Candida auris epidemiology and control: the role of multiuse equipment: Supplementary material

David W Eyre DPhil, Anna E Sheppard PhD, Hilary Madder FANZCA, Ian Moir, Ruth Moroney MSc, T Phuong Quan MSc, David Griffiths BSc(Hons), Sophie George MSc, Lisa Butcher MSc, Marcus Morgan MSc, Robert Newnham, Mary Sunderland BSc, Tiphanie Clarke BA, Dona Foster PhD, Peter Hoffman BSc, Andrew M Borman PhD, Elizabeth M Johnson PhD, Ginny Moore PhD, Colin S Brown FRCPath, A Sarah Walker PhD, Tim EA Peto FRCP, Derrick W Crook FRCPath, Katie JM Jeffery PhD

Supplementary Methods 2
Setting 2
Lookback exercise 2
Patient screening 2
Environmental screening 3
Antifungal susceptibility testing 3
Definitions 3
Statistical methods 3
Duration of colonisation 4
Whole-genome sequencing 5
Temperature probe decontamination 6
Supplementary Results 6
Carriage duration 6
Supplementary Figures 8
Supplementary Tables 17
References 35

Supplementary Methods

Setting

OUH consists of four teaching hospitals (total 1225 beds), providing secondary- and tertiary-level care to Oxfordshire, UK (population ~600000) and the surrounding region. OUH has 4 intensive care units providing care to adult patients, including a neurosciences intensive care unit (NICU), consisting of 16 beds, 13 in an open plan configuration and three dedicated side rooms. The NICU admits patients from neurosurgery, neurology, plastic surgery (excluding burns), and ear, nose and throat surgery, and has approximately 650 admissions per year. This study included patients colonised or infected with *C. auris* between 02 February 2015 and 31 August 2017.

Lookback exercise

All OUH microbiology results from 01 January 2012 to 31 August 2017 were reviewed. Additionally, all *Candida* spp. isolates from OUH that had been identified by the national reference laboratory were also reviewed. Within OUH, *Candida* identification was undertaken by MALDI-TOF from late 2013 (specific date not recorded), as described below. Nine patients were colonized (n=4) or infected (n=5) with *C. auris* between 02 February 2015 and 16 October 2016, with all isolates prospectively identified by the OUH microbiology laboratory or the national reference laboratory.

Additionally, sensitivity data for all candidaemia isolates from all OUH locations from 01 January 2012 to 31 August 2017 were reviewed. There were no non-*Candida auris* isolates identified with antifungal susceptibilities consistent with *Candida auris*, that did not have an alternative identification confirmed by the national reference laboratory.

We also reviewed the incidence of *Candida* spp. identification from NICU inpatients from 01 January 2010 onwards. Between 1 January 2010 and 23 October 2016, four CSF infections, and one bloodstream infection caused by any *Candida* spp. were identified, all susceptible to fluconazole.

Patient screening

Patients were screened for *C. auris* colonisation on admission to the NICU, weekly, and on discharge, from 24 October 2016. Screening sites were nose, axilla, groin, tracheostomy and wound swabs (Sigma Transwab [Sigma-Aldrich Company Ltd, Gillingham, UK]), and urine culture. In January 2017 this was amended to three times weekly screening via axilla and groin swabs, with a full screen on admission and discharge. Patients on the neuroscience ward (the main step-down ward for NICU) were also screened weekly with axilla and groin swabs. This was amended from 06 July 2017 to admission and weekly *C. auris* screens using axilla and groin swabs on NICU and admission screens on transfer from NICU to all ward areas, undertaken by the admitting ward. Patients continued to be screened after a positive swab.

Swabs were cultured on Sabouraud dextrose agar with chloramphenicol (SabC [Oxoid Ltd, Basingstoke, UK]) at 37˚C in air. Colonies with morphological characteristics of *C. auris* were identified using the Bruker MALDI Biotyper system (Bruker Billerica, MA, USA); resulting spectra were analysed with MALDI Biotyper RealTime Classification software, version 3.0.

Environmental screening

Various methodologies for environmental screening were used, focussing on sampling high-touch areas and multi-use devices. These included bacterial swabs in a liquid transport medium (Sigma Transwab [Sigma-Aldrich Company Ltd, Gillingham, UK]), sponges to sample larger surface areas (Polywipe [Medical Wire and Equipment, Corsham, UK]), and Sabouraud dextrose agar contact plates (55mm diameter [Oxoid Ltd, Basingstoke, UK]). Enrichment culture was performed in brain heart infusion (Oxoid Ltd, Basingstoke, UK) or malt extract broth (Oxoid Ltd, Basingstoke, UK) as specified in Table S2. The air was sampled using passive air sampling techniques (SabC settle plates) and two active air samplers (an Air Ideal [BioMérieux, Marcy-l'Étoile, France] sampling at 100 L/min onto 90mm Sab agar and a Coriolis air sampler [Bertin Instruments, Montigny-le-Bretonneux, France] sampling at 300 L/min into 15ml phosphate buffered saline containing 0.01% Tween 20). Confirmation of *C. auris* was achieved using the Bruker MALDI Biotyper system as above.

Antifungal susceptibility testing

Antifungal susceptibility testing was undertaken using broth microdilution plates, with the Sensititre YeastOne system (Thermo Fisher Scientific, East Grinstead, UK).

Definitions

We conducted a case-control study to determine risk factors for *C. auris* colonisation/infection in patients admitted to NICU. Cases were any patient admitted to NICU prior to a *C. auris* isolate being cultured, either from a positive-screen or a clinical sample, taking the first isolate as the diagnosis date. Controls were never colonised/infected and had a NICU admission prior to ≥1 negative *C. auris* screen(s), taking exposures at the last screen. Other than requiring all cases and controls to have had NICU exposure (since all but four cases had NICU exposure prior to diagnosis), we did not match controls to cases, because matching is used to control by design those factors already known to be associated with case-control outcome, and this is the first study to investigate risk factors for colonisation to date. We included all available controls in order to maximise the power of the study to determine risk factors for *C. auris* colonisation.

Statistical methods

Independent predictors of the first *C. auris* colonisation/infection per patient were identified using multivariable logistic regression, selecting a final model from factors shown in Table 1 using backwards elimination with an exit p value of >0.1. This analysis considered patient demographics, diagnoses, physiological measurements, biomarkers, antimicrobial exposure, and ICU procedures as potential predictors based on clinically plausible factors for which routinely collected electronic data were available. All continuous variables had absolute correlation <0.65 in magnitude, suggesting limited potential for collinearity problems in model fitting. Additionally, use of axillary temperature monitoring was included following environmental sampling results. There was limited prior information which could be used to restrict the number of factors considered; previous studies have examined risk factors for invasive *C. auris* infection only.[1] The analysis was therefore considered exploratory and used a p>0.1 exit criteria to improve control of confounding.

Patients with incomplete data for any of the factors in Table 1 were excluded from the study (0 cases and 2 controls). Multivariable fractional polynomials were used to allow for non-linear effects of continuous factors. Interactions between main effects in the final model were retained where interaction $p < 0.01$. All analyses were performed using Stata 14.1 (Stata Corp, College Station, TX).

Potential risk factors were assessed in the 90-days before the date of the first *C. auris* isolate in cases, and before the last negative *C. auris* screen in controls. For laboratory test results and physiological measurements, a time-weighted average was calculated across any days of hospital admission in the preceding 90-days using the trapezoid rule.

Duration of colonisation

Patients with multiple screening samples over time were used to estimate the duration of carriage following the first positive screen in each patient using a survival analysis approach, treating death without clearance of colonization as a competing risk.

As any one screen is imperfectly sensitive the first two consecutive negative screens after a positive screen were taken to define loss of colonisation. Loss of colonisation was set to have occurred at the first of the two negative screens. Patients who survived the duration of the study with only a single negative screen following their last positive screen had their follow up censored at the date of their last positive screen as it was not possible to determine if these patients had cleared their colonization or not. Similarly, for patients who survived with no further screens after their last positive screen, their follow up was censored at the date of their last positive screen. Death without clearance was modelled as a competing risk, that is, patients who died prior to clearing their colonization (i.e. died with their last screen positive or following a single negative screen) had their date of death treated as a competing event. These patients typically died shortly after their last positive screen (median [IQR] 9 [6-13] days). Data were available on patient survival until the end of the study from nationally mandated registration of deaths, data on which are available to hospitals via the National Health Service Spine. Possible outcomes were therefore clearance of colonisation (2 consecutive negative screens) or death without clearance, with those surviving without observed clearance censored at their last positive screen. The analysis was repeated requiring three consecutive negative screens to define loss of colonisation.

However, mortality was relatively high in this critically unwell population; there was no evidence that mortality differed in those with or without colonization, and therefore whether death should be considered as an administrative censoring event or a competing event can be debated. We therefore also present an additional supplementary analysis treating death while colonised as censoring event, rather than a competing risk.

In an additional analysis, following a positive screen we determined the proportion of next screens in a given patient that were positive, based on the number of preceding negative screens in the same patient, e.g. 0, 1, 2, 3, etc. In this analysis, each screen conducted in patients with more than one screen was included as a single data point. For example, a patient with a positive screen, followed by two negative screens and then a positive screen would contribute one data point for 0 preceding negative screens (at their second screen), one data point for 1 preceding screen (at their third screen), and one data point for 2 preceding screens (at their fourth screen). The 95% confidence intervals for each proportion across all screens were generated using the exact binomial distribution.

We also considered whether the time between consecutive *C. auris* screens affected the odds of the next screen being positive using logistic regression (allowing for non-linearity using fractional polynomials).

Whole-genome sequencing

Only a subset of *C. auris* isolates were stored by the OUH clinical microbiology laboratory. All available first and last isolates per patient were sequenced, with samples from multiple time points, and multiple samples from the same time point sequenced in a small simple random subset of patients. Sequences were obtained at one, two, three and four time-points in 18, 13, four and two patients respectively. Multiple samples obtained at the same time-point were also sequenced: two from a single time-point in four patients and six from two time-points in one patient. Available environmental isolates were also sequenced.

Starting with SabC plates from primary culture, 6-12 colonies were touched with a loop and sub-cultured on Columbia blood agar (Oxoid Ltd, Basingstoke, UK) for 48 hours at 37˚C in air. DNA was extracted from the resulting growth using a commercial kit, QuickGene (Fujifilm UK, Bedford, UK), with an additional mechanical lysis step using the FastPrep-24 system (MP Biomedicals Solon, OH, US) at 6.5m/s for 180 seconds.

Whole-genome sequencing was undertaken using the Illumina MiSeq (Ilumina Inc, San Diego, CA, USA) following Nextera XT library preparation according to the manufacturer's instructions, generating 2x 150bp reads. Short-read data generated are available from the NCBI SRA under BioProject number PRJNA415955.

An outbreak-specific reference sequence was generated by long-read sequencing one isolate (T26425) using the Oxford Nanopore MinION. Library preparation was performed using 1D Genomic DNA by ligation kit (SQK-LSK108, Oxford Nanopore Technologies, Oxford, UK) according to the manufacturer's protocol. Libraries were loaded onto flow cell versions FLO-MIN106 R9.4 SpotON and sequenced for 48 hours using MinKnow software version 1.5.12. Basecalling was performed after completion of the sequencing run using Albacore v1.0.4. This resulted in a total of 295079 reads, with mean read length 10065 bp, corresponding to >200x coverage. Reads were converted to fastq format using poretools version 0.5.1, and error-corrected reads were generated using canu version 1.5 with "correctedErrorRate=0.075 corOutCoverage=100 genomeSize=12.4m -nanopore-raw" and otherwise default parameters. Hybrid assembly was then performed using Unicycler version 0.4.0[2] with Illumina reads plus error-corrected nanopore reads as input, and default parameters. The resulting assembly contained 39 contigs, with a total size of 12,476,846 bp.

Illumina reads for all isolates were mapped to the T26425 reference using Stampy[3] version 1.0.23 (without Burrows-Wheeler Aligner pre-mapping, using an expected substitution rate of 0.01). Samples were compared using single nucleotide polymorphisms (SNPs) identified with Samtools[4] version 0.1.19 mpileup, with variant filtering as previously described.[5-7] A median (IQR) [range] of 99.0% (98.9-99.0%) [97.7-99.6%] reads mapped to the reference sequence, with a median (IQR) [range] of 95.1% (95.0-95.2) [74.4-95.4%] reference positions called as A, C, G or T (i.e. non-ambiguous).

To place the 78 study isolates in a global phylogenetic context, publicly available WGS data for 47 isolates from Lockhart *et al*[8] and four additional Indian isolates[9] (accession numbers ERR899743,

ERR1519359, ERR1519358 and ERR1519357) were subjected to mapping and variant calling as above. A maximum likelihood phylogeny was constructed from the resulting SNP data using IQ-TREE version 1.3.13.[10]

As sequences were generated from DNA originally based on pooling 6-12 fungal colonies, data could contain a mixture of different sequences from different colonies. We used a previously described probabilistic method to determine when sequence mixtures were present.[11] To determine the constituent sequences in each mixture, this method requires a pre-specified panel of potential sequences to be specified; these were generated from all sequences in the study without evidence of a mixture present.

BEAST version 1.8.2 was used to reconstruct time-scaled phylogenies. Three independent runs, each with 10 million iterations, were combined to determine the final trees, after discarding the first 1 million iterations of each run as burn-in, and ensuring similar convergence.

Temperature probe decontamination

Prior to their removal from use, skin surface temperature probes were cleaned between patients using quaternary ammonium compound pre-prepared wipes (Clinell Universal Sanitising Wipes, GAMA Healthcare Ltd, Watford, UK). Following cleaning, no additional disinfection step was undertaken.

This was accepted custom and practice, but differed from the manufacturer's instructions for use (http://incenter.medical.philips.com/doclib/enc/5874327/Autoclavable_Temperature_Probes_Instructions_for_Use_(IFU)_(multi-language).pdf%3Ffunc%3Ddoc.Fetch%26nodeid%3D5874327). These instructions recommend cleaning with a soft cloth moistened with 70% isopropanol, activated dialdehyde (Cidex) or sodium hypochlorite (1 part bleach diluted with 10 parts water minimum), followed by disinfection by immersing the probe into the same cleaning solution for up to 2 minutes. The probe manufacturer (Philips Medical Systems) does not make any claim as to the efficacy of the chemical cleaning set out for the purposes of infection control, and recommends the institution's infection control disinfection policies be consulted.

Supplementary Results

Carriage duration

Treating death while colonised as a competing risk, the median(IQR) carriage duration in patients remaining alive was 61 (IQR 33-[not estimable]) days (2 negatives) or 82 (37-[not estimable]) days (3 negatives) (Figure 2A and Figure S5). Treating death while colonised as censoring event, the median(IQR) carriage duration was 39 (24-62) days or 58 (29-82) days respectively (Figure S6).

In addition to the analysis in the main manuscript, we also considered if the time between screens was associated with the chance of the second screen being positive. We only observed 21 occasions where a patient was re-screened after 2 negative screens and 12 occasions where a patient was re-screened after 3 negative screens (shown in Figure 2B). Following 2 negative screens the median (IQR) [range] days to the next screen were 7 (3-8) [1-23], and 7 (4.5-24) [2-79] following 3 negative

screens. There was no evidence that the odds of the next screen being positive depended on the time since the last screen (p=0.24 and p=0.66 respectively).

We also investigated patients with a relapse after serial negative screens. Seven patients had a screen-positive recurrence after ≥2 negative screens, two patients with two such recurrences. Two recurrences occurred after ≥3 negative screens, one after three negative screens and one after six negative screens. In eight of the nine recurrences, the initial and subsequent isolates were whole-genome sequenced. Six of the eight recurrences with complete whole-genome sequencing were due to an identical or very closely related strain, suggestive of recurrence of the initial colonisation / transient false-negative screens (although re-infection with a very similar strain cannot be excluded given the high rates of colonisation in other patients with similar strains). Two recurrences (both in the same patient, patient 15) were genetically distinct suggestive of re-infection (Table S5). In patients with recurrences of the same colonising strain in some instances exposure to micafungin may have temporarily supressed detectable colonisation (patients 6 and 28), while other patients received broad spectrum antibiotics or had an invasive procedure during the screen negative period (patients 11, 22, 25, 27) which may have promoted *C. auris* colonisation subsequently. However, exposure to broad-spectrum antibiotics was relatively common in the whole study cohort. Therefore, a larger study is required to investigate the relationship between relapse and antimicrobial exposure formally.

Supplementary Figures

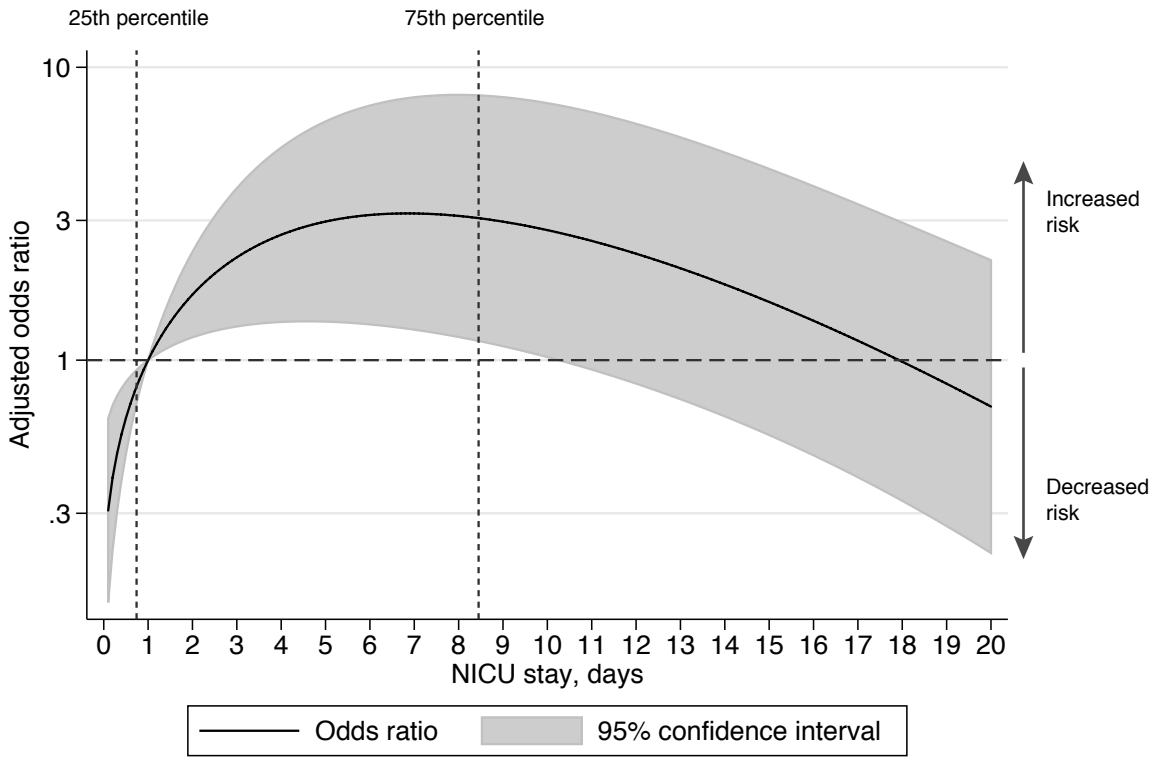

Figure S1. Adjusted odds ratio for non-linear predictors of *C. auris* colonization: NICU stay.

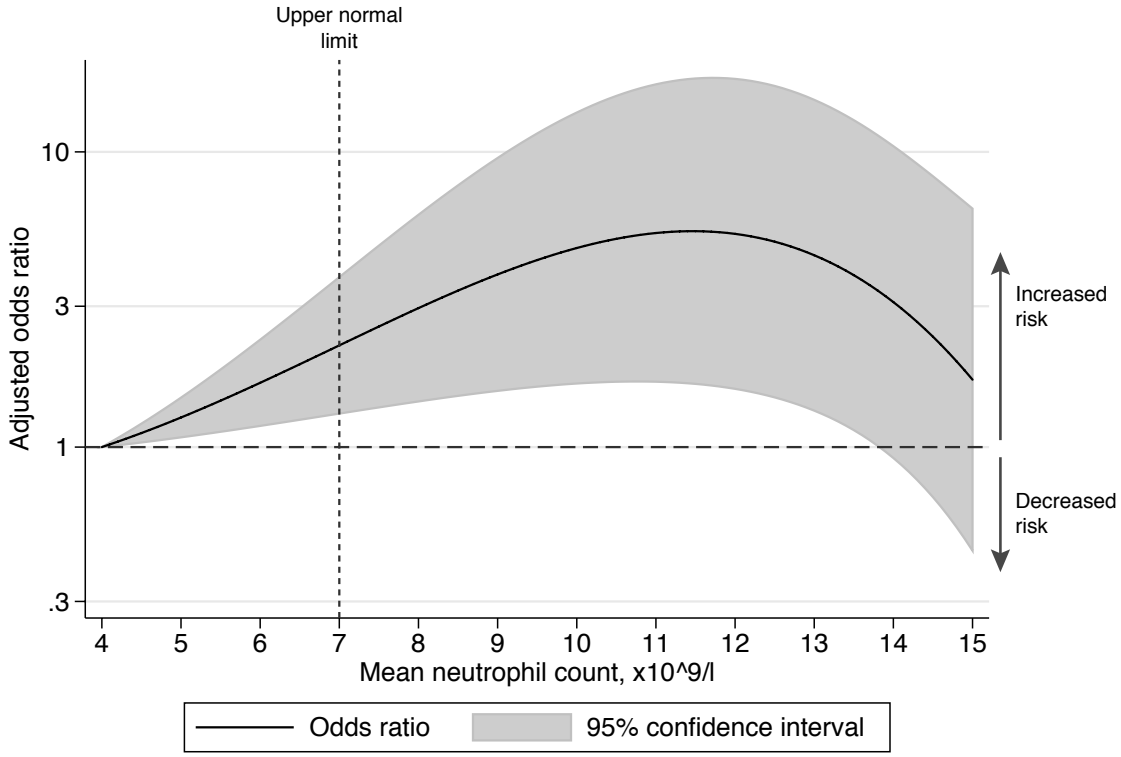

Figure S2. Adjusted odds ratio for non-linear predictors of *C. auris* colonization: neutrophil count.

Figure S3. Skin surface temperature probe.

http://www.philips.co.uk/healthcare/product/HC989803162641/skin-surface-temperature-probe-sensor; Philips Healthcare, Guildford, UK.

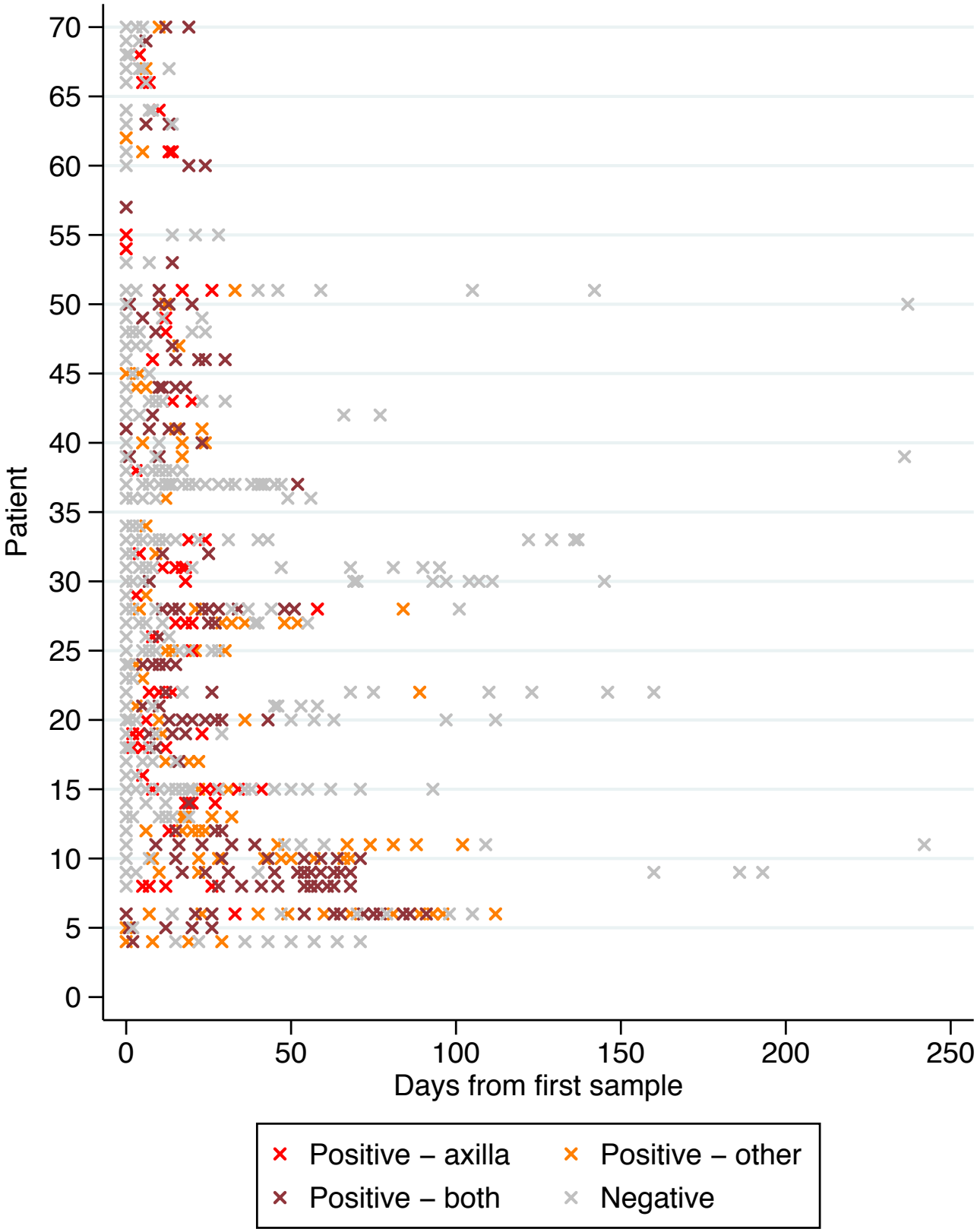

Figure S4. ***C. auris*** **screening results in 60 cases with ≥1 screen.**

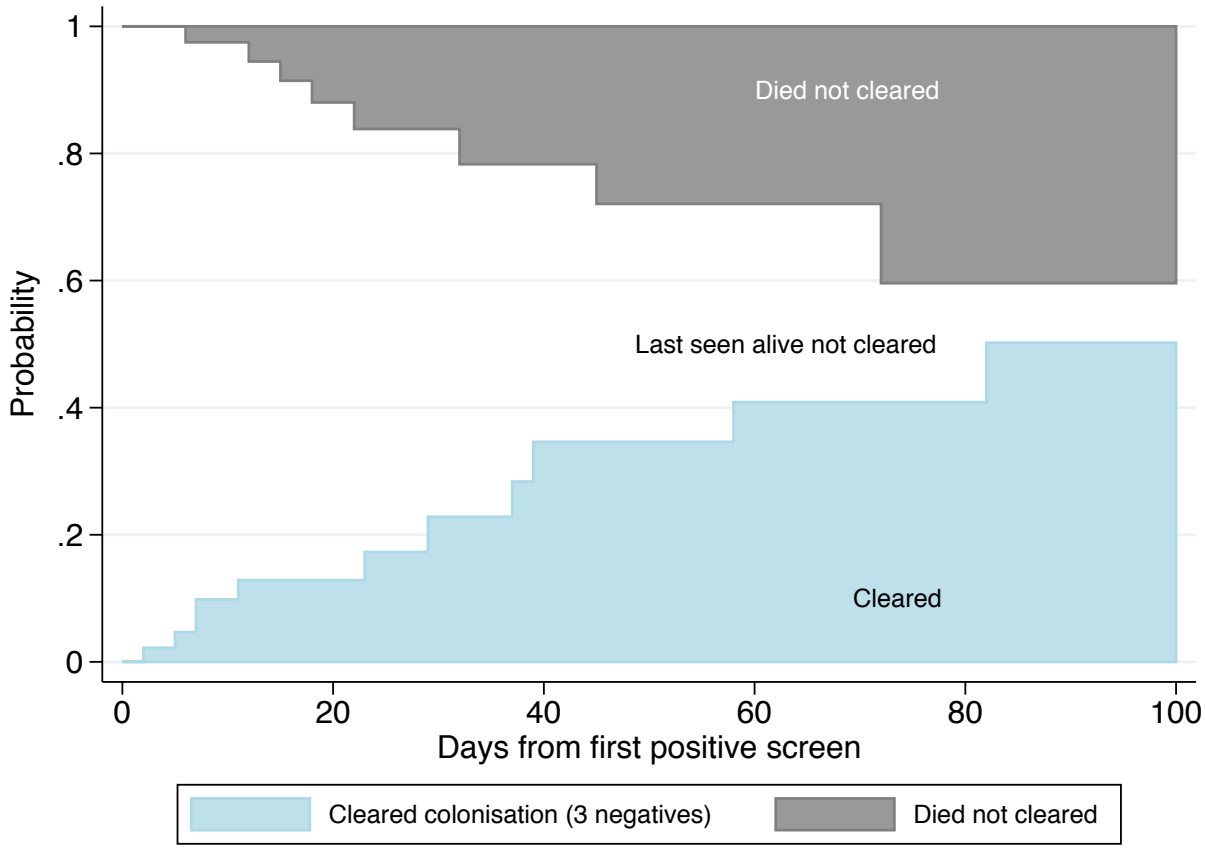

Figure S5. Duration of *C. auris* colonisation following a first positive screen. The figure shows proportion of patients clearing *C. auris* colonisation by the number of days since their first positive screen, treating death without clearance as a competing risk. As any one screen was imperfectly sensitive, clearance of colonisation was defined as 3 consecutive negative screens, timed from the day of the first negative screen. Of 11 patients clearing their colonisation, 2 had a subsequent relapse (one with 6 prior negative screens and one with 3 prior negative screens; details, including WGS comparisons, in Table S5). See Figure 2A for an alternative definition of 2 negative screens.

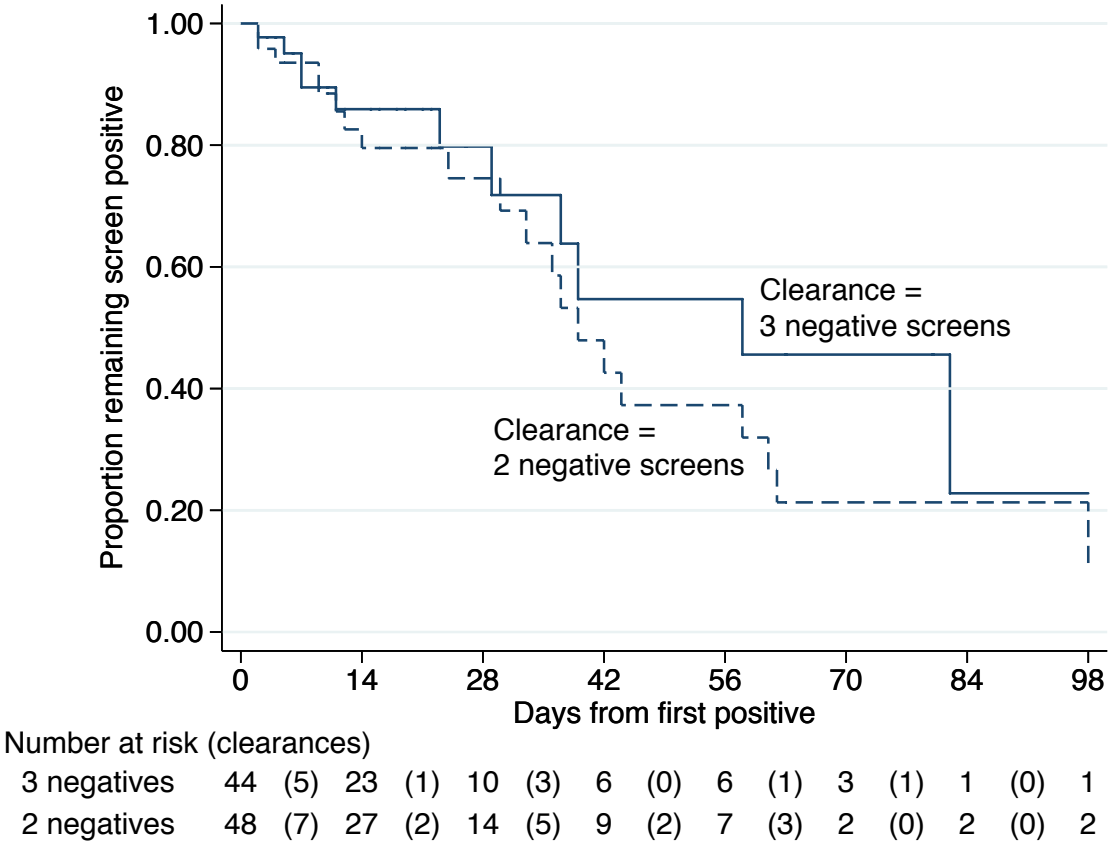

Figure S6. Duration of *C. auris* colonisation following a first positive screen, treating death while colonised as censoring event. Clearance of colonisation was defined as 2 (dashed line) or 3 (solid line) consecutive negative screens.

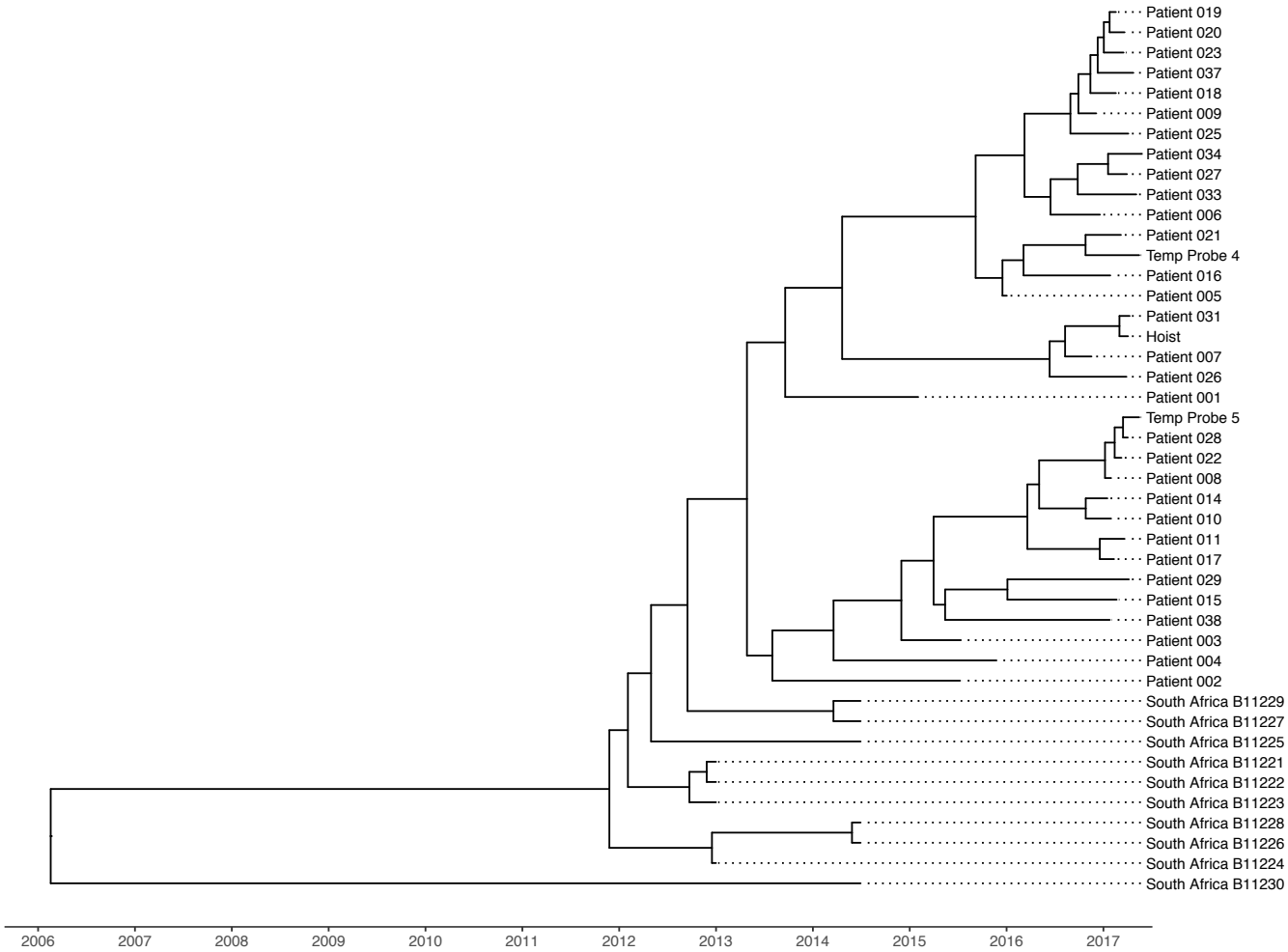

Figure S7. Time-scaled Bayesian phylogeny of 34 outbreak and 10 South African *C. auris* sequences. We used sequences previously obtained from the South African clade, combined with all Oxford outbreak sequences without any evidence of mixed infection to estimate the rate of *C. auris* mutation.

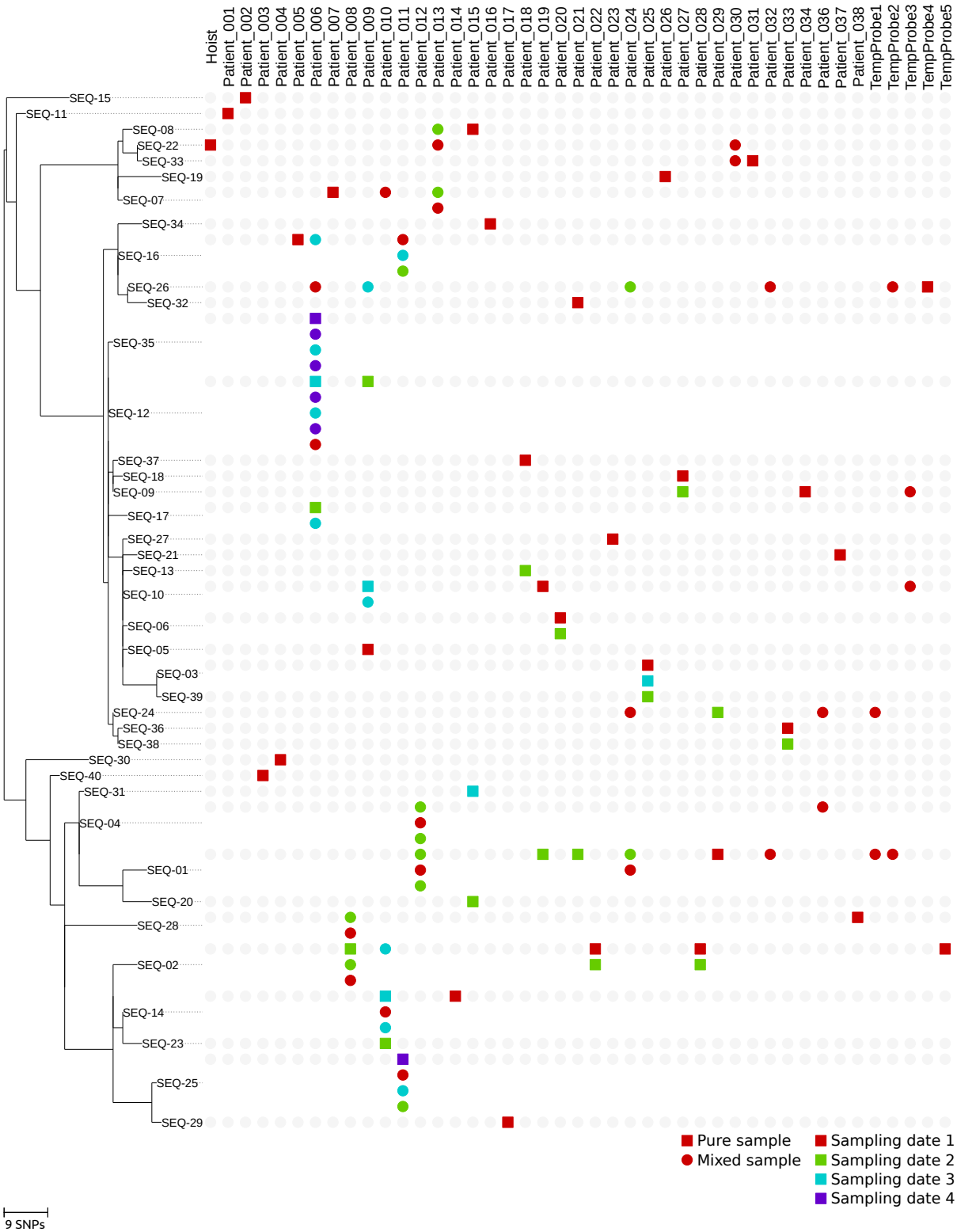

Figure S8. Maximum likelihood phylogeny of 40 unique sequences identified (SEQ01 to SEQ40) and presence of these sequences in 37 patients and 6 environmental samples. Based on 215 variable sites. SNPs, single nucleotide polymorphisms.

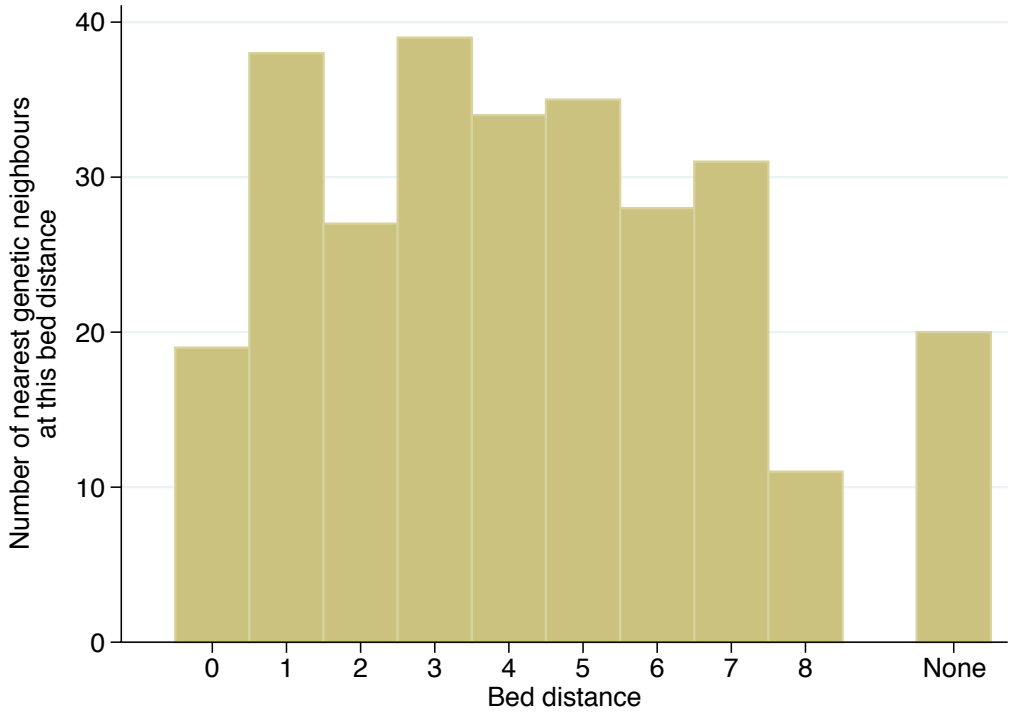

Figure S9. Distribution of bed proximity for genetically-related patients. The most closely genetically-related sequence or sequences from other patients were identified for each patient's colonisation/infection(s). For pairs of genetically-related patients, bed locations following the diagnosis of the first case, and prior to the diagnosis of the second are analysed. Bed distance is the number of bed spaces between the cases in this time period, where zero is sharing the same bed space, and one is adjacent beds within this time window, etc. Within the NICU beds are arranged in a circular layout, such that bed 1 is adjacent to bed 16 and 2, bed 2 is adjacent to bed 1 and 3, and so on. Where a patient pair did not both spend time on NICU in the time window of interest this is indicated as "None". Where patients were similarly closely genetically related to multiple patients, or where patients had multiple bed overlaps, all data points are plotted.

Supplementary Tables

	Controls (N=361)		Cases (N=66)		Univariable			Multivariable		
	n / median	% / IQR	n / median	% / IQR	Odds ratio	95% Confidence interval	p value	Odds ratio	95% Confidence interval	p value
Sex, female	173	48%	22	33%	0.54	(0.31, 0.94)	0.03			
Age	55.5	43.5 - 67.2	51.9	41.7 - 63.9	0.99	(0.97, 1.01)	0.19			
Primary Diagnosis										
- Other	89	25%	9	14%	1.00	Baseline	0.03			
- Benign neoplasm	54	15%	0	0%	*					
- CNS / ENT infection	32	9%	6	9%	1.85	(0.61, 5.62)				
- Intracranial haemorrhage	90	25%	21	32%	2.31	(1.00, 5.31)				

- Malignancy	34	9%	6	9%	1.75	(0.58, 5.27)				
- Trauma	62	17%	24	36%	3.83	(1.67, 8.80)				
Duration of NICU stay prior to *C. auris* diagnosis §	1.8	0.7 - 6.6	8.4	4.6 - 13.4						
- 1 day					1.00	Baseline	<0.001	1.00	Baseline	0.001
- 3 days					3.89	(2.38, 6.36)		2.24	(1.30, 3.86)	
- 5 days					7.37	(3.65, 14.89)		2.97	(1.35, 6.53)	
- 10 days					12.68	(5.38, 29.88)		2.78	(1.02, 7.54)	
- 20 days					6.75	(2.78, 16.40)		0.69	(0.22, 2.19)	
Emergency admission	277	77%	63	95%	6.37	(1.95, 20.80)	0.002			
Axillary temperature monitoring	122	34%	57	86%	12.41	(5.94, 25.90)	<0.001	6.80	(2.96, 15.63)	<0.001

Invasive ventilation	210	58%	63	95%	15.10	(4.65, 48.99)	<0.001			
Any central venous access	184	51%	51	77%	3.27	(1.77, 6.03)	<0.001			
Laboratory										
Albumin, g/L	29.3	25.0 - 32.5	23.8	21.6 - 27.3	0.82	(0.77, 0.87)	<0.001	0.92	(0.85, 1.00)	0.06
Creatinine, µmol/L	62.7	53.0 - 75.0	65.3	49.2 - 77.6	1.00	(0.99, 1.02)	0.62			
Potassium, mmol/L	4	3.8 - 4.1	4	4.0 - 4.1	2.76	(1.04, 7.31)	0.04			
Sodium, mmol/L	139.3	137.1 - 141.1	141.4	138.5 - 143.6	1.20	(1.09, 1.31)	<0.001	1.10	(0.99, 1.22)	0.07
Haemoglobin, g/L	119.5	106.7 - 134.0	106.8	93.0 - 119.3	0.96	(0.94, 0.98)	<0.001			
Neutrophils ‡	8.6	6.6 - 10.9	9.6	7.3 - 10.9						
- 4 x10^9/L					1.00	Baseline	0.003	1.00	Baseline	0.01
- 7 x10^9/L					2.18	(1.40, 3.41)		2.21	(1.30, 3.76)	

- 10 x10^9/L					4.41	(1.84, 10.59)		4.72	(1.64, 13.59)	
- 15 x10^9/L					1.17	(0.37, 3.71)		1.69	(0.45, 6.42)	
Physiology										
Temperature, per 0.5°c change	36.5	36.3 - 36.9	36.9	36.6 - 37.3	2.44	(1.78, 3.35)	<0.001	1.43	(0.96, 2.14)	0.08
Heart Rate	76.5	68.5 - 85.1	82.3	69.3 - 92.2	1.02	(1.00, 1.05)	0.06			
Respiratory Rate	16.7	16.0 - 17.7	17.1	15.7 - 19.0	1.17	(1.00, 1.37)	0.05			
Systolic BP, mmHg	132.4	122.8 - 145.2	135.8	127.6 - 143.3	1.01	(0.99, 1.03)	0.32			
Diastolic BP, mmHg	70	65.3 - 76.6	68.2	64.6 - 72.6	0.95	(0.92, 0.99)	0.01			
Antimicrobials										
Any antifungal (fluconazole)	3	1%	3	5%	5.68	(1.12, 28.79)	0.04	10.34	(1.64, 65.18)	0.01

Per day of broad spectrum antibiotics	2	1.0 - 6.0	7	4.0 - 11.0	1.12	(1.07, 1.17)	<0.001			

Table S1. Characteristics of cases and controls, univariable and multivariable predictors of *C. auris* colonisation. Complete data were available for all factors shown for all 66 cases and 361 controls. *As there were no cases with a primary diagnosis of a benign neoplasm this group is excluded from the odds ratio shown. § There was a non-linear relationship between risk of colonisation and duration of NICU stay, represented by the multiple fractional polynomial terms X, and X^0.5, where X = (duration of NICU stay)/10 (Figure S1 for multivariable model). ‡ There was a non-linear relationship between risk of colonisation and neutrophil count, represented by the multiple fractional polynomial terms Y^3 and Y^3*ln(Y), where Y=(neutrophil count)/10 (Figure S2 for multivariable model). All patients with a prior antifungal received fluconazole only. Multivariable model C statistic 0.88.

Site	Date	Sampling method	Culture (Sabouraud Dextrose Agar +/- Chloramphenicol)	Enrichment (brain heart infusion, BHI, or malt extract broth, MEB)
Pulse oximeter	23/11/16	Swab in liquid transport medium	**1/1 *C. auris***	
Dynamic mattresses x4	23/11/16	Swab in liquid transport medium	4/4 No growth	
Curtains	23/11/16	Contact plate	No growth	
Blood pressure cuff	23/11/16	Swab in liquid transport medium	No growth	
Air x 16§	24/11/16	SabC Settle plates	**1 colony *C. auris*^**	
			2 x 1 colony *C. guilliermondii*	
			1 colony *C. albicans*	
			12/16 plates No growth	
Pulse oximeters x 12	29/11/16	Swab in liquid transport medium	12/12 No growth	
Central ventilation ceiling tile	29/11/16	Swab in liquid transport medium	No growth	
Waste water x 4 sink P traps	03/02/17	50mls from P trap	4/4 No growth	
Sink surface x 4	03/02/17	Polywipe plus 5ml saline	4/4 No growth	
Ceiling fan/tile	03/02/17	Polywipe plus 5ml saline	1/1 No growth	1/1 No growth (BHI)
Computer keyboard*	03/02/17	Polywipe plus 5ml saline	4/4 No growth	4/4 No growth (BHI)
Suction apparatus*	03/02/17	Polywipe plus 5ml saline	4/4 No growth	4/4 No growth (BHI)

Bedrails*	03/02/17	Polywipe plus 5ml saline	4/4 No growth	4/4 No growth (BHI)
Trolley**	03/02/17	Polywipe plus 5ml saline	2/2 No growth	2/2 No growth (BHI)
Ventilator/controls***	03/02/17	Polywipe plus 5ml saline	2/2 No growth	2/2 No growth (BHI)
40 surfaces****	04/04/17	Contact plate	38/40 no growth (see below)	
Temperature probe x 1	04/04/17	Contact plate	**1/1 *C. auris***	
Patient mobile hoist	04/04/17	Contact plate	**1/1 *C. auris***	
Twelve surfaces[#]	04/04/17	Cotton-tipped swabs and malt extract broth	12/12 no growth	12/12 No growth (MEB)
Air[¥]	04/04/17	Air Ideal (sampling at 100 L/min)	No growth	
Air[¥]	04/04/17	Coriolis sampler (sampling at 300 L/min)	No Growth	
Temperature probe x 1^^	15/04/17	Swab in liquid transport medium	No growth	
Temperature probe x 10	16/05/17	Contact plates and enrichment culture	10/10 No growth	**4/10 *C. auris* (BHI)**

Table S2. *C. auris* environmental sampling results.

[§]Two settle plates were placed in 4 bedspaces for 5 hours during a day shift, and repeated during a nightshift.

^ plate initially in an empty bedspace; a colonised patient was moved into the bed during the period of observation.

[¥]Air samplers were positioned in a negative bed space; a positive bed space; a positive side room; an empty (previously positive) bed space and at the doctors/nursing station. Can assume that at the time of sampling, the number of *C. auris* in the air was < 1 cfu/m3 of air sampled.

*From 4 bedspaces, 2 occupied by colonised patients.

**From 2 bedspaces occupied by colonised patients.

***From 2 unoccupied bedspaces.

**** From 2 bedspaces, 1 occupied by a colonised patient

[#] From the wider NICU environment

^^Temperature probe in use, *C. auris* negative patient

Date	Interventions
September 2016	▪ Enhanced surveillance – full speciation of any *Candida* species from any site on NICU to identify *C. auris*
November 2016	▪ Patient screening for *C. auris* on admission, weekly and discharge. Sites: nose, axilla, groin, urine/urethral swab, endotracheal secretions, tracheostomy site/secretions, drain sites (eg. external ventricular drain, lumbar drain), any percutaneous endoscopic gastrostomy site, wounds (=full screen) ▪ Barrier nursing with isolation or cohorting of all positive patients. Long sleeved gowns ▪ Enhanced clean whole unit daily (Acticlor plus 2000ppm) floors and level surfaces ▪ Terminal cleans for all vacated bedspaces ▪ Enhanced monitoring of cleaning and hand hygiene ▪ General 'decluttering' of the unit, including removal of unnecessary paper stocks ▪ Restricted traffic - stopped use of unit as a 'corridor' to offices ▪ Removal of fans and forced air convection blankets ▪ Reduction of bedside stocks of equipment with stocks of single use items in the immediate patient environment discarded on discharge ▪ Single use equipment obtained where possible e.g. blood pressure cuffs ▪ Wipe clean pillows obtained (SleepAngel™) ▪ Regular staff meetings (formal and informal 'brain-storming') ▪ Introduction of Micafungin prophylaxis (single dose) for device related surgical procedures in colonised patients ▪ Addition of C. auris alert to patient transfer form ▪ On transfer out of NICU, *C. auris* positive patients to be admitted to a side-room for continued isolation ▪ *C. auris* negative patients discharged from NICU to be admitted to a side-room until 3 negative *C. auris* screens taken 24 hours apart
January 2017	Patient screening increased to three times weekly axilla and groin only, with 'full screen' as above on admission and discharge to NICU
April 2017	Withdrawal of multi-use axillary temperature monitors

Table S3. *C. auris* infection prevention and control measures on NICU.

	Fluconazole	Voriconazole	Posaconazole	Amphotericin	Micafungin	Flucytosine
n	79	80	73	79	77	79
Tentative resistance breakpoint	≥8	≥ 1	≥ 0.125	≥2	≥1	≥32
Resistant, n	79	78	66	14	0	0
Resistant, %	100%	98%	90%	18%	0%	0%
Minimum inhibitory concentration, mg/l						
<=0.03			1			6
0.06			6		7	6
0.125			32	1	58	44
0.25			29	2	11	22
0.5		2	2	5	1	
1		4		57		
2		19		14		1
4		46				
8		7	1			
16		2	2			
32						
64						
128	3					
256	3					
>256	73					

Table S4. Antimicrobial susceptibility testing data. Where testing was not done over the whole range the adjacent doubling dilution is shown, e.g. for posaconazole isolates with a minimum

inhibitory concentration >8 mg/l are shown as having a minimum inhibitory concentration of 16 mg/l. Breakpoints are based on those established for *Candida albicans* isolates (CLSI for all except Posaconazole [EUCAST breakpoint]): Fluconazole S ≤2 R ≥8, Voriconazole S ≤0.125 R ≥1.0, Micafungin S ≤0.25 R ≥1.0, Flucytosine S ≤4.0 R ≥32, Amphotericin B S ≤1.0 R ≥2.0, Posaconazole S ≤0.06 R ≥0.125. Two of the three isolates with higher Posaconazole MICs underwent WGS; these were found in the lower half of the phylogeny in Figure 4, in patients 8 and 10, but did not form a distinct sub-clade. The isolate with a higher flucytosine MIC was not sequenced.

Patient	Diagnosis	SNPs between initial and relapse strains	SNP interpretation	Number of negative screens prior to recurrence	Antimicrobial exposure and procedures around recurrence	Postulated summary
6	Intracerebral arteriovenous malformation	Relapse not sequenced	-	2	Micafungin for VP shunt insertion 7 days prior to 2 negative screens; possible temporary suppression of detection	Temporary colonisation suppression with micafungin
11	Extra-dural haemorrhage, decompressive craniotomy	0	Relapse	3	Co-amoxiclav and metronidazole while screen negative prior to relapse	Broad spectrum antibiotic promotion of existing colonisation
15	Guillain-Barre syndrome	2 relapses: 57 and 16 SNPs	Re-infection, twice	2 relapses: 6 and 2	No antibiotics around each recurrence	Re-infection, twice
22	Traumatic brain injury	0	Relapse	2	Co-amoxiclav during screen negative period prior to relapse	Broad spectrum antibiotic promotion of existing colonisation
25	Hypertensive intracerebral bleed	2 relapses: 1 and 1 SNPs	Relapse, twice	2 relapses: 2 and 2	Tracheostomy insertion immediately prior to first relapse, antibiotic, but no antifungal prophylaxis. (Topical nystatin for oral candidiasis during both screen negative periods)	Invasive procedure / antibiotic promotion of existing colonisation
27	Traumatic brain injury	2	Relapse	2	Piperacillin-tazobactam during screen negative period prior to relapse	Broad spectrum antibiotic promotion of existing colonisation
28	CNS device infection	0	Relapse	2	Micafungin as empiric treatment during screen negative period	Temporary colonisation

						suppression with micafungin

Table S5. Details of patients with a recurrence of colonisation after ≥2 negative screens. Patient numbering corresponds to numbering used in Figure S4 and Figure 4. SNPS, single nucleotide polymorphisms; CNS, central nervous system.

id	Proportion dominant sequence			Proportion of sites differing between mixed sequences			Deviance statistic	Constituent mixed sequence pairs, with probabilities	Most likely mixed or pure sequence
	Maximum likelihood estimate	2.5% centile	97.5% centile	Maximum likelihood estimate	2.5% centile	97.5% centile			
5353e0a5	0.813	0.734	0.871	1.76E-01	1.22E-01	2.34E-01	3.00E+03	SEQ-01 x SEQ-24: 0.99997	SEQ-01; SEQ-24
9be9119e	0.718	0.694	0.745	2.46E-01	1.90E-01	3.02E-01	5.20E+03	SEQ-01 x SEQ-24: 0.99999	SEQ-01; SEQ-24
417a7f10	0.657	0.620	0.691	2.66E-01	2.06E-01	3.28E-01	5.34E+03	SEQ-01 x SEQ-26: 1	SEQ-01; SEQ-26
d3c92e9a	0.785	0.678	0.940	1.88E-02	4.66E-03	3.81E-02	4.32E+02	SEQ-04 x SEQ-01: 0.99995	SEQ-04; SEQ-01
a9056c9e	0.964	0.945	0.980	4.93E-02	1.58E-02	9.40E-02	6.28E+01	SEQ-04 x SEQ-01: 1	SEQ-04; SEQ-01
015db648	0.784	0.693	1.000	1.40E-02	1.00E-10	3.26E-02	3.15E+02	SEQ-04 x SEQ-01: 1	SEQ-04; SEQ-01
03a9dfca	0.718	0.676	0.757	1.75E-01	1.23E-01	2.26E-01	1.35E+03	SEQ-04 x SEQ-24: 0.99923	SEQ-04; SEQ-24
bd55b7f6	0.930	0.889	1.000	1.65E-02	1.00E-10	3.89E-02	3.86E+01	SEQ-07 x SEQ-08: 0.97261, SEQ-07 x SEQ-22: 0.02605	SEQ-07; SEQ-08
45a123c9	0.871	0.853	1.000	6.11E-03	5.03E-14	2.29E-02	5.73E+01	SEQ-07 x SEQ-22: 0.89514, SEQ-07 x SEQ-08: 0.0978	SEQ-07; SEQ-22
51c54d83	0.574	0.500	0.910	1.86E-02	4.67E-03	4.21E-02	1.02E+03	SEQ-10 x SEQ-09: 0.99995	SEQ-10; SEQ-09
1ddd9db5	0.900	0.855	0.940	3.99E-02	1.47E-02	7.32E-02	2.79E+02	SEQ-12 x SEQ-26: 0.99988	SEQ-12; SEQ-26
dab356d7	0.852	0.852	1.000	4.73E-03	1.77E-12	1.42E-02	4.80E+01	SEQ-12 x SEQ-35: 1	SEQ-12; SEQ-35
05d95077	0.864	0.863	1.000	4.77E-03	3.55E-12	1.45E-02	5.56E+01	SEQ-12 x SEQ-35: 1	SEQ-12; SEQ-35

add7c228	0.829	0.786	1.000	9.36E-03	7.67E-11	9.90E-01	1.40E+02	SEQ-14 x SEQ-02: 1	SEQ-14; SEQ-02
9ad40612	0.887	0.827	0.926	1.60E-01	1.12E-01	2.15E-01	1.32E+03	SEQ-14 x SEQ-07: 0.99997	SEQ-14; SEQ-07
94d17e80	0.735	0.689	0.824	3.29E-02	1.38E-02	5.76E-02	9.41E+02	SEQ-16 x SEQ-17: 0.77501, SEQ-12 x SEQ-16: 0.12359, SEQ-17 x SEQ-16: 0.1014	SEQ-16; SEQ-17
be9e4b5c	0.650	0.629	0.669	2.54E-01	1.98E-01	3.13E-01	1.13E+04	SEQ-16 x SEQ-25: 0.96072, SEQ-16 x SEQ-29: 0.03928	SEQ-16; SEQ-25
2532dbd9	0.823	0.807	0.840	2.46E-01	1.90E-01	3.06E-01	4.71E+03	SEQ-16 x SEQ-25: 0.99951	SEQ-16; SEQ-25
8fda3b08	0.856	0.841	0.871	2.53E-01	1.96E-01	3.12E-01	3.77E+03	SEQ-25 x SEQ-16: 1	SEQ-25; SEQ-16
00037a8c	0.695	0.674	0.718	2.56E-01	2.01E-01	3.17E-01	7.06E+03	SEQ-26 x SEQ-01: 1	SEQ-26; SEQ-01
2c41e521	0.757	0.737	0.776	2.66E-01	2.09E-01	3.27E-01	6.87E+03	SEQ-26 x SEQ-01: 1	SEQ-26; SEQ-01
7676a0e8	0.868	0.798	0.921	4.12E-02	1.55E-02	6.94E-02	3.93E+02	SEQ-26 x SEQ-10: 0.98975, SEQ-26 x SEQ-27: 0.00617	SEQ-26; SEQ-10
ec225732	0.903	0.885	0.920	1.33E-01	8.63E-02	1.79E-01	1.03E+03	SEQ-28 x SEQ-02: 1	SEQ-28; SEQ-02
00ca4766	0.594	0.568	0.619	1.30E-01	8.84E-02	1.72E-01	7.33E+03	SEQ-28 x SEQ-02: 1	SEQ-28; SEQ-02
9896aa10	0.565	0.565	1.000	4.64E-03	4.24E-13	1.40E-02	1.66E+02	SEQ-33 x SEQ-22: 0.86143, SEQ-22 x SEQ-33: 0.13857	SEQ-33; SEQ-22
e67f27e8	0.501	0.500	1.000	4.66E-03	7.47E-14	1.39E-02	2.81E+02	SEQ-35 x SEQ-12: 0.50062, SEQ-12 x SEQ-35: 0.49938	SEQ-35; SEQ-12
73a5efef	1.000	1.000	1.000	3.05E-10	1.69E-12	3.05E-10	1.64E-05		SEQ-01
7ad4d665	1.000	1.000	1.000	4.24E-13	4.24E-13	3.76E-10	1.30E-05		SEQ-23
3707bbeb	1.000	0.986	1.000	5.93E-12	5.03E-14	2.14E-02	1.32E-05		SEQ-30
c100258d	1.000	1.000	1.000	1.15E-11	3.84E-12	1.15E-11	9.48E-06		SEQ-07

d8de07f5	1.000	1.000	1.000	1.70E-11	1.70E-11	1.70E-11	1.51E-05		SEQ-26
1905b963	1.000	1.000	1.000	1.69E-12	1.69E-12	3.05E-10	5.45E-06		SEQ-06
cf851e72	1.000	0.972	1.000	1.70E-11	1.70E-11	2.21E-02	1.20E-05		SEQ-05
31d909b2	1.000	1.000	1.000	1.83E-11	8.04E-15	1.83E-11	1.32E-05		SEQ-08
8afd8162	1.000	1.000	1.000	1.77E-12	1.77E-12	1.77E-12	1.42E-05		SEQ-09
d76610dc	1.000	1.000	1.000	1.77E-12	1.77E-12	1.77E-12	1.15E-05		SEQ-10
71179493	1.000	1.000	1.000	5.93E-12	5.93E-12	5.93E-12	1.32E-05		SEQ-03
38f490e6	1.000	1.000	1.000	1.83E-11	8.04E-15	1.83E-11	1.08E-05		SEQ-12
8e713eee	1.000	1.000	1.000	1.83E-11	8.04E-15	1.83E-11	1.18E-05		SEQ-02
c0100210	1.000	1.000	1.000	1.83E-11	8.04E-15	1.83E-11	1.42E-05		SEQ-01
7ee0cf9c	1.000	1.000	1.000	1.00E-10	1.00E-10	1.00E-10	1.77E-05		SEQ-13
78fcca41	1.000	0.999	1.000	3.05E-10	1.69E-12	9.99E-01	1.56E-06		SEQ-14
6b3d821b	0.916	0.914	1.000	3.82E-03	1.83E-11	1.42E-02	1.47E+00		SEQ-15
9534ddf6	1.000	0.962	1.000	1.77E-12	1.77E-12	1.68E-02	1.25E-05		SEQ-06
43b4a960	1.000	1.000	1.000	1.83E-11	8.04E-15	1.83E-11	1.50E-05		SEQ-16
29ebd8ab	1.000	1.000	1.000	2.07E-11	2.07E-11	2.07E-11	1.28E-05		SEQ-02
837752ac	1.000	1.000	1.000	1.83E-11	8.04E-15	1.83E-11	1.56E-05		SEQ-17
1489785f	1.000	1.000	1.000	5.93E-12	5.93E-12	5.93E-12	1.11E-05		SEQ-18

baf4dd9e	1.000	1.000	1.000	1.00E-10	1.00E-10	1.00E-10	1.38E-05		SEQ-19
eee58a72	1.000	1.000	1.000	3.76E-10	4.24E-13	3.76E-10	1.85E-05		SEQ-20
2332aefc	1.000	1.000	1.000	1.77E-12	1.77E-12	1.77E-12	1.55E-05		SEQ-21
bc390223	1.000	1.000	1.000	1.00E-10	1.00E-10	1.00E-10	1.47E-05		SEQ-22
ff4bc014	1.000	1.000	1.000	5.93E-12	5.93E-12	5.93E-12	1.04E-05		SEQ-09
ef3c6da5	1.000	1.000	1.000	1.15E-11	1.15E-11	1.15E-11	1.29E-05		SEQ-02
bd9754c8	1.000	1.000	1.000	3.76E-10	4.24E-13	3.76E-10	1.21E-05		SEQ-24
67f92200	1.000	1.000	1.000	1.00E-10	1.00E-10	1.00E-10	1.62E-05		SEQ-25
c6aca106	1.000	0.960	1.000	1.77E-12	1.77E-12	1.42E-02	1.69E-05		SEQ-40
ef28568f	1.000	0.979	1.000	1.77E-12	1.77E-12	2.85E-02	1.04E-05		SEQ-27
9109611	1.000	1.000	1.000	1.15E-11	3.84E-12	1.15E-11	5.91E-06		SEQ-02
6455f9ee	1.000	1.000	1.000	2.04E-10	3.33E-12	2.04E-10	1.11E-05		SEQ-12
e5d915ad	0.954	0.950	1.000	6.00E-03	1.00E-10	2.56E-02	1.13E+00		SEQ-01
112bf4d7	1.000	1.000	1.000	1.83E-11	8.04E-15	1.83E-11	6.02E-06		SEQ-37
5939429a	1.000	0.965	1.000	1.83E-11	8.04E-15	1.33E-02	1.80E-05		SEQ-03
8ef2aebd	1.000	1.000	1.000	3.05E-10	1.69E-12	3.05E-10	1.27E-05		SEQ-28
3ea730e4	1.000	1.000	1.000	1.69E-12	1.69E-12	3.05E-10	1.79E-05		SEQ-29
1df8c0f8	1.000	1.000	1.000	1.00E-10	1.00E-10	1.00E-10	1.49E-05		SEQ-31

73eac925	1.000	1.000	1.000	3.76E-10	4.24E-13	3.76E-10	1.41E-05		SEQ-32
dee255d7	1.000	1.000	1.000	3.76E-10	4.24E-13	3.76E-10	1.30E-05		SEQ-02
5cdf1c55	1.000	0.983	1.000	1.83E-11	1.32E-16	1.33E-02	1.81E-05		SEQ-33
0baa611a	1.000	1.000	1.000	1.00E-10	1.00E-10	1.00E-10	1.61E-05		SEQ-34
c23689e5	1.000	1.000	1.000	5.93E-12	5.03E-14	5.93E-12	1.46E-05		SEQ-11
6de79e62	1.000	1.000	1.000	1.69E-12	1.69E-12	3.05E-10	1.64E-05		SEQ-35
6a272250	1.000	0.968	1.000	1.77E-12	1.77E-12	1.76E-02	1.55E-05		SEQ-36
5346988c	1.000	1.000	1.000	1.00E-10	1.00E-10	1.00E-10	1.85E-05		SEQ-10
df7a245a	1.000	1.000	1.000	4.24E-13	4.24E-13	3.76E-10	1.16E-05		SEQ-02
c71b535a	1.000	0.976	1.000	1.62E-15	1.62E-15	2.61E-02	8.68E-06		SEQ-38
7d57d8ff	1.000	1.000	1.000	1.70E-11	1.70E-11	1.70E-11	1.27E-05		SEQ-39
e15b3d34	1.000	1.000	1.000	5.16E-11	5.16E-11	5.16E-11	1.02E-05		SEQ-14

Table S6. Mixed infection estimator outputs. The deviance statistic determines whether an infection is called mixed. The default value of >19.4 from a previous validation was used.

References

1 Rudramurthy SM, Chakrabarti A, Paul RA, *et al.* Candida auris candidaemia in Indian ICUs: analysis of risk factors. *J Antimicrob Chemother* 2017; **72**: 1794–801.

2 Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017; **13**: e1005595.

3 Lunter G, Goodson M. Stampy: A statistical algorithm for sensitive and fast mapping of Illumina sequence reads. *Genome Res* 2011; **21**: 936–9.

4 Li H, Handsaker B, Wysoker A, *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009; **25**: 2078–9.

5 Sheppard AE, Stoesser N, Wilson DJ, *et al.* Nested Russian Doll-Like Genetic Mobility Drives Rapid Dissemination of the Carbapenem Resistance Gene blaKPC. *Antimicrob Agents Chemother* 2016; **60**: 3767–78.

6 De Silva D, Peters J, Cole K, *et al.* Whole-genome sequencing to determine transmission of *Neisseria gonorrhoeae*: an observational study. *Lancet Infect Dis* 2016; **16**: 1295–303.

7 Eyre DW, Cule ML, Wilson DJ, *et al.* Diverse sources of *C. difficile* infection identified on whole-genome sequencing. *N Engl J Med* 2013; **369**: 1195–205.

8 Lockhart SR, Etienne KA, Vallabhaneni S, *et al.* Simultaneous Emergence of Multidrug-Resistant *Candida auris* on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses. *Clin Infect Dis* 2017; **64**: 134–40.

9 Sharma C, Kumar N, Meis JF, Pandey R, Chowdhary A. Draft Genome Sequence of a Fluconazole-Resistant *Candida auris* Strain from a Candidemia Patient in India. *Genome Announc* 2015; **3**: e00722–15.

10 Nguyen L-T, Schmidt HA, Haeseler von A, Minh BQ. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol Biol Evol* 2014; **32**: 268–74.

11 Eyre DW, Cule ML, Griffiths D, *et al.* Detection of Mixed Infection from Bacterial Whole Genome Sequence Data Allows Assessment of Its Role in *Clostridium difficile* Transmission. *PLoS Comput Biol* 2013; **9**: e1003059.