Host-inherent variability influences the transcriptional response of Staphylococcus aureus during in vivo infection

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The rise of antibiotic resistance calls for alternative strategies to treat bacterial infections. One attractive strategy is to directly target bacterial virulence factors with anti-virulence drugs. The expression of virulence traits by pathogens is, however, not constitutive but rather induced by the level of stress encountered within the host. Here we use dual RNA sequencing (RNA-seq) to show that intrinsic variability in the level of host resistance greatly affects the pathogen’s transcriptome in vivo. Through analysis of the transcriptional profiles of host and pathogen during Staphylococcus aureus infection of two mouse strains, shown to be susceptible (A/J) or resistant (C57BL/6) to the pathogen, we demonstrate that the expression of virulence factors is dependent on the encountered host resistance. We furthermore provide evidence that this dependence strongly influences the efficacy of anti-virulence strategies, highlighting a potential limitation for the implementation of these strategies.
The rise of antimicrobial resistance is one of the most challenging problems in modern medicine, causing an increase in morbidity and mortality associated with common bacterial infections. While available antibiotics are loosing their effectiveness, the introduction of novel bactericidal or bacteriostatic antibiotics cannot be considered a long-term solution because it is eventually followed by the emergence of resistant bacterial clones that become increasingly prevalent under selective drug pressure. Consequently, there is a pressing need for new anti-infective agents that do not impose similar levels of selection pressure on pathogens as classical antibiotics. In this regard, alternative approaches based on attenuating bacterial pathogenesis by targeting bacterial virulence, the so-called 'anti-virulence' strategies, are emerging as promising tools for the treatment of infections.

Bacterial pathogens express a large repertoire of different virulence factors to survive under the adverse conditions imposed by the host environment. Thus, anti-virulence strategies have been proposed that specifically target bacterial toxins produced by the pathogen to evade host defenses, bacterial factors mediating adhesion to the host, secretion systems as well as regulatory systems and quorum-sensing signalling. The key feature of anti-virulence drugs is the attenuation of the pathogen's virulence to aid clearance by the host's immune defenses. These drugs seem attractive, because it is believed that not killing the pathogen directly exerts less selective pressure for the development of resistance. However, such an approach will only confer therapeutic benefit if the targeted virulence factor(s) are actually expressed by the bacterium during infection and if the natural defense mechanisms of the host are strong enough to clear the pathogen, weakened by the anti-virulence treatment.

Bacterial pathogenesis, on the other hand, is strongly influenced by the strength of the host immune defense. For example, avirulent microorganisms can be pathogenic for immunocompromised hosts, whereas virulent microorganisms can be nonpathogenic in immune hosts. This situation is further complicated by the fact that, in addition to the immune status, inherent characteristics of the host, such as the genetic background, significantly influence the capability of the immune system to overcome invading pathogens. Thus, the response to a specific pathogen can range from weak in susceptible hosts, causing severe infections, to strong in more resistant individuals, resulting in milder diseases. These differences imply that pathogens will encounter stronger immune pressure in resistant than in susceptible hosts and virulence factors that are essential for counteracting a weak immune response may not be the same as those required under stronger immune pressure in resistant hosts. Therefore, the dependence of virulence factor expression on host resistance is a potential limitation for the effectiveness of anti-virulence drugs.

Here we investigate how intrinsic variability of host resistance to a pathogen affects the expression of virulence factors needed to successfully infect the host. We use Staphylococcus aureus, a human pathogen that can cause severe invasive infections and is notorious for its capacity to develop antibiotic resistances. These characteristics make S. aureus one of the most dangerous and intractable infectious pathogens worldwide. Despite numerous attempts to develop a vaccine that can prevent S. aureus infections, none of the vaccine candidates tested in clinical trials has succeeded so far. This failure, in combination with the increase of antibiotic resistance, has lead to an intensification of efforts to search for alternative treatment approaches in recent years. In this regards, anti-virulence strategies targeting crucial pathogenicity factors produced by S. aureus during infection have been proposed as an attractive therapeutic option. However, since the outcome of S. aureus infection is strongly influenced by the host factors such as racial origin, age and genetic makeup, the search for anti-virulence targets in S. aureus has to consider the inherent variability of the host responses to infection. Similar to humans, variability in the host response to S. aureus has been also observed among different inbred strains of mice. While some mouse strains (for example, A/J and DBA/2) are very susceptible to S. aureus infection, C57BL/6 mice are highly resistant and survive a bacterial dose that rapidly kills mice from susceptible strains. These differences in the capacity to control S. aureus infection provide a unique experimental system to explore the extent to which intrinsic host variability affects the expression of bacterial virulence factors during infection.

In this study, we use dual RNA sequencing (RNA-seq), and two mouse strains previously shown to display differential susceptibility to S. aureus infection (strain A/J is susceptible and strain C57BL/6 is resistant), to investigate how the intrinsic variability of host resistance to a pathogen affects the expression of virulence factors. Dual RNA-seq enables the simultaneous determination of the transcriptional response of the host and pathogen during infection and does not require physical separation of pathogen and eukaryotic RNA since the sequencing reads can be assigned to the host or to the pathogen genomes by in silico analysis. Although dual RNA-seq has been successfully applied to characterize the transcriptional signature of bacteria and host in several in vitro infection systems, the study presented here is one of the first using this technology in an in vivo system. Our results demonstrate the impact of the host genetic background on the transcriptional response of S. aureus during infection, and provide experimental evidence that host-dependent bacterial expression of virulence factors is a potential limitation for the efficacy of anti-virulence therapies.

Results

C57BL/6 and A/J mice differ in their resistance to S. aureus. For this study, we selected two strains of mice that differ in their susceptibility to S. aureus infection. Whereas A/J mice are very vulnerable to S. aureus, resulting in a significantly increased bacterial growth in the kidneys (Fig. 1a) and liver (Fig. 1b) and fatal infection outcome (Fig. 1c), C57BL/6 mice exhibit greater resistance to S. aureus and were capable to significantly restrict bacterial growth in the kidneys (Fig. 1a) and liver (Fig. 1b) with all mice surviving (Fig. 1c). Therefore, these two mouse strains provide an excellent tool for investigating the extent to which variability in the host response to infection affects the transcriptional response of S. aureus within the host.

Dual RNA-seq analysis of S. aureus and infected host tissue. A dual RNA-seq approach that enables simultaneous transcriptional profiling of bacteria and host tissue was used to characterize the host response to infection and investigate the impact of different levels of host resistance on the transcriptional response of S. aureus. The schematic representation of the experimental design is shown in Fig. 1d. Total RNA including host and pathogen RNA, was isolated from the kidneys of A/J and C57BL/6 mice at 48 h of infection and analysed by Illumina deep sequencing. Between 9 and 21 millions reads were uniquely assigned to the reference genome of Mus musculus assembly GRCm38.p3 (GCA_000001635.5), while between 32,228 and 5 millions reads were uniquely mapped to the revised reference genome of S. aureus strain 8325-4 (ref. 21).

Transcriptome analysis of S. aureus-infected mice. Hierarchical clustering (Supplementary Fig. 1a) and principal component analysis (PCA) (Supplementary Fig. 1b) of gene expression datasets from infected A/J and C57BL/6 mice, as well as of
infected intravenously with 4 × 10^7 CFU of S. aureus SH1000. Each symbol represents the bacterial counts determined in an individual mouse and the horizontal lines represent the average ± s.d. for each mouse strain. One representative experiment out of three independent experiments is shown (n = 6, t-test, *P < 0.05). (c) Survival curves of A/J and C57BL/6 mice intravenously infected with 4 × 10^7 CFU of S. aureus SH1000 (n = 5, log-rank test, P < 0.01). (d) Schematic summary of the experimental design for dual RNA-seq analysis. Susceptible A/J mice and resistant C57BL/6 mice were infected intravenously with 4 × 10^7 CFU of S. aureus SH1000, their kidneys removed at 48 h after bacterial inoculation and subjected to dual RNA-seq analysis to simultaneously determine the gene expression profile of the host and pathogen in the same sample. The genes differentially expressed by S. aureus in A/J and C57BL/6 mice were identified and related to the infection-associated transcriptional response of the corresponding mouse strain. The effect of targeting a virulence factor differentially expressed by S. aureus SH1000 between infection of A/J and C57BL/6 mice was also determined.

Figure 1 | A/J and C57BL/6 mice exhibit opposed levels of resistance to S. aureus. Bacterial loads in the kidneys (a) and liver (b) of A/J and C57BL/6 mice at 48 h after intravenous inoculation with 4 × 10^7 CFU of S. aureus SH1000. Each symbol represents the bacterial counts determined in an individual mouse and the horizontal lines represent the average ± s.d. for each mouse strain. One representative experiment out of three independent experiments is shown (n = 6, t-test, *P < 0.05). (c) Survival curves of A/J and C57BL/6 mice intravenously infected with 4 × 10^7 CFU of S. aureus SH1000 (n = 5, log-rank test, P < 0.01). (d) Schematic summary of the experimental design for dual RNA-seq analysis. Susceptible A/J mice and resistant C57BL/6 mice were infected intravenously with 4 × 10^7 CFU of S. aureus SH1000, their kidneys removed at 48 h after bacterial inoculation and subjected to dual RNA-seq analysis to simultaneously determine the gene expression profile of the host and pathogen in the same sample. The genes differentially expressed by S. aureus in A/J and C57BL/6 mice were identified and related to the infection-associated transcriptional response of the corresponding mouse strain. The effect of targeting a virulence factor differentially expressed by S. aureus SH1000 between infection of A/J and C57BL/6 mice was also determined.

Functional classification of the DEGs using KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis, revealed that a large group of host genes with increased expression in response to infection in both A/J and C57BL/6 mice belonged to the groups ‘cytokine-cytokine receptor interaction’ and ‘chemokine signalling pathway’ (Fig. 2b). Particularly, genes encoding inflammatory cytokines such as IL-6, IL-1α, IL-1β and TNF-α as well as chemokines involved in the chemotraction of monocytes/macrophages such as Cxc1l, Cxc2 and Cxc3, were upregulated in both A/J and C57BL/6 mice in response to S. aureus infection (Supplementary Data 1 and 2). Also, host genes encoding acute phase proteins such as Saa1, Saa2, haptoglobin and the calcium-binding proteins S100a8 and S100a9 were highly induced in infected A/J and C57BL/6 mice. Although the global analysis of the transcriptional data suggested that a ‘core’ set of inflammation-related genes was highly expressed in both A/J and C57BL/6 mice in response to S. aureus infection, the average fold change of expression in this set of genes was markedly higher in infected A/J than in infected C57BL/6 mice (Fig. 2). This suggested that A/J mice developed a more intense systemic inflammation than C57BL/6 mice in response to S. aureus infection, which is indicative of severe sepsis leading to death. Besides the systemic hyperinflammation, the increased expression of the gene encoding the coagulation activator tissue factor (F3) and of the gene encoding the fibrinolysis inhibitor PAI-1 (Serpine1) in A/J mice (Supplementary Data 1), revealed a net pro-coagulant status that is typical for severe sepsis23. Altered coagulation, coupled with microvascular dysfunction occurring during sepsis, decreases tissue perfusion. This leads to perturbations of oxygen supply, resulting in tissue hypoxia and the activation of the hypoxia-inducible factor alpha encoded by Hif1a24,25. The significant induction of Hif1a observed in A/J, but not in C57BL/6 mice, in response to infection (Supplementary Tables 2 and 3), indicated more severe tissue hypoxia in the kidneys of infected A/J mice than in those of C57BL/6 mice. Furthermore, the genes encoding arginase 1 (Arg1) and arginase 2 (Arg2) were expressed to a greater extent in infected A/J than in infected C57BL/6 mice
mice. (*S. aureus* with increased (left) or decreased (right) expression in response to up-regulated genes in response to system' category (left part). The corresponding numbers of significantly versus uninfected A/J and C57BL/6 mice within the KEGG 'immune significantly up-regulated genes determined between 4 NATURE COMMUNICATIONS | DOI: 10.1038/ncomms14268 | www.nature.com/naturecommunications A/J and C57BL/6 mice. Figure 2 | Gene expression analysis of *S. aureus* infecting A/J and C57BL/6 mice. (a) Venn diagram showing the number of DEGs with increased (left) or decreased (right) expression in response to *S. aureus* infection that are unique or common between A/J and C57BL/6 mice. (b) Heat map of the mean log2-fold change of gene expression of the significantly up-regulated genes determined between *S. aureus*-infected versus uninfected A/J and C57BL/6 mice within the KEGG 'immune system' category (left part). The corresponding numbers of significantly up-regulated genes in response to *S. aureus* infection in A/J (blue bars) and C57BL/6 (red bars) are shown in the right part of the figure. S. aureus transcriptome during infection of A/J or C57BL/6. In parallel, we analysed the transcriptome of *S. aureus* during the infection of resistant C57BL/6 and susceptible A/J mice to determine the impact of the different physiological conditions present at the site of infection on the pathogen's transcriptional response. Hierarchical ordination (Supplementary Fig. 2a) and PCA (Supplementary Fig. 2b) showed high within-group reproducibility, while PERMANOVA demonstrated that the transcriptional response of *S. aureus* infecting susceptible A/J mice differed significantly from that of *S. aureus* infecting resistant C57BL/6 mice (Supplementary Table 2). Transcript abundance was determined by normalizing the number of raw reads in each data set for gene length and expressed as transcripts per Kilobases per Million (TPM) (Supplementary Data 3). A total of 85 genes were identified as differentially expressed (probability value ≥ 0.95) by *S. aureus* between infection of A/J and infection of C57BL/6 mice using NOISeq (Supplementary Data 4). Of those, transcripts of 20 genes were more abundant in *S. aureus* infecting A/J mice (Fig. 3a, Table 1, Supplementary Table 4), 65 genes exhibited greater expression in *S. aureus* during infection of C57BL/6 mice (Fig. 3a, Table 2, Supplementary Table 5) and 594 were expressed at a similar level by *S. aureus* in A/J and C57BL/6 mice (Fig. 3a, Supplementary Data 4).

One of the most prominent operons expressed by *S. aureus* to a greater extent during infection of A/J mice, than during infection of C57BL/6 mice, was the arc operon, which encodes the arginine synthase uncoupling and consequently to the production of high levels of harmful reactive oxygen species (ROS)26. Taken together, these findings suggest that the micro-environment in the infected tissue is highly different between A/J and C57BL/6 mice, which could significantly affect the expression of virulence determinants by *S. aureus*.

**Table 1 | DEGs between *S. aureus* infecting A/J and C57BL/6 mice with greater transcript abundance during infection of A/J mice.**

| Locus tag       | Gene symbol | Description                                      |
|-----------------|-------------|--------------------------------------------------|
| SAOUHSC_00845   | yflI        | Hypothetical                                    |
| SAOUHSC_02853   | arcR        | Hypothetical                                    |
| SAOUHSC_00371   | gvpP        | Hypothetical                                    |
| SAOUHSC_02964   | rpmB        | Hypothetical                                    |
| SAOUHSC_01477   | arcD        | 50S ribosomal protein L28                       |
| SAOUHSC_01969   | rpmB        | Hypothetical                                    |
| SAOUHSC_00181   | xynA        | Hypothetical                                    |
| SAOUHSC_02967   | arcD        | Arginine/ornithine antipporter                  |
| SAOUHSC_01191   | rpmB        | 50S ribosomal protein L28                       |
| SAOUHSC_00868   | aapA        | Hypothetical                                    |
| SAOUHSC_01803   | clpL        | ATP-dependent Clp protease, ATP-binding subunit ClpC |
| SAOUHSC_02862   | cidB        | Hypothetical                                    |
| SAOUHSC_01403   | cspA        | Cold shock protein                              |
| SAOUHSC_02850   | qoxB        | quinol oxidase AA3 subunit II                   |
| SAOUHSC_01002   | groF        | Hypothetical                                    |
| SAOUHSC_01024   | tcyC        | Amino acid ABC transporter ATP-binding protein   |
| SAOUHSC_02697   |             | Hypothetical                                    |
| SAOUHSC_02665   |             | Hypothetical                                    |

DEG, differentially expressed gene. The complete data of the differentially expressed genes with higher expression by *S. aureus* during infection of the susceptible A/J mice are displayed in Supplementary Table 4.
deiminase (ADI) system (Fig. 3b, Supplementary Data 4). The arc operon comprises the genes coding for arginine deiminase (arcA), ornithine transcarbamylase (arcB), carbamate kinase (arcC), the arginine/ornithine antiporter (arcD) and the transcriptional regulator ArcR (arcR). These enzymes catalyse the conversion of arginine to ornithine, ammonia, and CO2, while producing regulator ArcR (arginine/ornithine antiporter (arcD), which can kill host cells by damaging the plasma membrane34, were also expressed by S. aureus to a higher extent during infection of C57BL/6 than during infection of A/J mice, which is most probably driven by the different growth phase of the bacteria in the two mouse strains.

The expression of these virulence factors is controlled by regulatory elements such as two-component regulatory systems (TCRS) and transcriptional regulatory systems in response to environmental cues encountered by the bacterium during infection35. The staphylococcal quorum-sensing system accessory gene regulator (agr) was found highly expressed by S. aureus during infection of both A/J and C57BL/6 mice. The agr system comprises two divergent transcripts, RNAII and RNAIII, which are under the control of two distinct promoters, P2 and P3, respectively36. RNA II encodes the quorum-sensing elements AgrB, AgrD, AgrC and AgrA that represent an autocatalytic sensory transduction system. RNAIII encodes delta-hemolysin (hld)37 and is a major regulator of virulence factors in S. aureus, inducing the transcription of various extracellular proteases and toxins38. Although the genes encoding the agr P2 operon

Figure 3 | Gene expression analysis of S. aureus during infection of A/J and C57BL/6 mice. (a) Venn diagram showing the number of unique and common expressed genes between S. aureus infecting A/J and C57BL/6 mice based on differential gene expression analysis determined with NOISeq. (b) Gene composition and organization of the genes of the ADI operon and their level of expression in S. aureus during infection of A/J (blue bars) or C57BL/6 (red bars) mice. Each bar represents the mean of TPM ± s.d. of triplicates.
were expressed by *S. aureus* to a similar level in both mouse strains, the expression level of RNAIII/*hld* was greater during infection of C57BL/6 mice (Fig. 4c, Supplementary Data 4). The gene encoding the transcriptional regulator SarR was also upregulated by *S. aureus* infecting C57BL/6 mice (Fig. 4c, Supplementary Data 4). This could explain the higher level of transcripts encoding proteases and toxins detected in *S. aureus* during infection of C57BL/6 mice, since both RNAIII (ref. 38) and SarR39 activate their transcription.

Antimicrobial peptides (AMPs) are an important part of the host innate immune defense against *S. aureus* by directly impairing the integrity of the bacterial cell wall40. The bacterial gene encoding phosphatidylglycerol lysyltransferase (*mprF*), which is part of the cell wall stress stimulon and mediates

### Table 2 | DEGs between *S. aureus* infecting A/J and C57BL/6 mice with greater transcript abundance during infection of C57BL/6 mice

| Locus tag         | Gene symbol | Description                      |
|-------------------|-------------|----------------------------------|
| SAOUHSC_02260     | hld         | Delta-hemolysin                  |
| SAOUHSC_00411.1   | psma1       | Alpha phenol soluble modulin     |
| SAOUHSC_00266     | sarR        | Hypothetical                     |
| SAOUHSC_002971    | aur         | Zinc metalloproteinase aureolysin|
| SAOUHSC_00435     | gitB        | Glutamate synthase large subunit |
| SAOUHSC_00788     | thrS        | Threonyl-tRNA synthetase         |
| SAOUHSC_00987     | sspB        | Cysteine protease                |
| SAOUHSC_00248     | lytM        | Peptidoglycan hydrolase          |
| SAOUHSC_002571    | ssaA        | Secretory antigen                |
| SAOUHSC_004427    | ste1        | Autolysin                        |
| SAOUHSC_002941    | nrgG        | Hypothetical                     |
| SAOUHSC_01001     | qoxA        | Quinol oxidase subunit I         |
| SAOUHSC_000640    |             | Hypothetical                     |
| SAOUHSC_000030    |             | Hypothetical                     |
| SAOUHSC_000717    |             | Hypothetical                     |
| SAOUHSC_000741    | nrdl        | Ribonucleotide reductase stimulatory protein |
| SAOUHSC_01942     | splA        | Serine protease SPlA             |
| SAOUHSC_000083    | sbn         | Hypothetical                     |
| SAOUHSC_00348     | rpsF        | 30S ribosomal protein S6         |
| SAOUHSC_00436     | gldD        | Glutamate synthase subunit beta  |
| SAOUHSC_000051    | plc         | 1-phosphatidylinositol phosphodiesterase |
| SAOUHSC_00411.2   | psma2       | Alpha phenol soluble modulin     |
| SAOUHSC_00112     | hla         | Alpha-hemolysin                  |
| SAOUHSC_000727    |             | Hypothetical                     |
| SAOUHSC_00681     | secG        | Preprotein translocase subunit SecG |
| SAOUHSC_00135     | splF        | Serine protease SPlF             |
| SAOUHSC_00269     | rpoE        | DNA-directed RNA polymerase subunit delta |
| SAOUHSC_000268    |             | Hypothetical                     |
| SAOUHSC_01110     | efb         | Fibrinogen-binding protein-like protein |
| SAOUHSC_01688     | lepA        | GTP-binding protein LepA         |
| SAOUHSC_002855    | amD2        | LysM domain-containing protein   |
| SAOUHSC_002762    |             | Hypothetical                     |
| SAOUHSC_002114    | dogK        | Putative lipid kinase            |
| SAOUHSC_002372    |             | Hypothetical                     |
| SAOUHSC_002430    | htsA        | ABC transporter periplasmic binding protein |
| SAOUHSC_001320    | dhoM        | Homoserine dehydrogenase         |
| SAOUHSC_000986    | sspC        | Cysteine protease                |
| SAOUHSC_00411.3   | psma3       | Alpha phenol soluble modulin     |
| SAOUHSC_002112    | isaB        | Hypothetical                     |
| SAOUHSC_000268    |             | Hypothetical                     |
| SAOUHSC_001326    | lysP2       | Hypothetical                     |
| SAOUHSC_002127    | sspB2       | Staphopain thiol proteinase      |
| SAOUHSC_001936    | splE        | Serine protease SPlE             |
| SAOUHSC_000728    | ltaS        | Hypothetical                     |
| SAOUHSC_000625    | mmnA        | Putative monovalent cation/H + antipporter subunit A |
| SAOUHSC_002763    | oppA-IF     | Peptide ABC transporter ATP-binding protein |
| SAOUHSC_000988    | sspA        | Glutamyl endopeptidase           |
| SAOUHSC_000771    |             | Hypothetical                     |
| SAOUHSC_005561    | vrX         | Hypothetical                     |
| SAOUHSC_002550    | FdhD        | Formate dehydrogenase accessory protein |
| SAOUHSC_000875    | ndr2        | Hypothetical                     |
| SAOUHSC_001359    | mprF        | Hypothetical                     |
| SAOUHSC_001192    | vfrA        | Hypothetical                     |
| SAOUHSC_002887    | isaA        | Immunodominant antigen A         |
| SAOUHSC_002254    | groEL       | chaperonin GroEL                 |
| SAOUHSC_002485    | rpoA        | DNA-directed RNA polymerase subunit alpha |
| SAOUHSC_001462    | gpsB        | Hypothetical                     |
| SAOUHSC_000367    | tcyP        | Hypothetical                     |
| SAOUHSC_001062    |             | Hypothetical                     |
| SAOUHSC_000893    | namA        | FMN oxidoreductase               |
| SAOUHSC_001044    | ausA        | Hypothetical                     |
| SAOUHSC_000652    | fhuA        | Iron compound ABC transporter ATP-binding protein |
| SAOUHSC_002883    | ssaA        | LysM domain-containing protein   |
| SAOUHSC_001431    | mxrB        | Methionine sulfoxide reductase B  |

DEG, differentially expressed gene.

The complete data of the differentially expressed genes with higher expression by *S. aureus* during infection of the resistant C57BL/6 mice are displayed in Supplementary Table S.
Target expression affects efficacy of anti-virulence approaches. After having demonstrated the influence of the levels of host resistance on both the quality and quantity of \( S. aureus \) transcriptional response, we sought to determine the consequences of this dependence on the effectiveness of anti-virulence strategies. For this purpose, we assessed the effect of neutralizing a virulence factor that differed in expression between \( S. aureus \) infecting A/J and C57BL/6 mice on the bacteria fitness during infection. We chose aureolysin, which has been shown to be important for full virulence of \( S. aureus \) in experimental infection models\(^{46,47} \) and was expressed to a significantly greater extent by \( S. aureus \) during infection of C57BL/6 than during infection of A/J mice. The overall amount of \( S. aureus \) bacteria counting both wild type and \( \Delta aur \) strains was significantly greater \((P = 0.0138, t\)-test\) in the kidneys of A/J \( (1.3 \times 10^6 \pm 4.3 \times 10^5) \) than in the kidneys of C57BL/6 mice \( (1.03 \times 10^7 \pm 3.4 \times 10^6) \). Moreover, while a lower amount of \( \Delta aur \) than wild type \( S. aureus \) was recovered from C57BL/6 mice, the amount of \( \Delta aur \) recovered from A/J mice was comparable to that of wild type strain (Fig. 6a).

Thus, the \( \Delta aur \) had a competitive disadvantage when co-administered with wild type \( S. aureus \) in C57BL/6 mice (mean competitive index for \( \Delta aur/wild type \ S. aureus \) of 0.013), while \( \Delta aur \) and wild type \( S. aureus \) were equally competitive after co-administration in A/J mice (mean competitive index for \( \Delta aur/wild type \ S. aureus \) of 1.595) (Fig. 6b). Taken together, these results indicate that the efficacy of targeting a virulence factor by anti-virulence strategies will strongly depend on its level of expression by the pathogen during infection, which in turn is highly influenced by the intrinsic levels of host resistance to infection.

Discussion
Anti-virulence strategies based on attenuation of bacterial pathogenesis by the specific inhibition of virulence factors essential for the pathogen’s survival during infection\(^{48} \), have received increasing attention as novel treatment options for infections caused by antibiotic-resistant pathogens\(^{48} \). The concept of anti-virulence therapy is still very much in its infancy and therefore more research is needed to explore its practicability. One important aspect that should be considered carefully when designing anti-virulence strategies is that the expression of virulence traits by the pathogens is not constitutive but therefore more research is needed to explore its practicability. Consequently, absent expression of the targeted virulence factors could render anti-virulence strategies completely ineffective. Therefore, it is essential to understand the impact of the wide-ranging, inter-individual variation of the host response on the pathogen’s expression of virulence determinants during infection.

Our study supports the idea that the host genetic background affects the transcriptional response of \( S. aureus \) during infection. The limited capability of the immune defense mechanisms of susceptible A/J mice to control \( S. aureus \) growth led to the development of an intense inflammatory response, apparent by the disproportionate expression of inflammatory cytokines and damage-associated molecular patterns. Transcriptional reprogramming of the eukaryotic cells, possibly resulting from the concomitant accumulation of acidic products and the lowered oxygen tension (hypoxia) in the infected tissue, involved the induction of \( \text{Hif1a} \) that encodes the central mediator of transcriptional responses to hypoxia HIF-1\( \alpha \)\(^{49,50} \). Therefore, the
major challenge faced by \textit{S. aureus} in susceptible A/J mice seems to be the adaptation to the adverse conditions imposed by the hyperinflammatory response and hypoxic microenvironments. To survive in the septic A/J mice, \textit{S. aureus} increased expression of the ADI operon (\textit{arcABDCR})27, which is generally induced under anaerobic conditions and is important for energy generation, but also protects \textit{S. aureus} against acidic stress28.

The superior resistance mechanisms of C57BL/6 mice against \textit{S. aureus} enabled a better control of bacterial multiplication. Therefore, \textit{S. aureus} faced the main challenge of counteracting the powerful host defense mechanisms of the resistant C57BL/6 mice. The transcription data indicated that \textit{S. aureus} responded to the adverse environment encountered within C57BL/6 mice by increasing the expression of cytoxins and extracellular proteases. Cytoxins such as alpha-hemolysin (\textit{hla}) and PSMs \textit{(psma1-3 and hld)} help \textit{S. aureus} to avoid phagocytic killing by inducing pores in the membrane of host cells, leading to cell death51 and can promote bacterial spreading by disrupting

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**Figure 4 | Expression of selected genes by \textit{S. aureus} during infection of C57BL/6 or A/J mice.**

(a) Expression levels of genes encoding proteases. (b) Expression levels of genes encoding toxins. (c) Schematic representation and level of expression of genes encoding the global regulator Agr and SarR. Red bars show expression values of the genes expressed by \textit{S. aureus} in C57BL/6 mice and blue bars show expression values of the genes expressed by \textit{S. aureus} in A/J mice. Each bar represents the mean TPM ± s.d. of triplicates.
epithelial barrier. The strong induction of genes encoding key exoproteases, including the metalloprotease aureolysin (aur), serine proteases (splA, spsA, splE and splF), staphostatin B (spsC) and cysteine protease (splP), further accentuates the importance of immune evasion for S. aureus survival in C57BL/6 mice. These proteases can cleave and degrade components of the complement system and can inhibit neutrophil chemotaxis. The expression of these virulence determinants is orchestrated through transcriptional and post-transcriptional regulation by regulatory systems. The expression of RNAIII, a major regulator of these factors, was higher expressed by S. aureus during infection of C57BL/6 mice than in A/J mice, while the expression of the autocatalytic sensory transduction system did not differ between both mouse strains. This apparent discrepancy could, however, be explained by greater transcript abundance of SarR in S. aureus infecting C57BL/6 mice, which mitigates the expression of the agr operon, while having no apparent effect on the expression of RNAIII (ref. 55). Moreover, SarR enhances the expression of genes encoding several extracellular proteases regulated by RNAIII (ref. 39) and, therefore, may act in synergy with RNAIII to boost the expression of virulence factors, required for survival under the strong immune pressure in C57BL/6 mice. The strong immune pressure in the resistant mice could also explain the greater expression of several genes of the cell wall stress stimulon by S. aureus infecting C57BL/6 mice. It has been shown that the magnitude of cell wall stimulus induction strongly

Figure 5 | Apolipoprotein B (ApoB) contributes to resistance against S. aureus bloodstream infection in C57BL/6 mice. (a) Relative fold change of Apob expression values in the kidneys of uninfected C57BL/6 respect to those in the kidneys of uninfected A/J mice determined by either RNA-Seq (white bars) or qRT–PCR (black bars). Each bar represents the mean relative fold change ± s.d. of triplicates. (b) Expression of ApoB in kidneys of A/J (left) and C57BL/6 (right) mice determined by immunostaining of kidney tissue using specific antibodies against ApoB. Magnification X40. (c) Relative fold change of Apob expression values in the kidney tissue using specific antibodies against ApoB. Magnification X40. (d) Relative fold change of Apob expression values in the kidneys of S. aureus-infected C57BL/6 in comparison to those in the kidneys of S. aureus-infected A/J mice at 48 h of infection determined by either RNA-Seq (white bars) or qRT–PCR (black bars). Each bar represents the mean relative fold change ± s.d. of triplicates. (d) Bacterial loads in the kidneys of A/J (blue symbols) and C57BL/6 (red symbols) mice treated with 4-Aminopyrazolo[3,4-d]pyrimidine (4-APP) (open symbols) or with vehicle alone (solid symbols) at 48 after intravenous inoculation with 2 × 10^7 CFU of S. aureus strain SH1000. Each symbol represents the bacterial counts determined in an individual mouse and the horizontal lines represent the average ± s.d. for each mouse strain (n = 6, t-test, **P < 0.01, ***P < 0.001).

Table 3 | S. aureus gene expression during infection of 4-APP-treated and MyD88-deficient C57BL/6 mice.

| Locus tag    | Gene symbol | Description                     | Relative fold change in 4-APP-treated to vehicle-treated C57BL/6 mice (mean (s.e.)) | Relative fold change in MyD88-deficient to wild type C57BL/6 mice (mean (s.e.)) |
|--------------|-------------|---------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| SAOUHSC_02265 | ogrA        | Accessory gene regulator protein A | -1.60 (0.22)                                                                     | -1.62 (0.5)                                                                     |
| SAOUHSC_02566 | sarR        | Accessory regulator R            | -1.83 (0.22)                                                                     | -1.23 (0.09)                                                                     |
| SAOUHSC_01121 | hla         | Alpha-hemolysin                  | -3.32 (1.56)                                                                     | -18.06 (0.16)                                                                   |
| SAOUHSC_02971 | aur         | Zinc metalloprotease aureolysin  | -2.21 (0.96)                                                                     | -2.59 (0.75)                                                                     |
| SAOUHSC_00988 | spsA        | Glutamyl endopeptidase           | -2.19 (0.01)                                                                     | -2.32 (0.46)                                                                     |
| SAOUHSC_00561 | vraX        | Protein VraX                     | -3.91 (2.76)                                                                     | 1.11 (0.04)                                                                     |
| SAOUHSC_00436 | gltD        | Glutamate synthase subunit beta  | -2.41 (1.11)                                                                     | -6.03 (1.66)                                                                     |
| SAOUHSC_01803 | oapA        | D-serine/D-alanine/glycine transporter | 1.21 (2.32)                                                                     | 1.69 (0.29)                                                                     |
| RNAIII      |             | Regulatory RNA                   | -1.80 (0.53)                                                                     | -2.52 (0.08)                                                                     |

Level of expression of a subset of genes by S. aureus during infection of 4-APP-treated compared with vehicle-treated C57BL/6, respectively MyD88-deficient compared with wild-type C57BL/6 mice determined by quantitative reverse transcription-PCR (qRT–PCR).
depends on the concentration of cell wall damaging agents which might indicate that S. aureus is exposed to a higher concentration of these damaging agents during infection of C57BL/6 than during infection of A/J mice.

To further corroborate the strong dependence of S. aureus expression of virulence factors on the strength of the host immune defense we investigated the effect of reducing the innate resistance of C57BL/6 mice to S. aureus either by chemotherapeutic reduction of ApoB concentration or by genetic deletion of the adaptor molecule MyD88 on the pathogen’s gene expression. The results showed that reduction of resistance to infection in C57BL/6 mice resulted in a shift of S. aureus gene expression. The genes encoding the virulence factors alpha-hemolysin (hla), aureolysin (aur) and a glutamyl endopeptidase (sspA) as well as the virulence regulators SarR (sarR) and RNAIII were expressed to a lesser extent by S. aureus infecting the more susceptible 4-APP-treated or MyD88-deficient C57BL/6 mice than during infection of normal C57BL/6 mice. Together these results provide evidence of a relationship between host resistance and pathogen expression of virulence factors.

After having demonstrated the differential expression of virulence genes by S. aureus in response to different levels of host resistance during infection, we investigated the impact of this dependence on the efficacy of anti-virulence approaches. Targeting aureolysin, a gene that was expressed by S. aureus to a higher extent during infection of C57BL/6 than during infection of A/J mice, resulted in significant reduction of S. aureus fitness in C57BL/6 mice (P < 0.05, t-test), but did not affect the bacterial fitness during infection of A/J mice. These findings highlight that the efficacy of an anti-virulence strategy against S. aureus will depend on the level of expression of the targeted virulence factor. Given that we show effects of differential expression of virulence factors on the efficiency of targeted interference in a defined model using two inbred mouse strains, it can be assumed that the more diverse situation in humans would even further complicate the application of anti-virulence strategies.

In summary, by combining the transcriptional response of the pathogen and of the resistant and susceptible hosts in a single experimental system, we provided evidence of the impact of host intrinsic variability on the gene expression of S. aureus during infection. Differences in the capacity of C57BL/6 and A/J mice to control S. aureus resulted in different amounts of bacteria in the organs, which might affect bacterial gene expression via growth phase as well as quorum-sensing-dependent regulation and consequently result in the different expression of virulence factors. This information is essential when searching for novel anti-virulence targets. Although a number of anti-virulence strategies targeting S. aureus toxins as well as the quorum-sensing regulator agr have been tested in pre-clinical mouse models with promising results a common limitation of these studies is the determination of treatment efficacy using a single host background. The findings of our study highlight the risk of drawing definitive conclusions for in vivo efficacy from such pre-clinical studies using a single host strain and emphasize the necessity to evaluate anti-virulence strategies in a range of host backgrounds.

Methods
Bacterial strains. The S. aureus strains used in this study were strain SH1000 (ref. 61), the progenitor strain 8325-4 and the aureolysin-deficient (8325-4-aur) S. aureus mutant strain. Bacteria were grown to mid-log phase in brain heart infusion medium (BHI) at 37 °C with shaking (120 r.p.m.), collected by centrifugation, washed with sterile PBS and diluted to the required concentration for injection. The number of viable bacteria was determined after serial dilution and plating on blood agar.

Mice and infection model. Pathogen-free 10 weeks old female C57BL/6 and A/J mice were purchased from Harlan-Winkelman (Envigo, The Netherlands) and had similar body weight (21 ± 1.9 g for A/J) and 20.5 ± 0.8 g for C57BL/6 mice). Mice were infected intravenously with 4 × 10^7 colony forming units (CFU) of S. aureus strain SH1000 in 100 μl of PBS via a lateral tail vein. For determination of bacterial numbers in the kidneys, mice were killed by CO2 asphyxiation at 48 h after bacterial inoculation, kidneys were removed and homogenized in PBS. Serial 10-fold dilutions of kidney homogenate were plated on blood agar plates. Bacterial colonies were counted after incubation at 37 °C for 24 h and calculated as CFU per kidneys.

For competition experiments, mice were intravenously inoculated with a 1:1 mixture of S. aureus strain 8325-4 and 8325-4-aur mutant strain containing a total of ~4 × 10^7 bacteria. Mice were killed at 48 h of infection and the amount of each bacterial strain was determined by plating kidney homogenates in the absence (wild type + aur strain) or in the presence (Aur strain) of 7.5 μg ml⁻¹ Erythromycin. The competitive index (CI) in the mixed infection was defined as the Aur/wild type ratio in the infected kidneys at 48 h of infection. The experiment was repeated independently three times.

In some experiments, mice were treated intraperitoneally with 2.5 μg ml⁻¹ of 4-Aminopyrazolo[3,4-d]pyrimidine (4-APP, Sigma-Aldrich) in 0.9% NaCl solution or with vehicle alone (0.9% NaCl) at 48 and 24 h before bacterial inoculation. Individual mice were randomly assigned to the treatment or control group. Bacterial injections were performed blindly. Mice were then infected with 2 × 10^7 bacteria of S. aureus strain SH1000, killed at 48 h of infection and the amount of each bacterial strain was determined by plating on blood agar. The experiment was repeated independently three times.

Figure 6 | In vivo competitive fitness of aureolysin-deficient (Δaur) and wild type S. aureus during infection of C57BL/6 or A/J mice. (a) C57BL/6 and A/J mice were injected intravenously with a 1:1 mixtures of Δaur and wild type S. aureus 8325-4 containing a total of approximately 4 × 10^7 bacteria. The number of bacterial cells in kidneys homogenates was determined at 48 h of infection. Symbols representing Δaur and wild type S. aureus 8325-4 bacteria from the same animal are connected by a broken line. (b) Competitive index (CI), representing the ratio of the recovered Δaur divided by the recovered wild type bacteria, within resistant C57BL/6 and susceptible A/J mice (n = 7, t-test; P < 0.001).
Animal experiments were performed in strict accordance with the German regulations of the Society for Laboratory Animal Science (GV - SOLAS) and the European Health Law of the Federation of Laboratory Animal Science Associations (FELASA) and animals were excluded from further analysis if killing was necessary according to the human endpoints established by the ethical board. All experiments were approved by the ethical board Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Oldenburg, Germany (LAVES; permit N. 33.3-42502-04-1195).

**Sample preparation and fixation for RNA-seq.** For RNA-seq experiments, three infected biological replicates from each mouse strain were analysed. Three uninfected, mock-treated replicates of each mouse strain were included as a reference to assess host gene expression changes induced by the infection. Each replicate consisted of pooled equimolar amounts of RNA extracted from the kidneys of five mice. To visualize the RNA expression profiles, kidneys were extracted from infected and uninfected A/J and C57BL/6 mice were stored in 3 ml of RNAAlater (Ambion) at 4 °C overnight.

**Total RNA extraction, purification and rRNA depletion.** Whole kidneys were mechanically disrupted using a Polytron disperser (Kinematica) in 2 ml of sterile PBS supplemented with 1% β-mercaptoethanol (Sigma-Aldrich) to denature RNA. Two microliters per ml of 50 cycles in one direction. Between 84 and 139 million reads (50 bp) on the illumina HiSeq 2500 platform utilizing the TruSeq S.R. cluster kit, RNA species. Library quality and size-selection was assessed using the Agilent 2100 using the QIAquick gel extraction kit to exclude remaining contamination by other indices for sample multiplexing and Illumina adaptor sequences. Following a polyonal rabbit anti-mouse apolipoprotein B-specific antibody (Abcam ab20737). Paraffin sections were rehydrated through graded alcohols. For blocking of the endogenous peroxidase, formalin-fixed, paraffin-embedded tissue sections were treated with 0.5% H2O2 diluted in methanol for 30 min at room temperature (RT). Subsequently, sections were treated in 10 ml Na-citrate buffer pH 6.0 for 20 min in a microwave oven (800 W). Following incubation with 20% goat serum (obtained from University of Veterinary Medicine Hannover) for 30 min to block non-specific binding sites, sections were incubated with polyonal rabbit anti- mouse apolipoprotein B-specific antibody (Abcam ab20737, dilution 1:50) for 1.5 h at RT. Rabbit serum (Sigma-Aldrich; R 4505) was used as negative control. Thereafter, sections were treated for 30 min at RT with the secondary goat anti-rabbit antibody (Vector Laboratories BA1000, dilution 1:200). Slides were subsequently incubated with the peroxidase-conjugated avidin-biotin complex (Vector Laboratories PK6100) for 30 min at RT. All antibodies were diluted in PBS. Following visualization of the positive antigen-antibody reaction by incubation with 3,3-diamobenzidine-tetrachloride (Fluka 32750) for 5 min, sections were counterstained with hematoxylin.

**Tissue ELISA.** Kidneys were taken from S. aureus-infected C57BL/6 and A/J mice at 48 h after bacterial inoculation or from mock-treated and homogenized in tissue lysis buffer containing 20 mM NaCl, 5 mM EDTA, 10 mM Tris, 1 mM PMSF, 1 μg/ml Leupeptin, 28 μg/ml Aprotinin at pH 7.4. Homogenized samples were centrifuged twice and the supernatant was collected and used for quantification of interleukin 6 (IL-6), interleukin 1β (IL-1β), interleukin 10 (IL-10), Cxcl1, Cxcl2 and Ccl2 concentrations using matched antibody pairs and recombinant proteins as standards. Briefly, 96-well microtiter plates were coated overnight at 4 °C, with the corresponding purified anti-mouse capture monoclonal antibody (Bioreagent 31305, 432002, 431412, R&D Systems DSY52, DY453, BD Bioscience 555260) in the coating buffer (Na2PO4, pH 6.5, Na2CO3, pH 9.5 or PBS). Plates were washed and blocked with 1% bovine serum albumin-PBS or 10% fetal bovine serum-PBS. Tissue supernatants as well as standard were added in technical triplicates at previously established dilutions. Monoclonal anti-mouse detection antibody was added incubated for 1 h at RT (Aldrich; R 4005) was used as negative control. This step was omitted for Ccl2 quantification since the detection antibody was already HRP coupled. Plates were developed using TMB as substrate.

**Data processing.** Raw sequenced reads were quality filtered and trimmed for Illumina-adapter contamination using fastq-mc1. Remaining reads were aligned either to the genome of the S. aureus strain 8325-4, manually revised for the changes of strain SH1000 (ref. 21), or to the Mus musculus reference genome GRCm38.p3 using STAR2. Minimal sequence matches of 93.33% were included. Mapped reads were collapsed using samtools6 and counted using HTSeq5. Raw mapped read counts were normalized to transcripts per million (TPM).

**Quantitative RT-PCR.** Total RNA was extracted from tissue samples using the phenolchloroformisooamyl alcohol method. Total RNA was obtained by RNAse-free kit (Invitrogen). RNA samples were reverse transcribed and amplified using a SensiFast SYBR No-ROX Kit (Bioline) following the manufacturer’s recommendations. The primers used for quantitative RT-PCR were provided in Supplementary Table 3. Thermal cycling conditions for hld/RNAIII, sarka, hla, spsa, aur, vraX, gtb, ppaq, apob and β-actin quantification consisted of reverse transcription for 20 min at 45 °C, initial denaturation for 5 min at 95 °C, followed by 40 cycles of 20 s at 95 °C (denaturation), 20 s at 60 °C (annealing) and 20 s at 72 °C (elongation). Annealing temperature was 70 °C. Data were normalized against the housekeeping gene 16S for S. aureus and β-actin for apob. Fold change values were calculated by the Pfaffl equation, in which the expression ratio is estimated by (Etarget/Expcontrol − experiment/Expcontrol) / (Etarget/Expcontrol + experiment/Expcontrol), where Etarget is the fold change between the expression of apoB in the kidneys of C57BL/6 relative to that in A/J mice. For S. aureus values were expressed as the fold change between the expression of a specific gene by S. aureus during the infection of 4-APP-treated C57BL/6 mice in respect to the expression by S. aureus during infection of vehicle-treated C57BL/6 mice.

**Apolipoprotein B immunohistochemistry.** Kidney samples were collected from infected C57BL/6 and A/J mice for immunohistochemistry. Tissues were fixed in 10% formalin and embedded in paraffin. Tissue sections were cut to 4 μm thickness and stained immunohistochemically using the polyclonal rabbit anti-mouse apolipoprotein B-specific antibody. Following visualization of the positive antigen-antibody reaction by incubation with 3,3-diamobenzidine-tetrachloride (Fluka 32750) for 5 min, sections were counterstained with hematoxylin.

**Statistical analysis.** Statistical analyses were performed using PRIMER (Version 6.1.1, PRIMER-E: Plymouth Marine Laboratory), GraphPad Prism (Version 5/04, GraphPad Software, Inc.) and R (http://www.r-project.org). To assess the global similarity between biological replicates, a sample-similarity matrix was generated using euclidean distance measurement. The transcriptional profiles were compared using PCA and group-average agglomerative hierarchically clustering. PERMA- NOVA was used to determine the statistical significance of differences between the transcriptional profiles of the different groups. For the one-way PERMANOVA, the established matrix was used distanced using euclidean distance using type III (partial) sums of squares with a fixed effects sum to zero for mixed models. Exact P values were generated using unrestricted permutation of the raw data. Pseudo-F statistic and generated P values were reported for each pair of conditions. Monte Carlo simulations were performed in the pairwise test function if low permutation values were obtained. Differences between groups were considered significant if P < 0.05. Low read counts were filtered from the murine datasets applying a strict LODR-cutoff (limit of detection of ratio) as determined for a two-fold increase in transcript abundance by analysis of the ERCC spike-in mixes. To avoid misinterpretation resulting from the low sequencing depth of the S. aureus transcriptions, a more stringent threshold was applied, where genes with transcripts levels below 0.05% (< 16 copies per 33,228 reads) were filtered from the bacterial gene expression datasets. Due to the differences in sequencing depth different strategies were used for determination of differential gene expression analysis for host and pathogen. To identify genes differentially expressed by S. aureus and/or Apob after infection of vehicle-treated C57BL/6 mice, the non-parametric NOISeq algorithm, whose sensitivity is less dependent on the sequencing depth compared to other methods64, was applied. A gene was considered differentially expressed by...
S. aureus between infection of A/J and C57BL/6 mice when the FDR was <0.05 (probability value >0.95). The DESeq2 algorithm was applied to identify genes differentially expressed between infected and S. aureus-infected A/J and C57BL/6 mice. A gene was considered significantly differentially expressed between mice if the Benjamini-Hochberg adjusted P value was <0.05 and the fold change ≥1. KEGG pathway enrichment analysis was performed using DAVID Informatics Resources. KEGG pathways with a FDR ≤0.05 were considered significantly enriched.

Data availability. The RNA-seq data that support the findings of this study have been deposited in the European Nucleotide Archive with the accession code PRJEB14649. All other relevant data are available from the corresponding author on request.

References
1. Spellberg, B. et al. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin. Infect. Dis. 46, 155–164 (2008).
2. Rasko, D. A. & Sperrandio, V. Anti-virulence strategies to combat bacteria-mediated disease. Nat. Rev. Drug Discov. 9, 117–128 (2010).
3. Wang, J. et al. Novel inhibitor discovery and the conformational analysis of inhibitors of lisserylisin O via protein-ligand modeling. Sci. Rep. 5, 8664 (2015).
4. Krachler, A. M. & Orth, K. Targeting the bacteria-host interface: strategies in oxidative stress and inflammation. Front. Cell. Infect. Microbiol. 2, 12 (2012).

27. Mahlkin, J. et al. Staphylococcus aureus ArcR controls expression of the arginine deiminase operon. J. Bacteriol. 189, 5976–5986 (2007).
28. Rasko, D. A. & Sperrandio, V. Targeting Host-Adapting to Anaerobic Conditions by Staphylococcus aureus. Adv. Appl. Microbiol. 84, 1–25 (2013).
29. Seidl, K. et al. Effect of a glucose impulse on the CcpA regulon in Staphylococcus aureus. BMC Microbiol. 9, 1 (2009).
30. Monnet, V. Bacterial oligopeptide-binding proteins. Cell Mol. Life Sci. 66, 2100–2114 (2003).
31. Kohler, S. L. et al. Extracellular proteases are key mediators of Staphylococcus aureus virulence via the global modulation of virulence-determinant stability. Microbiol. Ecol. 12, 18–34 (2013).
32. Jusko, M. et al. Staphylococcal proteases aid in evasion of the human complement system. J. Infect. Immun. 6, 31–46 (2013).
33. Ko, Y. P. et al. Phagocytosis escape by a Staphylococcus aureus protein that connects complement and coagulation proteins at the bacterial surface. PLoS Pathog. 9, e1003816 (2013).
34. Vandenesch, F., Lina, G. & Henry, T. Staphylococcus aureus hemolysins, bi-component leukocidins, and cytolytic peptides: a redundant arsenal of membrane-damaging virulence factors? Front. Cell. Infect. Microbiol. 2, 12 (2012).
35. Tormo, S., Monteil, H. & Prevost, G. Regulation of virulence determinants in Staphylococcus aureus: complexity and applications. FEMS Microbiol. Rev. 28, 183–200 (2004).
36. Peng, H. L., Novick, R. P., Kreiswirth, B., Kornblum, J. & Schlievert, P. Cloning, characterization, and sequencing of an accessory gene regulator (agr) in Staphylococcus aureus. J. Bacteriol. 170, 4365–4372 (1988).
37. Westermann, A. J., Gorski, S. A. & Vogel, J. Dual RNA-seq of pathogen and host–pathogen interactions. Cell. Microbiol. 17, 275–291 (2015).
38. Cassadavell, A. & Pirófski, L. Host-pathogen interactions: the attributes of virulence. J. Infect. Dis. 184, 373–344 (2001).
39. Lowy, F. D. Staphylococcus aureus infections. N. Engl. J. Med. 339, 520–532 (1998).
40. Casadevall, A. & Pirofski, L. Host-pathogen interactions: two novel alternatives. FEMS Immunol. Med. Microbiol. 65, 399–412 (2012).
41. Hiramatsu, K. et al. Multi-drug-resistant Staphylococcus aureus and future chemotherapy. J. Infect. Chemother. 20, 593–601 (2014).
42. Fowler, Jr V. G. & Proctor, R. A. Where does a Staphylococcus aureus vaccine stand? Clin. Microbiol. Infect. 20(Suppl 5): 66–75 (2014).
43. King, C., Neoh, H. M. & Nathan, S. Targeting Staphylococcus aureus toxins: a potential form of anti-virulence therapy. Toxins (Basel) 8, pii E72 (2016).
44. Fitzgerald-Hughes, D., Devoelle, M. & Humphries, H. Beyond conventional antibiotics for the future treatment of methicillin-resistant Staphylococcus aureus infections: two novel alternatives. FEMS Immunol. Med. Microbiol. 65, 399–412 (2012).
45. Mavromatis, C. H. et al. The co-transcriptome of uropathogenic Escherichia coli-infected mouse macrophages reveals new insights into host–pathogen interactions. Cell Microbiol. 17, 730–746 (2015).
46. Humphrys, M. S. et al. Simultaneous transcriptional profiling of bacteria and their host cells. PLoS ONE 8, e50957 (2013).
47. von Kockritz-Böckwede, M. et al. Immunological mechanisms underlying the genetic predisposition to severe Staphylococcus aureus infection in the mouse model. Am. J. Pathol. 173, 1657–1668 (2008).
48. Ahn, S. H. et al. Two genes on A/J chromosome 18 are associated with susceptibility to Staphylococcus aureus infection by combined microarray and QTL analyses. PLoS Pathog. 6, e1001088 (2010).
49. Westermann, A. J., Gorski, S. A. & Vogel, J. Dual RNA-seq of pathogen and host. Nat. Rev. Microbiol. 10, 618–630 (2012).
50. Westermann, A. J. et al. Dual RNA-seq unveils noncoding RNA functions in host–pathogen interactions. Nature 529, 496–501 (2016).
51. Baddal, B. et al. Dual RNA-seq of nontypeable Haemophilus influenzae and host cell transcriptomes reveals novel insights into host-pathogen cross talk. mBio 6, e01765–15 (2015).
52. O’Neill, A. J. Staphylococcus aureus SH1000 and 8325–4: comparative genome sequences of key laboratory strains in staphylococcal research. Lett. Appl. Microbiol. 51, 358–361 (2010).
53. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DSEAq2. Genome Biol. 15, 550 (2014).
54. Okamoto, K., Tamura, T. & Sawatsubashi, Y. Sepsis and disseminated intravascular coagulation. J. Intensive Care 4, 23 (2016).
55. Nizet, V. & Johnson, R. S. Interdependence of hypoxia and innate immune responses. Nat. Rev. Immunol. 9, 609–617 (2009).
56. Imamura, H. Z. & Simon, M. C. Hypoxia-inducible factors as essential regulators of inflammation. Curr. Top. Microbiol. Immunol. 345, 105–120 (2010).
57. Yang, Z. & Ming, X. F. Arginase: the emerging therapeutic target for vascular oxidative stress and inflammation. Front. Immunol. 4, 149 (2013).
58. Nakashima, Y. et al. Extracellular proteases are key mediators of Staphylococcus aureus virulence via the global modulation of virulence-determinant stability. Microbiol. Ecol. 12, 18–34 (2013).
59. Jusko, M. et al. Staphylococcal proteases aid in evasion of the human complement system. J. Infect. Immun. 6, 31–46 (2013).
60. Smagur, J. et al. Staphylococcal cytotoxic protease staphopain B (SspB) induces rapid engulfment of human neutrophils and monocytes by macrophages. Biol. Chem. 390, 361–371 (2009).
61. Reyes, D. et al. Coordinated regulation by AgrA, SarA, and SarR to control agr expression in Staphylococcus aureus. J. Bacteriol. 193, 6020–6031 (2011).
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Author contributions

R.T. and E.M. designed the study. R.T., O.G. and A.B. performed the experiments and analysis of the data. R.T. and E.M. wrote the manuscript.

Additional information

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