Impact of bacterial secondary resistome on antimicrobial susceptibility

Lancet Microbe 2025; 6: 101171
Published Online June 3, 2025
https://doi.org/10.1016/j.lanmic.2025.101171

Antimicrobial resistance (AMR) is a major global public health burden[1] and is mediated through five principal mechanisms: reduced permeability, active efflux, target modification, drug inactivation, and target bypass.[2] Each mechanism is primarily driven by specific resistance genes; however, the interplay between AMR determinants and other genetic elements within the bacterial genome remains poorly understood.

The current understanding of AMR restricts effective treatment options for bacterial infections because treatment options rely on antimicrobial agents that are losing effectiveness. Moreover, new drug development has been slow, with most available antimicrobials dating back to before the 1970s,[3] and many bacterial strains have already developed resistance to available antimicrobials. This scenario of antimicrobial resistance has driven research into alternative compounds such as antimicrobial peptides[3] and alkaloids,[4] which have showed efficacy in preventing but not treating infections. Another obstacle to antimicrobial drug discovery is the high financial cost. In 2016, the estimated cost of drug development was US$2·87 billion, with an annual increase of approximately 8.5%.[5] These economic challenges highlight the need for research strategies beyond identifying or designing new pharmaceuticals without an in-depth understanding of microbial physiology or only focusing on targeting AMR determinants. This perspective has led to studies focusing on the development of alternative approaches to resensitise bacteria to known drugs,[6] such as phage therapy[7] or targeted gene deletions.[8]

Advances in genomics have increasingly revealed the role of several non-primary resistance genes that contribute to AMR and, together, form the secondary resistome (appendix p 2).[2,8] For example, studies focusing on secondary resistome have shown that *ded*A, encoding a putative integral membrane protein whose deletion restores colistin susceptibility;[8] *ccd*B, associated with plasmid maintenance; and *rep*A2, encoding the replication initiation protein that controls IncFII plasmid copy number,[9] favour the expression of primary resistome in the plasmid. These findings highlight the underexplored role of non-essential genes (the secondary resistome) in AMR. In this Comment, we discuss secondary resistome as a potential alternative for identifying novel antimicrobial targets, developing alternative treatment strategies, and discovering new biomarkers for AMR diagnosis.[8] However, studies on secondary resistome are emerging and have focused on a few species such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The narrow range of known secondary resistome-associated genes hinders the comprehension of their functional behaviour, particularly how their expression varies across bacterial species, irrespective of their evolutionary relationships.

In a study of *Salmonella enterica* serovars, the acquisition of the IncB/O plasmid pH2332-166 via conjugation resulted in up to a 4096-fold increase in the minimal inhibitory concentration for β-lactams (from 4 μg/mL to 16 384 μg/mL for ampicillin) compared with the respective recipient bacteria. However, compared with the original donor (*E coli* H2332), the minimal inhibitory concentration values in transconjugant *Salmonella* strains increased by 16-fold (from 2 μg/mL to 32 μg/mL for ceftazidime).[10] The findings indicate that the same plasmid, harbouring AMR determinants, can confer elevated resistance levels when introduced into different bacterial genera. We hypothesise that such variable increases in resistance levels might be directly linked to the secondary resistome and its expression.

Nonetheless, the study[10] did not investigate the specific mechanisms underlying the increase in minimal inhibitory concentrations, which is a limitation of the study; however, the study generated the hypothesis that resistance determinants can interact with other genes—the secondary resistome—thereby influencing antimicrobial susceptibility. Although a previous study[6] investigated secondary resistome, the role of non-essential genes that contribute to AMR remains insufficiently explored. This gap in knowledge highlights the need for a comprehensive approach to thoroughly characterise most secondary resistome-associated genes and their functions in other pathogenic bacteria, especially those crucial for guiding strategies against AMR, such as *Salmonella* spp. Furthermore, the insufficient focus on non-essential genes emphasises the importance of developing a robust technical workflow to decipher secondary resistome and

See Online for appendix

identify potential targets for therapeutic intervention, which could subsequently be translated into clinical or practical applications.

We propose that more emphasis be placed on the role of non-essential genes in conferring or enhancing AMR to elucidate the potential clinical applications of non-essential genes in treating infections. Moreover, we intend to bring attention to this underexamined topic and explore a new approach to combat AMR through three key objectives: first, to identify the genes constituting the secondary resistome; second, to elucidate existing species-specific differences in secondary resistome mechanisms; and third, to understand how secondary and primary resistance genes interact. Advancing knowledge in this topic could inform the development of novel antimicrobial targets and treatment strategies, which are increasingly accessible through progress in genomics and ultimately contribute to mitigating the impact of AMR.

Authors declare no competing interests. VAF and MMSS contributed equally. We wish to express our thanks for the financial support through Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, financial code 001 and grant number 88887.107596/2025-00), São Paulo Research Foundation (FAPESP) [grant number 2024/10213-6], and Conselho Nacional de Pesquisa e Desenvolvimento (CNPq, Proc. 175352/2023-2).

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