ARTICLE

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A new antibiotic kills pathogens without detectable resistance

Losee L. Ling[1]*, Tanja Schneider[2,3]*, Aaron J. Peoples[1], Amy L. Spoering[1], Ina Engels[2,3], Brian P. Conlon[4], Anna Mueller[2,3], Till F. Schäberle[3,5], Dallas E. Hughes[1], Slava Epstein[6], Michael Jones[7], Linos Lazarides[7], Victoria A. Steadman[7], Douglas R. Cohen[1], Cintia R. Felix[1], K. Ashley Fetterman[1], William P. Millett[1], Anthony G. Nitti[1], Ashley M. Zullo[1], Chao Chen[4] & Kim Lewis[4]

Antibiotic resistance is spreading faster than the introduction of new compounds into clinical practice, causing a public health crisis. Most antibiotics were produced by screening soil microorganisms, but this limited resource of cultivable bacteria was overmined by the 1960s. Synthetic approaches to produce antibiotics have been unable to replace this platform. Uncultured bacteria make up approximately 99% of all species in external environments, and are an untapped source of new antibiotics. We developed several methods to grow uncultured organisms by cultivation *in situ* or by using specific growth factors. Here we report a new antibiotic that we term teixobactin, discovered in a screen of uncultured bacteria. Teixobactin inhibits cell wall synthesis by binding to a highly conserved motif of lipid II (precursor of peptidoglycan) and lipid III (precursor of cell wall teichoic acid). We did not obtain any mutants of *Staphylococcus aureus* or *Mycobacterium tuberculosis* resistant to teixobactin. The properties of this compound suggest a path towards developing antibiotics that are likely to avoid development of resistance.

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Triinu Kõressaar

Introduction (1/2)

Antimicrobial drug discovery is uniquely difficult

Primarily due to the poor penetration of compounds into bacterial cell

Most of the antibiotics introduced to the clinics are dicovered by screening cultivable soil microorganisms

About 99% of all species in external environment are uncultured

- promising source of new antibiotics

Introduction (2/2)

The discovery of a new cell wall inhibitor from environment, **teixobactin**.

Most antibiotics target proteins.
It is relatively easy for a microbe to become resistant to those drugs by accumulating mutations that alter the target protein's shape.

Unusually for an antibiotic, teixobactin is thought to attack microbes by binding to fatty lipids that make up the bacterial cell wall
It is difficult for a bacterium to alter such fundamental building blocks of the cell.

Antibiotics classification

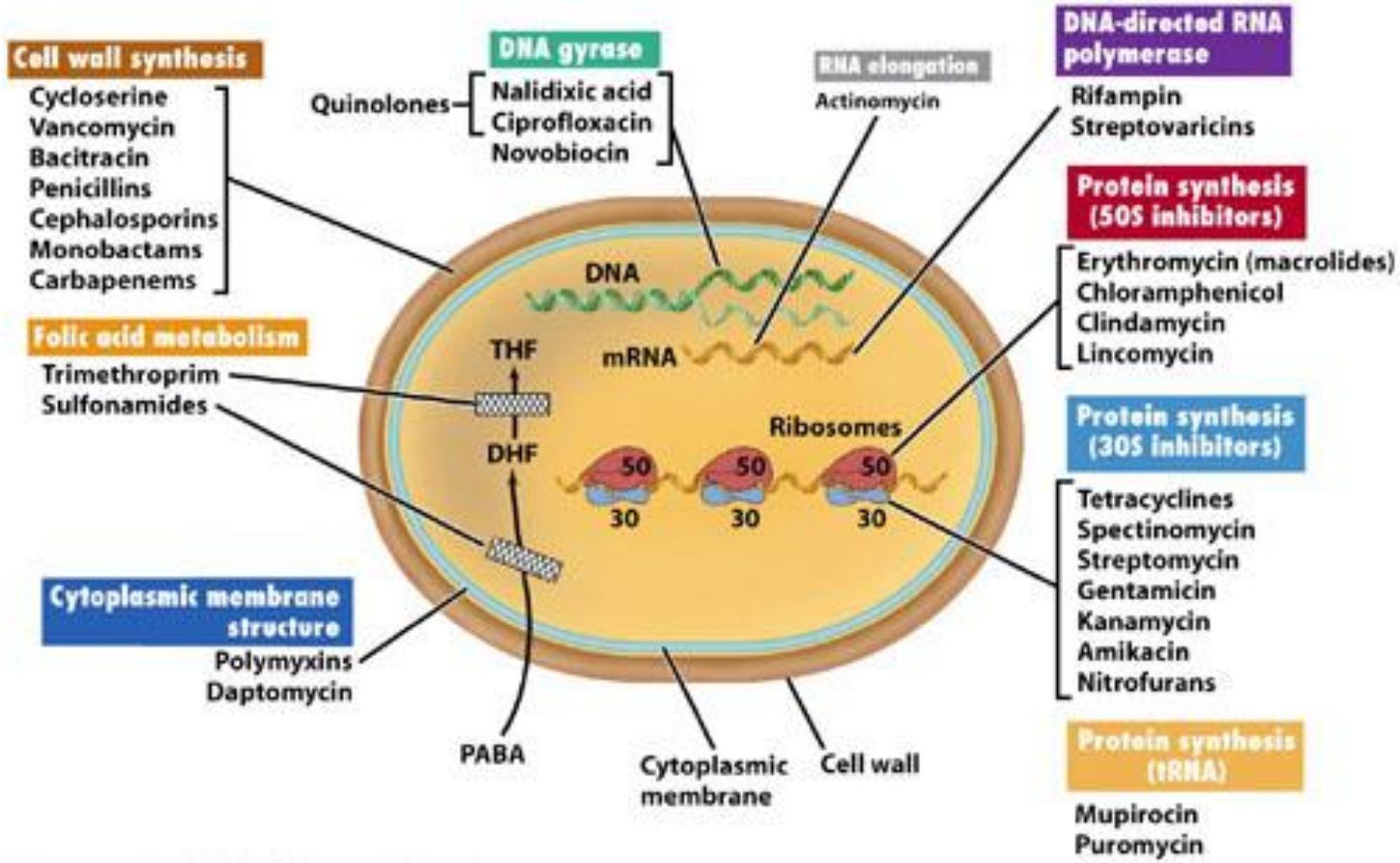
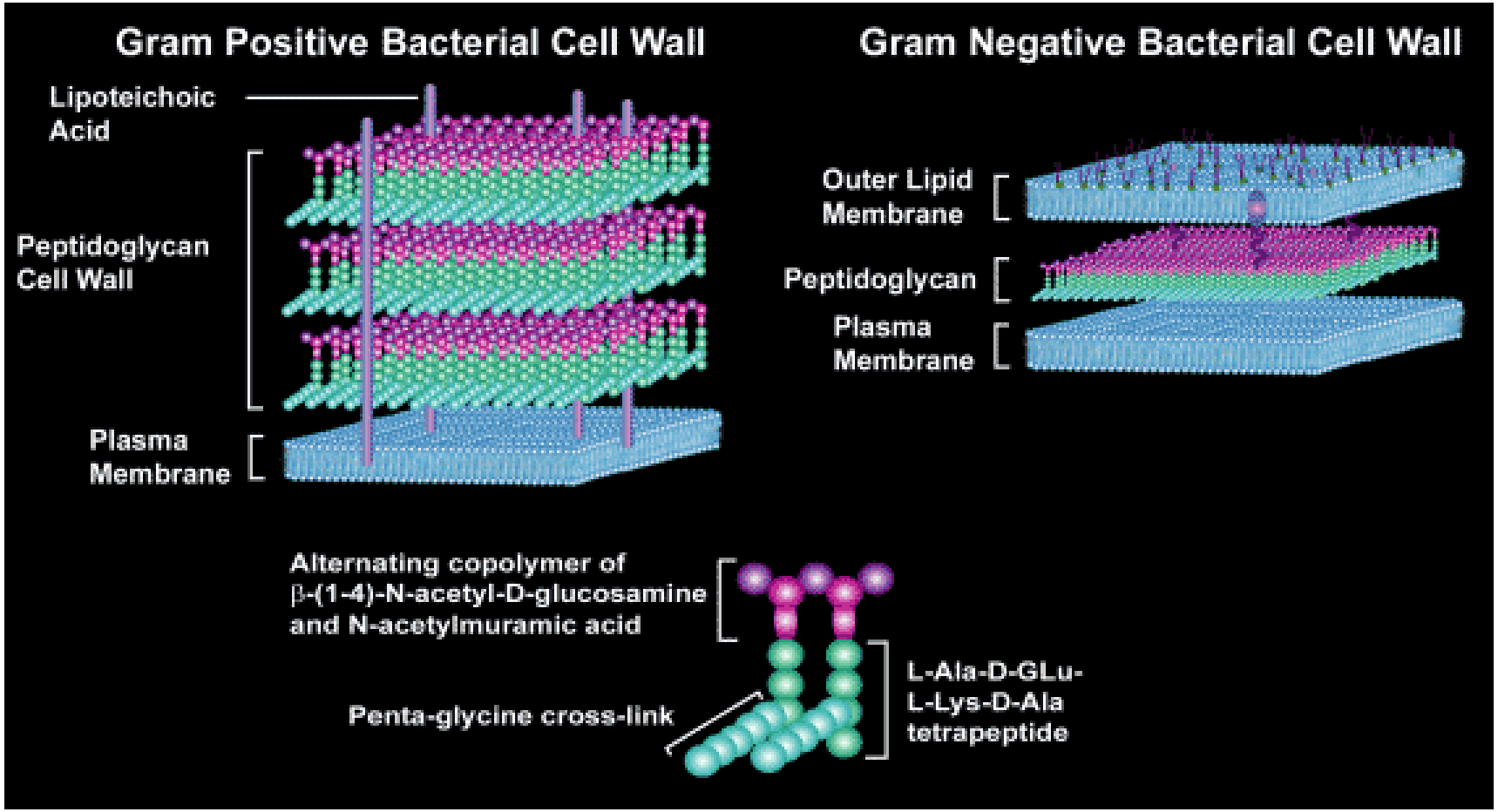


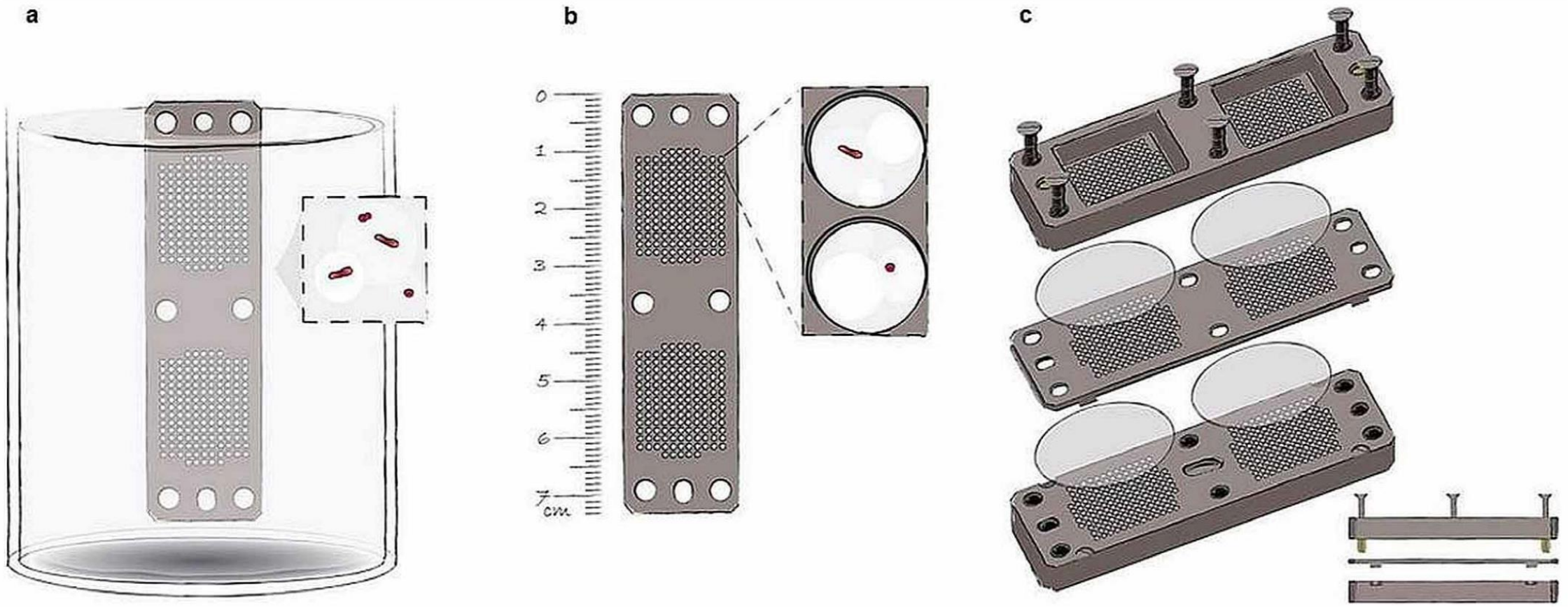
Figure 20-14 Brock Biology of Microorganisms 11/e

Cell wall


Gram Positive Bacterial Cell Wall
Lipoteichoic Acid
Peptidoglycan Cell Wall
Plasma Membrane
Gram Negative Bacterial Cell Wall
Outer Lipid Membrane
Peptidoglycan
Plasma Membrane
Alternating copolymer of β-(1-4)-N-acetyl-D-glucosamine and N-acetylmuramic acid
Penta-glycine cross-link
L-Ala-D-GLu-L-Lys-D-Ala tetrapeptide

Growing uncultivable bacteria

iChip – used for simultaneously isolate and grow uncultured bacteria
Growth recovery by this method approaches ca 50% vs 1% on a nutrient Petri dish



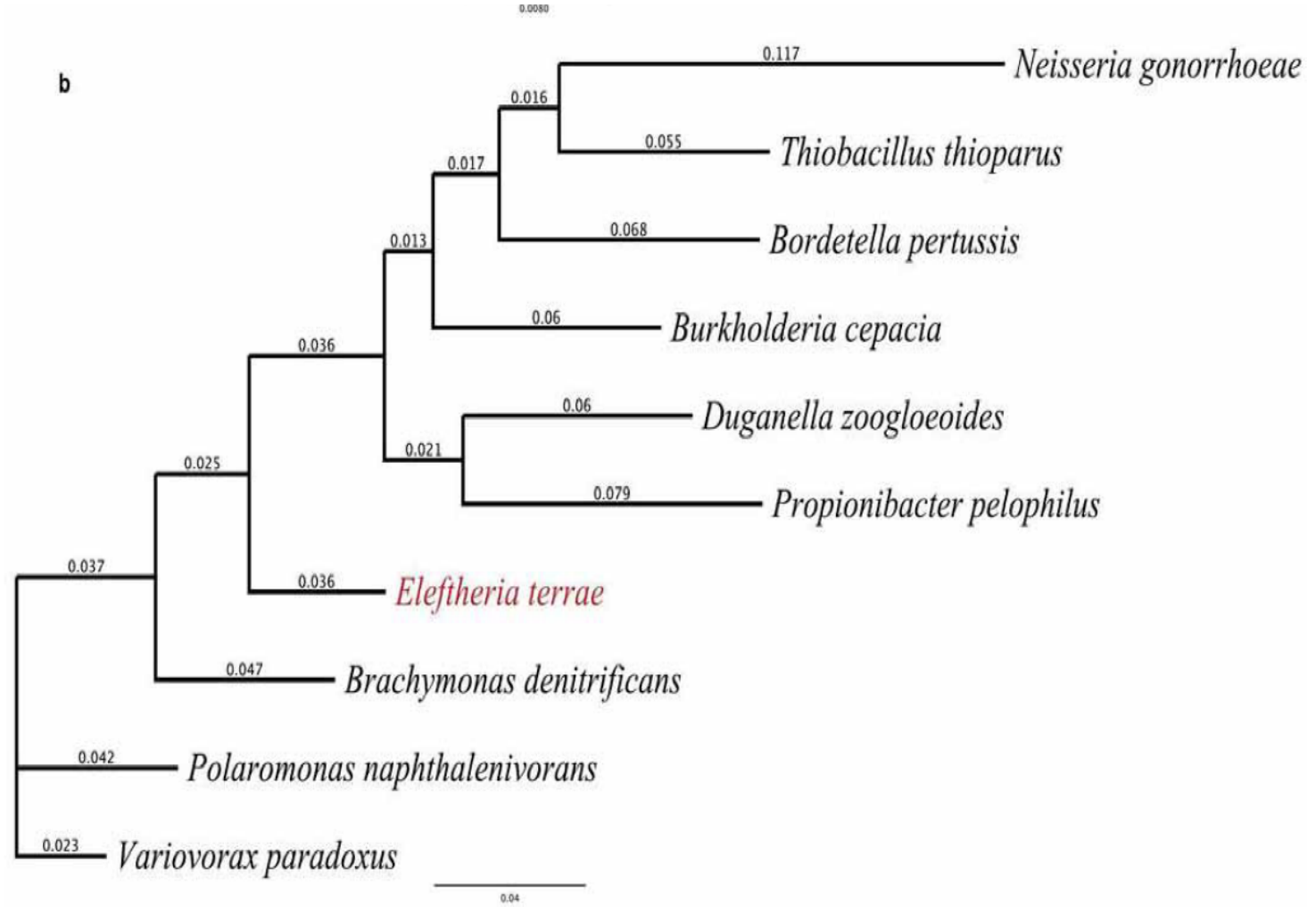
Extended Data Figure 1 | The iChip. **a–c**, The iChip (**a**) consists of a central plate (**b**) which houses growing microorganisms, semi-permeable membranes on each side of the plate, which separate the plate from the environment, and two supporting side panels (**c**). The central plate and side panels have multiple matching through-holes. When the central plate is dipped into suspension of cells in molten agar, the through-holes capture small volumes of this suspension, which solidify in the form of small agar plugs. Alternatively, molten agar can be dispensed into the chambers. The membranes are attached and the iChip is then placed in soil from which the sample originated.

Identification of teixobactin

A substantial number of uncultured isolates are able to grow in vitro

Extracts from 10,000 isolates obtained by growth in iChips were screened for antimicrobial activity on plates overlaid with *S.aureus*

Eleftheria terrae (β-proteobacteria) showd a good activity



Extended Data Figure 2 | 16S rRNA gene phylogeny of *Eleftheria terrae*. **a**, The phylogenetic position of *E. terrae* within the class β-proteobacteria. The 16S rRNA gene sequences were downloaded from Entrez at NCBI using accession numbers retrieved from peer-reviewed publications. **b**, The phylogenetic position of *E. terrae* among its closest known relatives. The sequences were downloaded from NCBI using accession numbers retrieved from the RDP Classifier Database. For both trees, multiple sequence alignments (MSA) were constructed using ClustalW2, implementing a default Cost Matrix, the Neighbour-Joining (NJ) clustering algorithm, as well as optimized gap penalties. Resulting alignments were manually curated and phylogenetic trees were constructed leveraging PhyML 3.0 with a TN93 substitution model and 500 Bootstrap iterations of branch support. Topology search optimization was conducted using the Subtree–Pruning–Regrafting (SPR) algorithm with an estimated Transition–Transversion ratio and gamma distribution parameters as well as fixed proportions of invariable sites.

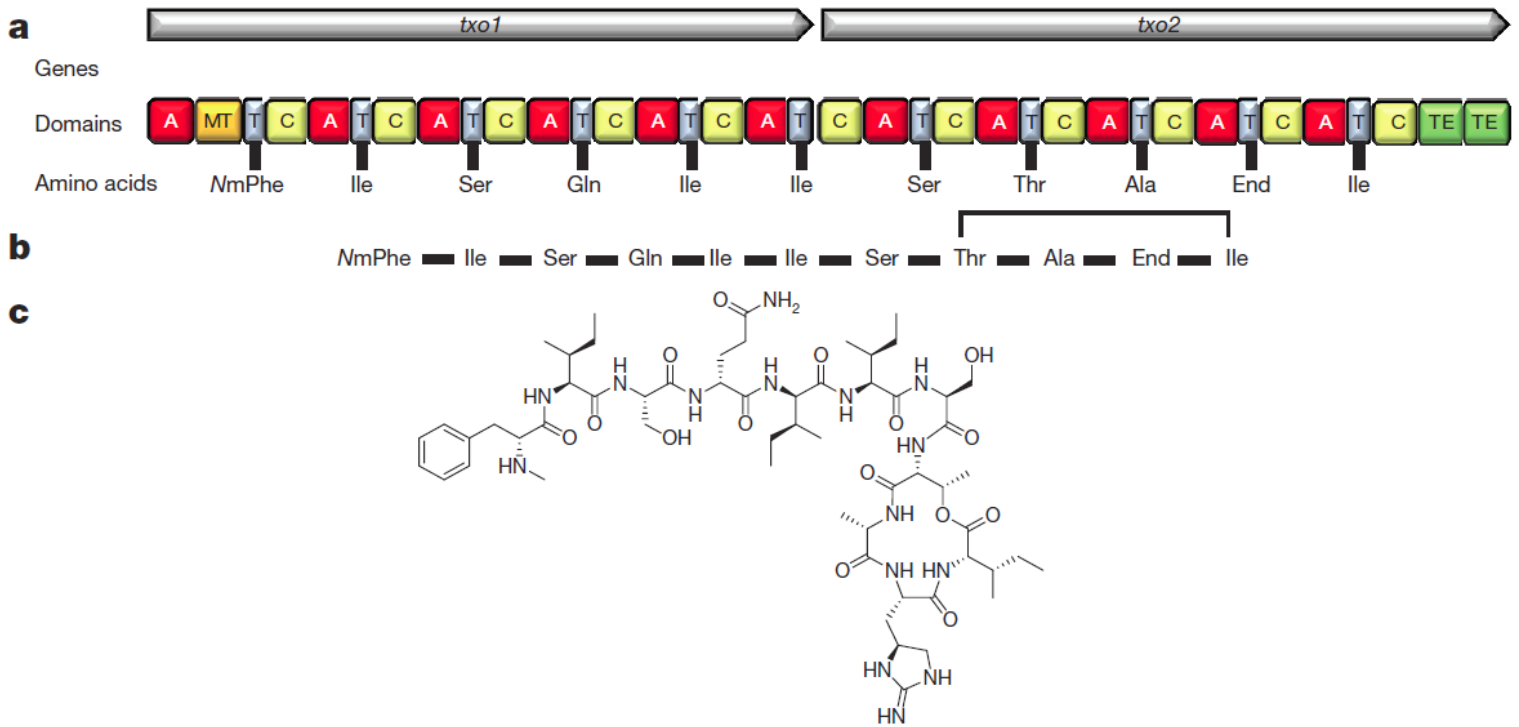


Figure 1 | The structure of teixobactin and the predicted biosynthetic gene cluster. **a**, The two NRPS genes, the catalytic domains they encode, and the amino acids incorporated by the respective modules. Domains: A, adenylation; C, condensation; MT, methylation (of phenylalanine); T, thiolation (carrier); and TE, thioesterase (Ile-Thr ring closure). *N*mPhe, *N*-methylated phenylalanine. **b**, Schematic structure of teixobactin. The *N*-methylation of the first phenylalanine is catalysed by the methyltransferase domain in module 1. The ring closure between the last isoleucine and threonine is catalysed by the thioesterase domains during molecule off-loading, resulting in teixobactin. **c**, Teixobactin structure.

Excellent activity against Gram-positiive pathogens including drug-resistant strains

Table 1 | Activity of teixobactin against pathogenic microorganisms

Organism and genotype	Teixobactin MIC ($\mu g\,ml^{-1}$)
S. aureus (MSSA)	0.25
S. aureus + 10% serum	0.25
S. aureus (MRSA)	0.25
Enterococcus faecalis (VRE)	0.5
Enterococcus faecium (VRE)	0.5
Streptococcus pneumoniae (penicillin^R)	≤ 0.03
Streptococcus pyogenes	0.06
Streptococcus agalactiae	0.12
Viridans group streptococci	0.12
B. anthracis	≤ 0.06
Clostridium difficile	0.005
Propionibacterium acnes	0.08
M. tuberculosis H37Rv	0.125
Haemophilus influenzae	4
Moraxella catarrhalis	2
Escherichia coli	25
Escherichia coli (*asmB1*)	2.5
Pseudomonas aeruginosa	>32
Klebsiella pneumoniae	>32

The MIC was determined by broth microdilution. MSSA, methicillin-sensitive *S. aureus*; VRE, vancomycin-resistant enterococci.

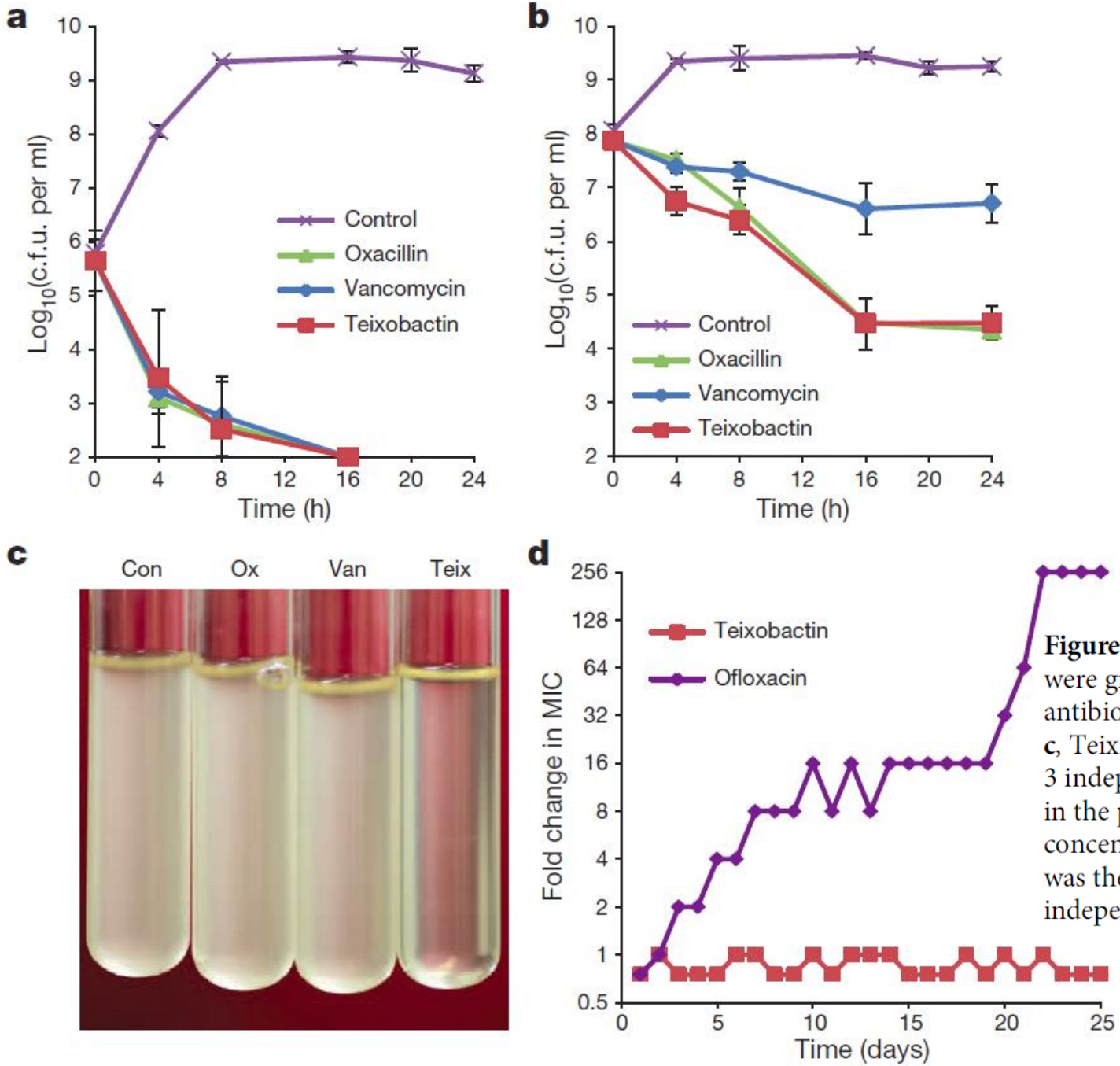


Figure 2 | Time-dependent killing of pathogens by teixobactin. **a**, **b**, *S. aureus* were grown to early (**a**), and late (**b**) exponential phase and challenged with antibiotics. Data are representative of 3 independent experiments ± s.d. **c**, Teixobactin treatment resulted in lysis. The figure is representative of 3 independent experiments. **d**, Resistance acquisition during serial passaging in the presence of sub-MIC levels of antimicrobials. The *y* axis is the highest concentration the cells grew in during passaging. For ofloxacin, 256 × MIC was the highest concentration tested. The figure is representative of 3 independent experiments.

Teixobactin mechanism

Teixobactin is a peptidoglycan synthesis inhibitor (like vancomycin)

Resistance is not developed to this compound, suggesting that the target is not a protein

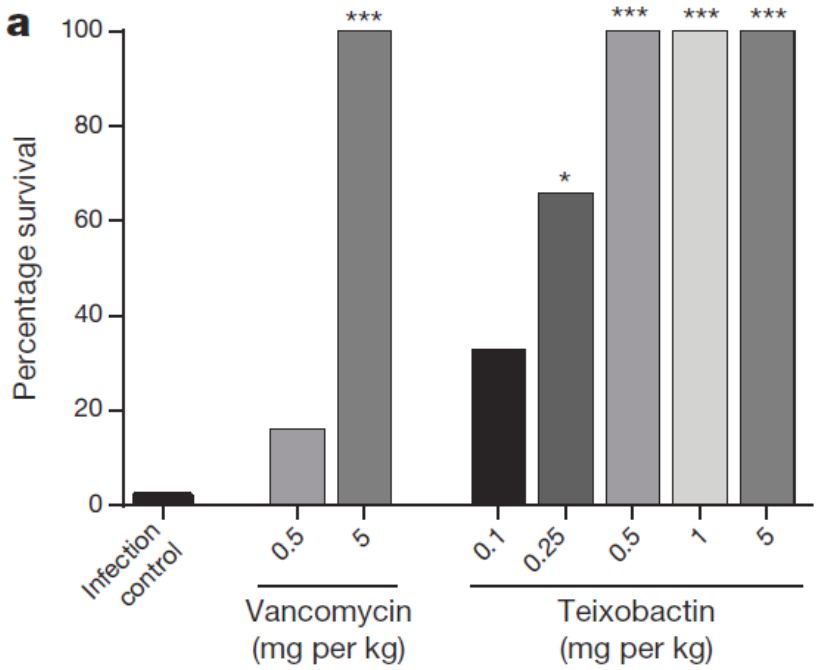
Teixobactin inhibits peptidoglycan biosynthesis with either lipid I, lipid II or lipid III as a substrate.

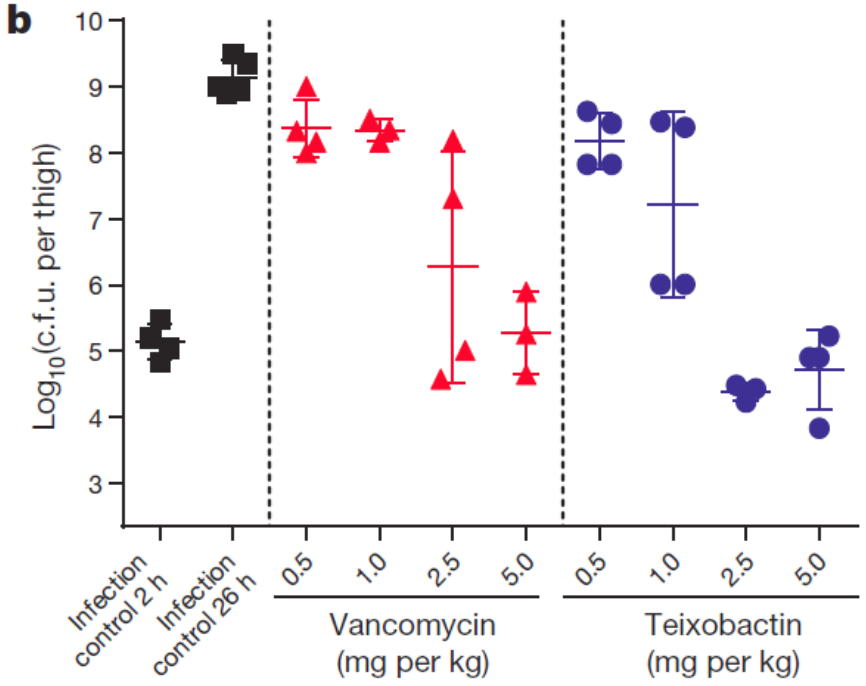
Vancomycin binds to lipid II

Teixobactin is active against vancomycin-resistant enterococci that have modified lipid II

Teixobactin also binds to the wall teichoic acid (WTA) precursor lipid III. Inhibition of WTA biosynthesis steps is lethal due to accumulation toxic intermediates.

In vivo efficacy





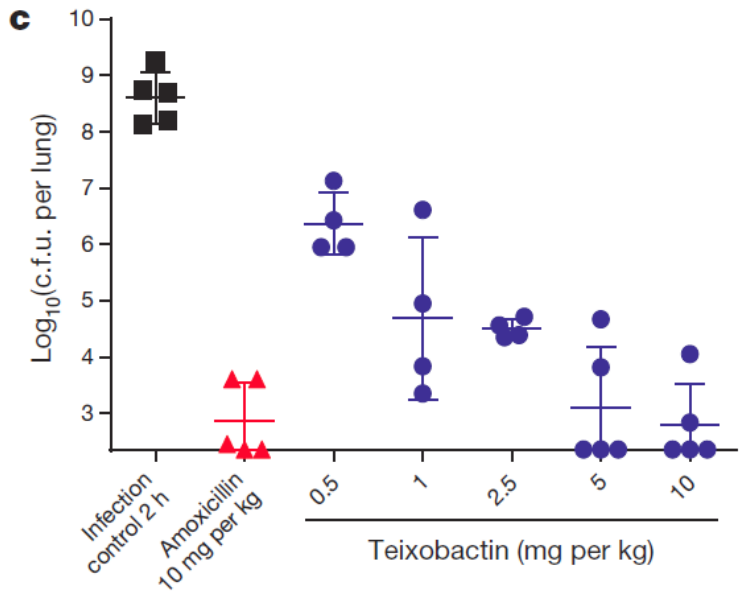


Figure 4 | Teixobactin is efficacious in three mouse models of infection. **a**, Single dose treatment (i.v., 1 h post-infection, 6 mice per group) with teixobactin and vancomycin in septicemia protection model using MRSA. Survival is depicted 48 h after infection. **b**, Single dose (i.v., 2 h post-infection, 4 mice per group) treatment with teixobactin and vancomycin in neutropenic mouse thigh infection model using MRSA. For drug-treated animals, thigh colony-forming units (c.f.u.) were determined at 26 h post-infection. For controls, c.f.u. in thighs were determined at 2 h and 26 h post-infection. **c**, Two dose treatment, 5 mice per group, with teixobactin (i.v., 24 h and 36 h post-infection) and single dose treatment with amoxicillin (subcutaneous, 24 h post-infection) in immunocompetent lung infection model using *S. pneumoniae*. Lung c.f.u. were determined at 48 h post-infection. The c.f.u. from each mouse are plotted as individual points and error bars represent the deviation within an experimental group. $^{*}P < 0.05$, $^{***}P < 0.001$ (determined by non-parametric log-rank test).

Concluding remarks

Very promising new bactericidal antibiotics was dicovered – teixobactin

There are signs that pathogens will be slow to evolve resistance to teixobactin

Teixobactin is active against the deadly bacterium MRSA (methicillin-resistant *Staphylococcus aureus)*

Antibiotic has yet to be tested in larger number of bacterial strains and peole.