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Genomic diversity of clinically relevant bacterial pathogens from an acute care hospital in Suva, Fiji

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Objectives: Antimicrobial resistance (AMR) is a global health threat, with third-generation cephalosporin–resistant (3GCR) and carbapenem-resistant infections of particular concern. There is currently a lack of genomic data on AMR organisms in the Pacific region.

Methods: We aimed to address this gap by examining the genetic diversity of a collection of 788 Gram-negative and Gram-positive clinical isolates collected between July 2020 and October 2022 from a single hospital in Suva, Fiji. We sampled sensitive and resistant isolates, focusing on 3GCR and carbapenem-resistant Gram-negatives, and methicillin-resistant *Staphylococcus* and vancomycin-resistant *Enterococcus*.

Results: We detected 29 distinct species across 12 different genera. Amongst Gram-negative genomes, *Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii* and *Pseudomonas aeruginosa* were the most common. Carbapenem resistance was mostly detected in *A. baumannii* ST2 and *P. aeruginosa* ST773, with both STs carrying NDM-1 and showing evidence of transmission within Fiji. Carbapenem resistance was relatively rare amongst the Enterobacterales; however, we observed evidence of transmission of OXA-232–carrying *K. pneumoniae* ST395 and NDM-7 *E. coli* ST410. For Gram-positive bacteria, *Staphylococcus aureus* ST1 was the dominant clone, and phylogenetic analysis revealed a single clade harbouring the majority of Fijian genomes, with close relationships to genomes from neighbouring Samoa. *Enterococcus* was relatively rare, with only 22 genomes detected.

Conclusions: This study provides crucial genomic data on AMR organisms in Fiji, highlighting the diversity of resistant species in the region. Local transmission of four carbapenem-resistant clones within Fiji was observed, underscoring the importance of local spread of these resistant strains.

Introduction

Antimicrobial resistance (AMR) is an increasingly serious issue. Frequently described as a 'hidden pandemic', bacterial infections with high levels of AMR lead to poor patient outcomes, and current estimates state that 1.27 million deaths per year can be directly attributed to AMR infections.[1] AMR determinants accumulate within bacterial genomes, creating MDR strains that lead to fewer treatment options. Bacteria of particular concern include Gram-negative bacteria that are third-generation cephalosporin–resistant (3GCR) or carbapenem-resistant, in addition to MRSA and vancomycin-resistant *Enterococcus faecium*.

Whereas prevalence of AMR is well studied in high-income regions of the world, until recently there have been limited data on AMR in the Pacific region, including Fiji.[2–5] A decade ago, the WHO reported that rates of 3GCR *Escherichia coli* were ~12%.[6] More recent studies have tried to address this data gap: one found that 40.7% of all Enterobacterales bloodstream infections in Fiji's main hospital were now 3GCR,[7] whereas another assessed almost 30 000 bacterial isolates across four calendar years and

demonstrated that rates of 3GCR among *Klebsiella pneumoniae* and *E. coli* had risen to 43% and 23%, respectively.[8] However, most of the data on AMR in Fiji are phenotypic, with limited genomic information available. Genomic data can provide important insights into AMR, through elucidating the specific mechanisms of interest and the bacterial clones that are driving spread.

Here, we present a collection of Gram-negative and Gram-positive bacterial genomes obtained from clinical bacterial cultures from a single hospital in Suva, Fiji, from a 15 month period during 2020–2022. We focused on genomes from bacteria with AMR phenotypes, but included a subset of susceptible genomes for comparison. By using genomics, we were able to accurately determine the species and clones circulating within Suva, and elucidate the diversity of AMR determinants leading to highly drug-resistant phenotypes, including 3GCR and carbapenem resistance. Finally, for common clones detected in Fiji, we placed Fijian genomes into a global context to better understand their local epidemiology.

Materials and methods

Ethics

Ethics approval was provided by the Alfred Hospital Ethics Committee (593/19) and the Fiji National Health Research Ethics Review Committee (88/2019). Both committees granted a waiver of patient consent.

Isolate sampling

A total of 1481 bacterial isolates were collected from clinical samples at Colonial War Memorial Hospital (CWMH) in Suva, Fiji, from July 2020 to May 2021, and then from May 2022 to October 2022 (a total of 15 months). The collection of bacterial isolates was paused in May 2021 due to a rapidly rising number of COVID-19 cases in Fiji and interruptions to laboratory workflow, with collection recommencing in May 2022. Only bacterial isolates belonging to the following taxonomic groups were targeted for inclusion: *Acinetobacter*, Enterobacterales (excluding *Salmonella* and *Shigella*), *Pseudomonas*, *Staphylococcus aureus* or *Enterococcus*. The full CWMH laboratory sampling strategy can be found in Table 1; however, in brief, target Gram-negative organisms that were from blood and/or carbapenem resistant were prioritized, with sampling of carbapenem-susceptible isolates from other sites. Gram-negative isolates were classified as 3GCR if there was evidence of either ESBL production or resistance to at least one third-generation or fourth-generation cephalosporin (ceftriaxone, ceftazidime, cefepime).[7] For *S. aureus*, blood isolates were prioritized, followed by sampling of methicillin-susceptible and -resistant organisms from other sites. Finally, each identified VRE isolate was matched with the next two vancomycin-susceptible isolates to provide comparisons. We collected only a single sample for a particular class of organism from each patient, per hospital admission, and all isolates were clinical isolates. When we restricted the number of samples collected per month, this was always the first *n* samples for that month until the limit was reached.

We selected 788/1481 isolates for WGS based on the following criteria: (i) all 3GCR Enterobacterales, plus five third-generation cephalosporin susceptible (3GCS) *Escherichia* and *Klebsiella* per month, with three 3GCS per month for other species; (ii) all carbapenem-resistant *Acinetobacter* and *Pseudomonas*, plus three carbapenem-susceptible isolates per month for each species; (iii) all MRSA; and (iv) all *Enterococcus*. All isolates and their metadata, including source of infection, can be found in Table S1 (available as Supplementary data at *JAC-AMR* Online).

Antimicrobial susceptibility testing

As described in Loftus *et al.*,[7] antimicrobial susceptibility testing (AST) on isolates was performed at CWMH using VITEK-2 GN AST cards (bioMérieux, Marcy-Étoile, France; version 9.01). Disc diffusion with cefotaxime and ceftazidime discs with clavulanate (CLSI M100-ED31) was used to assess for ESBL production when VITEK-2 was unavailable. Confirmatory AST testing on the initial shipment of isolates was performed in Melbourne, Australia using VITEK-2 GN AST cards (bioMérieux, version 8.01), revealing only a single discrepancy. Therefore, no confirmatory AST testing was performed on subsequent shipments.[7] Colistin susceptibility testing was carried out on a subset of *E. coli* isolates (24) using standard broth microdilution in cation-adjusted Mueller–Hinton broth (BD) with colistin sulphate salt (Sigma-Aldrich). EUCAST clinical breakpoints v13.1 were used.

Table 1. Laboratory sampling strategy for selecting isolates at Colonial War Memorial Hospital

Organism	Limit the number collected/mo?
Organisms to be collected regardless of site (i.e. any of blood, urine, pus/wound, etc.)	
Carbapenem-resistant isolate (in blood), e.g. *Acinetobacter, Pseudomonas, Klebsiella* Carbapenem-resistant Enterobacterales (from any site), e.g. *E. coli, Klebsiella*	No—collect all
Carbapenem-resistant *Acinetobacter* (non-sterile sites, e.g. urine)	Yes—maximum 10/mo
Carbapenem-resistant *Pseudomona* (non-sterile sites, e.g. urine)	Yes—maximum 10/mo
Acinetobacter—carbapenem susceptible	Yes—maximum 10/mo
Pseudomonas—carbapenem susceptible	Yes—maximum 10/mo
Vancomycin-resistant enterococcus (VRE)	No—collect all
Vancomycin-susceptible enterococcus (VSE)	Yes—collect two VSEs for each VRE
***S. aureus*—collecting isolates mostly from blood cultures plus a few non-blood samples**	
MRSA in blood	No—collect all
MSSA in blood	No—collect all
MRSA in non-blood (e.g. pus/wound)	Yes—maximum 10/mo
MSSA in non-blood (e.g. pus/wound)	Yes—maximum 10/mo
Enterobacterales—collecting isolates from blood cultures only	
Enterobacterales—ESBL	No—collect all
Enterobacterales—non-ESBL	No—collect all

Genome sequencing

Bacterial isolates were cultured in heart infusion broth (Oxoid) for 16 h at 37°C, shaking at 200 rpm. Genomic DNA was extracted using the GenFind V3 Reagent Kit (Beckman Coulter) as per manufacturer's instructions. Illumina libraries were prepared using the Illumina DNA Library Prep Kit (M) Tagmentation, and sequenced on the Illumina NovaSeq 6000 to produce paired-end reads. Nine isolates failed sequencing (depth <10×) and were excluded from further analysis.

Nine isolates were selected for additional long-read nanopore sequencing (Oxford Nanopore Technologies) to resolve the locations of carbapenemase genes. Long-read nanopore sequencing of native genomic DNA (using the same extraction as for Illumina sequencing) was performed on a GridION device, using FLO-MIN114 R10.4.1 flow cells. Multiplexed, barcoded sequencing libraries were with the native barcoding kit SQK-NBD114.96 in accordance with the protocol NBE_9171_v114_revO_15Sep2022. Reads were base-called with Guppy using the dna_r10.4.1_e8.2_400bps_sup@v4.3.0 model. All sequenced reads have been uploaded to BioProject PRJNA1182024; individual accessions can be found in Table S1.

Assigning species and STs and detecting AMR determinants

All Illumina genomes were assembled using Unicycler v0.5.0 with default parameters.[9] Draft genome assemblies were uploaded to Pathogenwatch (https://pathogen.watch) to identify species and determine STs for species with available MLST schemes. AMR determinants (including SNP-based determinants, for supported organisms) were detected using AMRFinderPlus v3.11.4 with database v2023-02-23.1[10] for Gram-negative genomes and *E. faecium*. Pathogenwatch was used to detect AMR determinants, including SNP-based ones, in *S. aureus* (accessed 7 July 2023). Full details of AMR calls can be found in Table S1. AMR determinants were linked to drug classes using either AMRFinderPlus class designations, or Pathogenwatch class designations, respectively. All AMR data were visualized using ggplot2.[11]

For the genomes additionally sequenced with Oxford Nanopore Technologies, we generated completed genome sequences by combining both long and short reads and assembling with the Clinopore pipeline (https://github.com/HughCottingham/clinopore-nf). Clinopore filtered the long-reads using Filtlong (https://github.com/rrwick/Filtlong) to keep the best 90% of long-reads, and then assembled the filtered long-reads with Flye v2.9.2.[12] Resulting long-read assemblies were short-read polished using Polypolish v0.5.0[13] and Polca[14] with default parameters. The completed genome sequences were annotated with Bakta v1.8.1[15] using the full v5 Bakta database. Accessions for completed genome assemblies can be found in Table S1.

Determining genetic relatedness within common STs

Phylogenetic trees were constructed for the most common STs using either RedDog (https://github.com/katholt/reddog-nf) to detect SNPs, or the core-gene tree feature of Pathogenwatch. For *A. baumannii, P. aeruginosa* and *E. coli*, we used RedDog and selected representative sets of publicly available genomes from diverse geographical sources (Table S2). For *A. baumannii* and *P. aeruginosa*, genomes were randomly selected from the full sets of genomes available on Pathogenwatch for those STs; for *E. coli*, genomes were selected from those available in Enterobase[16] or from Decano and Downing.[17] In all cases, we included all genomes available from India and the Oceanic region, to explore potential avenues of transmission, as residents of Fiji occasionally travel to these regions for medical treatment. For other regions, we selected one genome per unique year/country combination to minimize sampling bias due to outbreaks or sequencing of common drug-resistant lineages, and to reduce computational load for analysis. Reference genomes were as follows: FIJ0488 (ST410 *E. coli*), GCF_900448475 (ST131 *E. coli*), CP023142 (ST69 *E. coli*), CP026943 (ST2 *A. baumannii*) and CP075176 (ST773 *P. aeruginosa*). SNP alignments were cleaned of recombination with Gubbins v2.3.2,[18] and phylogenetic trees were constructed with IQTree v2.0[19] using the TEST model parameter to select the best substitution model for each alignment. We calculated pairwise SNP distances from the alignments for these clones, to determine if recent transmission had taken place, using pairsnp (https://github.com/gtonkinhill/pairsnp). For *K. pneumoniae* and *S. aureus*, we compared with all genomes from each ST within Pathogenwatch. We calculated pairwise SNP distances between our Fijian genomes using SKA2[20] for these two STs, using assemblies. Trees were visualized in R using the ggtree package.[21]

Results

Diversity of clinical isolates

From 1481 isolates in the collection, we sequenced 788 (53.2%), of which 780 passed quality control (98.9% of sequenced isolates). Of these, 78.8% were Gram-negative organisms ($n=615$), belonging to either *Acinetobacter, Pseudomonas* or Enterobacterales, and 21.2% ($n=165$) were Gram-positive, belonging to either *Staphylococcus* or *Enterococcus*. Genomes were obtained primarily from blood (58%, $n=449$), followed by wound swabs (22%, $n=170$) (Table S1). We found 29 distinct species across 12 genera in the sequenced genomes (Table 2). Within Enterobacterales, *K. pneumoniae* (35.6%, 128/360) and *E. coli* (44.2%, 159/360) were the most common, with the remaining Enterobacterales species represented by 22 or fewer genomes each (Table 2). *A. baumannii* and *P. aeruginosa* represented 41% of Gram-negative genomes ($n=255/615$). Of the 165 Gram-positive genomes, the vast majority belonged to *S. aureus* (79.4%, $n=131/165$). *E. faecium* was relatively rare, represented by only 22 genomes (13%).

For species with an MLST scheme, we determined the ST for each genome (Tables 2 and S1 for all STs). The *K. pneumoniae* and *E. coli* genomes were diverse, with 58% and 60% of STs represented by a single genome. For *K. pneumoniae*, no ST carried >11 genomes. In contrast, *A. baumannii* were less diverse, with the majority of genomes belonging to a single ST (ST2) (68%, $n=99/145$). The next most common *A. baumannii* ST, ST499, had only 10 genomes (7% of the total *A. baumannii* collection).

Genetic determinants of AMR

Gram-negative genomes were classified as 3GCR (Enterobacterales only) or carbapenem resistant (Enterobacterales, *Acinetobacter* and *Pseudomonas*) using phenotypic tests (see Methods). Of these, 176 (31%) Enterobacterales were classified as 3GCR only (Table S1). A total of 52 Enterobacterales genomes (13.5%) were classified as both 3GCR and carbapenem resistant (Table S1). We found a significantly higher number of AMR genes in Gram-negative genomes that were 3GCR or carbapenem resistant compared with susceptible genomes (mean 10–17 versus 1–6 AMR genes, depending on genus; $P=0.03$ to $P=4.2\times10^{-23}$, Wilcoxon test), with the exception of *Serratia* (range 1–15 for both resistant and susceptible, Figure 1a). We detected 21 distinct acquired ESBL genes, most commonly $bla_{\text{CTX-M-15}}$ (67% of genomes with an ESBL gene, $n=165/245$) (Figure 1b). $bla_{\text{CTX-M-15}}$ was found only in Enterobacterales. In contrast, $bla_{\text{PER-7}}$ was found exclusively in *A. baumannii* ST2 genomes, and $bla_{\text{CTX-M-27}}$ only in ST131 or ST38 *E. coli* (Figure 1b).

Table 2. Number of genomes and STs per species

Genus	Species	No. of genomes	No. of STs[a]
Enterobacterales			
Citrobacter	*C. koseri*	9	—
	C. freundii	4	3
	C. werkmanii	1	—
Enterobacter	*E. hormaechei*	5	5
	E. cloacae	2	2
	E. chengduensis	1	—
	E. kobei	1	1
Escherichia	*E. coli*	159	33
Klebsiella	*K. pneumoniae*	128	58
	K. quasipneumoniae	6	6
	K. variicola	5	5
	K. michiganensis	2	2
Morganella	*M. morganii*	1	—
Proteus	*P. mirabilis*	22	—
Providencia	*P. rettgeri*	2	—
Serratia	*S. marcescens*	12	—
Acinetobacter/Pseudomonas			
Acinetobacter	*A. baumannii*	145	20
	A. nosocomialis	15	—
	Other spp.	6	—
Pseudomonas	*P. aeruginosa*	87	29
	P. mosselii	1	—
	Other spp.	1	—
Gram-positive			
Enterococcus	*E. faecium*	22	4
	E. faecalis	11	7
Staphylococcus	*S. aureus*	131	20
	S. haemolyticus	1	1

[a]Dash (—) indicates that no MLST scheme is available for that species, therefore STs could not be determined.

The overwhelming majority of gene determinants encoding carbapenemases were found in either *Acinetobacter* or *Pseudomonas*. In *Acinetobacter*, the most frequent carbapenemase genes were bla_{OXA-23} and bla_{NDM-1} (85.5% and 52% of *Acinetobacter* genomes, respectively; Figure 1c), primarily driven by carriage in *A. baumannii* ST2. *A. baumannii* ST25, ST499 and ST149 also carried bla_{OXA-23}. *P. aeruginosa* frequently carried bla_{NDM-1} (34% of genomes, $n=30/87$; Figure 1c), almost exclusively in ST773 (with the exception of one ST235 genome). Both *A. baumannii* ST2 and *P. aeruginosa* ST773 were present across the full sampling period, consistent with recent studies of carbapenem-resistant organisms in Fiji.[4,5,22] *Acinetobacter* and *Pseudomonas* genomes that did not harbour carbapenemases or ESBL genes belonged to completely different STs, with no particular ST dominating this group.

Genes encoding carbapenemases were identified in the Enterobacterales (carbapenemase-producing Enterobacterales) but were less common (20 genomes, 5.5%) and, notably, most were identified in the more recent isolates from 2022. This was primarily due to variants of bla_{NDM} (NDM-1, -5 and -7; see Figure 1c and Table S1). We confirmed the locations of these carbapenemase genes using long-read sequencing (isolates with completed genomes are listed in Table S1). Two *E. coli* ST167 genomes carried three copies of bla_{NDM-5} each, on three separate plasmids—a 126 kbp (kilobase pairs) IncFIA/IncFIC plasmid, a 71 kbp IncN plasmid and a small 11 kbp plasmid. bla_{NDM-1} was found in one *K. pneumoniae* ST231 genome on the chromosome, and one *Klebsiella michiganensis* ST195 on an IncFII plasmid. bla_{NDM-7} was found in seven *E. coli* ST410 genomes on a 44 kbp IncX3 plasmid—this same plasmid was also found in a single *Klebsiella quasipneumoniae* genome, indicating potential plasmid sharing. Finally, a group of six ST395 *K. pneumoniae* genomes all carried $bla_{OXA-232}$ on a small, 6 kbp plasmid that harboured no other AMR genes. Similar to *Acinetobacter* and *Pseudomonas*, Enterobacterales genomes that did not carry carbapenemases or ESBL genes were distinct STs, with almost no overlap with STs that were highly drug resistant.

Amongst our selected Gram-positive bacteria, all 131 *S. aureus* genomes carried *blaZ* (conferring penicillin resistance) and 95% ($n=124$) carried *mecA* (conferring methicillin resistance). Seven *S. aureus* genomes were phenotypically tested as methicillin resistant, but carried no *mecA* gene. Overall, the *S. aureus* genomes harboured a median of 2 acquired AMR determinants (range 1–11). Aside from *blaZ* and *mecA*, other common resistance determinants were *aacA-aphD* (14%, $n=18$ genomes) conferring aminoglycoside resistance, followed by *msrA* and *ermC* (11%, $n=11$ genomes carrying *msrA*, $n=3$ carrying *msrA* with *ermC*) conferring erythromycin and clindamycin resistance (Figure 1d). The majority of *S. aureus* genomes ($n=108$, 82%) were considered non-MDR MRSA (resistant to less than three antibiotic classes, excluding methicillin). Amongst the 22 *E. faecium* genomes, the median number of AMR determinants was 6 (range 4–16), with all genomes carrying at least 1 resistance determinant to one or more of aminoglycosides, macrolides and tetracyclines (Figure 1e). Seven *E. faecium* genomes (32%) carried the vancomycin resistance operon, *vanA*, all of which belonged to ST80 (Figure 1e).

Exploration of common STs harbouring problematic AMR profiles

Five STs had >20 genomes within the collection: *A. baumannii* ST2 (68%, $n=99/145$), *P. aeruginosa* ST773 (38%, $n=33/87$), *S. aureus* ST1 (43%, $n=56/131$), *E. coli* ST131 and *E. coli* ST69 (33% and 14%, $n=52$ and $n=22$ of 159 genomes). There were two additional STs, *K. pneumoniae* ST395 and *E. coli* ST410, which despite having fewer than 20 genomes, were the only Enterobacterales clones with >2 genomes that harboured carbapenemase genes ($n=6/93$ and $n=7/159$, respectively). For all seven STs, we explored the context of the genomes from Fiji against a set of globally representative genomes.

Comparison of our 56 *S. aureus* ST1 genomes with 1804 publicly available ST1 genomes revealed that the majority of genomes ($n=54$) fell into a single clade, clustering with genomes isolated from Samoa in 2018 (Figure 2).[23] Two genomes from Fiji fell outside this clade. One, FIJ0374, clustered with genomes from Asia, and the other, FIJ0644, clustered with genomes from Europe (Figure 2). Genomes within the Fijian clade were not

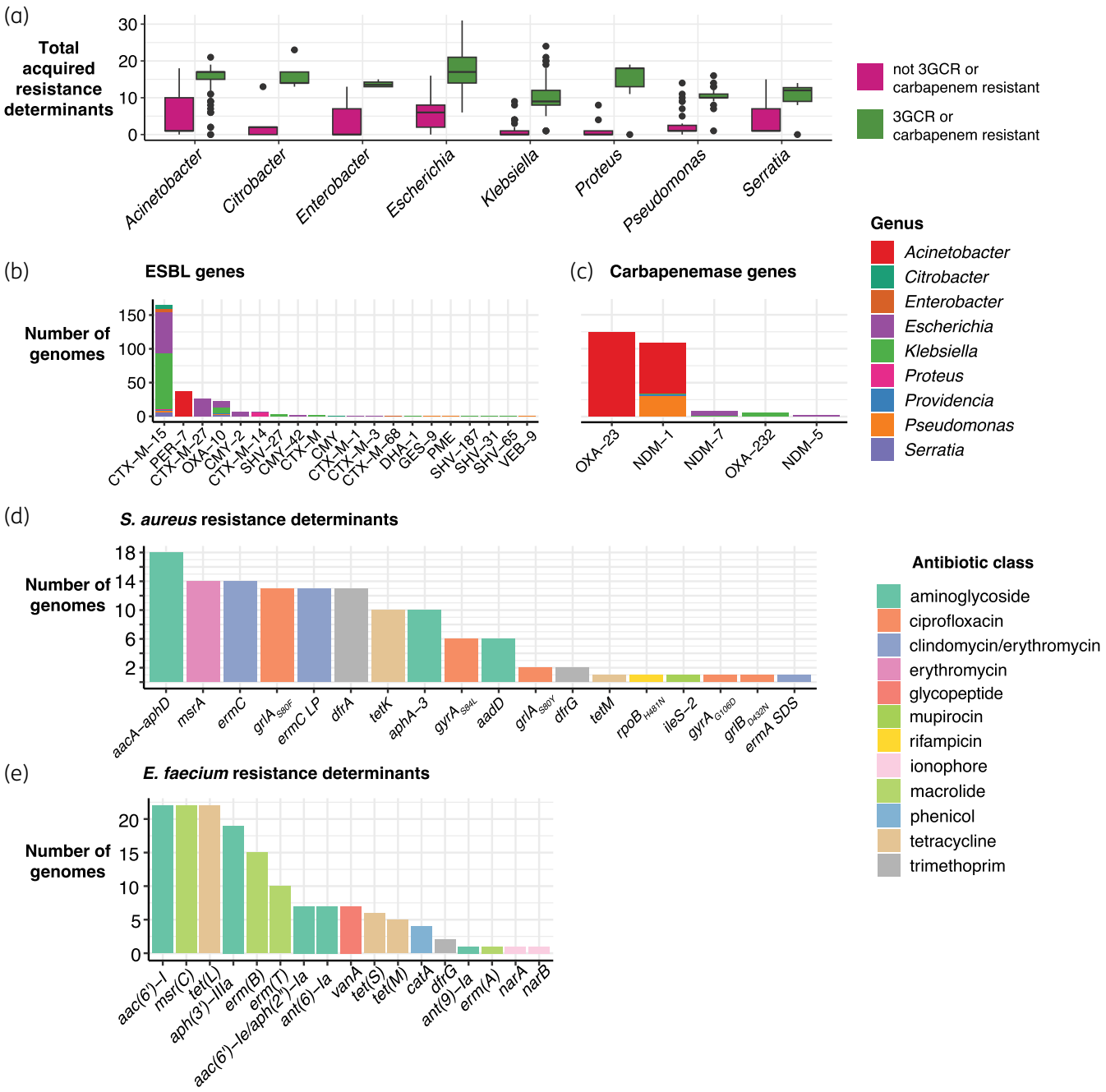

Figure 1. Number and diversity of AMR determinants across the collection. (a) Boxplots showing the distribution of the number of acquired AMR determinants by genus amongst Gram-negative bacterial genomes. Genomes were divided by their AMR phenotype as per legend. Black bars indicate median number of determinants, with boxes covering the 1st–3rd quartiles. Vertical lines cover from either the 1st or 3rd quartile to 1.5x the interquartile range. Black dots indicate outlier values (b, c) Bar plots showing total number of Gram-negative bacterial genomes carrying ESBLs (b) or carbapenemase (c) genes, with bars coloured by genus as per legend. (d, e) Bar plots showing total number of genomes carrying specific AMR determinants in *S. aureus* (d) and *E. faecium* (e), with bars coloured according to predicted resistance to a given antibiotic class, as per legend.

considered close enough to be considered recent transmission, with a median of 79 SNPs between pairs (range 14–183), similar to what was observed in Samoa.[23] All genomes from Fiji in the main clade carried *mecA* and *blaZ*, but were otherwise predicted sensitive to other antibiotics (Figure 2). FIJ0644 and FIJ0374 had expanded resistance profiles, predicted to be resistant to aminoglycosides (*aacA-aphD*), erythromycin (*msrA*) and trimethoprim (*dfrA*) (Figure 2).

Amongst the Enterobacterales, the two carbapenemase-positive clones (*K. pneumoniae* ST395 and *E. coli* ST410) both showed evidence of recent transmission within Fiji. The six *K. pneumoniae* ST395 genomes, which carried $bla_{OXA-232}$, were

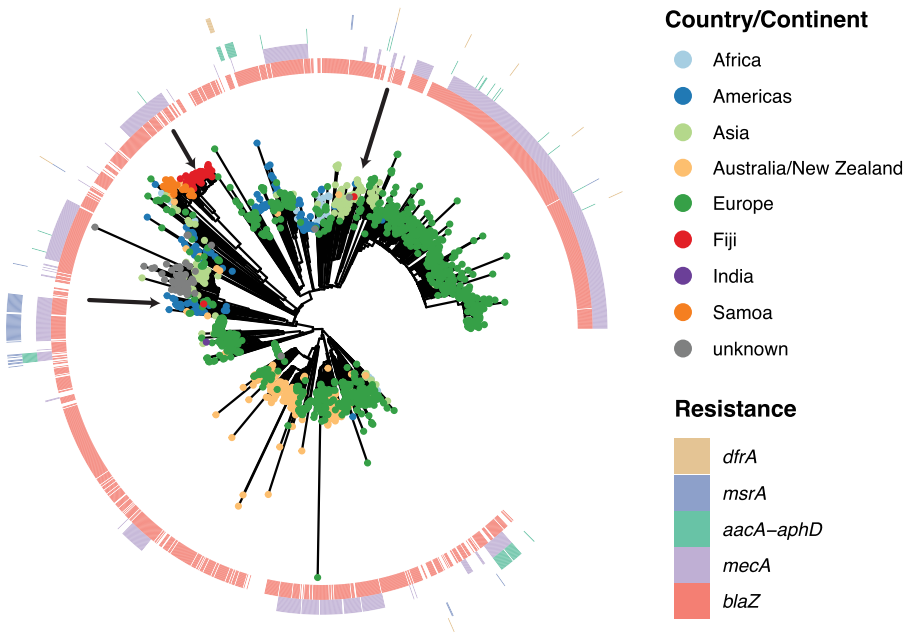

Figure 2. Global context of *S. aureus* ST1 genomes from Fiji. Core gene phylogeny generated by Pathogenwatch for 1804 ST1 genomes. Tips are coloured by country or continent as per legend. Black arrows indicate clades containing genomes from Fiji. Each ring around the phylogeny indicates the presence (coloured box) or absence (white) of a particular AMR determinant, coloured as per legend.

located in a sub-clade of the phylogeny that primarily consisted of genomes isolated from the USA and India (Figure 3a). The majority of genomes (82%) in this clade harboured $bla_{\text{OXA-232}}$ regardless of geographical origin, in contrast to the remainder of the genomes from Europe, which were $bla_{\text{OXA-232}}$ negative (Figure 3a). Pairwise SNP analysis of the *K. pneumoniae* ST395 genomes from Fiji revealed that these genomes were a median of 7 SNPs apart (range 2–11), indicative of a single import of this clone followed by local transmission. We observed ST395 only from May 2022 to August 2022 (1–2 isolates per month).

The seven *E. coli* ST410 genomes from Fiji were located in a single clade (Figure 3b), and were a median of 11 SNPs apart (range 2–19), suggesting recent transmission, as recently observed.[5,22] The first two ST410 genomes were isolated in April and May of 2021, with the remaining five isolated from May to July 2022. The closest genomes to this clade were from France (isolated in 2013, range 38–50 SNPs from the Fiji cluster) and from Australia (isolated in 2016, range 52–61 SNPs from the Fiji cluster) (Figure 3b).

The remaining two Enterobacterales clones, *E. coli* ST131 (n = 52 genomes from Fiji) and ST69 (n = 22 genomes from Fiji) did not carry carbapenemase genes. In both clones, the genomes from Fiji were spread across the full diversity of these STs (Figure S1), and were isolated throughout the full 15 month sampling period. Within ST131, there were 14 independent clades containing genomes from Fiji, with 5 of these clades showing evidence of recent transmission, including 1 carrying $bla_{\text{CTX-M-27}}$ (≤25 SNPs between pairs, marked with black bars in Figure S1). There were 10 independent clades in ST69, and 5 of these also showed evidence of recent transmission (Figure S1). As expected, ST131 was significantly more drug resistant than ST69, with ST131 genomes harbouring a median of 17 AMR genes (range 6–31) per genome (conferring resistance to a median of 10 drug classes, range 4–13) compared with a median of 9 AMR genes (range 2–13) per genome for ST69 (conferring resistance to a median of 7 drug classes, range 2–10). Only one ST69 genome from Fiji carried an ESBL ($bla_{\text{CTX-M-15}}$), and none of the ST69 genomes carried ciprofloxacin resistance determinants. In contrast, all but four ST131 genomes from Fiji carried a variant of the ESBL gene $bla_{\text{CTX-M}}$, either $bla_{\text{CTX-M-14}}$ (n = 1), $bla_{\text{CTX-M-15}}$ (n = 27) or $bla_{\text{CTX-M-27}}$ (n = 20) (Figure S1), as is typical for this clone.[17] All ST131 genomes from Fiji were predicted to be resistant to ciprofloxacin, due to either double or triple mutations in the quinolone resistance-determining regions of *parC* or *gyrA*, in addition to harbouring *qnr* or *aac(6')-Ib-cr5* (Table S1). Additionally, all ST131 genomes from Fiji had an E123D mutation in *pmrB*, which has been previously linked to colistin resistance.[24] However, additional colistin susceptibility testing of these isolates demonstrated that all were colistin susceptible.

Outside of Enterobacterales, two additional clones were found to be harbouring carbapenemase genes, *A. baumannii* ST2 and *P. aeruginosa* ST773, both of which were present throughout the sampling period, and both of which have been previously detected in Fiji, including as early as 2019 in the case of *A. baumannii* ST2.[4,5,22] We compared all 99 *A. baumannii* ST2 genomes from Fiji with a globally representative set of ST2 genomes, as well as 32 genomes from Fiji's close neighbour, Samoa.[5] We found that genomes from Fiji formed three tight clades amongst the global

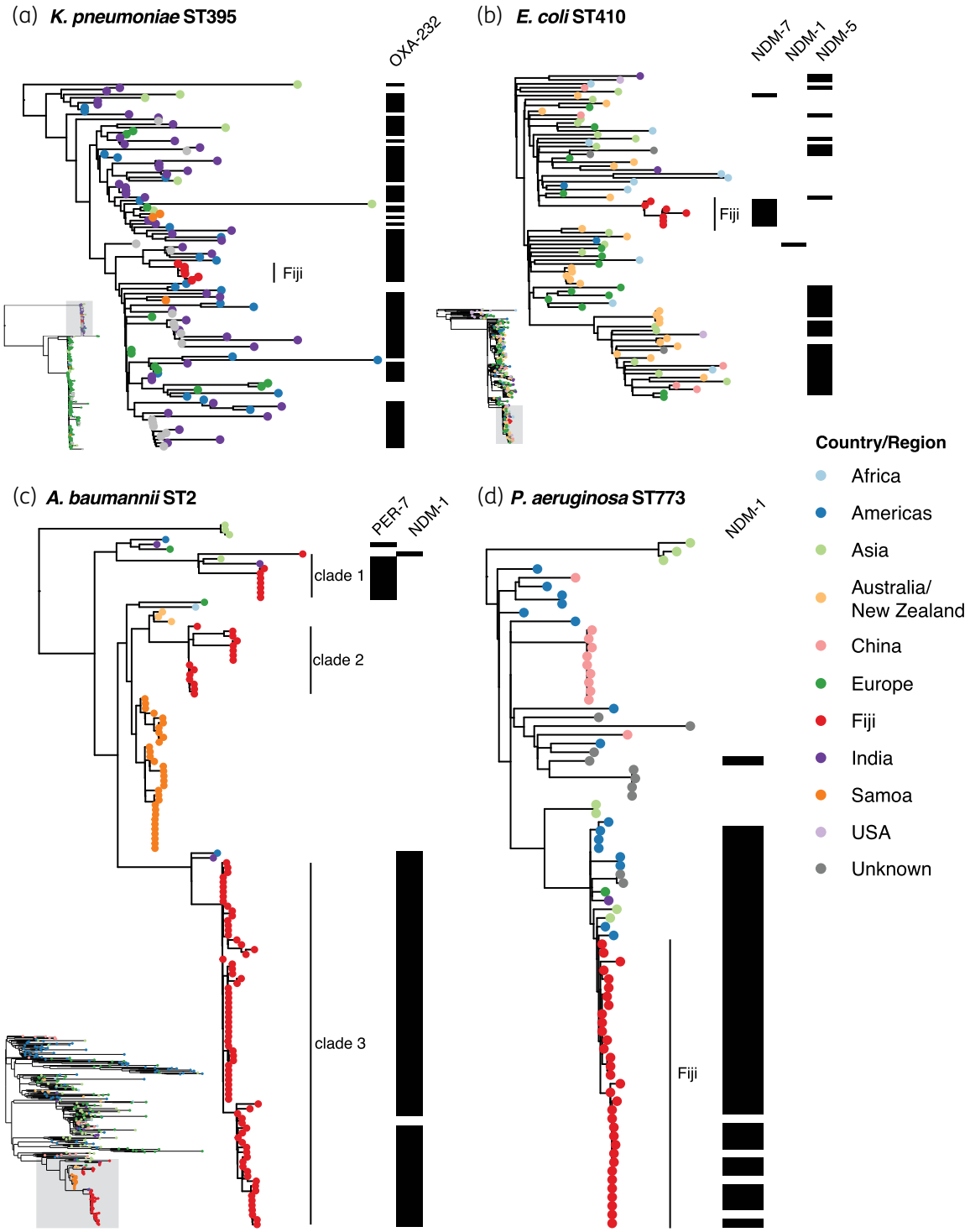

Figure 3. Global context of genomes from Fiji belonging to *K. pneumoniae* ST395, *E. coli* ST410, *A. baumannii* ST2 and *P. aeruginosa* ST773. All trees are maximum-likelihood phylogenies, midpoint rooted. Inset trees, where shown, are the full phylogenies of that ST, with a grey box indicating the location of the genomes from Fiji. Larger trees show the location of the genomes from Fiji in more detail. Tips are coloured by country or region, as per legend. Heatmaps next to the tree indicate the presence (black) or absence (white) of the relevant carbapenemase or ESBL genes for that ST. (a) *K. pneumoniae* ST395 ($n=496$ genomes, core-gene tree from Pathogenwatch). (b) *E. coli* ST410 ($n=335$ genomes, using reference genome FIJ0488 from this study). (c) *A. baumannii* ST2 ($n=281$ genomes, using public reference genome CP026943). (d) *P. aeruginosa* ST773 ($n=51$ genomes, using public reference genome CP075176).

diversity of the tree (inset, Figure 3c), with the closest genomes to each of these clades being Thailand and India (clade 2), Australia (clade 1) and the USA and India (clade 3) (Figure 3c). Within each clade, genomes from Fiji were highly related, with a median of 1 SNP between pairs (range 0–20), indicating local cross-transmission. ST2 genomes harboured a median of 19 AMR determinants (range 9–21) to 7 different antibiotic classes (range 5–9). Completed genomes of one representative isolate per clade (FIJ0290, FIJ0381 and FIJ0367) confirmed that all three clades carried the carbapenemase $bla_{\text{OXA-23}}$ in the Tn*2006* transposon on the chromosome. All three clades also harboured the AbaR resistance island, though this varied structurally depending on clade.[4] Clade 2 had the ESBL gene $bla_{\text{PER-7}}$ inside the AbaR resistance island, as well as an additional 11 AMR genes, conferring resistance to aminoglycosides, sulphonamides, chloramphenicol and macrolides. However, clade 1 harboured only three AMR genes in the AbaR island, whereas clade 3 carried a second copy of Tn*2006* with $bla_{\text{OXA-23}}$ within the AbaR island. Only clade 3 genomes carried the carbapenemase $bla_{\text{NDM-1}}$ (Figure 3c), which was found outside the AbaR island with ISAba125.

For *P. aeruginosa* ST773, all 33 genomes from Fiji were compared with a globally representative set of ST773 genomes (Figure 3d). All genomes from Fiji clustered together into a single clade, with a median of 1 SNP between pairs (range 0–18 SNPs), suggesting a single import into Fiji followed by local spread, as previously observed.[4,5,22] The closest non-Fiji genome to the clade with genomes from Fiji was SRR9988392 from the USA, isolated in 2017,[25] which was a median of 16 SNPs from the Fijian clade (range 11–17, Figure 3d). Notably, we detected $bla_{\text{NDM-1}}$ in all but 4 of the genomes from Fiji, as well as 13/14 global genomes in the same clade. The $bla_{\text{NDM-1}}$ gene was located on the chromosome, flanked by IS*91*, in a putative integrative conjugative element.

Discussion

Here, we present a genomic study of multiple bacterial pathogens in Suva, Fiji, which includes both drug-resistant and susceptible isolates. Overall, we found a high level of diversity amongst our target pathogens, with 29 different species detected. Each species was highly diverse, with the exceptions of *A. baumannii* (dominated by representatives from ST2) and *E. faecium* (two STs represented, ST80 and one novel ST). Carbapenem resistance was mostly driven by the presence of *A. baumannii* ST2 and *P. aeruginosa* ST773, both of which were likely actively transmitting throughout the sampling period. Baleivanualala *et al.*[5] undertook a similar study in Fiji with some overlap in sampling dates with our study (overlap period is July 2020 to May 2021). They included isolates from CWMH as well as other hospitals in Fiji and surrounding Pacific Island countries.[4,5] The findings of their studies are similar—the main circulating clones within carbapenem-resistant *A. baumannii* and *P. aeruginosa* were ST2 and ST773, respectively. In contrast, carbapenem resistance amongst the Enterobacterales was much less common, with only two clones likely transmitting—*K. pneumoniae* ST395 and *E. coli* ST410. The *E. coli* ST410 clone was also detected by Baleivanualala *et al.*,[5] and was present across all three major hospitals in Fiji. Amongst 3GCR Enterobacterales, we also observed some transmission of the globally circulating *E. coli* clones, ST131 and ST69, within Fiji.

Collection of isolates was interrupted during the COVID pandemic, and so we had two distinct sampling periods: one from July 2020 to May 2021, and a second from May 2022 to October 2022. We observed no major differences between these two periods—both *A. baumannii* ST2 and *P. aeruginosa* ST773 were present throughout both. However, the majority of genomes from the carbapenem-resistant Enterobacterales clones, *E. coli* ST410 and *K. pneumoniae* ST395, were found towards the end of the second sampling period, from May to August 2022.

Comparison of our genomes with publicly available genomes from the same STs indicated that these highly drug-resistant clones were likely imported into Fiji from elsewhere. However, it is extremely difficult to pinpoint the exact geographical origin of these importations due to inherent bias in the public databases. The metadata associated with most publicly available genomes only provide the location of sampling, and do not include recent travel history, and therefore the geographical origin of an isolate may not be entirely correct. For example, we observed that the closest genome to the *P. aeruginosa* ST773 Fijian clade was isolated in the USA. However, inspection of the publication describing this genome revealed that this isolate was taken from a patient who had recently undergone surgery in India.[25]

The reported incidence rates of *S. aureus* bacteraemia in the Pacific Islands are consistently some of the highest in the world, with up to 69 cases per 100 000 population per year.[7,26] Such elevated rates of this serious and life-threatening infection are likely driven by high levels of both diabetes and concomitant skin diseases.[27,28] The reported proportion of *S. aureus* infections caused by MRSA has shown significant variation across Pacific Island countries—from less than 5% to almost 50%—without a clear understanding of what has been driving this difference between similar countries with comparable climates.[29–31] Historically there have been very few data on MRSA STs in the Pacific Islands to help explain this variation, let alone WGS data—something our research has sought to address. We note that our Fiji *S. aureus* ST1 isolates clustered closely with isolates from Samoa, even though these countries have around a 5-fold difference in MRSA rates from hospital-based studies (~8% in Fiji and 40% in Samoa);[29,30] however, in the community-based Samoan study contributing WGS data, the rate of *mecA* gene carriage was just 6.8%.[23]

This study provides important contextual information about rising rates of resistance that have previously been observed in Fiji.[7] We demonstrate here that the majority of the resistance to carbapenems is primarily driven through the presence of two clones from *Acinetobacter* and *Pseudomonas*. These findings are in agreement with other recent studies from Fiji that have also detected the presence of these clones, including in other Fijian hospitals.[4,5,22] However, we additionally determined through comparison with publicly available global genomes that these clones have likely been introduced into Fiji from overseas. We also observed close relatedness between ST1 *S. aureus* genomes from both Fiji and Samoa, shedding further light on this pathogen of great importance to the region. Continued monitoring of these organisms in Fiji will be key for implementing appropriate controls to reduce their impact on patient health, as well as identification of future problematic clones that may arise in the future.

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Transparency declarations

The authors of this article have no conflicts of interest to declare.

Supplementary data

Figure S1 and Tables S1 and S2 are available as Supplementary data at *JAC-AMR* Online.

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