

EDITORIAL



## Prevalence of *Acinetobacter baumannii* strains expressing the Type 6 secretion system in patients with bacteremia

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**ARTICLE HISTORY** Received 16 June 2017; Accepted 20 June 2017

**KEYWORDS** bacterial competition; biofilm; *hcp*; serum-resistance; virulence factors

*Acinetobacter baumannii* (Ab) is an important nosocomial pathogen, of major concern worldwide due to its multi-drug resistance and the recent appearance of hyper-virulent strains in the clinical setting. Ab multi-drug resistant (MDR) strains are frequently associated to different types of infections, such as pneumonia, skin burns, endocarditis, meningitis and septicemia, prevalent in intensive care units.<sup>1,2</sup> For these reasons, Ab has just been included by the World Health Organization in the list of critical priority pathogens for further studies and development of novel therapeutic approaches.<sup>3</sup> In this respect, advanced knowledge of Ab physiology and mechanisms involved in environmental persistence, host colonization and virulence, all of which could be included in what is known as the physiopathology of the microorganism,<sup>4</sup> is required to reduce the socio-economic impact caused by Ab infections.



Antibiotic resistance and epidemiology have been the focus of much of the scientific work on Ab, but little is known about the strategies this bacterium uses for pathogenesis. The precise elements involved in the establishment and progression of Ab infections remain up to date poorly characterized, as well as the mechanisms related to the secretion and delivery of virulence factors (VF) to host cells. In this context, several protein secretion systems classified as type 1, type 2 and type 6 (T6SS) as well as other mechanisms such as outer membrane vesicles, have been implicated in these processes in Ab.<sup>5</sup>

The T6SS is a cell envelope-spanning machine that translocates toxic effector proteins into eukaryotic and prokaryotic cells and has a pivotal role in pathogenesis and bacterial competition.<sup>6</sup> Most *Acinetobacter* species encode a T6SS cluster consisting of 18 genes, showing 2 different genetic organizations depending on the

analyzed species.<sup>7</sup> A mutational analysis in *A. baylyi* ADP1 identified 14 of these genes as critical for the secretion into the supernatant of the T6SS-hallmark component Hcp, which is indicative of an active T6SS apparatus.<sup>8</sup> Homologous genes have been identified in several Ab strains and variable Hcp secretion profiles detected.<sup>7,9,10</sup> Moreover, the genes encoding the VgrG-associated components of the system and several secreted toxins and cognate immunity proteins have been recently identified in Ab ATCC17978.<sup>8</sup> This machinery is used to outcompete other Ab strains and even different bacterial species, including the nosocomial pathogens *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. This capacity may confer a competitive advantage against co-existing bacteria in particular niches.<sup>8,9,10</sup>

In this issue, Kim and coworkers<sup>11</sup> analyzed the prevalence of the T6SS on an important number (162) of Ab clinical isolates obtained from patients with bacteremia in 2 tertiary-care hospitals in Korea. The conclusions they have drawn are relevant to the understanding the regulation and the role played by this VF during Ab infections.

The *hcp* gene was detected in approximately one-third (51/162, 31.5%) of the Ab clinical isolates (*hcp*<sup>+</sup>), and its presence showed a clear affiliation to particular STs. This observation is in agreement with genomic comparative analysis of phylogenetically- and epidemiologically-related Ab MDR strains showing that the T6SS gene cluster is only present in particular populations.<sup>12,13</sup> Also, it suggests that this system is not critical in the conditions prevailing in the nosocomial environment. Possible hypotheses on favor of this genetic loss are higher chances of evasion from the host immune system and/or

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Comment on: Kim J, et al. Microbiological features and clinical impact of the type VI secretion system (T6SS) in *Acinetobacter baumannii* isolates causing bacteremia. Virulence 2017; <https://doi.org/10.1080/21505594.2017.1323164>

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the lower possibilities of interbacterial competition over the course of antibiotic therapy.

They also report that *hcp* gene transcriptional expression varied among the *hcp*<sup>+</sup> clinical isolates. This might obey to the presence of different transcriptional regulation mechanisms. In terms of regulatory mechanisms operating on the T6SS recent studies have shown that a group of Ab MDR strains bear a large plasmid conferring antimicrobial resistance, which carries 2 genes (locus tags ACX61\_19615 and ACX61\_19655) encoding the TetR1 and TerR2-T6SS transcriptional repressors.<sup>10</sup> The model proposes the existence of a mix bacterial population in which plasmid-bearing cells (T6SS<sup>-</sup>) will resist antibiotic therapy, whereas those subject to plasmid loss (T6SS<sup>+</sup>) will be better prepared to engage in bacterial competition. In this way, both populations would protect each other.<sup>10</sup> The information related to the prevalence of the *tetR1* gene obtained by Kim and coworkers<sup>11</sup> is relevant to the understanding of T6SS regulation in Ab. The authors report that a *tetR1* gene homolog was only detected in 4 out of 51 *hcp*<sup>+</sup> strains, all belonging to the ST229. For this group of isolates, *hcp* gene transcriptional levels are at least 30 times lower than for the reference strain ATCC19606, thus likely indicating that they do not bear a functional T6SS in laboratory conditions (T6SS<sup>-</sup>). Remarkably, another 4 strains classified as *hcp*<sup>+</sup> T6SS<sup>-</sup> do not carry a *tetR1* gene copy. It would be interesting to determine if this phenotype is associated to the presence of a *tetR2* gene copy. Overall, these data suggest that TetR-dependent regulation of T6SS genes is specific of particular lineages and that other regulatory mechanisms could be operative in other strains.

An important limitation of this study lies on the criterion used by the authors to classify strains as T6SS<sup>+</sup> or T6SS<sup>-</sup>. First, it is directly related to the transcriptional expression of the *hcp* gene in laboratory conditions, ruling out the possibility that *hcp* transcript levels might vary during the infection process. Furthermore, they assume that those strains with high *hcp* transcriptional rates will accordingly display high Hcp secretion profiles, obviating the possibility that other T6SS-core genes could remain silent or that a post-translational regulation mechanism could modulate the activation of the secretory apparatus. The latter hypothesis has been raised in a recent study<sup>9</sup> in which the role of this macromolecular complex has been characterized in other 3 Ab MDR strains (242, 244 and 825). It was shown that the *hcp* and *tssM* genes are actively transcribed and Hcp protein produced in the bacterial cytoplasm. Nevertheless, these strains do not display an active T6SS apparatus in laboratory conditions. These data suggest a post-translational mechanism of control operating on the T6SS. As reported for other bacteria,<sup>14</sup> several proteins encoded within the T6SS-core gene cluster might

be responsible for this regulation. It is also possible that specific signals lacking under laboratory conditions are necessary to induce Hcp secretion, *i.e.* contact with the target cell or environmental signals. Elucidating the basis of these discrepancies are of particular interest in the context of competition for a specific environmental niche but mainly of polymicrobial nosocomial infections, which could result in horizontal transfer of genetic material and spread of antibiotic resistance.

Furthermore, the authors performed experiments on a subgroup of 8 clinical isolates demonstrating that 3 T6SS<sup>+</sup> strains could better out-compete *Escherichia coli*, showed higher biofilm-forming activity and evidenced better survival in the presence of normal human serum than the 5 T6SS<sup>-</sup> isolates analyzed. However, it is important to note that a direct correlation between the capacity of forming biofilm structures and T6SS activity has never been demonstrated before in Ab. Noteworthy, no differences in biofilm amount or structure were observed when wild type and T6SS-mutant strains of the environmental isolate Ab DSM30011 were compared,<sup>9</sup> which suggests that the T6SS plays diverse roles depending on the niche occupied by the strain. More studies with a larger set of Ab strains are needed to clarify this point.

In addition, they conducted a retrospective study to determine the clinical impact of T6SS<sup>+</sup> strains in patients with bacteremia caused by Ab. In the 92% of the cases, infection was acquired within the hospital. They concluded that the presence of a functional T6SS was not a prognostic factor for mortality, but contributes to infections in immune-compromised patients and those with implanted medical devices. In this context, the implication of this system in host colonization using the *Galleria mellonella* infection model has been demonstrated.<sup>9</sup> Therefore, a T6SS-dependent secretion of unidentified extracellular proteins or effectors contributing to the colonization of this particular group of patients cannot be ruled out.

This study opens up numerous avenues for further investigation. Additional studies are now needed to determine which is the role played by the T6SS during Ab infections of these specific patients and why are they more susceptible to Ab infections than other hospitalized patients.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Acknowledgments

I am indebted to Dr. Suzana P. Salcedo for her generosity and insightful suggestions to improve this manuscript.

## Funding

This work was supported by a grant from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT); Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-PICT2015-1072). GDR is a staff member of CONICET.

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