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SUPPLEMENT ARTICLE

Global Gene Expression Profile of *Acinetobacter baumannii* During Bacteremia

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Background. *Acinetobacter baumannii* is a pathogen of major importance in intensive care units worldwide, with the potential to cause problematic outbreaks and acquire high-level resistance to antibiotics. There is an urgent need to understand the mechanisms of *A. baumannii* pathogenesis for the future development of novel targeted therapies. In this study we performed an in vivo transcriptomic analysis of *A. baumannii* isolated from a mammalian host with bacteremia.

Methods. Mice were infected with *A. baumannii* American Type Culture Collection 17978 using an intraperitoneal injection, and blood was extracted at 8 hours to purify bacterial RNA for RNA-Seq with an Illumina platform.

Results. Approximately one-quarter of *A. baumannii* protein coding genes were differentially expressed in vivo compared with in vitro (false discovery rate, ≤0.001; 2-fold change) with 557 showing decreased and 329 showing increased expression. Gene groups with functions relating to translation and RNA processing were overrepresented in genes with increased expression, and those relating to chaperone and protein turnover were overrepresented in the genes with decreased expression. The most strongly upregulated genes corresponded to the 3 recognized siderophore iron uptake clusters, reflecting the iron-restrictive environment in vivo. Metabolic changes in vivo included reduced expression of genes involved in amino acid and fatty acid transport and catabolism, indicating metabolic adaptation to a different nutritional environment. Genes encoding types I and IV pili, quorum sensing components, and proteins involved in biofilm formation all showed reduced expression. Many genes that have been reported as essential for virulence showed reduced or unchanged expression in vivo.

Conclusion. This study provides the first insight into *A. baumannii* gene expression profiles during a life-threatening mammalian infection. Analysis of differentially regulated genes highlights numerous potential targets for the design of novel therapeutics.

Keywords. *A. baumannii*; RNA-Seq; in vivo transcriptome; pathogenesis; virulence factors.

Acinetobacter baumannii is a gram-negative opportunistic pathogen responsible for a range of healthcare-associated infections, burn and wound infections, and community-acquired pneumonia [1, 2]. *A. baumannii* has a high degree of genome plasticity, conferring a propensity for the rapid acquisition of antibiotic resistance mechanisms. The escalating rate of outbreaks of disease in hospitals, particularly in intensive care units, combined with a diminishing armament of treatment options, has many investigators looking for novel means to prevent transmission and to treat *A. baumannii* infection [2].

Despite increasing research efforts, a clear picture of the mechanisms of *A. baumannii* pathogenesis is still elusive [2]. Numerous virulence-related factors have been described, although much about the pathogenesis remains unknown. One promising method for discovery is through the analysis of whole-genome transcriptional profiles under different growth and environmental conditions. Previous analyses of the *A. baumannii* transcriptome have provided important insights into *Acinetobacter* biology, including growth in different phases and states (planktonic cells vs biofilm) [3], and response to serum [4], iron limitation [5], antibiotics [6], and interaction with the *Candida* quorum sensing compound farnesol [7]. Regulatory networks of key transcriptional regulators (*gacA*, H-NS) have also been studied [8, 9]. However, no study has directly confronted the most important transcriptional response: what happens in vivo? In the current study, we analyzed the *A. baumannii* transcriptional response to the most important, life-threatening *A. baumannii* infection, bacteremia.

METHODS

Bacterial Strains and Culture Conditions

A. baumannii American Type Culture Collection (ATCC) 17978 was used for the mouse infection and transcriptomic analyses. Bacteria were grown overnight in LB medium at 37°C and were then washed and resuspended in phosphate-buffered saline before preparation of the infecting inoculum.

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Murine Infection Model and Bacterial RNA Preparation

Experiments were approved by the Monash University Animal Ethics Committee. Two independent groups of mice (Balb/c, n = 3 per group) were inoculated with 5×10^5 colony-forming units (CFUs) of *A. baumannii* in 5% pig gastric mucin. After 8 hours, 100–400 µL of blood was collected per animal and placed in Trizol (Qiagen) for purification with the mirVana RNA purification kit (Ambion), followed by DNA removal with the Turbo DNA-*free* kit (Ambion). Bacteria were not separated from host cells before RNA purification. The mean bacterial densities in blood were 5×10^9 CFUs/mL for mice from group 1 and 10^9 CFUs/mL for those from group 2. Two control cultures (same inoculum) were grown in heart infusion medium (Oxoid) with aeration for the equivalent period of time (mean final bacterial density of 10^9 CFUs/mL). Analysis of RNA preparations by Bioanalyzer revealed distinct peaks for prokaryotic RNA (16S and 23S ribosomal RNA [rRNA]), host RNA (18S and 28S rRNA), and mixed RNA (messenger RNA and small RNA). Samples were pooled (n = 3 per replicate), and then enriched for bacterial messenger RNA by depletion of prokaryotic rRNA, eukaryotic rRNA, and globin RNA with use of the TruSeq Stranded Total RNA with Ribo-Zero Globin kit (Illumina). RNA was sized (200–500 bases) for library preparation.

RNA Sequencing and Analysis

Library preparation, sequencing (Illumina HiSeq2000) and preliminary processing of reads was conducted at the Beijing Genomics Institute. Sequences were mapped to the ATCC 17978 genome (accession No. NC_009085) using BWA software's aln aligner [10], with SAMtools v 1.2 for converting .sam to .bam files and generating mapping statistics. Mapped reads were counted using featureCounts v1.5.0-p1 from the Subread package [11]. For the in vitro replicates 18 909 245 (89.38%) and 17 681 468 (90.83%) of the reads were mapped to the reference sequences, and for the in vivo replicates 794 326 (4.63%) and 191 628 (1.20%) of the reads were mapped for pools 1 and 2, respectively. Raw read counts were analyzed using Degust software at the Victorian Bioinformatics Consortium (http://www.vicbioinformatics.com/software.degust.shtml) with voom normalization [12] and limma v 3.26.8 differential expression analysis [13]. Biochemical pathways were explored using the resources at the BioCyc Web site [14]. Cluster of orthologous groups (COG) enrichment analysis was performed by dividing the percentage of genes up- or down-regulated for each category by the percentage of genes in that category across the whole genome [15].

RESULTS AND DISCUSSION

A. baumannii Global Gene Expression Profile During Bacteremia

A. baumannii ATCC 17978 gene expression differed substantially between in vitro and in vivo environments. A total of 886 protein-coding genes were identified as differentially expressed by ≥2-fold (557 with increased expression, 329 with decreased expression) (Figure 1). This accounts for approximately one-quarter of the *A. baumannii* ATCC 17978 genome (Supplementary Table 1). Analysis of the differentially expressed genes by COG category identified a high proportion with increased expression that encoded proteins involved in translation and ribosomal function (J), RNA processing (A), cell division (D), and DNA replication (L) (Figure 2). Together these data suggest an increased growth rate for the bacteria in vivo compared with the in vitro culture. In contrast, genes encoding chaperones (O) were overrepresented in the down-regulated set, suggesting that they are less important for adaptation to the challenges of growing in vivo. Interestingly, whereas approximately one-quarter of the genome was differentially regulated, genes of the COG category transcription (K) were underrepresented.

Up-regulation of Iron Uptake Genes In Vivo

The majority of the genes showing highly increased in vivo expression were involved in iron import. Iron is essential for most bacteria and is highly restricted in the host environment as an innate immune defense mechanism; consequently, bacterial pathogens have evolved specific mechanisms for the acquisition of iron in vivo, including the synthesis of high-affinity iron chelating molecules called *siderphores*. *A. baumannii* ATCC 17978 has 3 clusters of genes involved in siderophore biosynthesis, along with multiple predicted siderophore import proteins [5]. Siderophore cluster 1 (A1S_1647–57; 5.5–124-fold change), siderophore cluster 2 (A1S_2562–81, 21–800-fold change), and the acinetobactin cluster (A1S_2372–92, 12–128-fold change) were all highly up-regulated in vivo, as well as a number of putative siderophore transport genes (Figure 2); most of these genes showed very low or negligible expression in iron-replete medium in vitro. Similar transcriptional changes have been previously observed with iron limitation [5]; most up-regulated genes were involved in siderophore synthesis and iron uptake. There were other notable differences between our in vivo transcriptional analysis and the in vitro low iron response; for example the hemerythrinlike protein (A1S_0891) was heavily down-regulated under iron limitation but unchanged in vivo. Furthermore, fumarase C showed unchanged expression in vivo but showed strongly increased expression (27-fold) with iron limitation. This clearly shows that although low iron levels are a critical component of the in vivo environment, other important signaling processes in vivo are shaping the global transcriptional response. Many genes involved in iron uptake were also up-regulated during growth in serum [4] and in biofilms [3].

Expression of Genes Encoding Known Virulence Factors

Numerous *A. baumannii* virulence factors with diverse functions have been identified as necessary for full virulence in animal infection models (Supplementary Table 2). Surprisingly, the majority of these factors showed either reduced or unchanged expression in vivo. There are a number of plausible explanations for this observation. First, these factors are specifically required for virulence, but in tissues other than

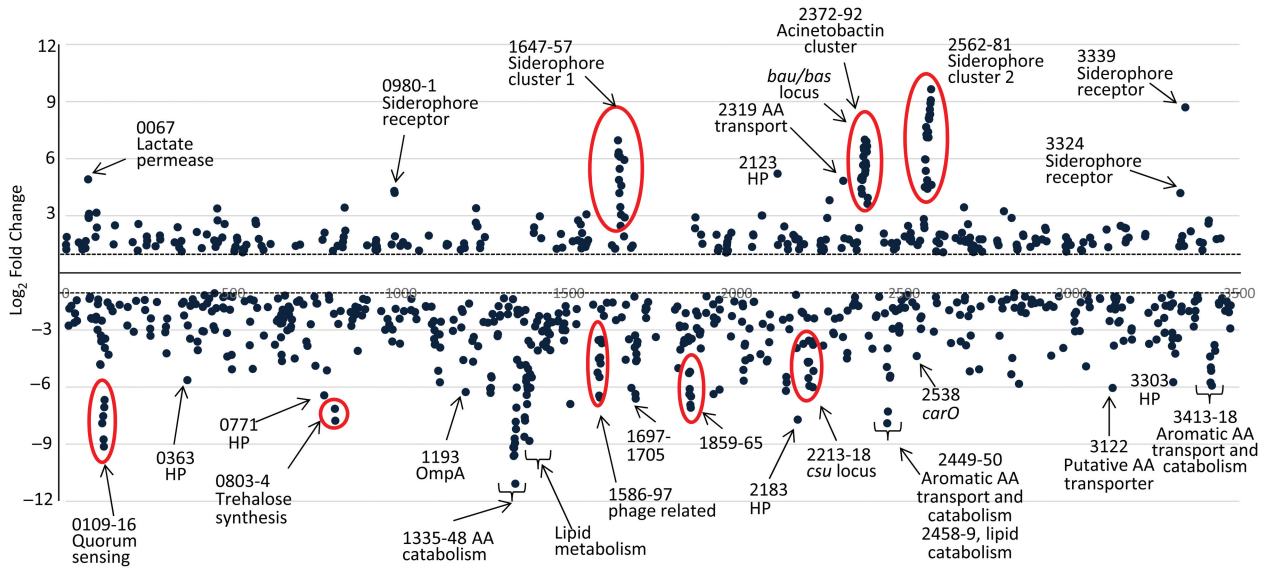

Figure 1. Plot of differential gene expression in vivo compared with in vitro with respect to gene locus tag number. The $\log_2$ fold change in expression for each gene meeting the study threshold ($\log_2$ fold change > 1, false discovery rate <0.001) was plotted against the gene locus tag number. Genes of interest are highlighted.

blood. Second, these factors are specifically required for disease in blood but are differentially expressed at an earlier stage of septicemia. Third, they are required in some but not all infection models [40], and the bacteremia model tested in the current study may differ from the model in which they were originally defined. Fourth, these factors may play a strain-specific role in virulence; the current study used ATCC 17978, whereas while other studies have used different strains of *A. baumannii*. For example, the type VI secretion systems of different strains of *A. baumannii* have different abilities to kill prokaryotic cells [22]. Finally, even expression at low levels might be sufficient to confer a virulence function. Indeed, reduced expression

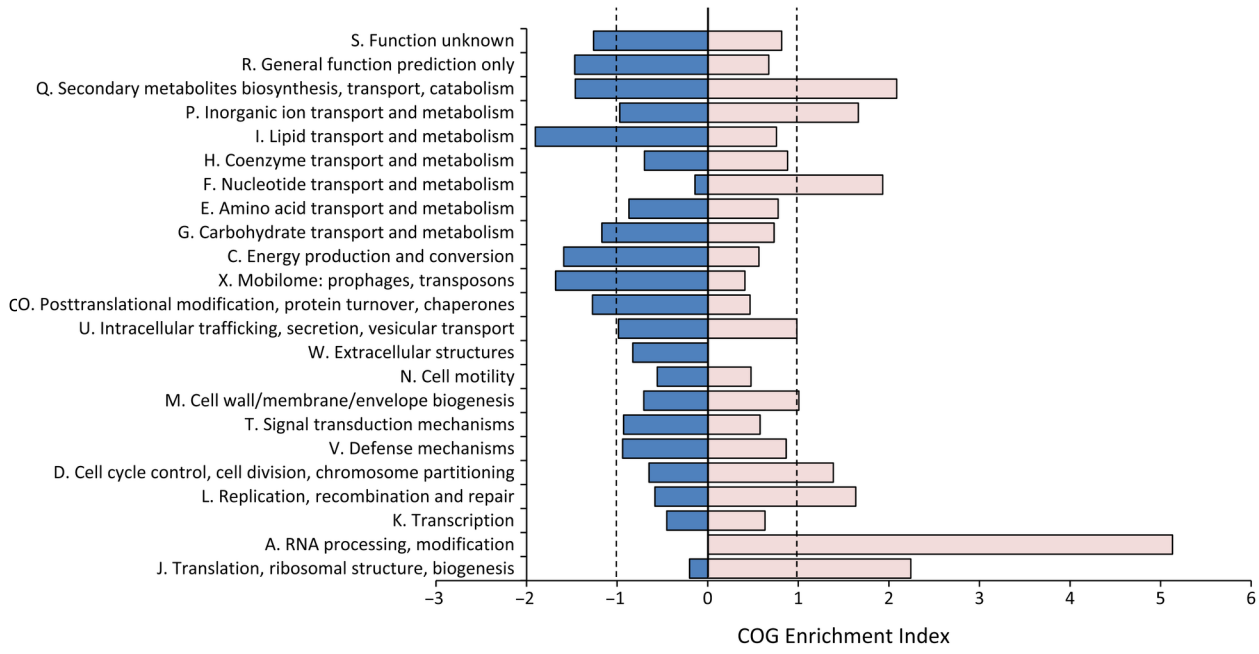

Figure 2. COG enrichment for differentially regulated genes. The COG enrichment index was calculated by dividing the percentage of genes up- or down-regulated for each category by the percentage of genes in that category across the whole genome. An index >1 indicates enrichment of the COG in the group. Negative numbers refer to the down-regulated gene set in the in vivo growth condition (when compared with in vitro growth); positive numbers, to the up-regulated genes.

may be necessary under some circumstances such as immune evasion. For instance, OmpA is an outer membrane porin required for disease with a diverse range of ascribed roles (Supplementary Table 2). OmpA is also immunogenic, and antibodies specific to OmpA can confer immunity [41]. Hence, moderate down-regulation of OmpA may strike a balance between virulence function and immune evasion, allowing growth in vivo.

Of particular interest are the genes that have a demonstrated association with survival in serum. Although *ptk* (capsule production) and *pld* (phospholipase D) were up-regulated, the genes *bfmSR* (2 component signal transduction system), *ompA* (multifunction porin), *cipA* (plasminogen binding), and *pglC* (protein glycosylation and capsule synthesis), surprisingly, were either unchanged or down-regulated in vivo (Supplementary Table 2).

Changes in the Expression of Lipopolysaccharide and Capsule Biosynthesis Genes

A. baumannii capsule and lipopolysaccharide are important virulence factors [23, 34]. There were few changes in the expression of lipid A biosynthesis genes, with *lpxC* (A1S_3330) the only gene showing increased expression (3.8-fold increase). In the capsule locus (A1S_0049–66), the genes A1S_0049 (*wzc*/*ptk*), A1S_0050 (tyrosine kinase/*wzb*), and A1S_0059 and A1S_0060 (glycosyltransferases) all showed enhanced transcription in vivo. Moreover, *bfmR*, which encodes a response regulator involved in the regulation (repression) of capsule biosynthesis, was down-regulated in vivo, which may have contributed to the expression changes observed in the capsule locus [42]. Notably, it has previously been shown that an increase in capsule synthesis through increased expression of several genes, including *wzc*/*ptk*, confers enhanced virulence [42], suggesting that the transcriptional changes observed in this study may contribute to virulence. Increased capsule production may confer resistance to the bactericidal activity of complement, antimicrobial peptides, and phagocytosis.

Down-regulation of Biofilm and Pili Biosynthesis Genes In Vivo

Adhesins, quorum sensing proteins, and other survival-related genes, such as trehalose synthesis genes, were strongly down-regulated in vivo. The type I pili assembly genes, *csuA/B* and *csuA–E* (A1S_2213–8), which are necessary for adhesion to abiotic surfaces and biofilm formation [43], were all down-regulated in vivo. However, the type I pilus biosynthesis locus A1S_1507-10 was not differentially regulated in vivo but has previously been shown to be expressed at increased levels during growth under iron-limiting conditions [5]. A second pilus biosynthesis cluster (A1S_2088–91) had 1 strongly down-regulated gene in vivo (A1S_2091; 25-fold down). The reduced expression of type 1 pilus adhesion genes is reflected in the overrepresentation of differentially expressed genes in the COG category W genes (extracellular structures). Unsurprisingly, most type 1 pilus biosynthesis genes have been identified as up-regulated in biofilms [3]. The biofilm-associated proteins Bap (of which there are 2 orthologues in ATCC 17978 [18]) were strongly down-regulated in the blood; in contrast, *bap* was overexpressed in biofilms [3]. Signaling pathways controlling biofilm formation were also down-regulated, including A1S_0748 (*bfmR*) [21], A1S_1377, and A1S_1687 [3]. Likewise, the quorum sensing homoserine lactone synthase gene (*abaI*) and nearby locus (A1S_0109–16) required for biofilm formation [3] were down-regulated. As expected, these observations indicate that bacterial cells in blood are in a planktonic state.

Transcriptional Changes Related to Metabolism

A number of amino acid catabolism pathways, such as the phenylalanine (*paa*), arginine (*ast*), and D alanine degradation (*dada*; A1S_0095) pathways were down-regulated. This may reflect the importance of amino acid catabolism as an energy source in protein-rich growth media such as heart infusion medium but less so in vivo. However, these data do not prove that these pathways do not play a role in virulence, because some down-regulated genes (such as those of the *paa* operon) have exceptionally high expression in vitro; hence, the reduced expression in vivo still corresponds to substantial levels of expression. Notably, many amino acid catabolism pathways have also been identified as up-regulated in biofilms [3]. The COG enrichment analysis indicated that many genes with predicted functions in lipid and carbohydrate transport/metabolism were expressed at reduced levels in vivo (Figure 2). These included 12 acyl-CoA dehyrogenases, beta-ketoadipyl-CoA thiolases (A1S_1849 and A1S_1891), 4 enoyl-CoA hydratase and/or isomerases, Acyl-CoA synthetase (A1S_0112) and long chain fatty-acid CoA ligase (A1S_1378). This suggests a metabolic shift away from fatty acids as an energy source in vivo.

In contrast, we observed a strong increase in expression of the lactate metabolism locus (A1S_0067–70) encoding a permease (30-fold increase), regulator and 2 lactate dehydrogenases (7–8-fold increase). Lactate dehydrogenase converts lactate to pyruvate, generating nicotinamide adenine dinucleotide, reduced. Pyruvate may enter the tricarboxylic acid cycle, although enzymes converting pyruvate to acetyl-CoA are down-regulated (pyruvate dehydorgenase/decarboxylase *aceE* genes A1S_2450 and A1S_1701 and pyruvate-ferredoxin oxidoreductase gene A1S_1368). Because some gluconeogenesis enzymes are up-regulated (eg, A1S_2596 fructose-1,6-bisphosphatase and A1S_2668 phophoenolpyruvate carboxykinase) it is possible that the lactate-to-pyruvate conversion is used for carbohydrate assimilation. Notably, lactate levels are elevated during human septicemia [44] and so may constitute an important carbon source.

Some components of other important biosynthetic pathways were also expressed at increased levels in vivo, including de novo nucleotide synthesis (reflected in COG category F, nucleotide transport and metabolism), which may reflect a change in

nucleotide availability, peptidoglycan biosynthesis, isoprenoid precurser biosynthesis (which may feed into pathways such as lipid and ubiquinone synthesis), and pseudouridine synthases (modification of transfer RNAs). These changes also suggest an increased growth rate.

Comparison With the Altered Expression Profiles in Serum

It is of interest to analyze how the transcriptomic changes observed in vivo compare with those observed during growth in serum [4]. In serum, in addition to the expected siderophore synthesis clusters, 2 heme binding loci (A1S_1608-9) were up-regulated suggesting a role for heme as an iron source in vivo. However, these genes were not differentially regulated in our study, suggesting that heme is not an important iron source; ATCC 17978 also has no identifiable heme oxygenase for heme utilization. Type IV pili genes were also up-regulated in serum, suggesting that bacteria are primed to anchor to host tissue and invade, however, only 1 such gene was up-regulated in vivo. It was also suggested that DNA uptake and repair processes are enhanced in serum, perhaps for the acquisition of virulence genes, but in vivo most of the genes involved in these processes showed unchanged expression. Thus, we predict that there are multiple drivers regulating expression in vivo, because transcriptional changes in serum do not closely reflect those seen in vivo. Interestingly, 22 efflux pump genes were up-regulated in human serum, suggesting an enhanced drug export capacity in vivo with important implications for antibiotic treatment of *A. baumannii* infections. However, the in vivo situation was clearly different; of these 22 genes, 15 were unchanged, 2 were down-regulated, and 5 were up-regulated, but these are all part of siderophore clusters and probably have a role in siderophore extrusion (A1S_1649, A1S_2375–8) [5].

CONCLUSION

Our results should be considered with the following caveats in mind. RNA sequencing measures RNA abundance, which is a product of both gene transcription and RNA stability. Differences in RNA abundance do not necessarily equate to differences in the levels of encoded product (ie, protein) or downstream metabolic processes due owing factors such as differences in translation efficiency and posttranscriptional regulation.

In conclusion, the current study illustrates the feasibility of in vivo transcriptomics for *A. baumannii*. As expected, there was a major shift in gene expression between the in vitro and in vivo growth conditions, emphasizing that in vitro growth is a poor reflection of a mammalian infection. Furthermore, it seems that in vivo growth only partly mirrors expression patterns seen in "in vivo–simulating" conditions, such as growth in serum or iron limitation. Future work should analyze transcriptomic changes in different tissues of the host and with different *A. baumannii* strains. Our findings should help future investigation of the virulence mechanisms for this problematic hospital-acquired pathogen.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Dexter C, Murray GL, Paulsen IT, Peleg AY. Community-acquired *Acinetobacter baumannii*: clinical characteristics, epidemiology and pathogenesis. Expert Rev Anti Infect Ther **2015**; 13:567–73.
2. Doi Y, Murray GL, Peleg AY. *Acinetobacter baumannii*: evolution of antimicrobial resistance-treatment options. Semin Resp Crit Care Med **2015**; 36:85–98.
3. Rumbo-Feal S, Gomez MJ, Gayoso C, et al. Whole transcriptome analysis of *Acinetobacter baumannii* assessed by RNA-sequencing reveals different mRNA expression profiles in biofilm compared to planktonic cells. PloS One **2013**; 8: e72968.
4. Jacobs AC, Sayood K, Olmsted SB, et al. Characterization of the *Acinetobacter baumannii* growth phase-dependent and serum responsive transcriptomes. FEMS Immunol Med Microbiol **2012**; 64:403–12.
5. Eijkelkamp BA, Hassan KA, Paulsen IT, Brown MH. Investigation of the human pathogen *Acinetobacter baumannii* under iron limiting conditions. BMC Genomics **2011**; 12:126.
6. Henry R, Crane B, Powell D, et al. The transcriptomic response of *Acinetobacter baumannii* to colistin and doripenem alone and in combination in an in vitro pharmacokinetics/pharmacodynamics model. J Antimicrob Chemother **2015**; 70:1303–13.
7. Kostoulias X, Murray GL, Cerqueira GM, et al. Impact of a cross-kingdom signaling molecule of *Candida albicans* on *Acinetobacter baumannii* physiology. Antimicrob Agents Chemother **2016**; 60:161–7.
8. Cerqueira GM, Kostoulias X, Khoo C, et al. A global virulence regulator in *Acinetobacter baumannii* and its control of the phenylacetic acid catabolic pathway. J Infect Dis **2014**; 210:46–55.
9. Eijkelkamp BA, Stroeher UH, Hassan KA, Elbourne LD, Paulsen IT, Brown MH. H-NS plays a role in expression of *Acinetobacter baumannii* virulence features. Infect Immun **2013**; 81:2574–83.
10. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics **2009**; 25:1754–60.
11. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics **2014**; 30:923–30.
12. Law CW, Chen Y, Shi W, Smyth GK. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol **2014**; 15:R29.
13. Ritchie ME, Phipson B, Wu D, et al. *limma* Powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res **2015**; 43:e47.
14. Caspi R, Billington R, Ferrer L, et al. The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. Nucleic Acids Res **2016**; 44:D471–80.
15. Jorth P, Trivedi U, Rumbaugh K, Whiteley M. Probing bacterial metabolism during infection using high-resolution transcriptomics. J Bacteriol **2013**; 195:4991–8.
16. Heindorf M, Kadari M, Heider C, Skiebe E, Wilharm G. Impact of *Acinetobacter baumannii* superoxide dismutase on motility, virulence, oxidative stress resistance and susceptibility to antibiotics. PloS One **2014**; 9:e101033.
17. Loehfelm TW, Luke NR, Campagnari AA. Identification and characterization of an *Acinetobacter baumannii* biofilm-associated protein. J Bacteriol **2008**; 190:1036–44.
18. Brossard KA, Campagnari AA. The *Acinetobacter baumannii* biofilm-associated protein plays a role in adherence to human epithelial cells. Infect Immun **2012**; 80:228–33.

19. Gaddy JA, Arivett BA, McConnell MJ, Lopez-Rojas R, Pachon J, Actis LA. Role of acinetobactin-mediated iron acquisition functions in the interaction of *Acinetobacter baumannii* strain ATCC 19606T with human lung epithelial cells, *Galleria mellonella* caterpillars, and mice. Infect Immun **2012**; 80:1015–24.
20. Liou ML, Soo PC, Ling SR, Kuo HY, Tang CY, Chang KC. The sensor kinase BfmS mediates virulence in *Acinetobacter baumannii*. J Microbiol Immunol Infect **2014**; 47:275–81.
21. Tomaras AP, Flagler MJ, Dorsey CW, Gaddy JA, Actis LA. Characterization of a two-component regulatory system from *Acinetobacter baumannii* that controls biofilm formation and cellular morphology. Microbiology **2008**; 154:3398–409.
22. Repizo GD, Gagne S, Foucault-Grunenwald ML, et al. Differential role of the T6SS in *Acinetobacter baumannii* virulence. PloS One **2015**; 10:e0138265.
23. Luke NR, Sauberan SL, Russo TA, et al. Identification and characterization of a glycosyltransferase involved in *Acinetobacter baumannii* lipopolysaccharide core biosynthesis. Infect Immun **2010**; 78:2017–23.
24. Rumbo C, Tomas M, Fernandez Moreira E, et al. The *Acinetobacter baumannii* Omp33-36 porin is a virulence factor that induces apoptosis and modulates autophagy in human cells. Infect Immun **2014**; 82:4666–80.
25. Smani Y, Dominguez-Herrera J, Pachon J. Association of the outer membrane protein Omp33 with fitness and virulence of *Acinetobacter baumannii*. J Infect Dis **2013**; 208:1561–70.
26. Lee JS, Choi CH, Kim JW, Lee JC. *Acinetobacter baumannii* outer membrane protein A induces dendritic cell death through mitochondrial targeting. J Microbiol **2010**; 48:387–92.
27. Gaddy JA, Tomaras AP, Actis LA. The *Acinetobacter baumannii* 19606 OmpA protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. Infect Immun **2009**; 77:3150–60.
28. Choi CH, Lee EY, Lee YC, et al. Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. Cell Microbiol **2005**; 7:1127–38.
29. Kim SW, Choi CH, Moon DC, et al. Serum resistance of *Acinetobacter baumannii* through the binding of factor H to outer membrane proteins. FEMS Microbiol Lett **2009**; 301:224–31.
30. Choi AH, Slamti L, Avci FY, Pier GB, Maira-Litran T. The *pgaABCD* locus of *Acinetobacter baumannii* encodes the production of poly-beta-1-6-N-acetylglucosamine, which is critical for biofilm formation. J Bacteriol **2009**; 191:5953–63.
31. Lees-Miller RG, Iwashkiw JA, Scott NE, et al. A common pathway for O-linked protein-glycosylation and synthesis of capsule in *Acinetobacter baumannii*. Mol Microbiol **2013**; 89:816–30.
32. Iwashkiw JA, Seper A, Weber BS, et al. Identification of a general O-linked protein glycosylation system in *Acinetobacter baumannii* and its role in virulence and biofilm formation. PLoS Pathogens **2012**; 8:e1002758.
33. Jacobs AC, Hood I, Boyd KL, et al. Inactivation of phospholipase D diminishes *Acinetobacter baumannii* pathogenesis. Infect Immun **2010**; 78:1952–62.
34. Russo TA, Luke NR, Beanan JM, et al. The K1 capsular polysaccharide of *Acinetobacter baumannii* strain 307-0294 is a major virulence factor. Infect Immun **2010**; 78:3993–4000.
35. Elhosseiny NM, Amin MA, Yassin AS, Attia AS. *Acinetobacter baumannii* universal stress protein A plays a pivotal role in stress response and is essential for pneumonia and sepsis pathogenesis. Int J Med Microbiol **2015**; 305:114–23.
36. Srinivasan VB, Vaidyanathan V, Rajamohan G. AbuO, a TolC-like outer membrane protein of *Acinetobacter baumannii*, is involved in antimicrobial and oxidative stress resistance. Antimicrob Agents Chemother **2015**; 59:1236–45.
37. Koenigs A, Zipfel PF, Kraiczy P. Translation elongation factor Tuf of *Acinetobacter baumannii* is a plasminogen-binding protein. PloS One **2015**; 10:e0134418.
38. Koenigs A, Stahl J, Averhoff B, et al. CipA of *Acinetobacter baumannii* is a novel plasminogen binding and complement inhibitory protein. J Infect Dis **2016**; 213:1388–99.
39. Stahl J, Bergmann H, Gottig S, Ebersberger I, Averhoff B. *Acinetobacter baumannii* virulence is mediated by the concerted action of three phospholipases D. PloS One **2015**; 10:e0138360.
40. Harper M, Boyce JD, Wilkie IW, Adler B. Signature-tagged mutagenesis of *Pasteurella multocida* identifies mutants displaying differential virulence characteristics in mice and chickens. Infect Immun **2003**; 71:5440–6.
41. Luo G, Lin L, Ibrahim AS, et al. Active and passive immunization protects against lethal, extreme drug resistant-*Acinetobacter baumannii* infection. PloS One **2012**; 7:e29446.
42. Geisinger E, Isberg RR. Antibiotic modulation of capsular exopolysaccharide and virulence in *Acinetobacter baumannii*. PLoS Pathogens **2015**; 11:e1004691.
43. Tomaras AP, Dorsey CW, Edelmann RE, Actis LA. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. Microbiology **2003**; 149:3473–84.
44. Faix JD. Biomarkers of sepsis. Crit Rev Clin Lab Sci **2013**; 50:23–36.