# nature research

Corresponding author(s):	Jesper Larsen	
Last updated by author(s):	Nov 7, 2021	

# Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Confirmed
	The exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
X	A description of all covariates tested
X	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
X	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

#### Software and code

Policy information about availability of computer code

Data collection

No software was used to collect data in our study.

Data analysis

SPAdes v3.15
PHASTER
k-mer alignment (KMA) tool v1.3
NASP v1.0
PhyML v3.0
ClonalFrameML v1.12
BactDating v1.0
Treedater v0.5
coda

msConvert

GraphPad Prism v8.3

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

#### Data

Replication

Blinding

Study description

Research sample

Sampling strategy

Data collection

Data exclusions

**Timing** 

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

LC-MS data in mzXML format and molecular networking results are available in the MassIVE repository with the identifier MSV000087335. S. aureus short-read sequence data have been deposited in the European Nucleotide Archive/NCBI Sequence Read Archive under BioProjects PRJEB15105, PRJEB21015, PRJEB2655, PRJEB2755, PRJEB2756, PRJEB28206, PRJEB3174, PRJEB32898, PRJNA596428, and PRJEB43456 and the genome accession numbers are provided in Supplementary Table 1. T. erinacei type strain IMI 101051 short-read sequence data have been deposited in the European Nucleotide Archive under BioProject PRJEB43453. Tree and genealogy files in Newick format are provided in Supplementary Data 1-10. Source data for Figs. 1-4 and Extended Data Figs. 1, 4, 6, 7, and 9 are provided with the

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Please select the one be	low that is the best fit for your research.	If you are not sure, read the appropriate sections before making your selection
Life sciences	Behavioural & social sciences	Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size Describe how sample size was determined, detailing any statistical methods used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.

Describe any data exclusions. If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the Data exclusions rationale behind them, indicating whether exclusion criteria were pre-established.

> Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.

Randomization Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.

> Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

# Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

State the research sample (e.g., Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.

Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.

Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.

If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

Non-participation

State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.

Randomization

If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

### Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

We first examined the distribution of mecC-MRSA and other S. aureus isolates in hedgehogs in ten European countries and New Zealand. We sequenced 244 isolates to determine the population structure of S. aureus in hedgehogs. We also sequenced 913 S. aureus isolates from other sources to infer the evolutionary histories, host dynamics, geographical dispersal patterns, and zoonotic potential of the major mecC-MRSA clones in Europe. The potential mechanisms for natural selection of mecC-MRSA by T. erinacei were assessed by sequencing and analysing the genome of T. erinacei for penicillin G and cephalosporin C biosynthetic genes and by screening T. erinacei for production of beta-lactam antibiotics and antibiotic activity against a panel of S. aureus strains.

Research sample

The hedgehog survey was conducted by staff members of the wildlife rescue centres who collected swab samples from the nasal area, skin, and feet of hedgehogs in connection with routine checks. The survey included 828 samples from 276 hedgehogs originating from 16 wildlife rescue centres in ten European countries and two wildlife rescue centres in New Zealand, which represent the geographic distribution of the two European hedgehog species, the west European hedgehog (Erinaceus europaeus) present in Western Europe and New Zealand and the northern white-breasted hedgehog (E. roumanicus) found in Eastern Europe. Of the 276 hedgehogs included in the survey, 251 belonged to E. europaeus and the remaining 25 to E. roumanicus. Determination of sex and age was not relevant and therefore not determined. This led to the identification of 244 S. aureus isolates. The survey is a convenience sample, because the number and geographic distribution of hedgehog samples were limited by the number of wildlife rescue centres that volunteered to participate in our study and the number of hedgehogs in care at the time of sampling. We also included a collection of 913 S. aureus CC130, CC425, and CC1943 isolates from other sources, which represents the known geographic distribution (mainly Western and Central Europe) and host repertoire (mainly humans, cattle, sheep, goats, and wild animals) of each clone.

Sampling strategy

We did not use statistical methods to predetermine sample size, because the number and geographic distribution of hedgehog samples were limited by the number of wildlife rescue centres that volunteered to participate in our study and the number of hedgehogs in care at the time of sampling. S. aureus CC130, CC425, and CC1943 isolates from other sources were selected to represent the known geographic distribution (mainly Western and Central Europe) and host repertoire (mainly humans, cattle, sheep, goats, and wild animals) of each clone.

Data collection

Personnel at the different wildlife rescue centres were instructed to obtain samples from the nasal area, skin, and feet of hedgehogs kept in separate enclosures using FLOQSwabs (Copan). Swabs were stored in liquid Amies medium at ambient temperature and sent to the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut in Denmark or the Department of Veterinary Medicine at University of Cambridge in the United Kingdom immediately after sample collection.

Timing and spatial scale

The hedgehog survey was conducted between 12th April and 21st November 2018 and included 16 wildlife rescue centres in ten European countries and two wildlife rescue centres in New Zealand, which represent the geographic distribution of the two European hedgehog species: Attica, Greece, Judeţul Cluj, Romania, Treviso, Italy, Prague, Czech Republic, Jutland, Denmark, Zealand, Denmark, Valencia, Spain, Catalunya, Spain, Norte Region, Portugal, Loire-sur-Rhône, France, Fouencamps, France, Cornwall, England, Berkshire, England, Cambridgeshire, England, Northumberland, England, Powys, Wales, South Island, New Zealand, and North Island, New Zealand. Swab samples were typically collected within 24 to 48 hours at each wildlife rescue centre. There were no specific instructions on when to collect swab samples, because S. aureus colonisation is not expected to display substantial seasonal fluctuations. The 913 S. aureus CC130, CC425, and CC1943 isolates from other sources were collected between 1975 and 2018 and originated from 16 European countries, which represent the geographic distribution of each clone: Denmark, England, Germany, Spain, Scotland, Sweden, France, Norway, Italy, Austria, Wales, Belgium, Switzerland, Finland, Greenland, and the Netherlands.

Data exclusions

No animals, samples, or isolates were excluded from our analyses.

Reproducibility

Each sequenced S. aureus genome was subjected to multilocus sequence typing and the derived clonal complex was compared to the spa type of the original isolate when available, including 63% (154/244) of the isolates from the hedgehog survey and 63% (576/913) of the isolates from other sources. The analysis showed that the two methods assigned 100% (730/730) of the isolates to the same clonal complex. Determination of T. erinacei inhibition zones against the panel of S. aureus strains was repeated four times for each strain. The dates of the most recent common ancestors of the major mecC-MRSA lineages were analysed by two different approaches (BactDating and Treedater), which produced very similar estimates.

Randomization

We did not perform randomization as our study was observational rather than experimental.

Blinding

We did not perform blinding as our study was observational rather than experimental.

Did the study involve field work?

Yes



### Field work, collection and transport

Field conditions

Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

Location

. State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

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Access	Χı	import/expor

Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

Disturbance

Describe any disturbance caused by the study and how it was minimized.

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experime	ental syste	ms Methods	
n/a Involved in the study		n/a Involved in the study	
Antibodies		ChIP-seq	
Eukaryotic cell lines		Flow cytometry	
Palaeontology and a	archaeology	MRI-based neuroimaging	
Animals and other o	organisms		
Human research pa	rticipants		
Clinical data			
Dual use research o	of concern		
Antibodies			
Antibodies used	Describe all	antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.	
Validation		validation of each primary antibody for the species and application, noting any validation statements on the er's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.	
	(		
Eukaryotic cell lin	ies		
Policy information about <u>ce</u>			
Cell line source(s)		e the source of each cell line used.	
Authentication	Desc	ribe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.	
Mycoplasma contaminat	Mycoplasma contamination  Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination.		
Commonly misidentified (See ICLAC register)	ommonly misidentified lines  Name any commonly misidentified cell lines used in the study and provide a rationale for their use.  LCLAC register)		
Dolocoptologyon	d Arobo	o allowy	
Palaeontology an			
Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).		
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.		
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.		
Tick this box to confir	m that the r	aw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.		
Note that full information on t	he approval o	f the study protocol must also be provided in the manuscript.	

### Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

The study did not involve laboratory animals.

Wild animals The hedgehog survey involved 251 west European hedgehogs (Erinaceus europaeus) and 25 northern white-breasted hedgehogs (E. roumanicus) that had been admitted to wildlife rescue centres for treatment of a diverse range of conditions. Determination of sex and age was not relevant and therefore not determined. The sampling procedure did not cause pain, suffering, distress or lasting harm, deprived the animal of usual care, or affected the fate of the animals. The survey did not involve wild hedgehogs captured for

scientific purposes.

Field-collected samples

Population characteristics

The study did not involve samples collected from the field.

Ethics oversight

The hedgehog survey was conducted by staff members of the wildlife rescue centres who collected swab samples in connection with routine checks. In accordance with the Animal Welfare Act 1999 administered by the New Zealand Ministry for Primary Industries and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, no ethical approval was required as sample collection did not cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice or deprived the animal of usual care. Ethical review was undertaken at the Department of Veterinary Medicine, University of Cambridge (ref. CR76).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### Human research participants

Policy information about studies involving human research participants

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

#### Clinical data

Ethics oversight

Recruitment

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.
Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.
Data collection	Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.
Outcomes	Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

### Dual use research of concern

Policy information about dual use research of concern

#### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
		Public health
		National security
		Crops and/or livestock
		Ecosystems
		Any other significant area

Experiments of concer	'n				
Does the work involve an	y of the	ese experiments of concern:			
Confer resistance t Enhance the virule Increase transmiss Alter the host rang Enable evasion of o Enable the weapor Any other potentia	to therapence of a libility of ge of a pa diagnost nization				
ChIP-seq					
Data deposition	1.0				
		nal processed data have been deposited in a public database such as GEO.			
Confirm that you have	e depos	sited or provided access to graph files (e.g. BED files) for the called peaks.			
Data access links May remain private before public	cation.	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.			
Files in database submiss	ion	Provide a list of all files available in the database submission.			
Genome browser session (e.g. <u>UCSC</u> )		Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.			
Methodology					
Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.				
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.				
Antibodies	Describ numbe	be the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot or.			
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index file used.				
Data quality	Describ	pe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.			
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.				
Flow Cytometry					
Plots					
Confirm that:					
The axis labels state the	he marl	ker and fluorochrome used (e.g. CD4-FITC).			
The axis scales are cle	arly vis	ible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).			
All plots are contour p	olots wi	th outliers or pseudocolor plots.			
A numerical value for	numbe	er of cells or percentage (with statistics) is provided.			
Methodology					
Sample preparation	Sample preparation  Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.				
Instrument	nstrument   Identify the instrument used for data collection, specifying make and model number.				

Software	Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.			
Cell population abundance	Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.			
Gating strategy	Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.			
Tick this box to confirm that	a figure exemplifying the gating strategy is provided in the Supplementary Information.			
Magnetic resonance in	naging			
Experimental design				
Design type	Indicate task or resting state; event-related or block design.			
Design specifications	Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.			
Behavioral performance measur	es State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).			
Acquisition				
Imaging type(s)	Specify: functional, structural, diffusion, perfusion.			
Field strength	Specify in Tesla			
Sequence & imaging parameters	Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.			
Area of acquisition	State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.			
Diffusion MRI Used	Not used			
Preprocessing				
Preprocessing software	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).			
Normalization	If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.			
Normalization template	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.			
Noise and artifact removal	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).			
Volume censoring	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.			
Statistical modeling & infere	nce			
Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).			
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.			
Specify type of analysis: W	hole brain ROI-based Both			
Statistic type for inference (See <u>Eklund et al. 2016</u> )	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.			
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).			

Models & analysis	
n/a   Involved in the study	
Functional and/or effective connectivity	
Graph analysis	
Multivariate modeling or predictive analysi	s
Functional and/or effective connectivity	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).
Graph analysis	Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).
Multivariate modeling and predictive analysis	Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.