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COMMENTARY

The Impact of Natural Transformation on the Acquisition of Antibiotic Resistance Determinants

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ABSTRACT Carbapenem and multidrug-resistant (MDR) *Acinetobacter baumannii* leads the World Health Organization's list of priority pathogens and represents an unmet medical need. Understanding the mechanisms underpinning the acquisition of antibiotic resistance in this pathogen is fundamental to the development of novel therapeutics as well as to infection prevention and antibiotic stewardship strategies designed to limit its spread. In their investigation, "Interbacterial Transfer of Carbapenem Resistance and Large Antibiotic Resistance Islands by Natural Transformation in Pathogenic *Acinetobacter*," Anne-Sophie Godeux and colleagues (mBio 13:e0263121, 2022, https://doi.org/10.1128/mBio.02631-21) delineate the unsuspected extent and circumstances under which natural transformation as a mechanism of intraspecies and interspecies exchange of genetic material occurs in *Acinetobacter* spp. This study offers key insights into how this notorious pathogen may have accelerated the development of its MDR phenotype via an unexpectedly robust and unnervingly casual approach to the acquisition of antibiotic resistance determinants through natural transformation.

KEYWORDS *Acinetobacter*, antibiotic resistance, multidrug resistance, natural transformation systems, pathogenicity islands

The transition from the 20th to the 21st century saw the global emergence of multidrug-resistant (MDR) *Acinetobacter baumannii* as a successful nosocomial pathogen (1). The outbreak of MDR *A. baumannii* associated with military operations in Iraq and Afghanistan generated special interest in this organism within the United States (2). At the same time, MDR and carbapenem-resistant *A. baumanni* became endemic in hospitals in New York and other locations in the country (3). Early on, enhanced infection control measures and improved use of antibiotics were adopted to control MDR *A. baumannii*; focus on the latter aspect represented an *avant la lettre* declaration of antibiotic stewardship principles (4). More recently, the disruption of these practices during the COVID-19 surge led to an increase in *A. baumannii* infections in a hospital in New Jersey (5).

As clinicians, we experienced first-hand the challenge that carbapenem-resistant *A. baumannii* posed to elderly patients with multiple comorbidities who frequently circulated between acute and long-term care facilities (6). We witnessed prolonged hospitalizations and devastating mortality because then, as now, effective antibiotics to treat MDR *A. baumannii* were lacking. While some patients died receiving ineffective empirical antibiotic therapy, others succumbed despite receiving "active" combinations of antibiotics such as polymyxins, meropenem, and tigecycline. Those who survived often persisted with colonization or were reinfected. Corresponding with other genetic descriptions of carbapenem-resistant *A. baumannii* in the United States, the acquired carbapenemases OXA-23 and OXA-24/40 often (though not always) underpinned the carbapenem-resistant phenotype. Similarly, genotyping tools available at the time

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revealed the predominance of strains related to the global clone 2, but also a large degree of heterogeneity.

The advent of whole-genome sequencing permitted rigorous analyses of these clinical strains and insights into their variation and dissemination (7). Multiple founder and independent strains occurred within the same patients, and the presumed persistence of *A. baumannii* was actually reinfection. Within a collection of clinical strains from the same health care system, a propensity toward extensive horizontal gene transfer was shown, including large genomic islands composed of transposable elements rife with antibiotic resistance determinants. Coinfecting strains within the same patient were implicated in horizontal gene transfer through plasmid exchanges. In another instance, coinfecting strains had differences in resistance islands and plasmid contents that could not be explained by gene loss alone. Nevertheless, the precise mechanism underlying such extensive gene transfer was elusive: perhaps natural transformation was occurring? The role of transformation as a process by which exogenous DNA is taken up and integrated into the bacterial chromosome is not as widely appreciated in *A. baumannii* as it is among other bacterial pathogens such as *Streptococcus pneumoniae*, *Haemophilus*, and *Neisseria*, where it is a crucial mechanism of horizontal gene transfer (8).

In the article titled "Interbacterial Transfer of Carbapenem Resistance and Large Antibiotic Resistance Islands by Natural Transformation in Pathogenic *Acinetobacter*," published in mBio, Anne-Sophie Godeux and collaborators report experimental evidence that natural transformation is a main driver of recombination events among *A. baumannii* clinical isolates (9). Godeux and her co-authors describe recombination events occurring spontaneously in mixed bacterial populations, which resulted in the exchange of resistance determinants to carbapenems among different clinical strains of *A. baumannii*, and even to a different but clinically relevant species of *Acinetobacter* like *A. nosocomialis*. These phenomena included the efficient acquisition of large resistance islands such as AbaR4 and AbaR1, and large recombination tracts similar to those observed in the genomes of clinical isolates. The authors concluded that natural transformation is a principal driver of genome recombination and the horizontal transfer of determinants of antibiotic resistance in *A. baumannii*. How was this insight possible? Understanding how the acquisition of antibiotic resistance determinants occurred through genomic recombination required developing an innovative experimental system, also by Godeux and collaborators (10). They engineered a translational fusion between *A. baumannii* nucleoprotein and fluorescent protein and used flow cytometry to reliably detect transformation events in *A. baumannii* isolates.

Previously, only certain species of *Acinetobacter* were studied for their ability to undergo natural transformation. For instance, *Acinetobacter baylyi* strain ADP1 displayed remarkable competence, being up to 100 times as competent as calcium chloride-treated *Escherichia coli* (11). Genomic sequencing of ADP1 detected gene clusters involved in competence (*comFECB* and *comQLONM*) which allow DNA uptake from the environment. Comparative genomic analysis of clinical *A. baumannii* strains revealed that some important genes involved in DNA uptake were absent, but many others were present. Thus, *A. baumannii* is likely endowed with a different type of molecular machinery required to transport foreign DNA through outer and inner membrane transporters than *A. bailey* ADP1 (12).

Further understanding of the role of natural transformation in *A. baumannii* became possible after the discovery by Maria Soledad Ramirez and collaborators of a naturally competent, non-MDR clinical isolate of *A. baumannii*, strain A118, in the bloodstream of a patient from Argentina (13). Comparative genomic analysis of A118 revealed genes presumptively related to competence that shared between 94% and 100% of amino acid identity among different *A. baumannii* genomes (14). An important exception was that A118 had an intact *comM* gene. Conversely, in almost all other clinical strains included in the comparison, *comM* was interrupted by the insertion of a resistance island. Experimentally, A118 was used to establish natural transformation as a

mechanism for *A. baumannii* to acquire mobile genetic elements and antimicrobial resistance genes from other species such as *Klebsiella pneumoniae* (15). Similarly, experiments using A118 as a model for natural transformation revealed that the expression of competence-related genes is increased with exposure to Ca^{2+} or serum albumin, present in blood and other human fluids (16). Interestingly, human serum albumin also altered genes which play roles in the persistence, pathogenicity, and antibiotic resistance of *A. baumannii* (17). Altogether, these observations in strain A118 contribute to our understanding of the virulence and resistance attributes of *A. baumannii* infection.

If natural transformation is a major mechanism of horizontal gene transfer which plays an important role in bacterial genomic diversification, it likely is because this process contributes to selective advantage and evolutionary success (8). This principle is illustrated by the observation that bacterial stress elicited by antibiotics of the fluoroquinolone and aminoglycoside classes induced natural transformation in *S. pneumoniae*, which lacks an SOS-like system (18). It has become a truism that selection and emergence of antibiotic resistant bacteria is a consequence of exposure to antibiotics; in the case of *A. baumannii*, natural transformation may be an important part of the story. Indeed, addition of meropenem to human serum albumin demonstrated synergistic enhancement of competence-associated genes as well as carbapenem-resistance genes in the A118 strain and, to a lesser extent, in a clinical strain (19). Polymyxins are cationic antimicrobial peptides that destabilize bacterial membranes and may facilitate both the release and uptake of DNA and thus potentiate horizontal gene transfer in *A. baumannii*; in a limited experimental model, polymyxins promoted low levels of transformation in *E. coli* not treated with calcium chloride (20). Furthermore, examination of microbial communities in different environments through metagenomic approaches provides fascinating insights into the role of antibiotics in the emergence of *A. baumannii*; cows untreated with antibiotics harbored communities of *Acinetobacter* spp. that were similar to those found in soil, whereas cows treated with antibiotics had overrepresentation of *Acinetobacter* taxa usually found in humans, including *A. baumannii* (21).

Since its irruption in the United States in the first decade of the 21st century, MDR *A. baumannii* may have peaked (22). Why is MDR *A. baumannii* retreating in the United States? We do not know, but we venture that fundamental changes may have occurred in the adaptability of *A. baumannii*. The work of Godeux and collaborators presents us with powerful tools and new knowledge to understand the roles of competence and natural transformation in the evolution of *A. baumannii*. Finally, while we still sorely need effective therapies against this devastating pathogen, a note of optimism may be introduced by the idea that perhaps we ourselves have changed *A. baumannii* by becoming more "competent" in infection control and antibiotic stewardship practices, a welcome, but frail and not at all "natural," transformation.

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REFERENCES

1. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 51:3471–3484. https://doi.org/10.1128/AAC.01464-06.
2. Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ, Ecker DJ, Massire C, Eshoo MW, Sampath R, Thomson JM, Rather PN, Craft DW, Fishbain JT, Ewell AJ, Jacobs MR, Paterson DL, Bonomo RA. 2006. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 50:4114–4123. https://doi.org/10.1128/AAC.00778-06.
3. Quale J, Bratu S, Landman D, Heddurshetti R. 2003. Molecular epidemiology and mechanisms of carbapenem resistance in *Acinetobacter baumannii* endemic in New York City. Clin Infect Dis 37:214–220. https://doi.org/10.1086/375821.
4. Urban C, Segal-Maurer S, Rahal JJ. 2003. Considerations in control and treatment of nosocomial infections due to multidrug-resistant *Acinetobacter baumannii*. Clin Infect Dis 36:1268–1274. https://doi.org/10.1086/374847.
5. Perez S, Innes GK, Walters MS, Mehr J, Arias J, Greeley R, Chew D. 2020. Increase in hospital-acquired carbapenem-resistant *Acinetobacter baumannii*

infection and colonization in an acute care hospital during a surge in COVID-19 admissions: New Jersey, February–July 2020. MMWR Morb Mortal Wkly Rep 69:1827–1831. https://doi.org/10.15585/mmwr.mm6948e1.
6. Perez F, Endimiani A, Ray AJ, Decker BK, Wallace CJ, Hujer KM, Ecker DJ, Adams MD, Toltzis P, Dul MJ, Windau A, Bajaksouzian S, Jacobs MR, Salata RA, Bonomo RA. 2010. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. J Antimicrob Chemother 65:1807–1818. https://doi.org/10.1093/jac/dkq191.
7. Wright MS, Haft DH, Harkins DM, Perez F, Hujer KM, Bajaksouzian S, Benard MF, Jacobs MR, Bonomo RA, Adams MD. 2014. New insights into dissemination and variation of the health care-associated pathogen *Acinetobacter baumannii* from genomic analysis. mBio 5:e00963-13. https://doi.org/10.1128/mBio.00963-13.
8. Smith JM, Dowson CG, Spratt BG. 1991. Localized sex in bacteria. Nature 349:29–31. https://doi.org/10.1038/349029a0.
9. Godeux A-S, Svedholm E, Barreto S, Potron A, Venner S, Charpentier X, Laaberki M-H. 2022. Interbacterial transfer of carbapenem resistance and large antibiotic resistance islands by natural transformation in pathogenic *Acinetobacter*. mBio 13:e0263121. https://doi.org/10.1128/mbio.02631-21.
10. Godeux A-S, Lupo A, Haenni M, Guette-Marquet S, Wilharm G, Laaberki M-H, Charpentier X. 2018. Fluorescence-based detection of natural transformation in drug-resistant *Acinetobacter baumannii*. J Bacteriol 200: e00181-18. https://doi.org/10.1128/JB.00181-18.
11. Metzgar D, Bacher JM, Pezo V, Reader J, Döring V, Schimmel P, Marlière P, de Crécy-Lagard V. 2004. *Acinetobacter* sp. ADP1: an ideal model organism for genetic analysis and genome engineering. Nucleic Acids Res 32: 5780–5790. https://doi.org/10.1093/nar/gkh881.
12. Smith MG, Gianoulis TA, Pukatzki S, Mekalanos JJ, Ornston LN, Gerstein M, Snyder M. 2007. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. Genes Dev 21:601–614. https://doi.org/10.1101/gad.1510307.
13. Ramirez MS, Don M, Merkier AK, Bistué AJS, Zorreguieta A, Centrón D, Tolmasky ME. 2010. Naturally competent *Acinetobacter baumannii* clinical isolate as a convenient model for genetic studies. J Clin Microbiol 48: 1488–1490. https://doi.org/10.1128/JCM.01264-09.
14. Traglia GM, Chua K, Centrón D, Tolmasky ME, Ramírez MS. 2014. Whole-genome sequence analysis of the naturally competent *Acinetobacter baumannii* clinical isolate A118. Genome Biol Evol 6:2235–2239. https://doi.org/10.1093/gbe/evu176.
15. Traglia GM, Place K, Dotto C, Fernandez JS, Montaña S, Bahiense CDS, Soler-Bistue A, Iriarte A, Perez F, Tolmasky ME, Bonomo RA, Melano RG, Ramírez MS. 2019. Interspecies DNA acquisition by a naturally competent *Acinetobacter baumannii* strain. Int J Antimicrob Agents 53:483–490. https://doi.org/10.1016/j.ijantimicag.2018.12.013.
16. Traglia GM, Quinn B, Schramm STJ, Soler-Bistue A, Ramirez MS. 2016. Serum albumin and Ca^{2+} are natural competence inducers in the human pathogen *Acinetobacter baumannii*. Antimicrob Agents Chemother 60: 4920–4929. https://doi.org/10.1128/AAC.00529-16.
17. Quinn B, Rodman N, Jara E, Fernandez JS, Martinez J, Traglia GM, Montaña S, Cantera V, Place K, Bonomo RA, Iriarte A, Ramírez MS. 2018. Human serum albumin alters specific genes that can play a role in survival and persistence in *Acinetobacter baumannii*. Sci Rep 8:14741. https://doi.org/10.1038/s41598-018-33072-z.
18. Claverys J-P, Prudhomme M, Martin B. 2006. Induction of competence regulons as a general response to stress in Gram-positive bacteria. Annu Rev Microbiol 60:451–475. https://doi.org/10.1146/annurev.micro.60.080805.142139.
19. Le C, Pimentel C, Tuttobene MR, Subils T, Nishimura B, Traglia GM, Perez F, Papp-Wallace KM, Bonomo RA, Tolmasky ME, Ramirez MS. 2021. Interplay between meropenem and human serum albumin on expression of carbapenem resistance genes and natural competence in *Acinetobacter baumannii*. Antimicrob Agents Chemother 65:e0101921. https://doi.org/10.1128/AAC.01019-21.
20. Viljanen P. 1987. The effect of polymyxin B nonapeptide (PMBN) on transformation. Biochem Biophys Res Commun 143:923–927. https://doi.org/10.1016/0006-291x(87)90338-x.
21. Garcia-Garcera M, Touchon M, Brisse S, Rocha EPC. 2017. Metagenomic assessment of the interplay between the environment and the genetic diversification of *Acinetobacter*. Environ Microbiol 19:5010–5024. https://doi.org/10.1111/1462-2920.13949.
22. Patel S, Wilson B, El Chakhtoura NG, Saade E, Donskey CJ, Perez F, Bonomo RA. 2017. Trends in the incidence of bacteremia with carbapenem non-susceptible Acinetobacter spp. in patients from the US Veterans Health Administration, 2000–2015. Open Forum Infectious Diseases 4 (Suppl):S152. IDWeek 2017, San Diego, CA, 4 to 8 October 2017.