nature research

Peer Review Information

Journal: Nature Microbiology
Manuscript Title: Emerging Staphylococcus epidermidis clones express Staphylococcus aureus-type wall teichoic acid to shift from a commensal to pathogen lifestyle
Corresponding author name(s): Andreas Peschel

Reviewer Comments & Decisions:

EA, duplicate for each version as needed, and then delete this instruction.

Decision Letter, initial version:

Dear Andreas,

Thank you for your patience while your manuscript "Emerging Staphylococcus epidermidis clones express Staphylococcus aureus-type wall teichoic acid to shift from commensal to pathogen behavior" was under peer-review at Nature Microbiology. It has now been seen by 4 referees, whose expertise and comments you will find at the of this email. As you will see from their reports, although the referees find your work of potential interest, they have raised a few concerns that will need to be addressed before we can consider publication of the work in Nature Microbiology.

In particular, you will see that referee #1 raises several concerns related to the evolutionary aspects of the paper, remarking that a "switch to a lifestyle where they instead preferentially exist in the transmission dead-end environment of the bloodstream (...) make no sense at all. Apart from the short-term 'within-patient' advantage described here, based on the these finding there is no 'between host' selective advantage to this, and so it is highly unlikely to be involved in the success of this clone. There may be other features involved and associated with the WTA shift, perhaps it off-sets some of the fitness costs associated with the more widely accepted reason for the success of this clone i.e. their enhanced AMR profile." Some of these concerns are shared by referee #3, who asks for several clarifications that are also mostly related to the evolutionary aspects of the paper, including on the genetic origin of tarIJM, how S. aureus may have acquired this in the first place, why only some S. epi lineages acquired the operon, and also whether this acquisition changes other resistant traits (which aligns with the aforementioned comment from referee #1 about whether this is linked to other features that could promote transmission). I would like to point out that we appreciate that the main point of the paper is the identification of the molecular determinants of virulence, rather than elucidating the benefits of these different evolutionary strategies. Nonetheless, we feel that it would still be important to try to address these concerns as best as possible, including by further discussing these issues in more detail. You will see that referee #1 also pointed out that they found the paper "(...) quite a clunky read because the authors have chosen to present it in a short format, requiring much of the critical data (...) to be removed from the main text. This really does their findings a disservice, where presenting it in a more cohesive manner would make a much stronger case for their findings." We appreciate that part of this is related to the paper being submitted to Nature in first instance, but you do have room to expand the article and hopefully this would help to address the concerns raised by the referees.

In addition to these concerns related to the evolutionary angles, the referees also ask that you tone down some of the claims about these WTAs being a vaccine target; clarify if other phi-11-transducible isolates were sequenced; look at bacterial dissemination to peripheral organs; and further probe the mechanisms involved in more detail - for example, referee #4 asks where does the SaPI integrate; what are the roles of tarL/tarK/tarM2; how does the biolfilm data fit with the proposed model; is the GroP-WTA ligand known; and does RboP-WTA physically obfuscates binding sites for host ligands).

Should further experimental data allow you to address these criticisms, we would be very happy to look at a revised manuscript.

We are committed to providing a fair and constructive peer-review process so please do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We strongly support public availability of data. Please place the data used in your paper into a public data repository, if one exists, or alternatively, present the data as Source Data or Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. For some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found at

https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data.

Please include a data availability statement as a separate section after Methods but before references, under the heading "Data Availability". This section should inform readers about the availability of the data used to support the conclusions of your study. This information includes accession codes to public repositories (data banks for protein, DNA or RNA sequences, microarray, proteomics data etc…), references to source data published alongside the paper, unique identifiers such as URLs to data repository entries, or data set DOIs, and any other statement about data availability. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see:
http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf

If revising your manuscript:

* Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already we suggest that you begin to revise your manuscript so that it conforms to our Letter format instructions at http://www.nature.com/nmicrobiol/info/final-submission. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Please use the link below to submit a revised paper:

{REDACTED}

Note: This url links to your confidential homepage and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this e-mail to co-authors, please delete this link to your homepage first.

Nature Microbiology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit <a

href="http://www.springernature.com/orcid">www.springernature.com/orcid.

If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision, even if a similar study has been accepted for publication at Nature Microbiology or published elsewhere (up to a maximum of 6 months).

In the meantime we hope that you find our referees' comments helpful and please let me know if you have any questions.

With best regards and wishes of happy holidays,

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Reviewer Comments:

Reviewer #1 (Remarks to the Author):

The Du et al manuscript contains an elegant series of experiments defining a shift in wall teichoic acid (WTA) structure in a clone of Staphylococcus epidermidis (ST23) that has achieved some notoriety in the past 10-15 years as a frequent cause of nosocomial infection. The authors present evidence that (in line with their previous work), the DNA on which the new WTA biosynthesis machinery is encoded has come from S. aureus; and they go on to characterise some of the consequences of the expression of this has for this S. epidermidis clone. They conclude that this switch alters the commensal V pathogenic lifestyle of this clone.

Despite the tome of high-quality data presented, I am of the opinion that this work would be more suited to a more specialised journal where the authors could present the data in a more comprehensive and cohesive manner without trying to over-interpret the findings. These concerns are described in detail below.

Major concern:
1: The major conclusion of the work is that the acquisition of this WTA encoding piece of DNA causes an organism that forms part of the normal skin flora of 100% (?) of the human population (from where they can readily transmit) to switch to a lifestyle where they instead preferentially exist in the transmission dead-end environment of the bloodstream. From an evolutionary perspective this make no sense at all. Apart from the short-term 'within-patient' advantage described here, based on the these finding there is no 'between host' selective advantage to this, and so it is highly unlikely to be involved in the success of this clone. There may be other features involved and associated with the WTA shift, perhaps it off-sets some of the fitness costs associated with the more widely accepted reason for the success of this clone i.e. their enhanced AMR profile.

While on an evolutionary theme, I'd like to see whether the shift to susceptibility to S. aureus phage means they are no longer susceptible to S. epidermidis phage (hopefully I didn't just miss this in the manuscript). As this would seem a very high-risk strategy to effectively accept DNA from distantly related organism while excluding that from within their own species.

2: While the writing style is clear throughout, I found it quite a clunky read because the authors have chosen to present it in a short format, requiring much of the critical data (9 additional figures, 4 additional tables plus 2 pages labelled 'supplementary results') to be removed from the main text. This really does their findings a disservice, where presenting it in a more cohesive manner would make a much stronger case for their findings.

3: I'm not convinced the data in supp fig. 5b supports the acquisition of the tarL2 cluster from S. aureus. While I agree with their conclusion that the S. epi. tarL2 is more similar to S. aureus tarL genes than the S. epi tarL1 cluster, the tree clearly shows it is most closely related to the S. epi tarL3, 4 and 5. So how do they conclude from this it was acquired from S. aureus?

Minor concerns:

4: I question the necessity of including the word 'no' in bar charts clearly reporting zero values.

5: To propose in manuscript abstract that this WTA represents a promising target for the development for new antimicrobial, while rightly dismissing it in the conclusion of the paper given it is only expressed by a sub-fraction of invasive HA-MRSE clones suggests it is an attempt to get this past the editors desk as opposed to being a suggestion of genuine intent.

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Reviewer #2 (Remarks to the Author):

In this manuscript "Emerging Staphylococcus epidermidis clones express Staphylococcus aureus-type wall teichoic acid to shift from commensal to pathogen behavior", Du et al. convincingly show that S. epidermidis ST23 strains contain a second functional WTA, encoded by the accessory genetic element tarIJLM2, which encodes RboP-WTA. tarL2 is found to be expressed in a number of isolates (E6, E45 and E73). Non-tarIJLM2 containing strains produced only GroP-WTA, whereas tarIJLM2 containing strains produced both GroP-WTA and Rbo-WTA. The authors elegantly demonstrate that tarIJLM2 is responsible for the production of Rbo-WTA by using genetic techniques to knock-in and -out the tarIJLM2 genes. tarL2-positive strains were exclusively observed in infection isolates, suggesting these genes are important in the infection settings, rather than colonization. The authors demonstrate that Rbo-WTA can bind to the endothelium, and suggest that this is leading to an increased bacterial burden in the bloodstream, during invasive infection. Overall, the manuscript is well-organized, contains rigorous experiments whereby the results support the conclusions, and the findings are very important for our understanding of the pathogenesis of S. epidermidis.

Minor comments:
1. Were any other phi-11-transducible isolates (apart from E73) sequenced? How was E73 chosen, given that other isolates, such as E50 have a higher transfer efficiency?
2. Did the increased bacterial burden in the blood resulted in bacterial burden at peripheral organs?

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Reviewer #3 (Remarks to the Author):

Staphylococcus epidermidis is a significant nosocomial pathogen, but also a main constituent of human skin and mucosa microbiota. Understanding the factors and processes that favour the one or the other lifestyle has major implications both for clinical diagnostics and for developing future therapeutic approaches. Many hospital-adapted S. epidermidis clonal lineages are characterized by the presence of a multitude of (acquired) antimicrobial resistance genes. The study by Du et al provides a new insight into the molecular basics of how nosocomial S. epidermidis clonal lineages may facilitate cross-species horizontal gene transfer (HGT) events and thereby adopt novel resistance and (maybe) virulence traits. The work is of extraordinary importance for understanding the evolution and adaptation power of this model opportunistic pathogen.

The study addresses the issue by focussing on the role of staphylococcal wall teichoic acids (WTA) as receptors for bacteriophages as well as as for the role of these structures as adhesins that mediate attachment to eukaryotic host cells. In their report, the authors can build on their own pioneering previous work, demonstrating that Staphylococcus aureus and S. epidermidis decorate their cell walls with structurally different WTAs (i.e. RboP-WTA in S. aureus, GroP-WTA in S. epidermidis) which serve as receptors for bacteriophages in a species-specific manner.

The authors show that common hospital-adapted S. epidermidis lineages have acquired an additional gene cluster (i.e. tarIJM) that enables synthesis of RboP-WTA. From the combined data it becomes evident that tarIJM is mobile and can spread by HGT among different S. epidermidis clonal lineages. Acquisition of the element by S. epidermidis renders these strains (i) susceptible to transduction by S. aureus bacteriophages and (ii) enables adhesion to endothelial rather than (nasal) epithelial cells. The latter mechanism is also associated with transition from a colonizing to a more invasive lifestyle of tarIJM-positive/ RboP-WTA-expressing S. epidermidis strains.

These are highly interesting findings and the experiments are well designed and conducted. I particularly

appreciate the comprehensive approach of the study, in which a broad range of adequate methods were used to answer the research question (i.e. NGS, comparative genomics & phylogenetic analysis; NMR/LC-MS for WTA detection and analyses; classical molecular & genetic analysis of tarIJM functions; phage adsorption assays, in vitro and in vivo adhesion & infection models etc.). The data obtained by these approaches are of high quality and convincing.

Nonetheless, while reading this well written and presented manuscript, some questions immediately came to my mind.

1. I understand from the data that tarIJM mediates synthesis of a S. aureus-type RboP-WTA, with the gene cluster itself being mobile. I wonder whether or not S. aureus is indeed the primary genetic origin of the S. epidermidis tarIJM? Thus, what about conservation of tarIJM-like genes in S. aureus and S. epidermidis on the nucleotide level? Also, did S. aureus (as a species) acquire RboP-WTA synthesis genes in the first place via HGT as well or do such genes belong to the S. aureus core genome?

Further, to tarIJM mobility:
2. Is there any (molecular/genetic) explanation why tarIJM was apparently acquired (exclusively?) by distinct S. epidermidis clonal lineages? Or is this, in evolutionary terms, a recent event and other clades will follow suit because all S. epidermidis have generally the capacity to integrate the element?

3. How does tarIJM generally travel? Apart from detection of tarIJM on a plasmid and an SCC element, are there other mobile genetic elements associated with the gene cluster?

3. Finally, with respect to the postulated increased capacity of tarIJM-carrying strains for HGT with S. aureus, do these strains harbour more antibiotic resistance genes than the other clades, and if so, is there a preference for specific resistance traits?

Minor points:

Line 122: 'lifestock' should read livestock; please correct
Line 123: Please add space to 'weremore'

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Reviewer #4 (Remarks to the Author):

In this study, the authors explore the genetic basis for the emergence of invasive Staphylococcus epidermidis (Sepi) isolates. Typically, Sepi colonizes the skin and nares of humans. Most strains synthesize wall teichoic acid with glycerol repeats (GroP-WTA) instead of ribitol repeats (RboP-WTA) as found in S. aureus and as synthesized by the tarIJL genes. Here, the authors use the specific binding of bacteriophages phi11 and phi187 to RboP-WTA and GroP-WTA respectively to identify Sepi clones that bind phi11. These clones carry an accessory tarIJLM gene referred as tarIJLM2. They demonstrate the tarIJL2 is responsible for the synthesis of RboP-WTA. By comparing skin and nasal isolates from healthy and infected humans, they report the exclusive association of tarIJLM2 genes with Sepi isolates from infections. They also note the presence of specific tarILJ elements in other healthcare associated clones of Sepi. The authors perform a series of experiment and conclude that (1) commonly used RP62A, 12228 and 1457 carry a non-functional tarIJL1 gene cluster and thus synthesize GroP-WTA only; (2) the GroP-WTA is better than RboP-WTA at promoting attachment of bacteria during colonization; specifically gain of tarIJLM2 genes leads to a reduced colonization potential; lastly acquisition of the tarIJLM2 genes results in (3) increased attachment to endothelial cells, (4) increased survival in a bloodstream model of infection, (5) increased ability to be recognized by phages and participate in HGT. Overall, the findings are interesting and support the authors' hypothesis. A few points could be further clarified.

Comments as they arise in the manuscript:

l. 31: here and elsewhere when referring to WTA: "expression" should be preferably changed to "production"

Fig. 1: where does the SaPI integrate on the chromosome of Sepi? Presumably, the insertion/integration site is a sequence shared between Sa and Sepi.

Extended Data Fig. 1: why does phi11 adsorption to isolates E1, E45 and E51 (Ext. Data Fig. 1a) does not

result in transduction (Fig. 1a)? Some of the Hamburg and Shanghai strains behave similarly. Do these strains lack the integration site for SaPI?

Line 82: What do the authors mean by "tarIJL1 is a pseudogene cluster"? is the coding sequence altered in such a way that the genes cannot be expressed or translated?

Fig. 1b:
- Do the authors concur with a model whereby TarL provides both the priming and polymerizing activity for Rbo? Or do the strains carry a tarK gene somewhere else on the chromosome?
- The authors do not say anything about TarM2; in S. aureus this enzyme adds O-linked α-GlcNAc at the C4 position of Rbo-P. Is this modification required for phi11 binding?

Extended Fig. 1d,e: it seems that these panels (while clear) are not described in the body of manuscript.

Fig. 3; Ext. Data Fig. 7cd:
The authors note that Sepi isolates from human skin and nose do not carry the tarIJLM2 gene cluster and posit that RboP-WTA may affect binding to epithelial surfaces. One test to examine this possibility compares biofilm formation of isogenic variants; presumably the presence of RboP WTA should reduce biofilm mass. But there isn't much of a biofilm to begin with, so the data is not terribly conclusive (see Ext. Fig. 7d that compares isogenic variants). Perhaps this section needs clarification.
Minor point: Line 155 and Ext. Data Fig. 7c: The figure panel does not use the ST nomenclature and the legend does not identify ST10.

Line 142: the authors write "GroP-WTA may be more capable of binding"? Capacity as in number of binding sites or affinity?
The authors posit that GroP-WTA is a better ligand than RboP-WTA. Yet S. aureus is a great colonizer. Is the GroP-WTA ligand known?

Ext. Data Fig. 8: here the title reads "RboP-WTA impairs Sepi binding to". In truth, the authors observe reduced binding to A549. Is it possible that the total amount of WTA in strain 1457(pBR-IJLM2) is unchanged and GroP-WTA is simply diluted away upon the competing synthesis of RboP-WTA? Or do the authors believe that RboP-WTA physically obfuscates binding sites for host ligands?

Lines 193-194: the authors conclude " RboP-WTA represents a promising antigen for future preventive or therapeutic vaccines". Previous work by the authors would suggest that this remains a challenge owing to the acquisition of galactosyl-transferases such as TarM and TarS that not only alter phage binding but also antigenicity of the molecule.

Author Rebuttal to Initial comments

Reviewer #1 (Remarks to the Author):

The Du et al manuscript contains an elegant series of experiments defining a shift in wall teichoic acid (WTA) structure in a clone of *Staphylococcus epidermidis* (ST23) that has achieved some notoriety in the past 10-15 years as a frequent cause of nosocomial infection. The authors present evidence that (in line with their previous work), the DNA on which the new WTA biosynthesis machinery is encoded has come from *S. aureus*; and they go on to characterise some of the consequences of the expression of this has for this *S. epidermidis* clone. They conclude that this switch alters the commensal V pathogenic lifestyle of this clone.

Despite the tome of high-quality data presented, I am of the opinion that this work would be more suited to a more specialised journal where the authors could present the data in a more comprehensive and cohesive manner without trying to over-interpret the findings. These concerns are described in detail below.

RE: We apologize for presenting our data in a very concise form to fit the Brief Communication format. The revised manuscript was adapted to the longer Article format with subheadings, better description of several of the findings, and inclusion of supplementary results, figure, and table that were previously included in the Supplementary Information. We also toned down the potential relevance for vaccine development in the Discussion. Nevertheless, RboP-WTA is regarded as a promising antigen for *S. aureus* vaccines and we feel that it is relevant to mention that such a vaccine could also potentially protect against many HA-MRSE clones as outlined above.

Major concern:

1: The major conclusion of the work is that the acquisition of this WTA encoding piece of DNA causes an organism that forms part of the normal skin flora of 100% (?) of the human population (from where they can readily transmit) to switch to a lifestyle where they instead preferentially exist in the transmission dead-end environment of the bloodstream. From an evolutionary perspective this make no sense at all. Apart from the short-term 'within-patient' advantage described here, based on the these finding there is no 'between host' selective advantage to this, and so it is highly unlikely to be involved in the success of this clone. There may be other features involved and associated with the WTA shift, perhaps it off-sets some of the fitness costs associated with the more widely accepted reason for the success of this clone i.e. their enhanced AMR profile.

RE: We appreciate the importance of this point, which was not sufficiently addressed in the original manuscript due to space limitations. We propose that RboP-WTA-producing *S. epidermidis* may use a currently unidentified niche as their major reservoir, where RboP-WTA may provide a fitness benefit, as outlined in more detail above.

While on an evolutionary theme, I'd like to see whether the shift to susceptibility to *S. aureus* phage means they are no longer susceptible to *S. epidermidis* phage (hopefully I didn't just miss this in the manuscript). As this would seem a very high-risk strategy to effectively accept DNA from distantly related organism while excluding that from within their own species.

RE: We performed additional experiments to address this interesting point using three GroP-WTA binding phages, two from *S. epidermidis* and one from the rare GroP-WTA expressing *S. aureus* lineage

ST395. The *S. epidermidis* strain with both, GroP- and RboP-WTA, still bound these phages, although with slightly reduced efficacy compared to the only-GroP-WTA expressing strains, which is in agreement with the fact that the overall WTA content of these strains remains constant and that those with both types of WTA have only ca. 50% of the binding sites for GroP-WTA specific phages. These new findings are shown in a new Fig. 2d and mentioned in the Results section (lines 115-120):

"The impact of *tarIJLM* on interaction with GroP-WTA-specific phages was studied using the previously described phages ΦPH15 and Φ187, plus a new bacteriophage ΦE72 that was isolated from *S. epidermidis* E72. E73Δ*tarIJLM2* had an increased capacity to bind the three phages compared to the E73 wild type and the complemented mutant (Fig. 2d), which supports our finding that E73 still produces GroP-WTA, albeit reduced amounts as a consequence of simultaneous RboP-WTA production."

2: While the writing style is clear throughout, I found it quite a clunky read because the authors have chosen to present it in a short format, requiring much of the critical data (9 additional figures, 4 additional tables plus 2 pages labelled 'supplementary results') to be removed from the main text. This really does their findings a disservice, where presenting it in a more cohesive manner would make a much stronger case for their findings.

RE: The manuscript style was changed to Article format with subheadings, better description of several of the findings, and inclusion of supplementary results, table, and figure that were previously included in the Supplementary Information.

3: I'm not convinced the data in supp fig. 5b supports the acquisition of the tarL2 cluster from S. aureus. While I agree with their conclusion that the S. epi. tarL2 is more similar to S. aureus tarL genes than the S. epi tarL1 cluster, the tree clearly shows it is most closely related to the S. epi tarL3, 4 and 5. So how do they conclude from this it was acquired from S. aureus?

RE: We agree that clear conclusions on the origin of *tarL2, 3, 4,* and *5* cannot be drawn on the basis of the comparisons shown in Supplemental Data Fig. 5b. All these genes are on mobile genetic elements and the closest core-genome encoded gene is the *S. aureus tarL*, which suggests that the genes have evolved at some point from the *S. aureus* gene. We modified the corresponding statement to clarify the point (Lines 170-174):

"The *tarIJLM* genes from the different clusters shared 66-89% nucleotide sequence identities, and the corresponding *tarL2*, *tarL3*, *tarL4*, and *tarL5* genes were more similar to the *S. aureus tarL* than the *S. epidermidis tarL1* gene (Extended Data Fig. 5a). These findings suggest that the *tarIJLM* gene clusters in *S. epidermidis* originated through HGT from an unknown donor rather than by gene duplication and diversification of the *S. epidermidis tarIJL1* cluster."

Lines 194-196:

"We have currently no indications for frequent mobility of any of the *tarIJLM* clusters suggesting that they might have evolved a long time ago, potentially originating from the core-genome-encoded *tar* genes of *S. aureus*."

Minor concerns:

4: I question the necessity of including the word 'no' in bar charts clearly reporting zero values.

RE: The 'NO' labels were removed from the figures as suggested. Values below detection limit of

qRT-PCR sample are denoted 'BD' (below detection limit) now.

5: To propose in manuscript abstract that this WTA represents a promising target for the development for new antimicrobial, while rightly dismissing it in the conclusion of the paper given it is only expressed by a sub-fraction of invasive HA-MRSE clones suggests it is an attempt to get this past the editors desk as opposed to being a suggestion of genuine intent.

RE: This statement was toned down at the end of the Discussion section as suggested. As stated above, protein-conjugated RboP-WTA is regarded as a promising antigen for *S. aureus* vaccines and it is of relevance to mention that such a vaccine could also potentially protect against many HA-MRSE clones.

Reviewer #2:

In this manuscript "Emerging Staphylococcus epidermidis clones express *Staphylococcus aureus*-type wall teichoic acid to shift from commensal to pathogen behavior", Du et al. convincingly show that *S. epidermidis* ST23 strains contain a second functional WTA, encoded by the accessory genetic element tarIJLM2, which encodes RboP-WTA. *tarL2* is found to be expressed in a number of isolates (E6, E45 and E73). Non-*tarIJLM2* containing strains produced only GroP-WTA, whereas *tarIJLM2* containing strains produced both GroP-WTA and Rbo-WTA. The authors elegantly demonstrate that *tarIJLM2* is responsible for the production of Rbo- WTA by using genetic techniques to knock-in and -out the *tarIJLM2* genes. *tarL2*-positive strains were exclusively observed in infection isolates, suggesting these genes are important in the infection settings, rather than colonization. The authors demonstrate that RboP-WTA can bind to the endothelium, and suggest that this is leading to an increased bacterial burden in the bloodstream, during invasive infection. Overall, the manuscript is well-organized, contains rigorous experiments whereby the results support the conclusions, and the findings are very important for our understanding of the pathogenesis of *S. epidermidis*.

RE: We are grateful for these encouraging comments.

Minor comments:

1. Were any other phi-11-transducible isolates (apart from E73) sequenced? How was E73 chosen, given that other isolates, such as E50 have a higher transfer efficiency?

RE: Indeed, all phi-11-transducible clones were sequenced and the corresponding accession numbers are given in Extended Data Table 1. These data confirmed the presence of the *tarIJLM* elements and allowed to assign their clonal identities. We created *tarIJLM* deletions in E73 and E1 because these strains could be most easily transformed with the gene inactivation plasmid vector.

2. Did the increased bacterial burden in the blood resulted in bacterial burden at peripheral organs?

RE: We present now additional experimental data in the new Fig. 4h showing that the bacterial burden of mice infected with the *tarIJLM*-deficient mutant are also significantly reduced. These findings are explained in the Results section as outlined above.

Reviewer #3 (Remarks to the Author):

Staphylococcus epidermidis is a significant nosocomial pathogen, but also a main constituent of human skin and mucosa microbiota. Understanding the factors and processes that favour the one or the other lifestyle has major implications both for clinical diagnostics and for developing future therapeutic approaches. Many hospital-adapted S. epidermidis clonal lineages are characterized by the presence of a multitude of (acquired) antimicrobial resistance genes. The study by Du et al provides a new insight into the molecular basics of how nosocomial S. epidermidis clonal lineages may facilitate cross-species horizontal gene transfer (HGT) events and thereby adopt novel resistance and (maybe) virulence traits. The work is of extraordinary importance for understanding the evolution and adaptation power of this model opportunistic pathogen.

RE: We are grateful for the positive assessment.

The study addresses the issue by focusing on the role of staphylococcal wall teichoic acids (WTA) as receptors for bacteriophages as well as for the role of these structures as adhesins that mediate attachment to eukaryotic host cells. In their report, the authors can build on their own pioneering previous work, demonstrating that *Staphylococcus aureus* and *S. epidermidis* decorate their cell walls with structurally different WTAs (i.e. RboP-WTA in *S. aureus*, GroP-WTA in *S. epidermidis*), which serve as receptors for bacteriophages in a species-specific manner.

The authors show that common hospital-adapted *S. epidermidis* lineages have acquired an additional gene cluster (i.e. *tarIJLM*) that enables synthesis of RboP-WTA. From the combined data it becomes evident that *tarIJLM* is mobile and can spread by HGT among different *S. epidermidis* clonal lineages. Acquisition of the element by *S. epidermidis* renders these strains (i) susceptible to transduction by *S. aureus* bacteriophages and (ii) enables adhesion to endothelial rather than (nasal) epithelial cells. The latter mechanism is also associated with transition from a colonizing to a more invasive lifestyle of *tarIJLM*-positive/ RboP-WTA-expressing *S. epidermidis* strains.

These are highly interesting findings and the experiments are well designed and conducted. I particularly appreciate the comprehensive approach of the study, in which a broad range of adequate methods were used to answer the research question (i.e. NGS, comparative genomics & phylogenetic analysis; NMR/LC-MS for WTA detection and analyses; classical molecular & genetic analysis of *tarIJLM* functions; phage adsorption assays, in vitro and in vivo adhesion & infection models etc.). The data obtained by these approaches are of high quality and convincing.

Nonetheless, while reading this well written and presented manuscript, some questions immediately came to my mind.

1. I understand from the data that tarIJLM mediates synthesis of a *S. aureus*-type RboP-WTA, with the gene cluster itself being mobile. I wonder whether or not *S. aureus* is indeed the primary genetic origin of the *S. epidermidis tarIJLM*? Thus, what about conservation of *tarIJLM*-like genes in *S. aureus* and *S. epidermidis* on the nucleotide level? Also, did *S. aureus* (as a species) acquire RboP-WTA synthesis genes in the first place via HGT as well or do such genes belong to the *S. aureus* core genome?

RE: We include now data on similarity of the nucleotide sequence level in the main manuscript, which were previously in the Supplemental Results. They show that *tarIJLM2/3/4/5* are related to the

corresponding *S. aureus tarL* but have evolved probably a long time ago as the similarities on nucleotide level are moderate. The *S. aureus tar* genes are part of the core genome. This point is explained now in the manuscript as outlined above.

Further, to *tarIJM* mobility:
2. Is there any (molecular/genetic) explanation why *tarIJLM* was apparently acquired (exclusively?) by distinct *S. epidermidis* clonal lineages? Or is this, in evolutionary terms, a recent event and other clades will follow suit because all *S. epidermidis* have generally the capacity to integrate the element?

RE: The *tarIJLM* genes have obviously been acquired by *S. epidermidis* clones several times as shown in Extended Data Fig. 5b. It is striking that they emerged exclusively in healthcare-associated clones and it is tempting to speculate that they would compromise the capacity of regular commensal clones to bind to epithelial surfaces while they may provide increased fitness to HA-MRSE clones for currently unknown reasons and, simultaneously, lead to increase virulence. This point is addressed now in the Discussion as outlined above.

3. How does *tarIJM* generally travel? Apart from detection of *tarIJLM* on a plasmid and an SCC element, are there other mobile genetic elements associated with the genecluster?

RE: These aspects were described in the Supplementary Results in the initial manuscript and were moved now to the main manuscript. We report that *tarIJLM* genes are also found in other CoNS species in addition to *S. epidermidis*, which supports the notion that *tarIJLM* can be exchanged between different *Staphylococcus* species and clones via horizontal gene exchange. For example, the *tarIJLM3* gene cluster and flanking IS*6*-like pseudogenes were found in a bovine *S. warneri* isolate. The high identity of the *tarIJLM3*- carrying plasmid-like contigs from *S. epidermidis* and *S. warneri* further underscores the mobility of *tarIJLM* elements. These findings are now described (lines 218-221):

“Nonetheless, our data support the hypothesis that the *tarIJLM* gene clusters were derived from other *Staphylococcus* species through exchange of genetic material. Furthermore, the identification of the *tarIJLM3* and *tarIJLM5* gene clusters in bovine *S. warneri* and *S. epidermidis* isolates, respectively, suggests the existence of an animal reservoir.”

3. Finally, with respect to the postulated increased capacity of *tarIJLM*-carrying strains for HGT with *S. aureus*, do these strains harbour more antibiotic resistance genes than the other clades, and if so, is there a preference for specific resistance traits?

RE: The *tarIJLM*-carrying strains are resistant to β-lactam and other antibiotics as indicated in Extended Data Table 1. However, we have no indications that they have more resistance genes than other *S. epidermidis* clones.

Minor points:

Line 122: 'lifestock' should read livestock; please

correct RE: Corrected as advised.

Line 123: Please add space to

'weremore' RE: Corrected as

advised

Reviewer #4:

In this study, the authors explore the genetic basis for the emergence of invasive *Staphylococcus epidermidis* (Sepi) isolates. Typically, Sepi colonizes the skin and nares of humans. Most strains synthesize wall teichoic acid with glycerol repeats (GroP-WTA) instead of ribitol repeats (RboP-WTA) as found in *S. aureus* and as synthesized by the *tarIJL* genes. Here, the authors use the specific binding of bacteriophages phi11 and phi187 to RboP-WTA and GroP-WTA respectively to identify *S. epi* clones that bind phi11. These clones carry an accessory *tarIJLM* gene referred as *tarIJLM2*. They demonstrate the *tarIJLM2* is responsible for the synthesis of RboP-WTA. By comparing skin and nasal isolates from healthy and infected humans, they report the exclusive association of *tarIJLM2* genes with *S. epi* isolates from infections. They also note the presence of specific *tarIJLM* elements in other healthcare-associated clones of *S. epi*. The authors perform a series of experiment and conclude that (1)commonly used RP62A, 12228 and 1457 carry a non-functional *tarIJL1* gene cluster and thus synthesize GroP-WTA only; (2) the GroP-WTA is better than RboP-WTA at promoting attachment of bacteria during colonization; specifically gain of *tarIJLM2* genes leads to a reduced colonization potential; lastly acquisition of the *tarIJLM2* genes results in (3) increased attachment to endothelial cells, (4) increased survival in a bloodstream model of infection, (5) increased ability to be recognized by phages and participate in HGT. Overall, the findings are interesting and support the authors' hypothesis. A few points could be further clarified.

Comments as they arise in the manuscript:

l. 31: here and elsewhere when referring to WTA: "expression" should be preferably changed to "production"

RE: These changes were made as advised.

Fig. 1: where does the SaPI integrate on the chromosome of *S. epi*? Presumably, the insertion/integration site is a sequence shared between Sa and Sepi.

RE: The capacity of SaPIbov1 to integrate into the chromosome of *S. epidermidis* has been analyzed before (Maiques *et al*, J Bacteriol, 2007, 189:5608-5616). SaPIbov1 was found to integrate at the same position of the core genome as in *S. aureus* in the *gmpS* gene. 15 of 18 conserved bp forming the attachment site att_C core are also found in the *S. epidermidis gmpS*. This information is mentioned now in the manuscript as outlined above.

Extended Data Fig. 1: why does phi11 adsorption to isolates E1, E45 and E51 (Ext. Data Fig. 1a) does not result in transduction (Fig. 1a)? Some of the Hamburg and Shanghai strains behave similarly. Do these strains lack the integration site for SaPI?

RE: The genomes of strains E1, E45, and E51 does not seem to differ in the putative integration site sequence. Several bacterial immunity mechanisms such as restriction/modification and CRISPR Cas systems are known to interfere with transduction and we have, unfortunately, no clear explanation at this time.

Line 82: What do the authors mean by "tarIJL1 is a pseudogene cluster"? is the coding sequence altered in such a way that the genes cannot be expressed or translated?

RE: We have no clear explanation for the fact that *tarIJL1* is not expressed and does not lead to RboP-WTA production. The sentence was changed to indicate that the reasons are currently unclear (lines 91-93):

"*tarL1* expression was at the detection limit at various growth conditions including broth, synthetic nasal medium, and human serum (Fig. 1c; Extended Data Fig. 2), suggesting that *tarIJL1* is non-functional or cannot be expressed."

Fig. 1b:

- Do the authors concur with a model whereby TarL provides both the priming and polymerizing activity for RboP? Or do the strains carry a *tarK* gene somewhere else on the chromosome?

- The authors do not say anything about TarM2; in *S. aureus* this enzyme adds O-linked α-GlcNAc at the C4 position of RboP. Is this modification required for phi11 binding?

RE: *S. epidermidis* bears also the WTA primase-encoding gene *tagB*, whose product is known to add two or three GroP residues to the WTA anchor structure. It can be assumed that it also serves as a primase for the RboP-WTA polymer. TarM2 is indeed related to the *S. aureus* TarM but we currently have no clear proof that it has the same activity in *S. epidermidis*. Its consistent presence in most of the *tarIJLM* clusters suggests a crucial role, which we plan to elucidate in future studies.

Extended Fig. 1d, e: it seems that these panels (while clear) are not described in the body of manuscript. RE: We apologize for not citing these figures, which was corrected in the revised manuscript.

Fig. 3; Ext. Data Fig. 7cd:

The authors note that Sepi isolates from human skin and nose do not carry the *tarIJLM2* gene cluster and posit that RboP-WTA may affect binding to epithelial surfaces. One test to examine this possibility compares biofilm formation of isogenic variants; presumably the presence of RboP WTA should reduce biofilm mass. But there isn't much of a biofilm to begin with, so the data is not terribly conclusive (see Ext. Fig. 7d that compares isogenic variants). Perhaps this section needs clarification.

RE: We agree that this experiment yields no clear indication but we feel it is important to demonstrate that RboP-WTA does not affect biofilm formation, which is thought to be a major virulence property of *S. epidermidis*. The roles of *tarIJLM* in the context of biofilm formation is now addressed in the Discussion as outlined above.

Minor point: Line 155 and Ext. Data Fig. 7c: The figure panel does not use the ST nomenclature and the legend does not identify ST10.

RE: We are sorry for omitting these data, which were added to the figure legend now.

Line 142: the authors write “GroP-WTA may be more capable of binding”? Capacity as in number of binding sites or affinity?

The authors posit that GroP-WTA is a better ligand than RboP-WTA. Yet *S. aureus* is a great colonizer. Is the GroP-WTA ligand known?

RE: The statement was modified because we have currently no more detailed information for this point. We propose that scavenger receptors may confer the GroP-WTA-dependent binding but several of these receptors are expressed on epithelial cells and it will be the focus of comprehensive future studies to reveal the specific receptor.

Ext. Data Fig. 8: here the title reads “RboP-WTA impairs Sepi binding to”. In truth, the authors observe reduced binding to A549. Is it possible that the total amount of WTA in strain 1457(pBR-IJLM2) is unchanged and GroP-WTA is simply diluted away upon the competing synthesis of RboP-WTA? Or do the authors believe that RboP-WTA physically obfuscates binding sites for host ligands?

RE: GroP-WTA amounts are indeed reduced in *tarIJLM2*-expressing *S. epidermidis* E73 compared to the RboP-WTA-deficient mutant because the overall WTA amount seems to remain constant. A possible explanation for reduced binding of the RboP-WTA producing strains can be either the reduced GroP-WTA
amount or obfuscation by RboP-WTA, which forms longer polymers than GroP-WTA because ribitol can form longer WTA backbones than glycerol. However, this point is very speculative and was, therefore, not further addressed in the manuscript.

Lines 193-194: the authors conclude “RboP-WTA represents a promising antigen for future preventive or therapeutic vaccines”. Previous work by the authors would suggest that this remains a challenge owing to the acquisition of galactosyl-transferases such as TarM and TarS that not only alter phage binding but also antigenicity of the molecule.

RE: We agree that many aspects need to be considered when using WTA variants for vaccine development. Nevertheless, WTA is a highly abundant, repetitive, and only moderately variable surface antigen, which holds in principle promise for inducing protective immunity, in particular when protein-conjugated WTA is used, which overcomes the low immunogenicity of TarM or TarP-modified RboP-WTA (van Dalen *et al*, Nature, 2019). We feel that it is relevant to mention that such a vaccine could also potentially protect against many HA-MRSE clones as outlined above.

Decision Letter, first revision:

Dear Andreas,

Thank you for submitting your revised manuscript "Emerging Staphylococcus epidermidis clones express Staphylococcus aureus-type wall teichoic acid to shift from commensal to pathogen behavior" (NMICROBIOL-20113387A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Microbiology, pending just some minor revisions to satisfy the referees'

final requests and to comply with our editorial and formatting guidelines.

We will now perform detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. As the current version of your manuscript is in a PDF format, please email us a copy of the file in an editable format (Microsoft Word or LaTex) as soon as you can - we can not proceed with PDFs at this stage for these final checks. Please do not upload the final materials or make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Microbiology and please do not hesitate to contact me if you have any questions.

With best regards,

{REDACTED}

****
Reviewer #1 (Remarks to the Author):

The revised manuscript is much improved. It reads better in its longer and more detailed format, and the concerns i had with regard to some of the over-selling of the finding have all been addressed or tempered. They have provided some additional data to address my query re phage susceptibility, and so on the whole I am satisfied as tot he quality of the work.

****
Reviewer #2 (Remarks to the Author):

The authors have addressed my prior concerns.

****
Reviewer #3 (Remarks to the Author):

The revised version of the manuscript includes additional data and clarifications and the authors appropriately addressed all isssues that I previously raised. Accordingly, I have no further comments on the manuscript.

****
Reviewer #4 (Remarks to the Author):

My comments have been addressed appropriately.

Decision Letter, final checks:

Dear Andreas,

Thank you again for your patience as we've prepared the guidelines for final submission of your Nature Microbiology manuscript, "Emerging Staphylococcus epidermidis clones express Staphylococcus aureus-type wall teichoic acid to shift from commensal to pathogen behavior" (NMICROBIOL-20113387A). Please carefully follow the step-by-step instructions provided in the personalized checklist attached, to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (ideally within two weeks). Please get in contact with us if you anticipate delays.

If you have not done so already, please alert us to any related manuscripts from your group that are

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In recognition of the time and expertise our reviewers provide to Nature Microbiology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Emerging Staphylococcus epidermidis clones express Staphylococcus aureus-type wall teichoic acid to shift from commensal to pathogen behavior". For those reviewers who give their assent, we will be publishing their names alongside the published article.

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Final Decision Letter:

Dear Andreas,

I am delighted to accept your Article "Emerging Staphylococcus epidermidis clones express Staphylococcus aureus-type wall teichoic acid to shift from a commensal to pathogen lifestyle" for publication in Nature Microbiology. Thank you for having chosen to submit your work to us and many congratulations to you and your co-authors.

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