We also identified this bio-serotype as the most frequent in a pork production chain in Brazil, being isolated mainly from lymphatic tissues and presenting high genetic-similarity by band matching after XbaI digestion and pulsed-field gel electrophoresis (PFGE) (MARTINS et al., 2018). The current study aimed to determine if these apparent “clonal” strains can persist over time in a large pork production chain in Brazil.

2. Material and Methods

After a two-year interval, the same slaughterhouse studied by MARTINS et al., (2018) was visited five times for sampling of different swine batches (three batches per visit, each batch from a different farm). At this company, animals are typically slaughtered with a mean age of 150 days and mean weight of 110 kg. Within swine batches, individual carcasses were randomly selected for sampling (tonsil = 100, palate = 30, head meat = 17) and subjected to *Y. enterocolitica* detection according to ISO 10273:2003 (ISO, 2003) and VAN DAMME et al., (2013). A portion of each sample (12.5 g) was transferred into a sterile bag with 25 mL of peptone-sorbitol-bile broth (PSB, Sigma-Aldrich, St. Louis, MO, USA). The material was homogenized for two minutes (230 rpm, Stomacher Seward 400®, Seward Limited, Worthing, England) and incubated at 25°C for 72 h. After this enrichment step, the obtained cultures were treated with 0.5% potassium hydroxide (KOH) for 20 seconds, streaked onto Yersinia Selective Agar (cefsulodin-irgasan-novobiocin - CIN, BD, Franklin Lakes, NJ, USA) and incubated at 30°C for 48 h.

Yersinia suspect colonies (approximately 3 per sample) were purified by successively (3X) streaking onto CIN, incubating at 30°C, and culturing in trypticase soya broth (TSB, Oxoid) at 30°C overnight. The obtained cultures were lysed by boiling (DIAS et al., 2016; PUI et al., 2011) and a PCR assay targeting a diagnostic 16S rRNA sequence and inv were used to confirm species identity (Garzetti et al., 2014). Confirmed isolates were subjected to a panel of biochemical tests and PCR assays targeting *per*, *wbbU*, *wbcA* and *wzt* for serotype identification (GARZETTI et al., 2014; HURST et al., 2011).

DNA from the *Y. enterocolitica* isolates was subjected to a panel of PCR assays targeting virulence related genes (*ail, ystB*, *virF*, *myfA*, *ystA*, *ystC*, *fepA*, *fepD*, fes, *tccC*, *ymoA*, *hreP* and *sat*) and multidrug resistance (MDR) related genes (*emrD*, *yfhD* and *marC*). PCR reactions were assembled by mixing 12.5 µL of GoTaq Green Master Mix (Promega), 0.5 µL of each primer (200 nMol), 1.0 µL of DNA (minimum of 40 ng), and 9.5 µL of nuclease free water (Promega). PCR cycles ranged from 30 to 35 repetitions, with varying values of the temperatures for denaturation (94-95°C), annealing (50-60°C) and extension (72°C). After reactions, PCR products were subjected to electrophoresis in 1.5% (w/v) agarose gel with GelRed™ (Biotium, Fremont, CA, USA). Further details of PCR assays were described by Martins et al. (2018).

Y. enterocolitica confirmed isolates (n = 24, 1 to 2 isolates per positive sample) were subjected to macro-restriction and PFGE following the protocols described by RIBOT et al., (2006) and recommended by PulseNet (Centers for Disease Control and Prevention, Atlanta, GA, USA). Briefly, bacterial plugs were prepared and treated as described by MARTINS et al., (2018) and subject to individual restriction with 50 U of XbaI (Promega), at 37°C for 1.5 h. The isolates and a selected panel of 13 isolates obtained in the same facility by Martins et al. (2018) were also subjected to individual restriction with 25 U of NotI (Cellco, São Carlos, SP, Brazil), at 37°C for 1 h (Wang et al., 2008). After restriction, plugs were subjected to PFGE (CHEF-DR III, Bio-Rad; for XbaI: initial switch-time of 2.2 s, final switch time of 63.8 s, 120° angle, 6 V/cm for 16 h; for NotI: initial switch time of 2.16 s, final switch time of 45 s, 120° angle, 6 V/cm for 16 h). Finished gels were stained with GelRed™ (0.015% v/v, Biotium) and "band sharing" among isolates was summarized by using an unweighted pair group method with arithmetic mean (UPGMA) cluster algorithm. Band matching was bounded by an optimization setting of 1% and a tolerance setting of 5% for the Dice coefficient (Bionumerics 7.6, Applied Maths, Ghent, Belgium). XbaI restricted *Salmonella* Braenderup ATCC BAA664 was used as marker for the band patterns.

3. Results and Discussion

Swine are considered the main reservoirs of pathogenic *Y. enterocolitica* for humans with pork products usually associated with yersinosis cases and outbreaks (BOTTONE, 2015). In the current study, *Y. enterocolitica* was detected in 14 (9.5%) of the samples, and 24 isolates were confirmed by PCR amplification of 16S rRNA and inv genetic markers (Table 1). As expected, tonsil and palate presented higher frequencies of *Y. enterocolitica* when compared to head meat (Table 1). These sites are usually used for *Y. enterocolitica* detection in swine due to the higher likelihood of isolation compared to other sites (GÜRTLER et al., 2005; MARTINS et al., 2018). Our finding confirms the importance of using care during the slaughtering process, especially in the head and mandibular areas to limit the spread of *Y. enterocolitica* (VAN DAMME et al., 2015).

All of the isolates were consistent with bio-serotype 4/O:3 based on both biochemical and PCR analyses (Table 1). This bio-serotype was already reported as being associated to swine and yersiniosis, and it is the most commonly associated bio-serotype responsible for human yersiniosis worldwide (EFSA, 2019; RUSAK et al., 2014; WANG et al., 2008). Also,

all *Y. enterocolitica* isolates from this study were positive for all virulence and MDR-related genes, which was identical to our earlier report (MARTINS et al., 2018). The screened panel of virulence related genes is usually considered for virulence characterization of *Y. enterocolitica* (BHAGAT; VIRDI, 2007; HUNTER et al., 2019a), and presumably confirms the pathogenic potential of these isolates.

Table 1. Frequency of positive *Y. enterocolitica* detection for the cranial region of pork carcasses and bio-serotyping of the obtained isolates.

sample	*Y. enterocolitica* detection		isolates	
	n	positive (%)	n[1]	bio-serotype[2]
tonsil	100	10 (10.0)	18	4/O:3
palate	30	3 (10.0)	4	4/O:3
head meat	17	1 (5.9)	2	4/O:3
total	*147*	*14 (9.5)*	*24*	-

[1] based on PCR amplification of *16s rRNA* and *inv*; [2] based on biochemical profiles and PCR amplification of *wbbU*.

In Brazil, there isn't a system for comprehensive investigation of foodborne illness, which makes it difficult to know the full extent of risk associated with any bacterial contaminants including *Y. enterocolitica* (DRAEGER et al., 2018). Without detailed information it is difficult to develop public policies and control programs to minimize the impacts of yersiniosis to public health. Besides helping with public health issues, more comprehensive information could help improve the microbial safety and quality of pork products produced in Brazil, strengthening this sector for the national and international trade (BAER et al., 2013), an issue with economic consequences considering that pork is the most consumed meat worldwide (GONZÁLEZ et al., 2020).

Macro-restriction digest with XbaI followed by PFGE revealed a high degree of similarity among *Y. enterocolitica* isolates (similar-sized DNA fragments, Figure 1). Interestingly, essentially the same XbaI band pattern was observed in most of the *Y. enterocolitica* isolates obtained in the same pork production chain two-years before the present

study (minor differences are attributable to different gel images and use of different parameter value for the image analyses) (MARTINS et al., 2018). NotI was included in the PFGE analysis to improve discriminatory power, but this assay did not alter our conclusions (Figure 1). Two clusters were formed: I, including most of the isolates (n = 33) and similarity ranging from 82.4 to 100%, and II, with four isolates obtained by (MARTINS et al., 2018) and similarity ranging from 83.3 to 100%. GILPIN et al. (2014) and RUSAK et al., (2014) have also reported low variability by PFGE of *Y. enterocolitica*. RUSAK et al., (2014) characterized *Y. enterocolitica* isolates from animal and human samples and indicated that all isolates identified as bioserotype 4/O:3 were grouped in a single cluster based on NotI digestion. CAMPIONI and FALCÃO (2014) reported that *Y. enterocolitica* biotype 4 isolates presented high similarity by PFGE, but there was significantly more diversity for biotype 1A.

The isolates collected from our study site are strong indicative of the persistence of *Y. enterocolitica* in this particular geographical area. Whole-genome sequencing (WGS) of the isolates is a logical next step to identify genetic variation that may allow detection of subpopulations of this important pathogen that are not apparent by macro-restriction digest-based assays. Genomic studies of *Y. enterocolitica* have demonstrated that approximately 71% of the genome can be considered “core” (SHI et al., 2016). Thus, while there is little apparent genetic variation based on current study, it is likely that more detailed sequence data can provide more insight into the evolution and molecular epidemiology of *Y. enterocolitica* in Brazil.

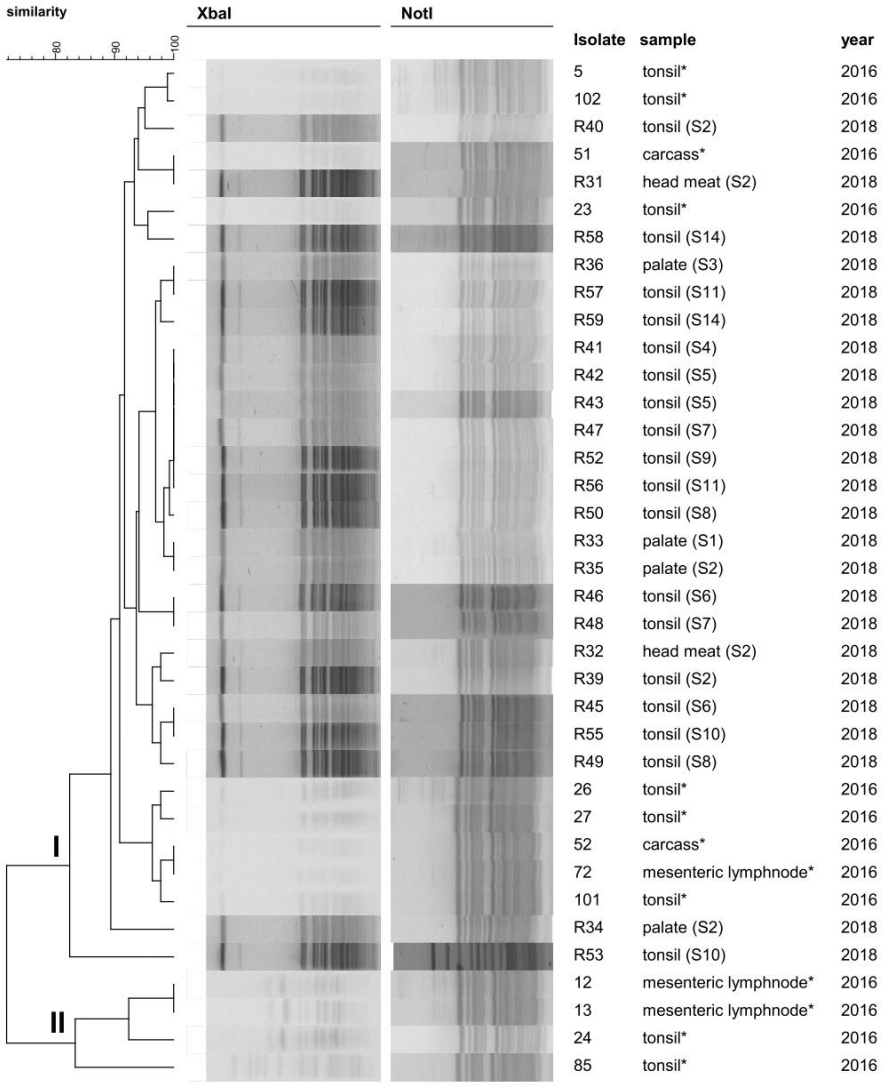

Figure 1: Results from macro-restriction (XbaI or NotI) followed by pulsed-field gel electrophoresis. *Yersinia enterocolitica* bio-serotype 4/O:3 were grouped into two clusters (I and II). The "sample" column indicates the isolates obtained for the current study ("S#") or from MARTINS et al., (2018)(*). Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA) method.

Acknowledgments

The present work was carried out with the support of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, DF, Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, DF, Brazil, Financial code

001), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, MG, Brazil) and Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo (FCF-RB, USP, Ribeirão Preto, SP, Brazil).

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CAPÍTULO 2. Comparative genomics and antibiotic resistance of *Yersinia enterocolitica* obtained from a pork production chain and human clinical samples in Brazil

Short title: WGS and AMR of *Y. enterocolitica* from Brazil

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**Manuscript submitted to Food Research International.
ISSN: 0963-9969

Abstract

Previous work found a high similarity of macro-restriction patterns for isolates of *Yersinia enterocolitica* 4/O:3 obtained at a pork production chain from Minas Gerais, Brazil. Herein we aimed to determine the clonality and the antibiotic resistance profiles of a subset of these isolates (n = 23) and human clinical isolates (n = 3). Analysis of whole genome sequence data showed that the isolates were distributed into two major clades based on single nucleotide polymorphisms (SNP) with one isolate defining Clade A (isolate R31) and remaining isolates (n = 25, 96.2%) defining Clade B. Seven clonal groups were identified. The inclusion of isolate R31 as a distinct clonal group was due to the presence of several phage-related genes, allowing its characterization as serotype O:5 by WGS. Disk-diffusion assays (14 antibiotics) identified 13 multidrug resistant isolates (50.0%). Subsequent sequence analysis identified 17 different antibiotic resistance related genes. The majority of isolates exhibited resistance to cephalosporins, but no corresponding beta lactamase gene was identified. The close genetic relationship among *Y. enterocolitica* obtained from a pork production chain and clinical samples of humans in Brazil was confirmed, and we can highlight the role of swine in the potential transmission of an antibiotic-resistant clones of a pathogenic bio-serotype to humans, or the transmission of these resistant bacteria from people to animals. The role of veterinary antibiotic use in this process is unclear.

Keywords: *Yersinia*, clonality, pork, antibiotic resistance, WGS

1. Introduction

Yersinia enterocolitica is an important human enteric pathogen that is associated with a number of clinical and immunological manifestations (FÀBREGA & VILA, 2012). Infections in people are mostly sporadic and related as a foodborne transmission (GUPTA et al., 2015). Pigs are the major reservoir for transmission to people, especially for those strains belonging to the bio-serotype 4/O:3 (LIANG et al., 2015). In Brazil, researchers have found this pathogen in pork production and in clinical samples obtained from yersiniosis cases (FRAZÃO & FALCÃO, 2015; MARTINS et al., 2021). Laukkanen-Ninios, Fredriksson-Ahomaa, & Korkeala, (2014) compiled data on the prevalence of *Y. enterocolitica* in pigs at slaughter and found that bio-serotype 4/O:3 is predominant globally, consistent with this bio-serotype being most commonly associated with yersiniosis.

A previous study found evidence for repeated recovery and, presumably, persistence of a *Y. enterocolitica* clone in the pork production chain of Minas Gerais based on XbaI and NotI macro-restriction and pulsed-field gel electrophoresis (PFGE) (Martins et al., 2021). In the case of *Y. enterocolitica*, studies have shown a high degree of genetic similarity within pathogenic bio-serotypes, including 4/O:3 (HALL et al., 2015), but there may be sufficient genetic variation to distinguish subtypes and assign them to different sources. In this respect, *Y. enterocolitica* makes a potentially valuable "source tracking" target for molecular epidemiology studies because organisms like *E. coli* encompass so much genetic variation that large numbers of isolates must be tested to gain sufficient power for relating isolates to sources. PFGE has been highly successful for strain matching with outbreak investigations where there are focused subsets of isolates for analysis (GUPTA et al., 2015) but as a technique it is ill-suited for characterizing the population genetic structure of bacterial populations (GILPIN et al., 2013).

As *Y. enterocolitica* is closely related to pork production, it is a foodborne pathogen that is highly susceptible to multiple selection pressures including antibiotics (BARTON, 2014). The use of antibiotics at subtherapeutic concentrations (e.g., growth promoters) is thought to contribute to selection for resistant strains whether extant or after newly acquiring resistance genes from other bacteria (HOLMES et al., 2016). Because of this, surveillance of antibiotic resistance in bacteria present in the animal production, including foodborne pathogens like *Y. enterocolitica*, is an important approach to develop strategies to mitigate this hazard (MAGOURAS et al., 2017).

Advances in genome sequencing have improved the resolution of epidemiological investigations related to foodborne diseases cases and outbreaks and the surveillance of antibiotic resistance in food related bacteria (ORLEK et al., 2017; SU et al., 2019). The phylogenetic and population structure of all *Yersinia* species has been studied through genomic sequences (HALL et al., 2015). The aim of the study was to study the genome sequences of *Y. enterocolitica* isolated in Minas Gerais and their clonal relationships including with isolates from three cases of yersiniosis in Brazil.

2. Material and Methods

2.1. Isolates and DNA extraction

Y. enterocolitica bio-serotype 4/O:3 isolates (n = 26) were obtained from a pork production chain (n = 23) and samples from humans diagnosed with yersiniosis (n = 3) (Supplementary Table). Isolates were selected based on their bio-serotype characterization as 4/O:3 and on their restriction profiles after digestion with XbaI and NotI (Martins et al., 2021; Martins et al., 2018). Representative isolates from human yersiniosis cases were kindly provided by Laboratório de Zoonoses Bacterianas (LABZOO) from Fundação Oswaldo Cruz (FIOCRUZ, Rio de Janeiro, RJ, Brazil).

Isolates were subjected to DNA extraction using the Wizard Genomic DNA Purification kit (Promega Corp., Madison, WI, USA), based on manufacturer's protocol. DNA concentration was estimated by spectrophotometry (NanoDrop 2000, Thermo Scientific, Wilmington, DE, USA) and DNA samples were sequenced at Neoprospecta (Florianópolis, SC, Brazil).

2.2. Whole genome sequencing (WGS), assembly and annotation

Libraries were prepared with a Nextera® XT (Illumina, Inc., San Diego, CA, USA) kit and quantified with Kapa Library Quantification (Roche Sequencing Solutions, Inc., CA, USA) according to manufacturer's recommendations. An Illumina MiSeq® System (Illumina) was used to generate raw reads based on 500 cycle, paired-end sequencing (2 x 250 bp reads). The reads were assembled with the pipeline OneShotWGS (Neoprospecta), a pipeline that uses the A5 software and Spades (COIL et al., 2015). An analysis in GMCloser was performed to improve the results, by processing for adapter trimming, quality filtering and error correction to generate contigs and scaffolds (TRITT et al., 2012). The quality of all sequences was evaluated by QUAST and reads with PHRED scores below of 20 were discarded (Huang &

Madan, 1999). The obtained sequences were deposited at National Center for Biotechnology Information (NCBI, Bethesda, MD, USA), and the accession numbers are indicated in the Supplementary Table.

2.3. *In silico* analysis

Contigs were annotated using Prokka (SEEMANN, 2014) and then imported into JSpecies (ver. 3.6.2) (RICHTER et al., 2016; RICHTER & ROSSELLÓ-MÓRA, 2009). A subset of the isolates was also analyzed in the JSpecies workspace using the Average Nucleotide Identity with Mummer (ANIm). neoSNV version 14 (Neoprospecta), which employs the NDtree algorithm (Leekitcharoenphon et al., 2014), was used to assess single nucleotide polymorphism (SNP) differences among the 26 isolate genomes in relation to a reference genome *Y. enterocolitica* IP29610 (NCBI: CPYE01). IP29610 was selected because of its close sequence similarity with our collection and with genome records from the European Nucleotide Archive. All reads were compared among themselves and only regions with support above 30 reads were considered reliable. Next, the Z-score test was applied to check the alignment positions and the number of reads was calculated. Only positions with Z-scores above 3.29 were considered accurate ($P < 0.001$). Next, a SNP counting matrix was obtained and used to define the groups of bacteria that can be considered clones. We grouped strains as clones if they differed by no more than 40 SNPs (BEKAL et al., 2016; RONHOLM et al., 2016; SALIPANTE et al., 2015). For each clonal group, we then identified a representative genome based on data from the assembly and annotation. For each group, a representative genome was selected based on having the highest scaffold, highest N50, highest N75, lowest number of scaffolds at L50, lowest number of scaffolds at L75, lowest N counts, and highest average of reads mapped by position of the scaffolds (Supplementary Table). The SNP counting matrix was also used to construct a phylogenetic tree using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (iTOL version 5.6.1) (LETUNIC & BORK, 2019).

Multi-locus sequence type (MLST) was determined using MLST software (ver. 2.0.4) and database (ver. 2.0.0) (HALL et al., 2015; LARSEN et al., 2012) of the CGE (https://cge.cbs.dtu.dk/services/MLST/)) and *Yersinia* MLST databases (https://pubmlst.org/yersinia/), respectively. For MLST, seven housekeeping genes were evaluated: *aarF* (Predicted protein kinase), *dfp* (fused 4'-phosphopantothenoylcysteine decarboxylase and phosphopantothenoylcysteine synthetase), *galR* (DNA-binding transcriptional dual regulator GalR), *glnS* (glutamine--tRNA ligase), *hemA* (Glutamyl-tRNA

reductase family protein), *rfaE* (fused heptose 7-phosphate kinase/heptose 1-phosphate adenyltransferase) and *speA* (arginine decarboxylase).

The organization of clonal groups was used to reduce data dimensionality through selection of representative genomes for the analysis of orthology and phylogeny. The orthology analyses were carried out in OrthoFinder version 2.4.1, which makes a systematic pairwise comparison of orthologous sequences (protein and DNA) (Emms & Kelly, 2019; Xu et al., 2019). Phage sequences were identified by using the PHASTER web server (https://phaster.ca/), and characterized as potentially functional or residual (Arndt et al., 2016). Visual comparison of the SNP matrix was performed using Blast Ring Image Generator (BRIG), based on BLASTALL v 2.2.25 and the comparisons were performed with default settings (Alikhan et al., 2011). WGSs were screened for antibiotic-resistance genes by using ABRIcate (ver. 1.0.1) (https://github.com/tseemann/abricate), which references several databases including NCBI, CARD, ARG-ANNOT, Resfinder, MEGARES, EcoH, PlasmidFinder, Ecoli_VF and VFDB (DOSTER et al., 2020; FELDGARDEN et al., 2019; GUPTA et al., 2014; INGLE et al., 2016; JIA et al., 2017; JIANG et al., 2019; LAURA VILLA , AURORA GARCÍA-FERNÁNDEZ, 2010; ZANKARI et al., 2012).

2.4. Antibiotic resistance

The selected isolates (n = 26) were subjected to antibiotic resistance characterization based on the disk diffusion assay, as described in CLSI (202). Antibiotics and concentrations included ampicillin (10 µg), ceftriaxone (30 µg), imipenem (10 µg), and meropenem (10 µg), sulfamethoxazole (300 µg), trimethoprim (5 µg), tetracycline (30 µg), nalidixic acid (30 µg), gentamycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), azithromycin (15 µg) and polymyxin B (300 UI). The antibiotic disk concentrations and interpretation of zone sizes followed CLSI (2021). Isolates that were resistant to three or more antibiotic classes were considered multidrug resistant (MAGIORAKOS et al., 2011). All antibiotics disks were purchased from Laborclin (Pinhais, PR, Brazil) and *Escherichia coli* ATCC 25922 was used as pansusceptible control.

3. Results

Based on WGS, the average genome size was 4.5 Mb, comprising between 35 and 120 contigs per genome and an average guanine and cytosine (GC) content of 46.97%

(Supplementary Table). As expected, the closest genomic match was *Y. enterocolitica* IP26656 (NCBI: SAMEA1486413) and *Y. enterocolitica* IP29610 (NCBI: SAMEA1486440) for all strains except for isolate R31, which more closely matched *Y. enterocolitica* YE208/02 (NCBI: SAMEA980137). Using the SNP matrix, we identified seven clonal groups relative to the reference strains (*Y. enterocolitica* IP26656 and *Y. enterocolitica* IP29610) (Table 1, Figure 1a).

Analysis of orthologous proteins showed that R31 was the outlier for the collection (Figure 1b). A primary difference for this strain was the presence of nine prophage sequences (Table 2) that ranged in size (10.8 – 46.1 kb) and had an average GC content of approximately 45.2%. Isolate R31 was most similar to *Y. enterocolitica* YE208/02 and predicted to be serotype O:5.

The SNP-based UPGMA included Clade A, comprised of only R31, and Clade B that was composed of the remaining isolates. Clade B was subdivided into subclade I, including 11 strains (1 from human clinical sample, and 11 from swine samples) and subclade II, composed of 14 strains (2 from human clinical samples and 12 from swine samples) (Figure 1c). MLST analyses identified ST-18 as the genotype (*aarF_6, dfp_2, galR_3, glnS_3, hemA_2, rfaE_3 and speA_4*) for 25 (96.2%) isolates, while R31 was identified as ST-3 (*aarF_2, dfp_4, galR_2, glnS_2, hemA_7, rfaE_2 and speA_2*).

All isolates were resistant to ampicillin, presumably due to intrinsic resistance (BONARDI et al., 2016) and to at least one additional antibiotic (Fig. 2). Isolates were mainly resistant to sulfamethoxazole (n = 24, 92.3%), while all isolates were susceptible to imipenem, amikacin and ciprofloxacin (Figure 2a). Thirteen isolates (50.0 %) were resistant to three or more antibiotic classes (excluding ampicillin), being characterized as multidrug resistant. One human isolate was resistant to meropenem or nalidixic acid while all three were resistant to sulfamethoxazole (Figure 2a).

A total of 18 different antibiotic resistance related genes were identified across all isolates (Figure 2b) including six antibiotic resistance-associated genes identified from the three yersiniosis isolates (Table 3). All isolates harbored *blaA, vatF, rosA, rosB, y56 beta-lactamase* and *crp*, while nine isolates harbored a high diversity of antibiotic resistance related genes (n = 14).

There was poor agreement between antibiotic resistance phenotypes and genotypes. For example, the majority of isolates (n = 25, 96.2%) presented phenotypic resistance to cephalosporins but no corresponding genes for hydrolyzing enzymes were observed. For other

antibiotics, such as macrolides and lipopeptides, around 75% of the isolates harbored the related genes but they were not resistant to the corresponding antibiotic. All isolates harbored a gene encoding a multidrug resistance pump, crp (Table 3).

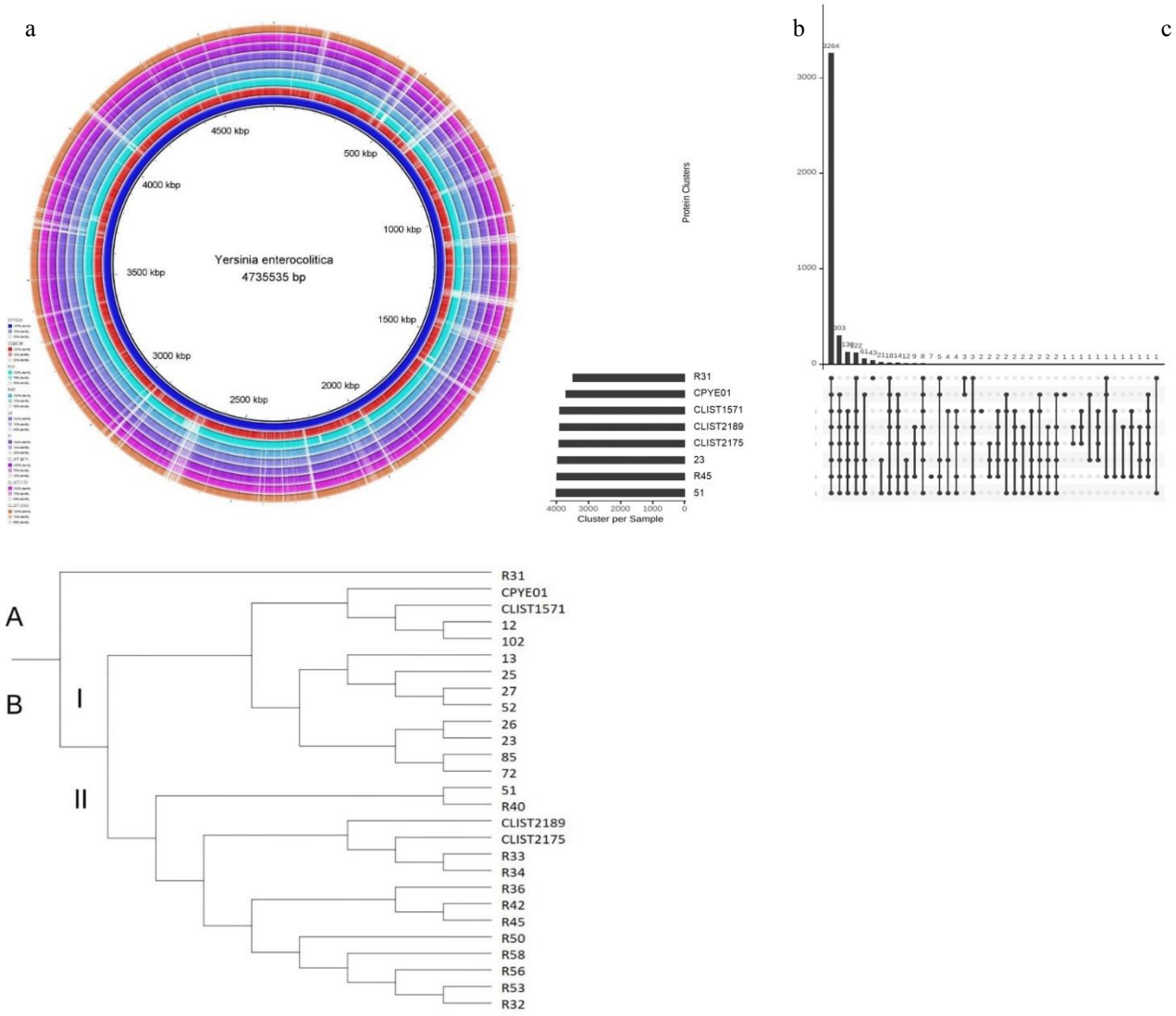
a
b
c
4500 kbp
500 kbp
4000 kbp
1000 kbp
Yersinia enterocolitica
4735535 bp
3500 kbp
1500 kbp
3000 kbp
2000 kbp
2500 kbp
Protein Clusters
R31
CPYE01
CLIST1571
CLIST2189
CLIST2175
23
R45
51
4000 3000 2000 1000 0
Cluster per Sample
3264
3000
2000
1000
0
A
B
I
II
R31
CPYE01
CLIST1571
12
102
13
25
27
52
26
23
85
72
51
R40
CLIST2189
CLIST2175
R33
R34
R36
R42
R45
R50
R58
R56
R53
R32

Figure 1. a) Visual comparison of the *Yersinia enterocolitica* groups isolated in Brazil from 2008 – 2018 in Blast Ring Image Generator (BRIG), the software uses BLASTALL v 2.2.25. The inner ring (navy) corresponds to the reference strain *Y. enterocolitica* IP29610 - CPYE01 with the corresponding genetic coordinates, the next four rings denote the orthologous groups. b) The OrthoFinder software use of the identification of orthologous for the construction of phylogenetic trees of genes present in each orthogroup by means of DendroBLAST, generating an un-rooted tree for each orthogroup. The numbers above the bar represent the number of groups of orthologous that are shared by the samples where the point is connected (in black). c) The SNP counting matrix was introduced in the neighbor program version 3.697 of the Phylip package to generate a tree of Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The Interactive Tree Of Life version 5.6.1 (https://itol.embl.de/) was used to display the phylogenetic tree. Comparative genome analysis of *Yersinia enterocolitica* through the orthologous genes. CPYE01: reference strain (*Yersinia enterocolitica* IP29610, NCBI: CPYE01).

Table 1. Clonality of *Y. enterocolitica* isolates obtained from a pork production chain and human clinical isolates compared with references genomes of *Y. enterocolitica* IP26656 (NCBI: SAMEA1486413) and *Y. enterocolitica* IP29610 (NCBI: SAMEA1486440).

Clonal group	number of isolates	isolates included*
group 1	5	**51**, 52, 72, 85, 102
group 2	6	12, 13, **23**, 25, 26, 27
group 3	3	**CLIST2175**, R33, R34
group 4	9	R32, R36, R40, R42, **R45**, R50, R53, R56, R58
group 5	1	**CLIST1571**
group 6	1	**CLIST2189**
group 7	1	**R31**

* sequences of the bold typed isolates were considered as reference in the genomic comparison

Table 2. R31 and *Y. enterocolitica* YE208/02 (NCBI: SAMEA980137) genomic prophage prediction results.

Strain	Phage assemblies*				Phages genomes most similar**			
	Genome size of assembly (kb)	Completeness	Score	Region position	***Acession number	Species name	Genome size of specie (kb)	GC%
R31	46.1	intact	140	216804-262924	NC_031940	*Salmonella* phage 118970_sal3	77.4	46.3
	14.8	intact	140	866296-881191	NC_019932	*Erwinia* virus ENT90	29.6	52.5
	30.3	incomplete	40	52418-82813	NC_001609	Enterobacteria phage P4	11.6	45.4
	10.8	incomplete	40	26655-37515	NC_022747	*Vibrio* phage VPUSM 8	34.1	43.7
	14.7	incomplete	40	1-14714	NC_027984	*Escherichia* virus 24B	57.7	43.1
	16.6	intact	100	33351-50046	NC_003444	Enterobacteria phage SfV	37.1	46.4
	11.6	incomplete	30	21151-32828	NC_001609	Enterobacteria phage P4	11.6	45.8
	23.5	incomplete	30	1370-24954	NC_028685	*Shigella* virus VASD	62.8	45.2
	14.2	incomplete	30	9358-23633	NC_019522	*Pectobacterium* phage ZF40	48.4	38.4
Y. enterocolitica YE208/02	25.0	incomplete	40	557701-582709	NC_029025	*Staphylococcus* phage IME-SA4	41.8	46.7
	28.2	incomplete	10	35025-63321	NC_047854	*Cronobacter* phage ESSI-2	28.8	46.4
	53.3	intact	120	378633-432175	NC_049459	*Salmonella* phage SW9	31.1	45.7
	23.5	incomplete	40	104376-127946	NC_049342	*Escherichia* phage 500465-1	39.2	45.4
	37.7	intact	150	39984-77692	NC_001317	*Escherichia* phage 186	30.6	48.0
	78.4	intact	150	23937-102353	NC_021857	*Shigella* phage SfII	41.5	46.7

*The identified prophage-like sequences with PHASTER web server.

**Retrieved from GenBank database

***Predict by PHASTER

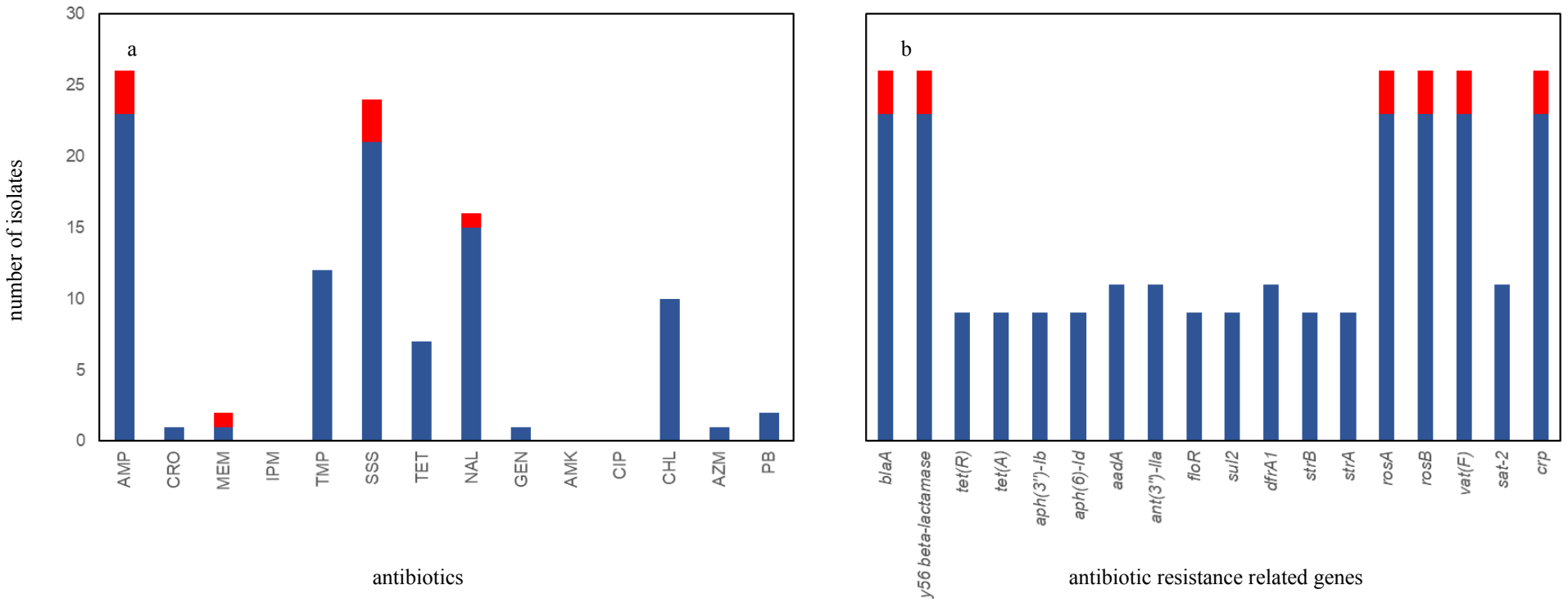

Figure 2. a) *Yersinia enterocolitica* phenotypical resistance by disk diffusion test from pork production chain samples (blue) and human samples (red). AMP: ampicillin - 10 µg, CRO: ceftriaxone - 30 µg, MEM: meropenem - 10 µg, IPM: imipenem - 10 µg, TMP: trimethoprim - 5 µg, SSS: sulfamethoxazole - 300 µg, TET: tetracycline - 30 µg, NAL: nalidixic acid - 30 µg, GEN: gentamycin - 10 µg, AMK: amikacin - 30 µg, CIP: ciprofloxacin - 5 µg, CHL: chloramphenicol - 30 µg, AZM: azithromycin - 15 µg, PB: polymyxin B - 300 UI. b) Y. enterocolitica genotypical resistance by ABRIcate software from pork porduction chain samples (blue) and human samples (red).

Table 3. Antibiotic resistance profiles (phenotypical and genotypical) of *Yersinia enterocolitica* isolates obtained from a pork production chain and human clinical isolates in Brazil.

Method	n of simultaneous resistance	profile *
disk diffusion	11	AMP - CRO - MEM - TMP - TET - NAL - SSS - GEN - CHL - AZM - PB **
	5	AMP - TET - NAL - SSS - CHL **
		AMP - TMP - NAL - SSS - PB **
	4	AMP - MEM - NAL - SSS **
		AMP - TMP - NAL - SSS **
	3	AMP - NAL - SSS
		AMP - SSS - CHL
		AMP - TMP - NAL
		AMP - TMP - SSS
	2	AMP - SSS
		AMP - TMP
WGS	14	*vat(F) - floR - tet(A) - tet(R) - strB - strA - sul2 - crp - y56 beta-lactamase - rosA - rosB - aph(3")-lb - aph(6)-ld - blaA*
	10	*drfA1 - sat-2 - aadA - vat(F) - crp - y56 beta-lactamase - ant(3")-lla - rosA - rosB - blaA*
	6	*vat(F) - crp - y56 beta-lactamase - rosA - rosB - blaA*

* AMP: ampicillin - 10 µg, CRO: ceftriaxone - 30 µg, MEM: meropenem - 10 µg, IPM: imipenem - 10 µg, TMP: trimethoprim - 5 µg, SSS: sulfamethoxazole - 300 µg, TET: tetracycline - 30 µg, NAL: nalidixic acid - 30 µg, GEN: gentamycin - 10 µg, AMK: amikacin - 30 µg, CIP: ciprofloxacin - 5 µg, CHL: chloramphenicol - 30 µg, AZM: azithromycin - 15 µg, PB: polymyxin B - 300 UI

** Multidrug resistant isolates, due to phenotypical resistance to three or more antibiotic classes, except for ampicillin (beta-lactam)

4. Discussion

Y. enterocolitica was responsible for 15 outbreaks and 149 cases of the reported in 2019 for food-borne diseases in Europe (EFSA, 2021). This foodborne pathogen is usually associated with pork products and fresh vegetables (ESPENHAIN et al., 2019; GUPTA et al., 2015), and pathogenic strains are commonly identified based on their bio-serotype characterization (ZADERNOWSKA et al., 2014). The majority of yersiniosis cases are associated with the bio-serotype 4/O:3, being often isolated and identified in clinical and food samples (BOTTONE, 2015).

Our analysis shows that there is limited genetic variation amongst isolates of *Y. enterocolitica* biotype 4/O:3 circulating in a Minas Gerais pork production chain (Figure 1, Table 1). *Yersinia* isolates naturally share a central genome with approximately 71% sequence identity (SHI et al., 2016). The genetic stability of *Y. enterocolitica* was also demonstrated by GARZETTI et al. (2012), who found that bio-serotype 1B/O:8 isolates were largely homogenous except for the presence or absence of pathogenic and prophage islands. In our study, R31 was the only isolate that did not present close similarity with other isolates, but it was highly similar to a *Y. enterocolitica* isolates obtained from a swine farm in the United Kingdom (*Y. enterocolitica* YE208/02, Table 3). Despite the European origin of both groups, it may be an indicative of infection of non-Brazilian origin.

Hunter et al. (2019) characterized 158 isolates of *Y. enterocolitica* from human clinical samples from England and found 86 different STs; most isolates were ST-18 (n = 25) and one was ST-3, as in the present study. In England, *Y. enterocolitica* from different bio-serotypes and STs were already described in clinical samples (HUNTER et al., 2019), but in Brazil a few reports only describe bio-serotype 4/O:3 as the causative agent of diagnosed yersiniosis (FRAZÃO & FALCÃO, 2015).

Based on *Y. enterocolitica* IP29610 genome, all isolates were characterized as part of a group, except for R31 (Figure 1). Pathogenic biotypes of *Y. enterocolitica* present low gene variability, suggesting little functional or metabolic differences are present (HALL et al., 2015). The differences presented by the isolate R31 after WGS analysis can be explained in part by the presence of phages in its genome (Table 2), as previously reported (LIANG et al., 2019). Phages are closely related to the acquisition of new phenotypic elements by bacteria (PLEŠKA et al., 2018), and their stable presence in the genome can be a useful tool for epidemiological surveillance of pathogens. R31 also presented high similarity to *Y. enterocolitica* YE208/02

genome by ortholog and SNP's analyses, typical of serotype O:5 and corroborating the MLST analysis. However, these results are in not in agreement with the previous bio-serotyping of this isolate by phenotypical and molecular assays, that lead its identification as bio-serotype 4/O:3 (MARTINS et al., 2021). The limitations of phenotypic characterization of enterobacteria, including *Y. enterocolitica*, are widely known, especially for serotyping (PORNSUKAROM et al., 2018).

Yersiniosis is characterized as a gastrointestinal disease, usually with no need for antibiotic therapy except severe cases are often treated with ciprofloxacin (BOTTONE, 2015). Notably, all isolates from the current study were susceptible to this antibiotic although over half were resistant to nalidixic acid (a quinolone). *Y. enterocolitica* is usually susceptible to aminoglycosides, chloramphenicol, tetracyclines, third-generation cephalosporins and fluoroquinolones (FÀBREGA & VILA, 2012). β-lactams, such as ampicillin, and first-generation cephalosporins are not indicated to be used in patients with yersiniosis due to the intrinsic production of β-lactamases by this species (CLSI, 2021).

Antibiotic selection pressure in Brazilian swine production may contribute to the emergence and maintenance of antimicrobial resistance in *Y. enterocolitica* (SIMONOVA et al., 2008), including previously documented use of amoxicillin, sulfamethoxazole, tetracycline, chloramphenicol and nalidixic acid (SCHMITHAUSEN et al., 2018; Tang et al., 2017). Although some authors consider the intensive use of antibiotics an important selective factor for resistant bacteria in animal production (VAN BOECKEL et al., 2017), the presence of antibiotic-resistant bacteria can reflect transmission dynamics within and between host populations more than antibiotic selection pressure (SUBBIAH et al., 2020; CAUDELL et al., 2018).

The resistance to ampicillin was consistent with the presence of *bla(A*) for all of the genome sequences. The expression of this gene varies among *Yersinia* strains, depending mainly on the subtype and geographical origin of the isolates. *Y. enterocolitica* isolates obtained from swine and poultry usually present resistance to ampicillin; similarly, isolates from humans also present a high rate of resistance to ampicillin (ÖZDEMIR et al., 2020). All isolates harbored y56-beta-lactamase, a heterogeneous *blaA* gene among *Y. enterocolitica* from biotype 1A, and presenting low homology to other Enterobacteriaceae β-lactamase-related genes (SHARMA et al., 2006). These results are in accordance with other studies, especially because of the intrinsic resistance to β-lactams by *Y. enterocolitica* (PENG et al., 2018).

Our panel of *Y. enterocolitica* isolates presented a diversity of aminoglycosides resistance related genes (Figure 2b, Table 3). *aph(3'')-Ib* was identified in nine isolates, being associated

to aminoglycoside resistance and often detected in conjunction with extended-spectrum beta-lactamase (ESBL) expressing bacteria. This genes is frequently associated with *aph(3")-Ib* and *aph(6)-Id* that confer resistance to streptomycin through a phosphotransferase (ASHENAFI et al., 2014). *aadA* also confers resistance to aminoglycosides due to the expression of an adenyltranspherase and commonly identified in *Salmonella* isolates from swine (LOPES et al., 2016). *ant(3")-lla* codes for nucleotidyltransferases and it is horizontally transferred in *Acinetobacter* (ZHANG et al., 2017). Despite the presence of various genes, only a few isolates presented phenotypical resistance to aminoglycosides, such as gentamicin (Figure 1a). The identification of various aminoglycosides related genes in the *Y. enterocolitica* isolates can be associated with their usual localization in mobile genetic elements and the use of this antibiotic class in the yersiniosis treatment over time (YOUNIS et al., 2021).

The resistance to tetracycline and the presence of *tet(A)* in *Y. enterocolitica* were described in some studies that associate these results with the intensive use of this antibiotic class in animal production (PENG et al., 2018). *tet(A)* encodes a tetracycline efflux pump in Gram-negative bacteria, especially *Salmonella*, and it is highly associated with bacteria isolated from animal production, however, its solely presence does not reflect tetracycline resistance in the isolates, demanding a deep analysis to elucidate the resistance mechanisms in the studied organisms (OLIVEIRA et al., 2020).

strA–strB cluster has been identified in *E. coli* isolated from the intestine of healthy pigs with a history of antibiotic treatment. These genes are associated with streptomycin resistance in Gram-negative bacteria, including commensal organisms from animals, humans and plants and indicating the environmental distribution of such resistance (SALINAS et al., 2019). *sul2* confers resistance to sulfonamides, and it is often detected in the upstream region of the *strA–strB* cluster (OKUBO et al., 2019). Also, *sul2* can be located in plasmids and chromosomes, indicating efficient ways for transferring among Gram-negative bacteria (JIANG et al., 2019). *drfA1* is usually identified in plasmids and integrons, and it is often identified associated to *sul*, a finding that we did not observe in this study (Table 3); it is associated with resistance to sulfonamides and trimethoprim, increasing the emergence, evolution and dissemination of resistance to sulfonamides, especially in aquatic environments (DOMÍNGUEZ et al., 2019).

floR is capable of exporting florfenicol and chloramphenicol by efflux pumps, the most important mechanism associated with multi-drug resistance cluster (MICHAEL et al., 2008). *rosA* and *rosB* genes confers resistance to cationic antimicrobial peptides (CAMPs), commonly described in pathogenic bacteria, and associated with an efflux pump/potassium antiporter

system; these systems are active at 37 °C, but can be induced due the presence of CAMPs, such as polymyxin B (SKURNIK & BENGOECHEA, 2003).

vat(F) and *sat-2* confer resistance to macrolides, such as virginiamycin that is widely used as a growth promoter in pigs (SEOANE & GARCÍA LOBO, 2000; TEKEDAR et al., 2020). All *Y. enterocolitica* isolates harbored *vat(F)* but *sat-2* was not fully associated (Figure 2b). *sat-2* is commonly identified in *Y. enterocolitica* and also in plasmids of Gram-positive bacteria, suggesting a common origin for this gene (SEOANE & GARCÍA LOBO, 2000). Despite the presence of macrolides resistance related genes, *Y. enterocolitica* isolates did not present resistance to azithromycin at high frequency (Figure 2a, Table 3).

crp is associated with resistance to macrolides, fluoroquinolones and β-lactams in *E. coli*, being enrolled in a multi-drug efflux pump system, being therefore a good indicator of multidrug resistance in bacteria (NISHINO et al., 2008). *crp* role and activity in *Y. enterocolitica* need to be further investigated but based on the obtained results it can also be a reliable indicative of multidrug resistance (Table 3). Deeper analysis based on genomics can reveal some evolutionary aspects of the persistent and multidrug resistant strains, allowing a proper comprehension about how this feature is successfully present in the pork production chain and human samples, and how it can reach humans through pork products (PENG et al., 2018). These studies must be able to relate phenotypic to genotypic characteristics, seeking to clarify the multidrug resistance mechanisms in *Y. enterocolitica* (TYSON et al., 2015).

Isolates from swine production samples presented high frequencies of antibiotic resistance features (Figure 2, Table 3), what can contribute significantly with the increase of horizontal gene transferring among pathogens and commensal bacteria naturally present in this production chain (KOCH et al., 2017). Adequate and conscient use of antibiotics in the animal production can contribute with the maintenance of the effectivity of such substances to control animal diseases, as well as reducing the risk of bacterial resistance.

The close genetic relationship among *Y. enterocolitica* isolates obtained from the pork production chain and human samples was confirmed in Brazil. In addition, the results obtained in this study showed the highly related proximity among isolates of the pork production chain and clinical human samples, suggesting the potential for transmission among these sources in Brazil. The antibiotic resistance profiles of *Y. enterocolitica* were characterized through a phenotypical approach and based on WGS, indicating the remarkable multidrug resistance of isolates, a worldwide concern.

Acknowledgments

The authors are thankful for the financial support provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, DF, Brazil - financial code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, DF, Brazil) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, MG, Brazil).

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5. Conclusões gerais

Nesse trabalho foi possível confirmar a relevância da região cranial da carcaça como ponto de contaminação por *Y. enterocolitica* no abate de suínos e ambiente de processamento. Ainda, um clone de *Y. enterocolitica* 4/O:3 foi caracterizado na cadeia produtiva de suínos na região estudada, com alta similaridade genética com isolados obtidos em amostras de humanos com o mesmo bio-sorotipo em diferentes regiões do Brasil. Esses achados evidenciam uma possível associação entre a cadeia produtiva de suínos e casos de yersioniose humana no Brasil. Em complementação, os isolados de *Y. enterocolitica* obtidos da cadeia produtiva de suínos apresentaram variados perfis de resistência a antimicrobianos, sugerindo uma possível interferência de práticas de manejo durante a vida produtiva dos animais para desenvolvimento dessas características. Além de contribuir com informações epidemiológicas sobre *Y. enterocolitica* no Brasil, esse estudo deixa evidente a importância da associação de metodologias para avaliações robustas sobre inocuidade de alimentos, filogenética, epidemiologia molecular, resistência a antibióticos e saúde única.

Supplementary Table

Supplementary Table. Genome sequencing and assembly parameters of *Yersinia enterocolitica* bio-serotype 4/O:3 whole genome sequencing obtained from pork production chain a

isolate id	Isolates information				Genome Assembly Statistics	
	origin	Brazilian state	year	reference	number of reads (R1 and R2)	size in pb (R1 and R2)
CLIST1571	human blood bag	Paraná	2008	FIOCRUZ	1802852	150
CLIST2175	human feces	Bahia	2009	FIOCRUZ	1980133	150
CLIST2189	human rectal swab	Minas Gerais	2011	FIOCRUZ	1512997	150
12	mesenteric lymph node	Minas Gerais	2016	Martins et al., (2018)	554968	38-251
13	mesenteric lymph node	Minas Gerais	2016	Martins et al., (2018)	1867113	150
23	tonsil	Minas Gerais	2016	Martins et al., (2018)	1077893	38-251
25	tonsi	Minas Gerais	2016	Martins et al., (2018)	2413852	150
26	tonsil	Minas Gerais	2016	Martins et al., (2018)	587626	36-251
27	tonsil	Minas Gerais	2016	Martins et al., (2018)	1324205	150
51	carcass after bleeding	Minas Gerais	2016	Martins et al., (2018)	1037892	150
52	carcass after bleeding	Minas Gerais	2016	Martins et al., (2018)	1677094	150
72	mesenteric lymph node	Minas Gerais	2016	Martins et al., (2018)	808263	35-251
85	tonsil	Minas Gerais	2016	Martins et al., (2018)	1663398	35-251
102	tonsil	Minas Gerais	2016	Martins et al., (2018)	919728	35-251
R31	head meat	Minas Gerais	2018	Martins et al., (2021)	2115566	237-251
R32	head meat	Minas Gerais	2018	Martins et al., (2021)	1291317	150
R33	palate	Minas Gerais	2018	Martins et al., (2021)	1592792	76-251
R34	palate	Minas Gerais	2018	Martins et al., (2021)	1690157	150
R36	palate	Minas Gerais	2018	Martins et al., (2021)	1967380	150
R40	tonsil	Minas Gerais	2018	Martins et al., (2021)	1580713	150
R42	tonsil	Minas Gerais	2018	Martins et al., (2021)	2121619	150
R45	tonsil	Minas Gerais	2018	Martins et al., (2021)	1488317	150
R50	tonsil	Minas Gerais	2018	Martins et al., (2021)	1934074	40-251
R53	tonsil	Minas Gerais	2018	Martins et al., (2021)	1919174	150
R56	tonsil	Minas Gerais	2018	Martins et al., (2021)	2545884	150
R58	tonsil	Minas Gerais	2018	Martins et al., (2021)	1813365	150

nd human cases in Brazil.

total of scaffolds	larger scaffold	total size in pb	% GC	N50	N75	L50	L75	N's / 100 kbp	mean of mapped reads
109	219911	4436469	46,96	70831	43486	19	39	4,51	111
105	259522	4430495	46,95	76011	47470	17	35	2,96	121
107	441534	4430889	46,95	74165	46168	17	36	3,7	92
98	270305	4404079	46,93	72258	43372	19	38	4,63	36
113	223659	4471759	46,88	76039	43389	19	38	3,82	113
94	406131	4472312	46,88	85242	50173	16	33	4,45	66
104	221753	4471798	46,88	76039	45575	18	37	4	140
90	680614	4403604	46,92	83934	50173	15	32	4,54	38
101	222900	4474823	46,89	76039	46546	18	37	4,34	294
112	263009	4514006	46,9	86739	43323	18	35	4,36	188
120	195665	4511635	46,9	70831	40690	20	41	3,83	282
92	272448	4440892	46,94	87038	56401	17	33	4,26	51
98	219698	4504483	46,9	84720	49017	19	36	4,95	52
94	280823	4443401	46,94	84720	49924	17	34	6,46	29
35	661877	4554533	47,14	240245	146668	7	13	2,2	64
107	220065	4623924	47,05	77580	45989	18	37	3,61	77
96	226056	4495404	46,91	74913	48019	18	37	5,56	50
105	224050	4494238	46,91	73697	49017	18	36	3,94	100
113	223302	4621318	47,05	72654	46315	19	38	3,42	118
108	218928	4621409	47,05	73696	43486	19	38	3,68	296
110	218845	4620124	47,05	73710	46039	18	38	4,29	124
116	219543	4625131	47,06	71748	43486	19	39	3,74	91
104	222540	4618145	47,05	76011	48019	19	38	4,35	56
105	220041	4621623	47,05	88200	47453	18	36	4,35	113
114	224167	4620734	47,05	73802	46325	19	38	4,13	141
116	220328	4622284	47,05	72264	43486	18	37	4,39	103

Species Identification with Jspecies	
Z-score	**Groups JS**
0,99975 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99995 (Yersinia enterocolitica IP10393)	Yersinia enterocolitica IP10393, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656 e Serrati
0,99995 (Yersinia enterocolitica IP10393)	Yersinia enterocolitica IP10393, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656 e Serrati
0,99994 (Yersinia enterocolitica IP10393)	Yersinia enterocolitica IP10393, Yersinia enterocolitica IP22276, Yersinia enterocolitica IP29610 e Serrati
0,99995 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP22276 e Serrati
0,99995 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP10393 e Serrati
0,99995 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP10393 e Serrati
0,99994 (Yersinia enterocolitica IP10393)	Yersinia enterocolitica IP10393, Yersinia enterocolitica IP22276, Yersinia enterocolitica IP29610 e Serrati
0,99995 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP10393 e Serrati
0,99996 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP10393 e Serrati
0,99996 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP22276 e Serrati
0,99994 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP10393, Yersinia enterocolitica IP26656 e Serrati
0,99997 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP10393 e Serrati
0,99995 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP10393, Yersinia enterocolitica IP26656 e Serrati
0,99985 (Yersinia enterocolitica YE208/02)	Yersinia enterocolitica YE208/02, Yersinia enterocolitica ERL053435, Yersinia enterocolitica IP26618 e S
0,99976 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99996 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP10393 e Serrati
0,99997 (Yersinia enterocolitica IP29610)	Yersinia enterocolitica IP29610, Yersinia enterocolitica IP26656, Yersinia enterocolitica IP10393 e Serrati
0,99974 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99975 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99975 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99974 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99974 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99975 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99975 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati
0,99975 (Yersinia enterocolitica IP26656)	Yersinia enterocolitica IP26656, Yersinia enterocolitica IP29610, Yersinia enterocolitica IP21447 e Serrati

Access code of the most similar genomes on GenBank and ENA	Mean size of the genomes in pb	Mean scaffolds of the similarity genomes in pb
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001354595.1, GCA_001124705.1, GCA_001354615.1 e GCA_001006005.1	4443858	131
GCA_001354595.1, GCA_001124705.1, GCA_001354615.1 e GCA_001006005.1	4443858	131
GCA_001354595.1, GCA_001107025.1, GCA_001124705.1 e GCA_001006005.1	4416665	122
GCA_001124705.1, GCA_001354615.1, GCA_001107025.1 e GCA_001006005.1	4455495	131
GCA_001124705.1, GCA_001354615.1, GCA_001107025.1 e GCA_001006005.1	4443858	131
GCA_001124705.1, GCA_001354615.1, GCA_001107025.1 e GCA_001006005.1	4443858	131
GCA_001354595.1, GCA_001107025.1, GCA_001124705.1 e GCA_001006005.1	4416665	122
GCA_001124705.1, GCA_001354615.1, GCA_001107025.1 e GCA_001006005.1	4443858	131
GCA_001124705.1, GCA_001354615.1, GCA_001354595.1 e GCA_001006005.1	4443858	131
GCA_001124705.1, GCA_001354615.1, GCA_001107025.1 e GCA_001006005.1	4455495	131
GCA_001124705.1, GCA_001354595.1, GCA_001354615.1 e GCA_001006005.1	4443858	131
GCA_001124705.1, GCA_001354615.1, GCA_001354595.1 e GCA_001006005.1	4443858	131
GCA_001124705.1, GCA_001354595.1, GCA_001354615.1 e GCA_001006005.1	4443858	131
GCA_001217745.1, GCA_001085265.1, GCA_001135745.1 e GCA_001006005.1	4644053	30
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001124705.1, GCA_001354615.1, GCA_001354595,1 e GCA_001006005.1	4443858	131
GCA_001124705.1, GCA_001354615.1, GCA_001354595.1 e GCA_001006005.1	4443858	131
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130
GCA_001354615.1, GCA_001124705.1, GCA_001220085.1 e GCA_001006005.1	4503172	130

Mean GC percentage of similar genomes	Quantity of N's	ANIb	Probable species according to ANIb	total of bp aligned with ANIb	ANIm
46,9	57 (198)	99,97	Yersinia enterocolitica IP26656	4423023 (95,73%)	99,97
46,92	61 (131)	99,97	Yersinia enterocolitica IP26656	4375157 (98,75%)	99,97
46,92	61 (164)	99,97	Yersinia enterocolitica IP26656	4368763 (98,60%)	99,97
46,94	78 (204)	99,97	Yersinia enterocolitica IP29610	4309223 (97,85%)	99,97
46,93	94 (171)	99,96	Yersinia enterocolitica IP29610	4380814 (97,97%)	99,97
46,92	61 (199)	99,96	Yersinia enterocolitica IP29610	4374753 (97,82%)	99,97
46,9	61 (179)	99,97	Yersinia enterocolitica IP29610	4377161 (97,88%)	99,97
46,94	79 (200)	99,96	Yersinia enterocolitica IP29610	4313288 (99,96%)	99,97
46,9	61 (194)	99,96	Yersinia enterocolitica IP29610	4374637 (97,76%)	99,97
46,92	61 (197)	99,96	Yersinia enterocolitica IP29610	4423515 (98,00%)	99,97
46,93	94 (173)	99,96	Yersinia enterocolitica IP29610	4420874 (97,99%)	99,86
46,92	61 (189)	99,96	Yersinia enterocolitica IP29610	4350571 (99,96%)	99,97
46,92	61 (223)	99,96	Yersinia enterocolitica IP26656	4430242 (98,35%)	99,97
46,92	61 (287)	99,96	Yersinia enterocolitica IP26656	4370152 (98,35%)	99,97
47,12	63 (100)	99,68	Yersinia enterocolitica YE208/02	4276456 (93,89%)	99,83
46,9	57 (167)	99,96	Yersinia enterocolitica IP26656	4435567 (95,93%)	99,97
46,92	61 (250)	99,97	Yersinia enterocolitica IP26656	4426874 (98,48%)	99,97
46,9	61 (177)	99,97	Yersinia enterocolitica IP26656	4434966 (98,68%)	99,97
46,89	57 (158)	99,97	Yersinia enterocolitica IP26656	4428064 (95,82%)	99,97
46,9	57 (170)	99,96	Yersinia enterocolitica IP26656	4433233 (95,93%)	99,97
46,9	57 (198)	99,97	Yersinia enterocolitica IP26656	4423023 (95,73%)	99,97
46,9	57 (173)	99,96	Yersinia enterocolitica IP26656	4431922 (95,82%)	99,97
46,9	57 (201)	99,97	Yersinia enterocolitica IP26656	4425179 (95,82%)	99,97
46,9	57 (201)	99,96	Yersinia enterocolitica IP26656	4425729 (95,76%)	99,97
46,9	57 (191)	99,96	Yersinia enterocolitica IP26656	4432477 (95,93%)	99,97
46,9	57 (203)	99,96	Yersinia enterocolitica IP26656	4428999 (95,82%)	99,97

Probably species according to ANIm	Total of bp aligned with ANIm	species confirmation	NCBI Accession number
Yersinia enterocolitica IP26656	4484507 (97,06%)	Yersinia enterocolitica	SAMN20061779
Yersinia enterocolitica IP26656	4426274 (97,97%)	Yersinia enterocolitica	SAMN20061780
Yersinia enterocolitica IP26656	4427394 (99,92%)	Yersinia enterocolitica	SAMN20061781
Yersinia enterocolitica IP29610	4367992 (99,18%)	Yersinia enterocolitica	SAMN19671755
Yersinia enterocolitica IP29610	4436728 (99,22%)	Yersinia enterocolitica	SAMN19671756
Yersinia enterocolitica IP29610	4436885 (99,21%)	Yersinia enterocolitica	SAMN19671757
Yersinia enterocolitica IP29610	4436738 (99,22%)	Yersinia enterocolitica	SAMN19671758
Yersinia enterocolitica IP29610	4367812 (99,19%)	Yersinia enterocolitica	SAMN19671759
Yersinia enterocolitica IP29610	4440164 (99,23%)	Yersinia enterocolitica	SAMN19671760
Yersinia enterocolitica IP29610	4478098 (99,20%)	Yersinia enterocolitica	SAMN19671761
Yersinia enterocolitica IP29610	4476634 (99,22%)	Yersinia enterocolitica	SAMN19671762
Yersinia enterocolitica IP29610	4405773 (99,21%)	Yersinia enterocolitica	SAMN19671763
Yersinia enterocolitica IP29610	4469806 (99,23%)	Yersinia enterocolitica	SAMN19671764
Yersinia enterocolitica IP29610	4407847 (99,20%)	Yersinia enterocolitica	SAMN19671765
Yersinia enterocolitica YE208/02	4302863 (94,47%)	Yersinia enterocolitica	SAMN19671766
Yersinia enterocolitica IP26656	4488376 (97,07%)	Yersinia enterocolitica	SAMN19671767
Yersinia enterocolitica IP29610	4474232 (99,53%)	Yersinia enterocolitica	SAMN19671768
Yersinia enterocolitica IP26656	4473576 (99,54%)	Yersinia enterocolitica	SAMN19671769
Yersinia enterocolitica IP26656	4485386 (99,06%)	Yersinia enterocolitica	SAMN19671770
Yersinia enterocolitica IP26656	4427394 (99,92%)	Yersinia enterocolitica	SAMN19671771
Yersinia enterocolitica IP29610	4436728 (99,22%)	Yersinia enterocolitica	SAMN19671772
Yersinia enterocolitica IP26656	4484821 (96,97%)	Yersinia enterocolitica	SAMN19671773
Yersinia enterocolitica IP26656	4482657 (97,07%)	Yersinia enterocolitica	SAMN19671774
Yersinia enterocolitica IP26656	4485573 (97,06%)	Yersinia enterocolitica	SAMN19671775
Yersinia enterocolitica IP26656	4484577 (97,05%)	Yersinia enterocolitica	SAMN19671776
Yersinia enterocolitica IP26656	4487217 (97,08%)	Yersinia enterocolitica	SAMN19671777