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Virulome and pathogenicity of carbapenem- and polymyxin-resistant priority pathogens

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Simplified version

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RESUMO

ESPOSITO, F.R.S. **Viruloma e patogenicidade de patógenos prioritários resistentes a carbapenem e polimixina**. 2023. 93f. Tese (Doutorado) - Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2023.

A convergência de hipervirulência e resistência a carbapenêmicos e polimixinas em patógenos considerados prioritários pela Organização Mundial da Saúde (OMS), tem começado a ser reportado mundialmente em isolados de origem humana-animal-ambiental, o que constitui um problema de saúde única. Como parte do projeto OneBR (<http://onehealthbr.com/>), no presente estudo foram sequenciados um total de 119 genomas completos de isolados de *Escherichia coli* ($n=1$), *Klebsiella pneumoniae* ($n=101$) e *Pseudomonas aeruginosa* ($n=17$) de origem humana, animal, ambiental e vegetal, a fim de identificar o fenômeno da convergência de virulência e resistência. Esta tese foi subdividida em seis capítulos, conforme apresentado a seguir: **Capítulo I - Revisão Bibliográfica e justificativa** - Apresenta a revisão de literatura atualizada sobre a patogenicidade, viruloma e convergência de virulência e resistência de isolados de *E. coli*, *K. pneumoniae* e *P. aeruginosa* resistentes aos carbapenêmicos e/ou polimixinas. **Capítulo II – Materiais e métodos** – Descrição dos materiais e métodos utilizados no decorrer do projeto. **Capítulo III - Análise genômica e fenotípica dos isolados de *E. coli* - Anexo 1 - Mucoviscosity induced by ciprofloxacin in CTX-M-15-producing *Escherichia coli* carrying the *Klebsiella pneumoniae* K23 gene cluster.** Artigo submetido no periódico *International Journal of Antimicrobial Agents* (Fator de Impacto 15.441), apresentando pela primeira vez o fenótipo de mucoviscosidade induzida pela presença de fluoroquinolonas, principalmente a ciprofloxacina, em um isolado multirresistente de *Escherichia coli* produtor de β -lactamase de espectro-estendido (ESBL), recuperado de folhas de espinafre. Interessantemente, realizando a análise do genoma deste isolado, genes de virulência responsáveis pela síntese de cápsula bacteriana pertencente ao sorotipo K23 foram identificados, corroborando o fenótipo observado anteriormente. Adicionalmente, através de experimentos realizados com célula semelhante a macrófagos do tipo RAW foi possível identificar maior evasão do sistema fagocitário por células previamente expostas à ciprofloxacina, sugerindo que cápsula sintetizada é funcional. Notavelmente, este se trata do primeiro relato de fenótipo de mucoviscosidade em isolados de *E. coli* induzido por fluoroquinolonas, bem como a identificação do sorotipo capsular K23 em isolados de *E. coli*. **Capítulo IV - Análise genômica e fenotípica dos isolados de *K. pneumoniae* - Anexo 2 - Genomic analysis of a Kpi (pilus system)-positive and CTX-M-15-producing *Klebsiella pneumoniae* belonging to the high-risk clone ST15 isolated from an impacted river in Brazil.** Artigo publicado (<https://doi.org/10.1016/j.ygeno.2021.12.007>) no periódico *Genomics* (Fator de Impacto 4.31, Qualis CAPES A2), em 2022. **Anexo 3 - Expansion of healthcare-associated hypervirulent KPC-2-producing *Klebsiella pneumoniae* ST11/KL64 beyond hospital settings.** Artigo publicado (<https://doi.org/10.1016/j.onehlt.2023.100594>) no periódico *One Health Journal* (Fator de Impacto 9.00, Qualis CAPES A1), em 2023. **Capítulo V - Análise genômica e fenotípica dos isolados de *P. aeruginosa* - Anexo 4 - Genomic analysis of carbapenem-resistant *Pseudomonas aeruginosa* isolated from urban rivers confirms spread of clone sequence type 277 carrying broad resistome and virulome beyond the hospital.** Artigo publicado (<https://doi.org/10.3389/fmicb.2021.701921>) no periódico *Frontiers in Microbiology* (Fator de Impacto 6.064, Qualis CAPES A1), em 2021. **Capítulo VI – Considerações finais** – Principais resultados, contribuições e aplicações obtidas com o presente estudo.

Palavras-chave: Virulência, resistência antimicrobiana, WGS, *Galleria mellonella*, Saúde Única.

ABSTRACT

ESPOSITO, F.R.S. **Virulome and pathogenicity of carbapenem- and polymyxin-resistant priority pathogens.** 2023. 93p. Thesis (PhD.) - School of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2023.

The convergence of hypervirulence and resistance to carbapenems and to polymyxins in pathogens classified by the World Health Organization (WHO) as priority has been increasingly reported for human-animal-environmental isolates around the world lately, thereby indicating a one health problem. As an integral part of the OneBR project (<http://onehealthbr.com/>), in the present study, 119 whole genomes of human, animal, environmental, and plant isolates of *Escherichia coli* ($n=1$), *Klebsiella pneumoniae* ($n=101$), and *Pseudomonas aeruginosa* ($n=17$) were sequenced with the aim of identifying the convergence between virulence and resistance. This dissertation is organized into six chapters, as follows: **Chapter I – Literature review and research justification** – Up-to-date literature review on pathogenicity, virulome, and convergence in the virulence and resistance of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* isolates that are resistant to carbapenems and/or polymyxins. **Chapter II – Materials and methods** – Brief description of the materials and methods used in the research project. **Chapter III - Genomic and phenotypic analyses of *E. coli* isolates – Anexo 1 - Mucoviscosity induced by ciprofloxacin in CTX-M-15-producing *Escherichia coli* carrying the *Klebsiella pneumoniae* K23 gene cluster.** Written article, in the process of finalizing corrections for submission to the International Journal of Antimicrobial Agents (Impact Factor 15.441), presenting for the first time the mucoviscosity phenotype induced by the presence of fluoroquinolones, chiefly ciprofloxacin, in a multiresistant strain of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* isolated from spinach leaves. Interestingly, the genomic analysis of this isolate detected virulence genes in charge of the synthesis of the K23 capsule were identified, confirming the presence of the phenotype observed previously. Moreover, by way of experiments with cells that resembled RAW macrophages, it was possible to identify a larger escape from phagocytosis by cells that had been previously exposed to ciprofloxacin, suggesting that the synthesized capsule is functional. Notably, this is the first report of fluoroquinolone-induced mucoviscosity phenotype in *E. coli* isolates, and identification of K23 serotype in *E. coli* isolates. **Chapter IV – Genomic and phenotypic analyses of *K. pneumoniae* isolates** – the *K. pneumoniae* genome bank, known as KpBR (<http://onehealthbr.com/bacteria/KpBr>), was developed so as to contribute to the epidemiological surveillance health systems in the management and control of antimicrobial resistance at the human-animal-environmental interface in Brazil. **Appendix 2 - Genomic analysis of a *Kpi* (pilus system)-positive and CTX-M-15-producing *Klebsiella pneumoniae* belonging to the high-risk clone ST15 isolated from an impacted river in Brazil.** Article published (<https://doi.org/10.1016/j.ygeno.2021.12.007>) in *Genomics* (Impact factor: 4.31, Qualis CAPES A2), in 2022. **Appendix 3 - Successful expansion of healthcare-associated hypervirulent KPC-2-producing *Klebsiella pneumoniae* ST11/KL64 to impacted aquatic environment.** Article published (<https://doi.org/10.1016/j.onehlt.2023.100594>) in *One Health Journal* (Impact factor: 9.00), in 2023. **Chapter V – Genomic and phenotypic analysis of *P. aeruginosa* isolates** – The genome bank of *P. aeruginosa*, known as PaBr (<http://onehealthbr.com/bacteria/PaBr>), was developed to contribute to epidemiological surveillance health systems in the management and control of antimicrobial resistance at the human-animal-environmental interface in Brazil. **Appendix 4 - Genomic analysis of carbapenem-resistant *Pseudomonas aeruginosa* isolated from urban rivers confirms spread of clone sequence type 277 carrying broad resistome and virulome beyond the hospital.** Article published (<https://doi.org/10.3389/fmicb.2021.701921>) in *Frontiers in Microbiology* (Impact factor: 6.064, Qualis CAPES A1), in 2021. **Chapter VI – Final remarks** – Major findings, contributions, and applications obtained from the present study.

Keywords: Virulence, antimicrobial resistance, WGS, *Galleria mellonella*, One Health approach.