Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens

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Mobile genetic elements (MGEs) encoding virulence and resistance genes are widespread in bacterial pathogens, but it has remained unclear how they occasionally jump to new host species. *Staphylococcus aureus* clones exchange MGEs such as *S. aureus* pathogenicity islands (SaPIs) with high frequency via helper phages. Here we report that the *S. aureus* ST395 lineage is refractory to horizontal gene transfer (HGT) with typical *S. aureus* but exchanges SaPIs with other species and genera including *Staphylococcus epidermidis* and *Listeria monocytogenes*. ST395 produces an unusual wall teichoic acid (WTA) resembling that of its HGT partner species. Notably, distantly related bacterial species and genera undergo efficient HGT with typical *S. aureus* upon ectopic expression of *S. aureus* WTA. Combined with genomic analyses, these results indicate that a ‘glycocode’ of WTA structures and WTA-binding helper phages permits HGT even across long phylogenetic distances thereby shaping the evolution of Gram-positive pathogens.
Major parts of bacterial genomes consist of genetic material originating from other organisms. Many of these elements can be mobilized and exchanged by horizontal gene transfer (HGT) thereby shaping bacterial genome plasticity and permitting rapid adaptation to changing environmental challenges. HGT of mobile genetic elements (MGEs) usually occurs at high frequency only among closely related bacterial clones because the transfer mechanisms, phage-mediated transduction or plasmid conjugation, rely on specific recognition of cognate recipient strains. However, HGT also occurs between members of different species or even genera albeit with lower frequency. Such rare events are responsible for the import of new genes into the species’ genetic pool along with the emergence of new phenotypic properties; they are particularly important for evolution of new bacterial pathogen lineages with new virulence and antibiotic resistance traits.

The major human pathogen Staphylococcus aureus represents a paradigm for studying the roles of ‘short-distance’ HGT between strains of the same species and ‘long-distance’ HGT with other species or genera. MGEs and non-mobile genomic islands constitute ca. 22% of the S. aureus genomes and govern the virulence and colonization capacities, host-specificity and antibiotic resistance of the various clonal complexes.

Methicillin-resistant S. aureus carrying staphylococcal cassette chromosomes with mecA genes represent the most frequent cause of severe community- or healthcare-associated infections in many developing and developed countries. While conjugation and uptake of naked DNA by natural transformation seem to occur

![Figure 1](image_url)

**Figure 1 | ST395 is resistant to Φ11 and Φ80α-mediated HGT of SaPIs.** (a) Various S. aureus sequence types and ST395 mutants lacking restriction modifications systems SauUSI or SauUSI plus HsdR were analysed for capacities to acquire SaPIbov1 or SaPI1 via helper phages Φ11 or Φ80α, respectively. SaPI donor strains were JP1794 (SaPIbov1) and JP3602 (SaPI1). Values represent the ratio of transduction units (TRU; transductants per ml phage lysate) to plaque-forming units (PFU; plaques per ml phage lysate on S. aureus RN4220 w.t.) given as means (n = 3) ± s.d. No TRU were observed in controls lacking phages or SaPI particles. (b) S. aureus PS187 w.t. and mutants lacking restriction modification systems were analysed for capacities to acquire the tetracycline resistance plasmid pT181 via electroporation. Values represent the CFU per microgram DNA and given as means (n = 3) ± s.d. CFU, colony forming units. No CFU were observed in controls lacking donor DNA. ΔhsdR, no type I restriction modification system. ΔsauUSI, no type IV restriction modification system, lacks SAOUHSC_02790 homologue of NCTC8325. Statistically significant differences compared with wild type (w.t.) calculated by the unpaired two-tailed Student’s t-test are indicated: NS, not significant, *P < 0.05; **P < 0.01 to < 0.05; ***P < 0.001 to 0.01; ****P < 0.001.
rarely, staphylococcal HGT of MGEs is generally believed to depend largely on transducing helper phages. Certain temperate phages of serogroup B such as Φ11 or Φ80ξ have been shown to be capable of transducing DNA between S. aureus clones and to employ the N-acetyl-β-glucosamine (GlcNAc) residues on wall teichoic acid (WTA), a surface-exposed glycopolymer, as receptor. WTA is produced by most Gram-positive bacteria and usually has species- or strain-specific structure. S. aureus produces a WTA polymer composed of ca. 40 ribitol-phosphate (RboP) repeating units modified with α- and/or β-linked GlcNAc and β-alanine, while the various coagulase-negative staphylococcal species (CoNS) produce WTA with glycerophosphate (GroP) or hexose-containing, complex repeating units modified with different types of sugars.

S. aureus pathogenicity islands (SaPIs) are exchanged among S. aureus lineages with high frequency by SaPI particles consisting of SaPI genomes and structural proteins from helper phages. While such ‘short-distance’ HGT events occur with high frequency, antibiotic resistance-mediating MGEs have been acquired only occasionally from other bacterial species. Of note, antibiotic resistance genes from CoNS have frequently been imported into S. aureus whereas enterococcal vancomycin resistance genes have emerged in staphylococci only in a few exceptional cases suggesting that there are mechanisms involved that favour or disfavour specific ‘long-distance’ HGT events. Restriction modification systems (Fig. 1a). In contrast, several restriction modification systems (Fig. 1a). In contrast, several restriction modification systems have been shown to interfere with HGT efficiency in staphylococci but the major determinants permitting HGT with other bacterial species and genera have remained unknown.

We demonstrate here that the variable structure of glycosylated WTA constitutes a ‘glycocode’ that is sensed by transducing phages thereby defining the routes of HGT. Similar WTA structures enable DNA exchange via helper bacteriophages even across the boundaries of species or genera, whereas S. aureus clones producing altered WTA become separated from the species’ genetic pool and may initiate new routes of HGT with other bacterial species and genera that share related WTA. Thus, related WTA structures are sufficient to initiate HGT even across long phylogenetic distances.

### Results

**ST395 cannot undergo HGT with other S. aureus lineages.** The various S. aureus clonal complexes differ largely in their epidemic potential and number of MGEs. We compared several S. aureus lineages for capability to acquire SaPI1 or SaPI187 originating from sequence types ST8 and ST151, respectively. Derivatives of these SaPIs with antibiotic resistance gene markers were transferred from S. aureus ST8 to a variety of potential recipient strains using helper phages Φ11 (for SaPIbov1) or Φ80ξ (for SaPI1). The majority of the sequence types acquired SaPIs albeit with varying efficiency, probably as a consequence of different restriction modification systems (Fig. 1a). In contrast, several independent clones of the ST395 lineage from various parts of the world including isolates from the lung or blood stream infections and nasal swabs (Supplementary Table S1) were completely resistant to HGT of SaPIs (Fig. 1a). Restriction modification systems were obviously not responsible for HGT resistance of ST395 because consecutive inactivation of the genes for type I (AhsdR) and type IV (AsauUSI) restriction systems in the ST395 strain PS187 did not enable transfer of SaPIs (Fig. 1a), whereas it considerably increased the rates of plasmid electroporation (Fig. 1b).

**Φ187 mediates HGT between ST395 and other bacterial species.** While strain PS187 was resistant to infection by Φ80ξ or Φ11 (Supplementary Fig. S1a), it has previously been shown to be susceptible to phage Φ187 (refs 21,24). Interestingly, most ST395...
but none of the other \textit{S. aureus} sequence types could be infected by \textit{F}187 (Supplementary Figs S1a and S2a). When \textit{F}187 was analysed for its capacity to transfer MGEs, it was found to facilitate indeed the exchange of SaPI187\textsubscript{b} (found in the PS187 genome, see below) and SaPIbov1 between different ST395 isolates but not to other \textit{S. aureus} sequence types (Fig. 2). Surprisingly, it also mediated HGT of SaPIbov1 and SaPI187\textsubscript{b} from ST395 to the CoNS species \textit{Staphylococcus epidermidis} and \textit{Staphylococcus carnosus} and even to \textit{Listeria monocytogenes} serotype 4e (Fig. 2). Thus, ST395 can participate in HGT with other species and genera while it is separated from phage-dependent HGT with typical \textit{S. aureus}.

**ST395 has a unique WTA gene cluster and WTA structure.** PS187 was sequenced to obtain a prototype ST395 genome. The draft sequence was assembled into 16 large contigs plus two plasmids encompassing 2,529 open reading frames along with 43 and 10 coding sequences for transfer RNAs and ribosomal ribonucleic acid RNAs, respectively. PS187 was found to be a true \textit{S. aureus} but to branch deeply in the \textit{S. aureus} lineage (Fig. 3a). It did not encode CRISPR determinants indicating that the unusual HGT behaviour of ST395 is probably not mediated by ‘adaptive immunity’ to foreign DNA\textsuperscript{19}. Among several unusual MGEs (see below) PS187 contained a novel genomic element, which encompassed transposon-related sequences plus four genes with similarity to WTA-biosynthetic genes (\textit{tagV}, \textit{tagN}, \textit{tagD}, \textit{tagF}) (Fig. 3b). Of note, the new element replaced the 11-kb \textit{tarIJLFS} cluster for biosynthesis of GlcNAc-modified RboP (RboP-GlcNAc) WTA\textsuperscript{10}, which is found in all other so far known \textit{S. aureus} genomes. Different ST395 clones were found to be related because they exhibited similar DNA fragment patterns in pulsed field gel electrophoreses albeit with some variation in the size of certain DNA bands (Supplementary Fig. S2b). Moreover, all of the 10 available ST395 isolates were tested positive for the \textit{tagN} gene encoded on the new genetic element indicating that the new WTA gene cluster is probably a common feature in the ST395 lineage (Supplementary Fig. S2c). WTA was isolated from PS187 and NMR-based structural elucidation demonstrated that PS187

![Figure 3](image-url)
produces a unique WTA type with an N-acetyl-\(n\)-galactosamine (GalNAc) modified GroP backbone (GroP-GalNAc) (Fig. 3c; Supplementary Fig. S3a and Supplementary Table S2), which was in agreement with an earlier analysis of PS187-related strains11.

Some unusual MGEs from ST395 may be originating from CoNS. Because of its inability to exchange DNA with typical \textit{S. aureus}, we assumed that ST395 may be genetically isolated. ST395 clones have been occasionally described as human commensals and invasive pathogens\textsuperscript{22,23} and a recent study has found that ST395 accounts for 5 and 2% of \textit{S. aureus} nasal and blood culture isolates, respectively, in Northeastern Germany\textsuperscript{23}. However, the actual numbers may be higher because ST395 isolates are usually methicillin susceptible and such clones are hardly collected and typed. Interestingly, early reports from the 1960s have pointed to a canine reservoir of PS187-related \textit{S. aureus}\textsuperscript{25}. In accord with this notion, we found the sequences of some of the host range-determining proteins of PS187 (IsdB and VwBP)\textsuperscript{26} to differ from typical human strains whereas the presence of the strictly human-specific chp, scn and sak genes\textsuperscript{26} suggests that PS187 is at least in part adapted to the human host.

Most MGEs of PS187 were distinct in synten and composition from those found in other \textit{S. aureus} genomes and some were even more related to MGEs found in CoNS. The genomic islands vSA\textsubscript{A} and vSA\textsubscript{B} found in all previously sequenced \textit{S. aureus} genomes were also present in PS187 but lacked typical enterotoxin or lantibiotic gene clusters, respectively (Fig. 4a and Supplementary Fig. S4). Two novel PS187 SaPIs named SaPI\textsubscript{187A} and SaPI\textsubscript{187B}
shared attachment (att) sites and some similarity with SaPI3 and SaPI122, respectively13, but differed with regard to enterotoxin, multidrug resistance transporter and serine protease genes (Fig. 4b,c and Supplementary Fig. S4). PS187 was found to encode two cryptic prophages named FPs187a and FPs187b (Supplementary Fig. S4), which were similar to previously described S. aureus phages Ψ77 (ref. 8) and Ψ187 (refs 21,24) and were integrated in the genes for sphingomyelinase (hbl) and the giant surface protein Ebb, respectively. However, both prophages were defective as no infective phages could be obtained from PS187 upon treatment with the prophage-inducing agent mitomycin C (Supplementary Fig. S5). Ψ77 was found to have entirely different host binding specificities from Ψ187 with efficient adsorption to S. aureus with RboP-GlcNAc WTA and inefficient binding to bacteria with other WTA types such as ST395 (Supplementary Fig. S1b) suggesting that the defective Ψ77-related prophage of PS187 may have originated from an ancient HGT event before the new WTA-biosynthetic genes had been acquired.

Notably, some of the PS187 MGEs shared higher similarity with genes from CoNS than from other S. aureus. This was found for mercury and cadmium resistance operons on a large plasmid, which were most similar to corresponding genes from an S. epidermidis SCC island27 and the chromosomally integrated S. epidermidis plasmid vSe128 (Fig. 4d), respectively, and for the new WTA-biosynthetic genes, whose products exhibited highest similarity with proteins from Staphylococcus pseudintermedius (TagV), S. carnosus (TagN), Staphylococcus lugdunensis (TagD), and Staphylococcus simiae (TagF) (Supplementary Fig. S6). Thus, the genome sequence confirms that ST395 clones are separated from frequent HGT with typical S. aureus but have access to MGEs from CoNS.

SaPI particles adopt receptor requirements of helper phages. The unusual WTA structure of PS187 resembled that of the CoNS strains accepting SaPI DNA from ST395 via Ψ187 (Table 1) suggesting that similar WTA structure may be a crucial determinant for the initiation of phage-dependent gene transfer even between distantly related bacteria. However, while S. aureus Ψ80z or Ψ11 phage particles are known to require a defined WTA structure for adsorption to host bacteria3, the receptor requirements of SaPI particles, which have a much broader host range than helper phages25, has remained unknown. Using defined mutants of the ST8 strains RN4220 (refs 8,10) and Newman with altered teichoic acids, the susceptibilities to phage Ψ80z or Ψ11 particle binding and infection were compared with their capacities to acquire SaPI DNA from SaPI particles derived from the same helper phages. Infection by both, phage particles and SaPI particles, was dependent on the presence of WTA and WTA glycosylation (Fig. 5a; Supplementary Fig. S7) whereas lipoteichoic acid was dispensable (Fig. 5a) indicating that SaPI particles adopt the receptor requirements of the corresponding helper phage. In accord with this finding, inactivation of tagO encoding the first enzyme of the WTA-biosynthetic pathway rendered strain PS187 resistant to Ψ187 infection because of impaired adsorption (Supplementary Fig. 1a,b) and to Ψ187-dependent SaPI transfer (Fig. 2) indicating that Ψ187 and Ψ187-derived SaPI particles require GroP WTA for binding to host bacteria.

Table 1 | Correlation of WTA structure and capacity to mediate HGT of SaPIs.

| Species/Strain | WTA type† | Glycosylation* | Reference | Transduction from ST8 via Ψ187 | Transduction from ST395 via Ψ187 |
|---------------|---------------|---------------|-----------|-----------------|-----------------|
| *S. aureus* RN4220 (ST8) | RboP | GlcNAc (α), GlcNAc (β) | 38 | + | - |
| *S. aureus* PS187 (ST395) | GroP | GalNAc (α) | This study | - | + |
| *S. epidermidis* 14577 | GroP | GlcNAc (α), Glic | 11 | - | + |
| *S. carnosus* TM300 | GroP | Glc, (GalicAc) | 42 | - | + |
| E. faecalis VRE392† | GalNAc-GroP | absent | 30 | - | + |
| E. faecalis V5831 | GalNAc-RboP, WTA1 | Rha (α), WTA1 Glc (α), WTA2 | 31 | - | - |
| L. monocytogenes ATCC191818, Serotype 4e | Glic-GalNAc-RboP, WTA2 | Gal | 43 | + | - |
| L. grayi ATCC25401† | RboP | GlcNAc | 43 | + | - |

*Bold printed WTA structure correlates with phage and SaPI particle receptor specificity.
†WTA structure published for another isolate is likely to be the same in the strain used here.
‡Expresses two different WTA as indicated.
§WTA glycosylation is likely to be GlcNAc because of the presence of close homologues of the S. aureus WTA glycosyltransferase genes tarM and tarS in the L. grayi DSM20601 genome sequence.
In accord with this finding, a systematic analysis with several Gram-positive bacteria revealed a strong correlation between WTA structure and the capacity to exchange resistance and virulence genes with either typical S. aureus via Φ11 or Φ80α (for example, Listeria grayi) or with ST395 via Φ187 (for example, S. epidermidis and other CoNS) (Table 1). The susceptibility of L. monocytogenes serotype 4e to Φ187-mediated HGT was reflected by efficient binding of Φ187 (Supplementary Fig. S1b).

It may be due to the decoration of WTA with galactose, which may facilitate binding of Φ187-derived SaPI particles in a similar way as GalNAc. Of note, vancomycin-resistant Enterococcus faecium or Enterococcus faecalis could not undergo HGT with any S. aureus probably because of the very complex enterococcal WTA structures30,31.

**Discussion**

HGT between S. aureus and other bacterial species and genera contributes substantially to the evolution of new epidemic clones, but it has remained unclear if it occurs accidentally or follows certain rules. While restriction modification and CRISPR systems have been shown to limit HGT efficiency16–19, no data on the criteria that need to be fulfilled by the HGT partners to initiate phage-dependent MGE exchange have been available. Our studies with naturally occurring and engineered bacterial strains with atypical WTA demonstrate that related WTA structures of MGE donor and recipient are sufficient to permit HGT even across long phylogenetic distances. While helper phage particles are known to have quite narrow host ranges for parasitic reproduction29, we found that their receptor specificities govern the capacity to

**Figure 5 | WTA structure determines the capacity of SaPIs to traverse even long phylogenetic distances.** S. aureus RN4220 (ST8) strains with, with altered or without WTA (a) or S. aureus PS187 (ST395) or other Gram-positive bacterial species expressing genes for biosynthesis of RboP-GlcNAc WTA (b) were analysed for capacities to acquire SaPIbov1 or SaPI1 via helper phages Φ11 or Φ80α, respectively. SaPI donor strains were JP1794 (SaPIbov1) and JP3602 (SaPI1). Values represent the ratio of transduction units (TRU; transductants per ml phage lysate) to plaque-forming units (PFU; plaques per ml phage lysate on S. aureus RN4220 w.t.) given as means (n = 3) ± s.d. No TRU were observed in strains expressing WTA other than RboP-GlcNAc. ΔtagO, no WTA; ΔltaS (45S), no lipoteichoic acid; c-ΔtagO, ΔtagO complemented with tagO; ΔtarM ΔtarS, no WTA glycosylation; c-tarM and c-tarS, ΔtarM ΔtarS complemented either with tarM or tarS. WTA hybrid strains expressing additional RboP-GlcNAc WTA are indicated with H.

### Table 1: WTA structure susceptibility of S. aureus and other bacterial species

| Species      | Strain         | WTA structure | SaPIbov1 (Φ11) TRU/PFU | SaPI1 (Φ80α) TRU/PFU |
|--------------|----------------|---------------|------------------------|----------------------|
| S. aureus    | PS187 w.t.     | None          | 10^-3                  | 10^-3                |
|              | PS187-H        | None          | 10^-2                  | 10^-2                |
|              | NRS111 w.t.    | None          | 10^-1                  | 10^-1                |
|              | NRS111-H       | None          | 10^-0                  | 10^-0                |
| S. camosus   | TM300 w.t.     | None          | 10^-3                  | 10^-3                |
|              | TM300-H        | None          | 10^-2                  | 10^-2                |
| S. capitis   | ATCC27840 w.t.| None          | 10^-1                  | 10^-1                |
|              | ATCC27840-H    | None          | 10^-0                  | 10^-0                |
| S. epidermidis | 1457 w.t.     | None          | 10^-3                  | 10^-3                |
|              | 1457-H         | None          | 10^-2                  | 10^-2                |
| L. monocytogenes | ATCC19118 w.t.| None          | 10^-1                  | 10^-1                |
|              | ATCC19118-H    | None          | 10^-0                  | 10^-0                |
| L. grayi     | ATCC25401 w.t.| None          | 10^-3                  | 10^-3                |
|              | None obtained  | None          | 10^-2                  | 10^-2                |

**Figure 5** WTA structure determines the capacity of SaPIs to traverse even long phylogenetic distances. S. aureus RN4220 (ST8) strains with, with altered or without WTA (a) or S. aureus PS187 (ST395) or other Gram-positive bacterial species expressing genes for biosynthesis of RboP-GlcNAc WTA (b) were analysed for capacities to acquire SaPIbov1 or SaPI1 via helper phages Φ11 or Φ80α, respectively. SaPI donor strains were JP1794 (SaPIbov1) and JP3602 (SaPI1). Values represent the ratio of transduction units (TRU; transductants per ml phage lysate) to plaque-forming units (PFU; plaques per ml phage lysate on S. aureus RN4220 w.t.) given as means (n = 3) ± s.d. No TRU were observed in strains expressing WTA other than RboP-GlcNAc. ΔtagO, no WTA; ΔltaS (45S), no lipoteichoic acid; c-ΔtagO, ΔtagO complemented with tagO; ΔtarM ΔtarS, no WTA glycosylation; c-tarM and c-tarS, ΔtarM ΔtarS complemented either with tarM or tarS. WTA hybrid strains expressing additional RboP-GlcNAc WTA are indicated with H.
transmit MGIs to a broad range of recipient strains expressing cognate surface ligands. On the other hand, changes in helper phage receptor structures can enable binding of new types of helper phages and redirect the routes of HGT, which may facilitate the development of new clonal lineages and species.

The structurally diverse WTA molecules represent a species- or lineage-specific signature at the surface of Gram-positive bacteria. WTA has several important roles for bacterial physiology such as the control of autolytic and peptidoglycan-biosynthetic enzymes, buting to phage-mediated HGT. In contrast, recently developed methods likely the transfer of new antibiotic resistance genes across the bacterial cell wall. WTA has several important roles for bacterial physiology such as the control of autolytic and peptidoglycan-biosynthetic enzymes,9,10 lineagespecific signature at the surface of Gram-positive bacteria. WTA has several important roles for bacterial physiology such as the control of autolytic and peptidoglycan-biosynthetic enzymes,11

**Methods**

**Bacterial strains and growth media.** The various bacterial strains listed in Supplementary Table S1 were grown in BM broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% KHP04, 0.1% glucose) or Luxia Bertani Broth supplemented with appropriate antibiotics at a concentration of 10 ng ml−1 (chloramphenicol), 3 µg ml−1 (tetracycline) or 100 µg ml−1 (ampicillin). In order to assess the growth and microscopic properties of mutant strains, phenotypic characterization of WTA hybrid strains overnight cultures were diluted to OD578 0.1 in BM and grown at 37°C on a shaker. Samples for microscopy were analysed after 3 h growth using a Leica DMRE microscope and Leica HCX 100 × objective. Bacterial growth was monitored for 8 h.

**Molecular genetic methods.** For the construction of marker-less ΔtspQ, ΔwvaUSI and ΔsuSUSI MoXdr mutants in S. aureus PS187, the pKOR1 shuttle vector was used according to standard procedures. For knockout plasmid construction, primers listed in Supplementary Table S4 were used. To label SaPI187 with an antibiotic resistance marker, the ermB gene was integrated into SaPI187 using the pKOR1 system. Knock-in primers are listed in Supplementary Table S4.

**Por3 dialysis membrane (MWCO of 3.5 kDa; VWR International GmbH, Darmstadt).** Samples were concentrated in a SpeedVac concentrator at 45°C to 5°C and applied to DEAE-Sephadex A25 matrix according to Xia et al.28 For elution from DEAE-Sephadex A25 matrix 20 mM Tris–HCl pH 7.2 0.35 M NaCl was used. The eluate was dialyzed against water and concentrated to 1 ml. The purified WTA samples were stored at 20°C for further analysis. All general analytical chemistry and NMR spectroscopy methods for WTA characterization are described in detail in the supplementary section.

**Genome sequencing and analyses.** S. aureus PS187 was sequenced initially by Roche 454 pyrosequencing (Roche GS-FLX system), then resequenced by using Illumina technology (Illumina HiSeq2000) at a 150-fold coverage. Details are described in supplementary section.

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Acknowledgements
We thank Jacques Schrenzel, Gabriele Bierbaum, Christiane Wolz, Angelika Grundling, Matthias Marschal and Ian Monk for providing bacterial strains and phages and Peter Bauer and Claudia Bauer for help with genome sequencing. This work was supported by grants TRR34 to A.P., B.M.B., and T.D. and SFB766 to G.X. and A.P. from the German Research Council, grants from the German Center for Infectious Disease Research to A.P. and G.X., and SkinStaph and Menage grants to A.P. from the German Ministry of Education and Research.

Author contributions
V.W., C.L., P.S.C., G.X., M.S. and M.M. performed the experiments and analysed the data; B.M.B. and J.R.P. provided essential materials; V.W., J.R.P., U.N., O.H., T.D., A.P. and G.X. conceived the study; V.W., A.P. and G.X. wrote the manuscript.

Additional information
Accession codes: The Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number ARP00000000 (BioProject PRJNA197438). The version described in this paper is the first version, ARP010000000.

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Winstel, V. et al. Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens. Nat. Commun. 4:2345 doi: 10.1038/ncomms3345 (2013).

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