Molecular and clinical epidemiology of carbapenem resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacterales in Fiji: a multicentre prospective observational study

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Summary

Background Carbapenem resistant organisms (CROs) such as *Acinetobacter baumannii* (CR*Ab*), *Pseudomonas aeruginosa* (CR*Pa*), *Escherichia coli* (CR*Ec*), and *Klebsiella pneumoniae* (CR*Kp*) have been identified by the World Health Organization (WHO) as global priority pathogens. The dissemination of these pathogens and clonal outbreaks within healthcare facilities are of serious concern, particularly in regions with limited resources. In Fiji, where healthcare services are primarily provided by public hospitals, understanding the extent and nature of this problem is essential for the development of effective patient management, prevention interventions and control strategies.

Methods CROs isolated from 211 (77.3%) non-sterile (urinary catheters, urine, sputum, wound swab, and endotracheal tube) and 62 (22.7%) normally sterile (blood, cerebrospinal fluid, intravascular catheter, and aspirates) body sites of 272 patients treated at the three major hospitals in Fiji, the Colonial War Memorial Hospital (CWMH), Lautoka Hospital (LTKH), and Labasa Hospital (LBSH), and outer peripheral health centres around Fiji, were analysed. Clinical and demographic patient data such as age, sex, admission diagnosis, admission and discharge dates, patient outcomes, date of death, start and end date of meropenem and colistin treatment were reviewed. These CRO isolates comprised *A. baumannii*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*, that were prospectively collected at the microbiology laboratory of CWMH and LBSH from January 2020 through August 2021 and at the LTKH from January 2020 to December 2021. In addition, 10 retrospectively stored CR*Pa* isolates collected from patients at the CWMH from January through December 2019, were also included in the study. All isolates were characterised using mass spectrometry, antimicrobial susceptibility testing, and whole genome sequencing. Phylogenetic relationships among the CROs were assessed through core genome single nucleotide polymorphism (SNP) analysis. The CR*Ab* isolates were also compared to the CR*Ab* isolates from CWMH isolated in 2016/2017 and 2019, along with CR*Ab* isolates obtained from Fijian patients admitted to New Zealand hospitals in 2020 and 2021 from our retrospective study.

Findings Of 272 patients, 140 (51.5%) were male, the median (range) age of patients was 45 (<1–89) years, 161 (59.2%) were I-Taukei, 104 (38.2%) Fijians of Indian descent, and 7 (2.6%) were from other ethnic backgrounds. 234 (86.0%) of these 272 patients, had their first positive CRO sample collected ≥72 h following admission and the remaining 38 (14.0%) were isolated within 72 h following admission. Of the 273 CROs, 146 (53.5%) were collected at the CWMH, 66 (24.2%) LTKH, and 61 (22.3%) LBSH, while 62 (22.7%) were isolated from normally sterile sites and 211 (77.3%) from sites that are not sterile. Of 273 isolates, 131 (48.0%) were CR*Ab*, 90 (33.0%) CR*Pa*, 46 (16.8%) CR*Ec*, and 6 (2.2%) CR*Kp*. Of 131 CR*Ab*, 108 (82.4%) were ST2, with three distinct clones, all encoding $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$,

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while clone 3 also encoded bla_{NDM-1}; bla_{OXA-23} was associated with two copies of IS*Aba1* insertion element, forming the composite transposon Tn*2006*. The first two CR*Ab* ST2 clones were genetically linked to those isolated at CMWH 2016 through 2019, while the third was genetically linked to isolates from Fijian patients admitted to New Zealand hospitals in 2020 and 2021. Of CR*Pa*, 65 (72.2%) were ST773 and carried β-lactamase genes bla_{NDM-1}, bla_{OXA-50}, and $bla_{OXA-395}$. Of 10 retrospective CR*Pa* isolates, all belonged to CR*Pa* ST773 and carried bla_{NDM-1}, bla_{OXA-50}, and $bla_{OXA-395}$. Of 46 CR*Ec*, 44 (95.7%) were ST410 and encoded bla_{NDM-7} on an IncX3 plasmid. Of 6 CR*Kp*, 4 (66.7%) were ST16 and carried bla_{NDM-5} on an IncX3 plasmid. Other sequence types of CR*Pa* (ST9, ST357, ST654, ST664), CR*Ab* (ST25, ST374, ST499), CR*Ec* (ST167), and CR*Kp* (ST45, ST336) were also detected. Of those receiving meropenem treatment in the prospective study, 30 (57.7%) received it inappropriately. Of 272 patients, 65 (23.9%) died within the 30 days after first positive CRO isolation.

Interpretation We identified nosocomial transmission of distinct clones of CR*Ab* ST2, CR*Pa* ST773, CR*Ec* ST410, and CR*Kp* ST16 within and between the three major hospitals in Fiji. Moreover, community onset infections associated with CR*Pa*, CR*Ec*, and CR*Ab* were also detected. Of note, cross-border transmission of CR*Ab* ST2 clone 3 strain between Fiji and New Zealand was also detected. These clones encoded an array of carbapenem resistance genes associated with mobile genetic elements, including plasmids, transposons, and integrative and conjugative elements, signifying their potential for increased mobility, further acquisition of resistance genes, and spread. Inappropriate use of meropenem was common. Of note, the majority of patients who died had acquired CRO during their hospital stay. These findings highlight the need for stringent IPC strategies focusing on catheter and ventilator management, meticulous wound care, rigorous sepsis control, consistent hand hygiene, effective use of disinfectants, and thorough sanitisation of both hospital environments and medical equipment in the three major hospitals in Fiji. Additionally, diligent surveillance of AMR and robust antimicrobial stewardship are crucial for effectively managing nosocomial infections.

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Keywords: Carbapenem resistant; *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; *Escherichia coli*; *Klebsiella pneumoniae*

Introduction

Since carbapenems are a last-line treatment for severe bacterial infections caused by otherwise resistant bacteria,[1] carbapenem resistant organisms (CROs) pose a major global public health threat. CROs can cause severe and often life-threatening infections, leading to increased morbidity, mortality, and healthcare costs.[1,2] CROs include carbapenem resistant strains of *A. baumannii* (CR*Ab*), *P. aeruginosa* (CR*Pa*), *E. coli* (CR*Ec*), and *K. pneumoniae* (CR*Kp*). The dissemination of these major pathogens within healthcare settings and their potential for inter-hospital and community transmission is of major concern. However, there are limited data regarding their epidemiology and prevalence in many countries, including those in the Pacific.

In recent years, the incidence of CROs has been on the rise globally, posing a serious challenge to antimicrobial treatment strategies.[1] Carbapenem resistance is primarily driven by the acquisition of carbapenemase genes, including serine β-lactamases (bla_{KPC}, bla_{GES}), metallo β-lactamases (bla_{NDM}, bla_{VIM}, bla_{IMP}), and carbapenem-hydrolysing class D β-lactamases (bla_{OXA}). These genes encode enzymes that inactivate all, or almost all β-lactams, including carbapenems.[3] Additional mechanisms, including efflux pumps, alterations in porins, and other resistance genes contribute to the overall resistance phenotype.[4,5] Mobile genetic elements, such as integrons, transposons, plasmids, and integrative and conjugative elements (ICE), carrying these resistance genes can readily transfer between bacterial strains and species, fuelling the rapid spread of resistance in healthcare settings.[6–9] Of note, the clones CR*Ab* sequence type 2 (ST2),[10] CR*Pa* ST654,[11] CR*Pa* ST773,[12] CR*Ec* ST410,[13] and CR*Kp* ST16[14] warrant special attention, having been recognised internationally as common hosts of carbapenemase genes.

Located in the Pacific region, Fiji is an upper middle-income country (UMIC) and holds a central position as an economic, educational, and technological hub in the South Pacific.[15] While Fiji provides vital medical services to its population, our recent study[16] has revealed that Fiji may also be a source for dissemination of AMR pathogens in the Oceania region. This underscores the strategic importance of studying AMR in Fiji, as the

Research in context

Evidence before this study

We systematically searched Google Scholar, PubMed, and Web of Science using the term "carbapenem resistant" "*Acinetobacter baumannii*", "*Pseudomonas aeruginosa*", "Enterobacterales", "*Escherichia coli*", "*Klebsiella pneumoniae*", "carbapenemase", "antimicrobial resistance", "molecular epidemiology", "Fiji", "Pacific" and "Oceania". We identified reports on carbapenem resistant *A. baumannii* in Fiji, New Caledonia, French Polynesia, New Zealand, and Australia. To the best of our knowledge, there have been no comprehensive molecular epidemiological studies on CR*Ab*, CR*Pa*, CR*Ec*, and CR*Kp* that include the major hospitals in Fiji.

Added value of this study

Our study is important as it is the first to provide comprehensive molecular epidemiological data on specific CROs in Fiji across multiple healthcare facilities. Addressing this critical knowledge gap, the study aims to enhance our understanding of CROs that pose a growing threat to global public health. Utilising molecular epidemiology, it offers invaluable insights into the genetic relatedness of CROs, identifying potential transmission routes within and between hospitals, thus guiding effective infection control measures. Moreover, the study's findings will contribute to the global understanding of resistance gene dissemination and the emergence of new strains with heightened resistance, vital for combating AMR worldwide. The data generated from this study will be valuable to public health authorities, healthcare professionals, and researchers working to address AMR and safeguard public health. Ultimately, this study holds the potential to inform evidence-based strategies for mitigating the impact of CROs and advancing the fight against AMR on a global scale.

Implications of all the available evidence

The study's findings reveal a serious and emerging health crisis in Fiji, marked by the dissemination of multiple clones of CROs within healthcare facilities. Urgent implementation of multifaceted infection control measures is necessary to contain CRO spread. The identification of AMR genes and mobile genetic elements, including plasmids, driving resistance underscores the need for continuous monitoring and surveillance. The high proportion of nosocomial CRO infections acquired during hospital stays highlights the role of healthcare settings as reservoirs of outbreak clones. Stringent adherence to infection prevention and control measures, alongside improved communication, and coordination among healthcare providers, is crucial. Equally concerning is the emergence of community-onset CRO infections, indicating that the spread of these resistant organisms extends beyond hospital walls, posing a broader public health threat. This aspect necessitates expanding the scope of surveillance and control strategies to include community settings, highlighting the need for a holistic approach to combat the challenge of AMR both within hospitals and in the wider community.

nation's interconnected healthcare system and the mobility of patients seeking treatment abroad could facilitate the spread of AMR pathogens, impacting regional and global health.

While previous reports have offered valuable insights into AMR, shedding light on issues such as the high prevalence of AMR, unrecognised nosocomial outbreaks, and the high case fatality ratios associated with AMR in Fiji, they have primarily focused on CWMH.[16–20] This single facility focus has prevented assessment of the movement of strains between facilities in the country's wider healthcare landscape. To address this, we undertook a molecular epidemiologic and phylogenetic study of CR*Ab*, CR*Pa*, CR*Ec*, and CR*Kp* strains collected from the three major hospitals in Fiji, and CR*Ab* strains isolated from Fiji patients admitted to New Zealand hospitals. This approach aimed to ascertain the transmission dynamics of these pathogens throughout Fiji's healthcare network and beyond.

Methods

Study design and setting

We undertook a prospective observational study across the three major hospitals in Fiji: the Colonial War Memorial Hospital (CWMH), Lautoka Hospital (LTKH), and Labasa Hospital (LBSH) (see map, Appendix Fig. 1). In addition, retrospectively collected isolates from the CWMH were accessed. CWMH, with 523 beds, is Fiji's largest and most advanced hospital, serving the central and eastern part of the country.[21] It offers specialised care and serves as a referral center for LTKH and LBSH. The LTKH, with 305 beds, serves the western part of Fiji and also operates as a referral center for smaller healthcare facilities in the area.[21] LBSH, with 180 beds, caters to the northern part of Fiji and serves as a referral center for healthcare facilities in the north.[21] These three major hospitals also serve as microbiology laboratory hubs and perform all microbiological culture received from other centers across the country, excluding one private hospital in the central and western regions that conducts microbiological testing exclusively for its own patients. In Fiji, antimicrobial susceptibility testing (AST) is performed using Clinical and Laboratory Standards Institute (CLSI) guidelines (editions 29–31).[22] Meropenem susceptibility testing is initiated only if the isolate is resistant to all antimicrobials tested in the first two rounds of testing (Appendix Table 1) or upon specific request from a consultant medical doctor. These actions depend on availability of laboratory consumables

and reagents essential for conducting the tests. Isolates demonstrating resistance to meropenem, are routinely stored at –80 °C.

Sample and clinical data collection

Patients who provided samples, either from normally sterile sites, such as blood, cerebrospinal fluid (CSF), intravascular catheter tips (IVC), and chest drains, or from normally non-sterile sites, including urine, urinary catheter tips (IUDC), sputum, wound swabs, and aspirates, for culture and antimicrobial susceptibility testing as part of routine clinical care from 1 January 2020 through 31 August 2021 at the CWMH and LBSH, and up to 31 December 2021 at Lautoka Hospital (LTKH), from which a CR*Ab*, CR*Pa*, CR*Ec*, or CR*Kp* was isolated were included in the study. These samples were all resistant to meropenem and were stored at the –80 °C. To avoid duplication, one isolate per species per patient irrespective of sources was included, selecting the earliest isolate from each patient. If a patient had multiple isolates of different species, each species was included in our study. Inclusion of isolates in the study was driven by their availability rather than random sampling. Isolates obtained for surveillance and screening purposes were excluded. Clinical data, including demographics, date of admission and discharge, date of sample collection, specimen type, date of the first positive culture, duration of meropenem and colistin exposure, and date of death, were accessed from the laboratory information management system (LabIMS), microbiology laboratory registers (MLR), pharmacy department records, and patient information system (PATIS) at Fiji's CWMH, LTKH, and LBSH. All carbapenem susceptibility results were obtained from CWMH, LTKH, and LBSH from LabIMS and respective MLRs. Ten (52.6%) of the 19 CR*Pa* isolates collected between 1 January through 31 December 2019 at the CWMH were also accessed for testing. No data on patient outcomes was collected these isolates.

Clinical data analysis

All clinical data were integrated with the genomic results for all CRO isolates. The hospital setting was classified into four categories: intensive care unit (ICU), inpatient, outpatient, and outer centre. Inpatients were defined as patients who had been admitted to hospital settings other than the ICU. Outpatient settings were defined as those where patients received care without being admitted to the hospital. Outer centres were defined as healthcare facilities located outside the CWMH, LTKH, and LBSH, serving patients in remote areas.

We used admission date, sample collection date, and sample results to determine whether the first CRO isolation was from a patient sample collected before or after the first 72 h of admission to classify infections as hospital-acquired or community-onset. Hospital-acquired CRO infections were defined as patients from whom the first positive CRO sample was collected from a patient ≥72 h following admission.[23] Community-onset CRO infections were defined as patients from whom the first positive CRO sample was collected <72 h after admission.[23] In-hospital mortality was determined at 30 days following the date of first isolation of CRO. We evaluated the appropriateness of meropenem and colistin use in CRO-positive patients across the three hospitals based on the timing and type of antimicrobial therapy initiation relative to the first positive culture dates. Appropriate therapy was defined as administering meropenem prior to first isolation of a CRO or administration of colistin following isolation of a CRO. Meropenem therapy was deemed inappropriate in situations where patients remained on meropenem therapy following the isolation of a CRO, or when it was initiated subsequent to the initial detection of a CRO. However, due to the absence of device day or bed day data, inconsistencies in total admission data, and the lack of information on the total number of patients at risk, estimating incidence becomes challenging. As a result, we were only able to determine the proportion of isolates that were CROs.

Isolate identification, and antimicrobial susceptibility testing

All clinical isolates underwent identification confirmation using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Biotyper; Bruker Daltonics, Billerica, MA, USA). Antimicrobial susceptibility testing (AST) was conducted following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines through the disk diffusion method (Appendix Table 2).[24] The minimum inhibitory concentration (MIC) of meropenem was determined using the E-test (bioMérieux). The MIC of colistin was determined using the broth microdilution method (Liofilchem) in accordance with EUCAST recommendations.[25] Carbapenem resistance by species was defined as follows: *A. baumannii*, meropenem zone <15 mm or MIC >8 mg/L; *P. aeruginosa*, meropenem zone <14 mm or MIC >8 mg/L; Enterobacterales, including *E. coli* and *K. pneumoniae*, meropenem zone <16 mm or MIC >8 mg/L. Phenotypic screening for carbapenemase production using the adjusted modified carbapenemase inactivation method (AmCIM)[26] was performed on all carbapenem resistant isolates.

Genomic DNA extraction and whole genome sequencing

The genomic DNA was extracted from overnight cultures using the NucleoSpin® Tissue kit (MACHEREY-NAGEL, Düren, Germany) according to the manufacturer's instructions (Appendix page 5). Subsequently, the DNA samples were sent to BGI Technical Solutions, Hong Kong, for WGS using a BGISEQ-500 sequencer (MGI, China).[27] The sequencer generated paired-end reads of 150 base pairs for further analysis. To evaluate the quality

of the reads, identify the species, and determine the multi-locus sequence type (MLST) using the Pasteur scheme,[28] the Nullarbor bioinformatics pipeline v2.0 was employed.[29] The presence of AMR genes was investigated using ResFinder (v4.3.3) (with criteria of 90% identity and 60% coverage) at the Center for Genomic Epidemiology (CGE) and Resistance Gene Identifier (RGI) (v6.0.2) at the Comprehensive Antibiotic Resistance database (CARD).[30,31] Additionally, the presence of *ampC* genes was also determined using the PubMLST database.[32–34] In silico serotyping of *P. aeruginosa* and *E. coli* isolates was performed using *P. aeruginosa* serotyper (PAst v1.0)[35] and SerotypeFinder v2.0,[36] respectively, at the CGE.

Representative isolates from each sequence type and cluster underwent supplementary sequencing using the MinION sequencer (Oxford Nanopore Technologies, ONT) (Appendix Table 3, Appendix page 5–6). DNA libraries were prepared using the ONT Rapid Barcoding Sequencing kit (SQK-RBK110.96) and sequenced using R9.4 (FLO-MIN106) flow cells.[37] The resulting ONT reads were combined with the BGI short reads using the Unicycler hybrid assembly pipeline v0.4.9b to generate complete genomes.[38] The representative complete genomes were used as reference genomes and also to detect the existence of plasmids and other mobile genetic elements (MGEs). Plasmids, and other MGEs, such as transposons and insertion elements, were identified using the PlasmidFinder (v2.1) and Mobile Element Finder (v1.0.3) tools, with criteria of 90% identity and 60% coverage, from the Center for Genomic Epidemiology.[39] Subtypes of incompatibility F group (IncF) plasmids were determined using pMLST (v2.0). Mobility characteristics of the plasmid were predicted using MOB-typer.[40] Integrative and conjugative elements (ICE) were identified using ICE finder in the ICEberg (v2.0) web based tool.[41] A comparative analysis of putative ICE and plasmids was performed using the Basic Local Alignment Search Tool (BLAST) database.[42] The AMR genes, transposons, and insertion elements were annotated using the BacAnt database (v3.3.3),[43] and visualised with SnapGene (v6.2.1)[44] and the BLAST Ring Image Generator (BRIG).[45]

Phylogenetic analysis and transmission inference

To determine the phylogenetic relationships among the isolates based on the core genome, the sequences were aligned to a complete reference genome of the same sequence type. Snippy (v.4.6.0)[46] was utilised to identify core genome variants, and Gubbins (v.2.3.4)[47] was employed to remove recombinant regions from the aligned core genome sequences for all isolates. SNP-sites was used to extract the non-recombinant core genome single nucleotide polymorphisms (SNPs).[48] Genetic clustering of isolates was determined using Fast hierarchical Bayesian analysis of population structure (fastBAPS)[49] in RStudio (v.4.1.2). The pairwise SNP distance threshold for genetic relatedness and potential transmission linkages were set at ≤17 for CR*Pa*[50] and CR*Ec*,[51] ≤19 for CR*Ab*,[16] and ≤25 for CR*Kp*.[51] Outbreak clustering and transmission analysis was performed and visualised using GraphSNP (v1.0).[52] Those below the SNP threshold were classified as highly likely to be involved in putative transmission while those above were classified as not likely to be involved. The non-recombinant core genome SNPs were subjected to maximum-likelihood (ML) phylogeny inference with FastTree (v2.1.10, double precision [No SSE3]).[53] The resulting trees were visualised using the online Interactive Tree of Life (iTOL) (v6.0).[54,55] To understand the evolution and spread of CROs in Fiji, a phylogenetic relationships analysis was conducted by comparing all prospective and retrospective CR*Pa* isolates of the same ST. A similar comparative analysis was conducted on our prospective CR*Ab* isolates and CR*Ab* isolates previously identified at CWMH in 2016/2017 and 2019.[16] Moreover, all these CR*Ab* isolates were also compared to those obtained from Fiji patients admitted to the New Zealand hospitals during 2020–2021, as detailed in our recent study.[16]

Further detail on bioinformatics analyses is provided in Appendix page 5–6.

Mapping putative CRO transmission networks

To map potential CRO transmission network in Fiji's healthcare system, we constructed a transmission tree based on SNP distances and chronological collection sequence of CRO isolates from CWMH, LTKH, and LBSH using GraphSNP (v1.0).[52] No data were collected on bed placement or movement of patients within facilities.

Research ethics

Approvals for the study were obtained from the College Health Research Ethics Committee, Fiji National University (reference number 183.20) together with facility approvals from CWMH, LTKH, and LBSH, and the Human Ethics Committee (Health) at the University of Otago (reference numbers H20/174).

Results

CROs by species, hospital, and years

During the study periods January 2020 through August 2021 for CWMH and LBSH, and January 2020 through December 2021 for LTKH, 17,161 non-duplicate isolates of *A. baumannii* complex, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* were collected at the CWMH, LTKH, and LBSH. Of these isolates, 7,134 (41.6%) were from CWMH, 7,068 (41.2%) from LTKH, and 2,959 (17.2%) from LBSH, and 1,913 (11.1%) were *A. baumannii*, 3,043 (17.7%) *P. aeruginosa*, 5,641 (32.9%) *E. coli*, and 6,564 (38.2%) *K. pneumoniae*. Meropenem susceptibility was assessed in 5,311 (30.9%) of the 17,161 isolates.

Among these isolates meropenem resistance was observed in 488 (41.4%) of 1,178 *A. baumannii*, 175 (56.2%) of 311 *P. aeruginosa* tested, 187 (12.1%) of 1,534 *E. coli*, and 68 (2.9%) of 2,288 *K. pneumoniae* tested. Among 1,128 *P. aeruginosa* retrospective isolates from CWMH in 2019, 19 (40.7%) of 39 tested against meropenem were resistant (Appendix Table 4).

Of the 918 CROs that were prospectively identified, 273 (29.7%) were available for analysis. Of the 273 isolates analysed, 146 (53.5%) were from CWMH, 66 (24.2%) from LTKH, and 61 (22.3%) from LBSH. 90 (32.9%) of the 273 isolates were CR*Pa*, 131 (47.9%) were CR*Ab*, 46 (16.8%) were CR*Ec* and 6 (2.2%) were CR*Kp*. Of the 273 isolates, 211 (77.3%) were isolated from non-sterile sites and the remaining 62 (22.7%) were isolated from normally sterile sites.

Patient demographics

Of the 272 patients with 273 CROs, the median (range) age was 45 (<1–89) years, 140 (51.5%) were male, 161 (59.2%) were I-Taukei or indigenous Fijians, 104 (38.2%) were Fijians of Indian descent, and the remaining seven (2.6%) were from other ethnic backgrounds (Table 1). One patient had both a CR*Pa* and a CR*Ab* isolated. Of the 273 isolates collected, 131 (48.2%) were *A. baumannii*, 90 (33.1%) were *P. aeruginosa*, 46 (16.9%) were *E. coli*, and 6 (2.0%) were *K. pneumoniae*.

Of 272 patients, 234 (86.0%) had been hospitalised for ≥72 h at the time the first sample yielding a CRO was collected. Among these 234 hospital-acquired infections, the median (range) duration from ward admission to first positive CRO was 10 (3–96) days and 235 CROs were isolated, one (0.37%) patient being coinfected with CR*Ab* and CR*Pa*. Of the 235 isolates, 121 (51.5%) were CR*Ab*, 73 (31.1%) were CR*Pa*, 37 (15.7%) were CR*Ec* and 4 (1.7%) were CR*Kp*. Among the 235 CROs, 175 (74.5%) were isolated from a non-sterile site and the remaining 60 (25.5%) from a normally sterile site. Of these non-sterile site samples, 58 (33.1%) were wound swabs, 54 (30.9%) urine, 34 (19.4%) indwelling urinary catheter tip (IDUC), 25 (14.3%) sputum, and 4 (2.3%) from an endotracheal tube (ETT). Of samples from normally sterile sites, 37 (61.7%) were intravascular catheter tip (IVC), 21 (35.0%) were blood, one (1.7%) was from a chest drain, and one (1.7%) was cerebrospinal fluid (CSF). Of note, one patient who had been co-infected with CR*Ab* and CR*Pa* had these isolates obtained from IDUC and blood respectively. The median (range) duration of hospital stay of those with a nosocomial CRO infection was 21 (3–134) days. Of the 38 patients with a CRO isolated within 72 h of admission, CR*Pa* was isolated from 17 (44.7%) patients, CR*Ab* from 10 (26.3%), CR*Ec* from 9 (23.7%), and CR*Kp* from two (5.3%). Thirty-six (94.7%) of the 38 CROs were isolated from non-sterile sites, of which 20 (55.6%) were from urine, 11 (30.6%) from wounds swabs, four (11.1%) from indwelling tips and one (2.8%) from sputum. The remaining two (5.6%) were isolated from intravascular tip.

In addition to findings from prospectively identified patients, we also identified 10 retrospective CR*Pa* infections in 10 patients at CWMH from 2019. Of these patients, the median (range) age was 34 (5–82) years, 3 (30.0%) were males, 4 (40.0%) were I-Taukei or indigenous Fijians, 5 (50.0%) were Fijian of Indian descent, and 1 (100.%) was from another ethnic background. Seven (70%) of the 10 patients had their first positive CR*Pa* sample ≥72 h after hospitalisation and three (30%) within 72 h. Of the 10 CR*Pa*, four (40.0%) were isolated from urine, two (20.0%) from blood, and one (10.0%) each from wound swab, sputum, IDUC tip and pericardial fluid.

Antimicrobial treatment

Of 272 prospectively identified patients, 9 (3.3%) were treated with meropenem and colistin combination therapy, none with colistin monotherapy, 43 (15.8%) with meropenem monotherapy, and 136 (50.0%) with neither meropenem nor colistin; treatment records were unavailable for 84 (31%) (Appendix Table 5). Of 52 patients treated with either meropenem and colistin combination therapy or meropenem alone, the CRO was isolated from a normally sterile site in 21 (40.4%), while in 31 (59.6%), it was isolated from a non-sterile site. Of the 136 patients who were not treated with either meropenem or colistin, 20 (14.7%) had a CRO isolated from a normally sterile site, and 116 (85.3%) from a non-sterile site. Among the 52 patients, 22 (42.3%) were initially appropriately treated with meropenem as meropenem monotherapy was given prior to CRO isolation. However, of these, meropenem was continued after first isolation of a CRO in 9 (40.9%) for a median (range) of 8 (1–14) days. The median (range) time to initiation of meropenem monotherapy prior to first positive CRO was 6.5 (2–38) days. Meropenem use was deemed inappropriate at initiation in 21 (40.4%) patients; the median (range) time to initiation of meropenem monotherapy after collection first positive culture yielding a CRO was 5 (1–25) days. Of nine patients treated with meropenem and colistin combination therapy, eight (88.9%) were initiated prior to CRO isolation and one (11.1%) after CRO isolation.

Mortality

Of the 272 prospectively identified patients, 76 (27.9%) died, of which 33 (43.4%) were from CWMH, 22 (28.9%) from LTKH, and 21 (27.6%) from LBSH. Of the 76 decedents, 65 (85.5%) died within 30 days after first positive CRO isolation, of which 18 (27.7%) received monotherapy with meropenem, and 47 (72.3%) were not treated with either meropenem or colistin. Of the 18 decedents who received meropenem, six (33.3%) were initiated on meropenem after isolation of the CRO and nine (50.0%) continued with meropenem after initial

Variables	Total, number (%) (by isolate; n = 273)	CRO isolates n (%) N = 273			
		CRA*b* (n = 131)	CRP*a* (n = 90)	CRE*c* (n = 46)	CRK*p* (n = 6)
Organism					
Hospital					
CWMH	146 (53.5)	83 (63.3)[b]	47 (52.2)[a]	16 (34.8)	0
LTKH	66 (24.2)	21 (16.0)	10 (11.1)	29 (63.0)	6 (100.0)
LBSH	61 (22.3)	27 (20.6)	33 (36.7)	1 (2.2)	0
Setting					
ICU	93 (34.1)	65 (49.6)	17 (18.9)	8 (17.4)	3 (50.0)
Medical	85 (31.1)	27 (20.6)	34 (37.8)	20 (43.5)	3 (50.0)
Surgical	60 (22.0)	22 (16.8)	24 (26.7)	14 (30.4)	
Maternity	7 (2.6)	6 (4.6)		1 (2.2)	
Paediatrics	6 (2.2)	4 (3.1)	2 (2.2)	1 (2.2)	
Outpatients	6 (2.2)	3 (2.3)	2 (2.2)	1 (2.2)	
Outer health center	16 (5.9)	4 (3.1)	11 (12.2)	1 (2.2)	
Infection					
Hospital acquired	235 (85.7)	121 (92.4)[a]	73 (81.1)[a]	37 (80.4)	4 (66.7)
Community-onset	38 (14.3)	10 (7.6)	17 (18.9)	9 (19.6)	2 (33.3)
Initial isolation site[b]					
Blood	21 (7.7)	10 (7.6)	6 (6.7)	4 (8.7)	1 (16.7)
CSF	1 (0.4)		1 (1.1)		
Intravascular catheter tip	39 (14.3)	26 (19.8)	11 (12.2)	2 (4.3)	
Chest drain	1 (0.4)	1 (0.8)			
Urine	74 (27.1)	16 (12.2)	27 (30.0)	30 (65.2)	1 (16.7)
IDUC tip	36 (13.2)	22 (16.8)	9 (10.0)	4 (8.7)	1 (16.7)
ETT tip	6 (2.2)	2 (1.5)	1 (1.1)		3 (50.0)
Wound	69 (25.3)	36 (27.5)	27 (30.0)	6 (13.0)	
Sputum	26 (9.5)	18 (13.7)	8 (8.9)		
	Total, number (%) (by patient; n = 272)				
Sex					
Male	140 (51.5)	57 (43.5)[a]	57 (63.3)[a]	22 (47.8)	5 (83.3)
Female	132 (48.5)	74 (56.5)	33 (36.7)	24 (52.1)	1 (16.7)
Ethnicity					
I-Taukei (Indigenous Fijians)	161 (59.2)	84 (52.2)	52 (32.3)	23 (14.3)	2 (1.2)
Fijian of Indian descendants	104 (38.2)	46 (43.8)[a]	34 (32.4)[a]	21 (20.2)	4 (3.8)
Others	7 (2.6)	1 (14.3)	4 (51.7)	2 (28.6)	
Age					
Median (range), years	45 (<1–89)	44.5 (<1–82)	45 (<1–89)	54 (<1–79)	47 (46–54)
Time to first positive CRO after hospital admission					
Median (range), days	8 (1–96)	8 (1–85)	10 (0–96)	4 (0–57)	9.5 (2–45)
Length of hospital stay					
Median, day (range)	19 (1–134)	19 (3–134)	23 (0–133)	14 (0–94)	14.5 (11–75)
Treatment					
Meropenem monotherapy	43 (15.8)	24 (18.3)	17 (18.9)	1 (2.8)	1 (16.7)
Meropenem and colistin combination therapy	9 (3.3)	5 (3.8)	4 (4.4)		
Outcome (n = 272)					
Deceased	76 (27.9)	40 (30.5)	20 (22.2)	13 (28.2)	3 (50.0)
30-days mortality after date of first positive CRO (n = 65)	65 (23.8)	35 (26.7)	17 (18.9)	10 (21.7)	3 (50.0)

CWMH, Colonial War Memorial Hospital; LTKH, Lautoka Hospital; LBSH, Labasa Hospital; CRP*a*, carbapenem resistant *Pseudomonas aeruginosa*; CRA*b*, carbapenem resistant *Acinetobacter baumannii*; CRE*c*, carbapenem resistant *Escherichia coli*; CRK*p*, carbapenem resistant *Klebsiella pneumoniae; CSF, cerebrospinal fluid; IDUC, indwelling urinary catheter; ETT, endotracheal tube.* [a]Both CRP*a* and CRA*b* isolated from the same patient. [b]In 25 cases, a CRO was initially identified in a sample from a non-sterile site (urine, IDUC, ETT, sputum, or wound swab) and subsequently detected in a sample from a sterile site (20 [80%] from blood cultures and 5 [20%] from an intravascular catheter tip) (Appendix Table 6).

Table 1: **Demographic and clinical characteristics of patients with CRO, Fiji, 2020–2021.**

isolation of CRO. None of the decedents received colistin. Of the 272 patients, 35 (26.7%) of the 131 with CR*Ab*, 17 (18.9%) of 90 with CR*Pa*, 10 (21.7%) of 46 with CR*Ec*, and three (50.0%) of six patients with CR*Kp* died within 30 days. Seventy three (96.1%) of the 76 deceased patients had acquired CRO infection 72 h or more after being admitted to the hospital.

Patient demographics and clinical characteristics, are shown in Table 1, Appendix Table 5.

Distribution of clinical sample types across different hospital settings are shown in Appendix Table 6.

Bacterial identification and antimicrobial susceptibility

All 131 *A. baumannii* were resistant to meropenem, gentamicin, amikacin, trimethoprim/sulfamethoxazole, and ciprofloxacin. All 90 prospectively and 10 retrospectively collected *P. aeruginosa* were resistant to piperacillin/tazobactam, ceftazidime, meropenem, gentamicin, and ciprofloxacin. All 46 *E. coli* and six *K. pneumoniae* were resistant to ampicillin, piperacillin/tazobactam, ceftriaxone, meropenem, gentamicin, amikacin, trimethoprim/sulfamethoxazole, and ciprofloxacin. Moreover, all organisms tested had meropenem MIC values of >32 mg/L. Of the 90 prospective CR*Pa* isolates, 22 (24.4%) were AmCIM negative while the remaining 68 (75.6%) were positive; all retrospective CR*Pa* isolates were AmCIM positive. All CR*Ab*, CR*Ec*, and CR*Kp* isolates were AmCIM positive, suggesting the presence of a carbapenemase.

Carbapenem resistant *A. baumannii*

Phylogenetic analysis

Phylogenetic analysis of the 131 CR*Ab* isolates showed eight distinct clusters that corresponded to four known sequence types. Of 131 *A. baumannii*, 108 (82.4%) belonged to international clone IC-2 (ST2), 12 (9.2%) to ST25, 10 (7.6%) to ST499, and 1 (0.8%) to ST374 (Appendix Fig. 2a). The ST2 isolates formed three distinct clusters, indicating different clonal lineages within the clone (Fig. 1a).

Of the 25 isolates in the first cluster of CR*Ab* ST2 (clone 1), 10 (40.0%) came from CWMH and 15 (60.0%) from the LTKH. All isolates in this cluster were closely related with a median (range) difference of 4 (0–24) SNPs, using a complete genome of CR*Ab* ST2 (FJ16) isolated from CWMH in 2019 as the reference genome (NCBI accession: SAMN37733145) (Appendix Fig. 2b).[16] Of the CWMH isolates, six (60.0%) were from ICU patients, including five (50.0%) from adult ICU and one (10.0%) from neonatal ICU (NICU). Three (30.0%) were from other inpatient settings, and one (10.0%) from outpatients. Among the LTKH isolates, six (40.0%) were from ICU patients, with four (26.7%) from adult ICU and two (13.3%) from NICU. Moreover, nine (60.0%) were from other inpatient settings with three (20.0%) from surgical and six (40.0%) from medical wards.

Of the 27 isolates in the second cluster of CR*Ab* ST2 (clone 2), nine (33.3%) came from CWMH, three (11.1%) from LTKH, and 15 (55.6%) from LBSH. These isolates had a median (range) difference of 3 (0–28) SNPs when compared to the 2019 CWMH CR*Ab* ST2 reference genome (NCBI accession: SAMN37736705) (Appendix Fig. 2c).[16] Of the CWMH isolates, five (55.6%) were from ICU patients, including three (33.3%) from maternity ICU (MICU) and two (22.2%) from adult ICU while four (44.4%) were from two inpatient settings including three (33.3%) from medical and one from (11.1%) maternity ward. In LTKH, all clone 2 isolates were from the medical inpatient setting. Among the 15 isolates from LBSH, four (26.7%) were from adult ICU patients and the remaining 11 (73.3%) were from three other inpatient settings including six (40.0%) from medical, three (20.0%) from paediatrics and two from (13.3%) surgical wards.

The largest of the CR*Ab* ST2 clusters, clone 3, comprised 56 isolates, 43 (76.8%) of which were isolated from CWMH, three (5.4%) from LTKH, and 10 (17.9%) from LBSH. Analysis of these isolates showed that they were closely related with a median (range) difference of 3 (0–11) SNPs using a complete genome of a representative isolate of CR*Ab* ST2 clone 3 (FJ104_CW) as the reference genome (Appendix Fig. 2d). Of the 43 isolates from CWMH, 25 (58.1%) were from ICU, including 13 (30.2%) from adult ICU, 10 (23.3%) from MICU, and one (2.3%) each from paediatric ICU (PICU) and NICU. 15 (34.9%) were from other inpatient settings including six (14.0%) each from surgical and medical wards, and three (7.0%) from maternity ward. Moreover, one (2.3%) isolate was from outpatient and 2 (4.7%) from outer centres. Of the three clone 3 isolates identified at LTKH, two (67.3%) were from inpatients (medical and surgical wards) and one (33.3%) from an outer centre. In LBSH, of the 10 isolates, four (40.0%) were from ICU patients, including two (20.0%) each from PICU and adult ICU. Five (50.0%) were from other inpatient setting, including four (40.0%) from surgical and one (10.0%) from maternity wards. Moreover, one (10.0%) isolate was from an outer centre.

The putative transmission networks delineating the connections among all isolates belonging to the three distinct CR*Ab* ST2 clones within the three healthcare facilities are shown in Fig. 1b.

When comparing all prospective CR*Ab* ST2 isolates, regardless of clone, to all the CR*Ab* ST2 isolates identified in our recent study at CWMH from 2016/2017, 2019, and isolates collected from Fijian patients admitted to the New Zealand hospitals,[16] distinct relationships emerged. All CR*Ab* ST2 clone 1 isolates from the prospective study were closely related to the 2016/2017 CR*Ab* ST2 and group 1 outbreak strain from CWMH in 2019, with a median (range) difference of 7 (0–24) SNPs (Appendix Fig. 2e). Similarly, all the prospective clone 2 isolates clustered with the CWMH

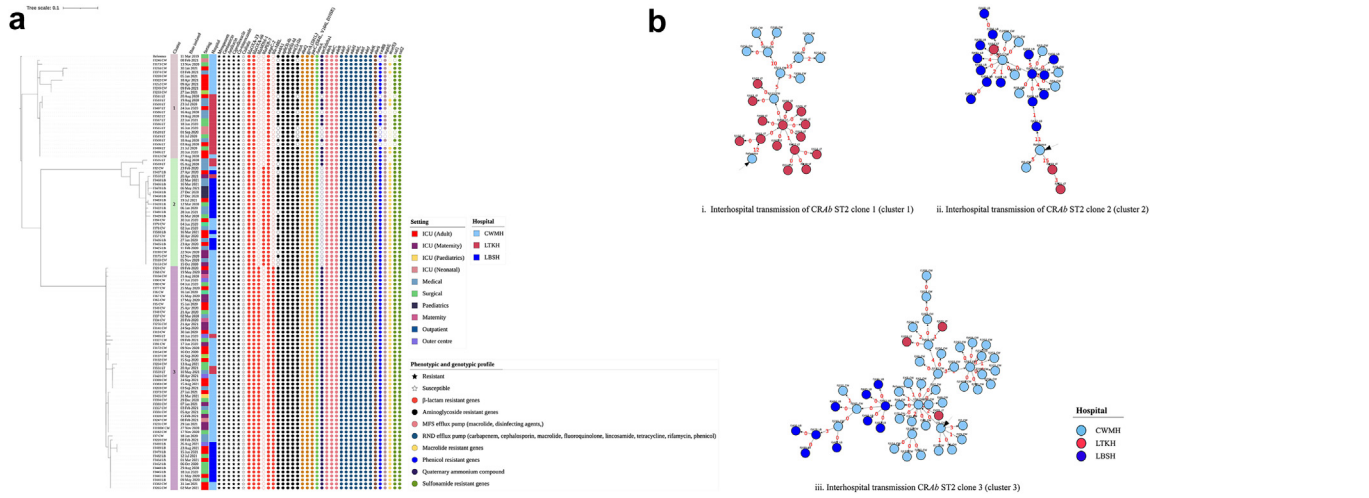

Fig. 1: Phylogenetic analysis, transmission networks, and mobile genetic elements of carbapenem resistant *A. baumannii* ST2 isolates from Fiji (2020–2021). a) Core genome SNP phylogeny of carbapenem resistant *A. baumannii* ST2 isolates from Fiji (2020–2021). Phylogenetic tree inferred from core genome SNPs of 108 CR*Ab* ST2 isolates. The core genome was generated by aligning at least 92% of the available sequence data for all isolates. The core SNP density, representing the average number of SNPs per base pair in the core genome, was 163 SNPs across 4,062,284 base pairs in the reference genome. The reference genome used was from CR*Ab* ST2 (SAMN37733145) isolated from CWMH in 2019.[16] The tree was rooted using the earliest isolate (reference) from CWMH. Isolation dates, patient admission locations, and AMR phenotypic and genotypic profiles are annotated on the tree. The scale bar on the tree represents the frequency of mutations per site, providing a measure of genetic distance between isolates. Clusters were determined by fastbaps.[49] b) Putative transmission trees of CR*Ab* ST2 clones 1–3 among CWMH, LTKH, and LBSH, 2020–2021. Transmission trees showing inter-hospital transmission directions of CR*Ab* ST2 clones 1 (i), 2 (ii), and 3 (iii) based on sample SNP distance and collection timeline. Numbers in red signify the SNP distance. Transmission tree generated and visualised using GraphSNP.[52] The black arrows represent the earliest positive isolate. c) Comparative distribution of mobile genetic elements and AMR genes in CR*Ab* ST2 isolates (clone 1–3) from Fiji, 2020–2021. The figure presents a circular depiction of the completed genomes of Fiji's CR*Ab* ST2 clone 1 (FJ567_LT), clone 2 (FJ437_LB), and clone 3 (FJ104_CW) isolates, alongside the dispersion of MGEs carrying AMR genes, visualised using BRIG. The innermost circle corresponds to CR*Ab* ST2 clone 1, the middle circle to CR*Ab* ST2 clone 2, and the outermost circle to CR*Ab* ST2 clone 3. Arrows indicate the existence of an MGE at that particular genome location. The detailed representation of MGEs can be found in the boxes (a–n). AMR abbreviation: *aph*, aminoglycoside phosphotransferase; *arm*, aminoglycoside resistance methylase; *ant*, aminoglycoside nucleotidyltransferase; *aadA*, aminoglycoside adenylyltransferase; *abaQ*, *A. baumannii* quinolone resistance transporter; *ade*, adenine deaminase; *ampC*, AmpC beta-lactamase; *amvA*, *Acinetobacter baumannii* AmvA efflux pump; *ble-MBL*, bleomycin resistance gene; bla_{NDM}, New Delhi metallo-beta-lactamase; bla_{OXA}, OXA-type beta-lactamase; bla_{PER}, PER beta-lactamases; *cat*, chloramphenicol acetyltransferase; *gyr*, DNA gyrase; *par*, Type IV topoisomerase; *qnrS1*, quinolone resistance gene; *qacE*, quaternary ammonium compounds; MFS, major facilitator superfamily; *mph*, macrolide phosphotransferase; *msr(E)*, macrolide resistance gene; RND, resistance nodulation-division; *sul*, sulfonamide resistance gene.

2019 group 2 outbreak strain with a median (range) difference of 5 (0–22) SNPs. Notably, and supporting the hypothesis from our recent study,[16] the prospective clone 3 isolates from the prospective study were closely related to the isolates from New Zealand patients who had been previously treated in Fiji, with a median (range) difference of 3 (0–10) SNPs.

Two distinct CR*Ab* ST25 clusters were identified within the 12 CR*Ab* ST25 isolates, each showing different degrees of genetic relatedness (Appendix Fig. 3). All isolates in cluster 1 and 2 were closely related within their cluster, with a median (range) difference of 0 (0–23) SNPs and 12 (0–37) respectively, using the earliest CR*Ab* ST25 (NCBI accession: SAMN38749311),[16] isolated from CWMH in 2019 as reference genome. Of the five isolates in the first cluster, four (80.0%) were from CWMH and one (20.0%) from LBSH's adult ICU. Of the four CWMH isolates, three (75.0%) were from adult ICU and one (25.0%) from the medical ward. Of the seven isolates within the second cluster, six (85.7%) were from CWMH and one (14.3%) from LBSH's maternity ward. Of the six CWMH isolates, four (66.7%) were from ICU setting, including two (33.3%) from adult ICU, and one (16.7%) each from NICU and PICU wards.

The 10 CR*Ab* ST499 isolated from CWMH formed two clusters, with 5 isolates in each cluster (Appendix Fig. 4). All isolates in cluster 1 and 2 were closely related within their cluster, with a median (range) difference of 2 (0–4) and 1 (0–3) SNPs, respectively, using a CR*Ab* ST499 isolate (FJ275_CW) from CWMH in 2020

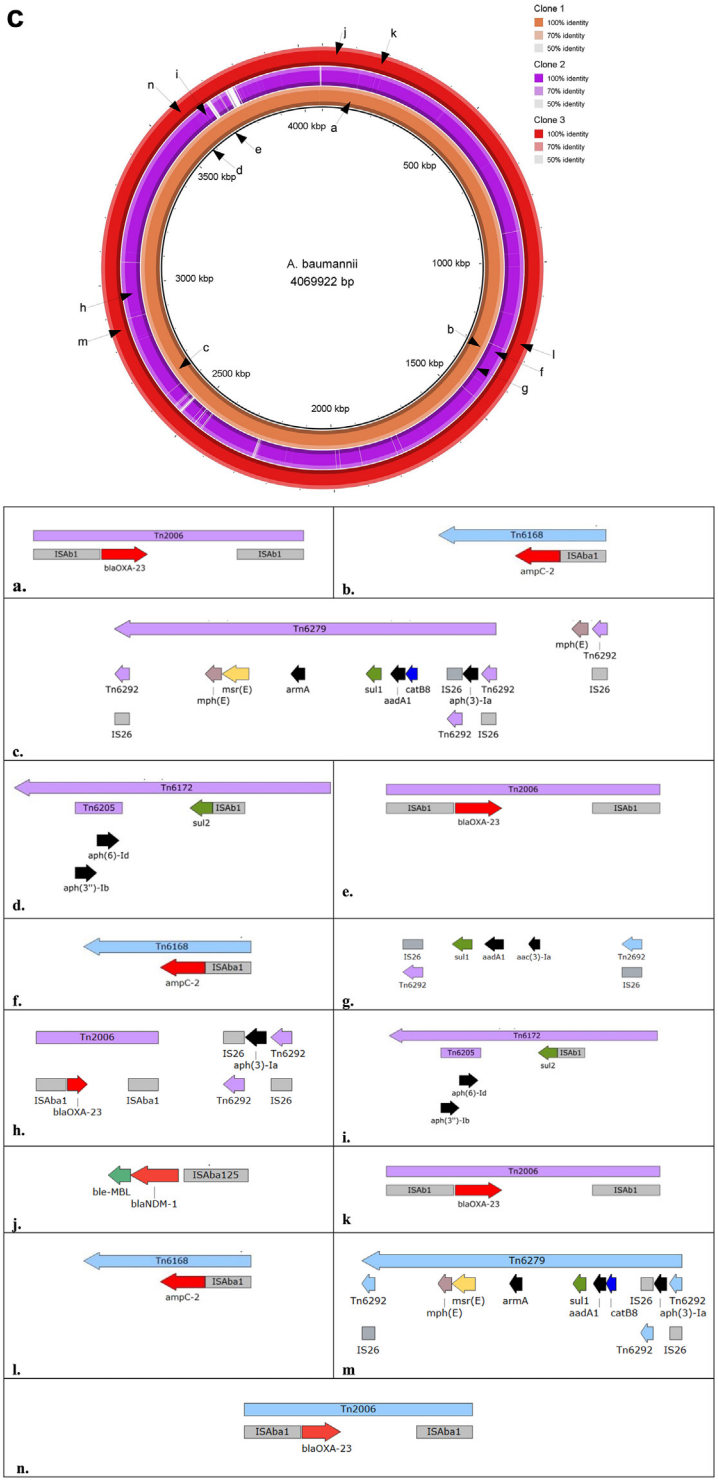

Fig. 1: (continued)

as a reference genome. Of the first cluster, four (80.0%) were from ICU and one (20.0%) from surgical ward. Of the four isolates from ICU, two (50.0%) were from adult ICU and one (25.0%) each from MICU and PICU. Moreover, cluster 2 comprised three (60.0%) isolates from ICU settings including two (40.0%) from adult

ICU and one (20.0%) from MICU; the remaining two (40.0%) isolates were from inpatient surgical ward. The only CR*Ab* ST374 was isolated from the adult ICU ward in the CWMH.

Detection and distribution of resistance genes

In the analysis of CR*Ab* ST2 isolates across three different clones, several shared resistance genes were detected. These included bla_{OXA-23}, bla_{OXA-66}, and $ampC_{-2}$, which mediate resistance to beta-lactams, including carbapenems. Additionally, aminoglycoside resistance genes (*aph(3')-Ib*, *aph(6')-Id*, *ant(3)-IIa*) were identified in all CR*Ab* ST2 isolates. These isolates also contained mutations in *gyrA* (S81L) and *parC* (S84L, V104I, D105E), which confer high-level resistance to fluoroquinolones. The isolates expressed several multidrug efflux pump genes such as *adeAC*, *FGHI*, *JKL* (resistance-nodulation-division, RND), *abaQ* (major facilitator superfamily, MFS), and *abeS* (small multidrug resistance, SMR), which contribute to resistance against tetracycline, fluoroquinolones, macrolides, and aminocoumarins. Lastly, all CR*Ab* ST2 isolates harboured the *sul2* gene, which mediates resistance to sulfonamides.

Differences in resistance gene carriage between the CR*Ab* ST2 clones were also evident. Notably, metallo β-lactamase gene bla_{NDM-1}, which mediates resistance to carbapenems, was found only in all clone 3 isolates. The bla_{PER-7} gene, encoding for an extended-spectrum beta-lactamase (ESBL), was exclusively found in CR*Ab* ST2 clone 2 isolates. Among the 25 isolates of clone 1, 18 (72%) harboured a subset of aminoglycoside resistance genes, such as *aadA1*, *aph(3')-Ia*, and *armA*. Of the 27 isolates of clone 2, 20 (74.1%) harboured *aadA1*, while all isolates carried both *aph(3')-Ia* and *armA* genes. Moreover, *qacEdelta1*, a gene mediating resistance to common hospital disinfectants, was found in 19 (76.0%) clone 1 isolates, 2 (7.4%) of clone 2 isolates, and all clone 3 isolates. The *catB8* and *sul1* genes, which mediate resistance to chloramphenicol and sulfonamides respectively, were present in all clone 2 and 3, and 18 (72.0%) of clone 1 isolates. The macrolide resistance gene, *mph(E)* was found in 21 (84.0%) clone 1 isolates and all isolates of clones 2 and 3.

In other CR*Ab* isolates, ST25 isolates harboured bla_{OXA-23}, bla_{OXA-66}, $bla_{OXA-396}$, and $ampC_{-25}$ genes, while ST499 isolates contained bla_{OXA-23}, bla_{OXA-95}, and $ampC_{-70}$, all mediating resistance to beta-lactams and carbapenems.

Mobile genetic elements

All the complete genomes of representative isolates of CR*Ab* ST2 (clones 1–3) contained composite transposon Tn*2006* (4,806 bp), incorporating two copies of the IS*Aba1* (1,180 bp) insertion element flanking bla_{OXA-23}, on the chromosome, indicative of carbapenem resistance (Fig. 1c).[6] While clone 1 and 3 contained two copies of Tn*2006* (clone 1: a and e, clone 3: k and n), clone 2 had only one (h). In addition, only clone 3 demonstrated the IS*Aba125* element upstream of the bla_{NDM-1} and ble_{MBL} genes (j) suggesting a further mechanism for carbapenem resistance. Only clone 1 and 3 representative isolates contained an IS*26*-composite transposon Tn*6279* (20,960 bp), housing various resistance genes such as *mph(E)*, *msr(E)*, *armA*, *sul1*, *catB8*, and *aph(3)-Ia* (c and m).[56] The genomes of all three clones contained IS*Aba1* upstream of the chromosomal $ampC_{-2}$ (b, f, and l), while only clones 1 and 2 contained *A. baumannii* resistance genome islands 1 (AbGR1), which encompassed a partial region of Tn*6172* (11,720 bp) containing the aminoglycoside resistance genes *aph(6)-Id* and *aph(3)-Ib* (d and i).[57]

Carbapenem resistant *P. aeruginosa*

Phylogenetic analysis

Of 90 CR*Pa* isolates from the prospective study, 65 (72.2%) belonged to ST773, 18 (20.0%) to ST664, three (3.3%) to ST654, two (2.2%) to ST9, and one (1.1%) isolate each to ST357 and ST1057.

All carbapenemase producing ST773 isolates were associated with serotype O11 and were closely related when independently analysed using a CR*Pa* ST773 representative isolate (FJ453_LB) as the reference genome, forming a single cluster with a median (range) difference of 8 (0–19) SNPs (Fig. 2a, Appendix Fig. 5a). Of 65 ST773 isolates, 28 (43.1%) were from CWMH, 29 (44.6%) from LBSH, and eight (12.3%) from LTKH. Of the 28 ST773 from CWMH, 18 (64.3%) were from inpatient wards with 11 (39.3%) from medical wards, seven (25.0%) from surgical, while nine (32.1%) were from ICU, including five (17.9%) from adult ICU, two (7.1%) each from MICU and PICU, and the remaining one (3.6%) from an outer centre. At LBSH, 22 (75.9%) of the 29 ST773 strains were from inpatient wards, with 11 (37.9%) each from surgical and medical wards, while adult ICU and outer centres had three (10.3%) each, and one (3.4%) from outpatients. At the LTKH, three (37.5%) of the eight ST773 isolates were isolated from inpatient wards including two (25.0%) from surgical and one (12.5%) from paediatrics; two (25.0%) were from adult ICUs and three (37.5%) from outer centres. All the ten retrospective study CR*Pa* isolates from CWMH in 2019 belonged to ST773. When compared with all prospective CR*Pa* ST773 isolates, they were closely related and clustered together with a median (range) difference of 7 (0–19) SNPs using CR*Pa* ST773 representative isolate (FJ453_LB) as the reference genome (Appendix Fig. 5b). Four (40.0%) of the ten retrospective CR*Pa* ST773 isolates were isolated from CWMH inpatient wards with one (10.0%) from surgical and three (30.0%) from medical wards, two (20.0%) from adult ICU, three (30.0%) from outpatients, and one (10.0%) from an

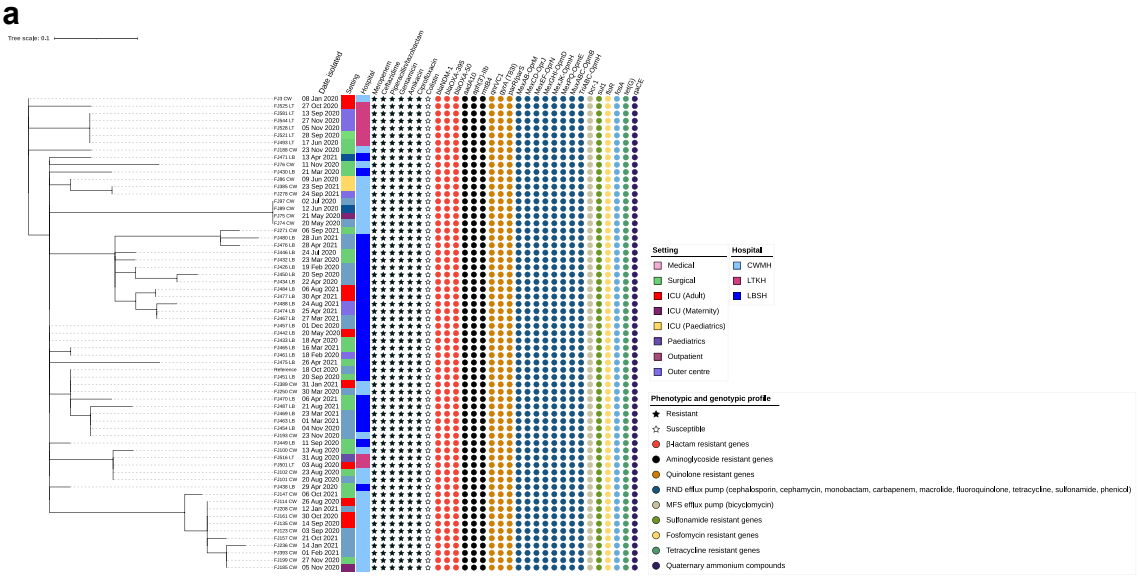

Fig. 2: Phylogenetic analysis, transmission networks, and mobile genetic elements of carbapenem resistant *P. aeruginosa* ST773 isolates from Fiji, 2020–2021. a) Core genome SNP phylogeny of carbapenem resistant *P. aeruginosa* ST773 isolates from Fiji (2020–2021). Phylogenetic tree inferred from core genome SNPs of 65 CR*Pa* ST773 prospective isolates. The core genome was generated by aligning at least 97% of the available sequence data for all isolates. The core SNP density, representing the average number of SNPs per base pair in the core genome, was 84 SNPs across 6,825,462 base pairs in the reference genome. The reference genome used was CR*Pa* ST773 (FJ453_LB), which was isolated from LBSH in 2020. The tree was rooted using the earliest isolate from Fiji (FJ03_CW) isolated from CWMH on 8th January 2020. Isolation dates, patient admission locations, and AMR phenotypic and genotypic profiles are annotated on the tree. The scale bar on the tree represents the frequency of mutations per site, providing a measure of genetic distance between isolates. b) Putative transmission tree of CR*Pa* ST773 among CWMH, LTKH and LBSH, 2020–2021. A transmission tree showing inter-hospital transmission directions of CR*Pa* ST773 based on sample SNP distance and collection timeline. Red numbers between nodes signify the SNP distance. Transmission tree generated and visualised using GraphSNP.[52] The black arrow represents the earliest positive isolate, FJ03_CW (isolated 8th January 2020). c) Distribution of MGE on the representative CR*Pa* ST773 (FJ453_LB) genome. AMR abbreviation: *aph*, aminoglycoside phosphotransferase; *aadA*, aminoglycoside adenylyltransferase; *bcr*, bicyclomycin; bla_{NDM}, New Delhi metallo-beta-lactamase; bla_{OXA}, OXA-type beta-lactamase; *floR*, florfenicol resistance gene; *fos*, fosfomycin resistance gene; *gyr*, DNA gyrase; MFS, major facilitator superfamily; *mex*, multidrug efflux system; *muxABC-opmB*, an aminocourmarin-specific efflux pump; *opr* and *opm*, outer membrane factors that form part of efflux pump complexes; *parR/S*, a two-component regulatory system; *qacE*, quaternary ammonium compounds; *qnrv*, quinolone resistance gene; *rmtB*, 16S rRNA methyltransferase; RND, Resistance-Nodulation-Division; *sul*, sulfonamide resistance gene; *tet*, tetracycline resistane gene; *triABC-opmH*, triclosan efflux pump.

outer centre. The putative transmission networks of prospective CR*Pa* ST773 within and between the three healthcare facilities are shown in Fig. 2b.

All ST664 isolates were also closely related with a median (range) difference of 16 (0–40) SNPs when analysed as a group using the ST664 representative isolate (FJ260_CW) as reference genome (Appendix Fig. 5c).

The three ST654 isolates, also all of serotype O11, were closely related, having all been isolated from CWMH and showing a median (range) difference of 2 (0–2) SNPs when analysed independently using CR*Pa* ST654 representative isolate (FJ237_CW) as a reference genome.

The two ST9 isolates from LBSH were genetically closely related with two SNP differences using ST9 representative (FJ473_LB) as reference genome.

Detection and distribution of resistance genes

Of the six CR*Pa* sequence types identified, all CR*Pa* ST773 and ST654 isolates showed a positive AmCIM test result. Moreover, all CR*Pa* isolates of all STs harboured a diverse array of resistance genes.

Shared across CR*Pa* ST773, ST664, ST654, ST9, ST357, and ST1047 were an *ampC* β-lactamase (bla_{PAO}), an aminoglycoside resistance gene (*aph(3)-IIb*), and a fosfomycin resistance gene (*fosA*). With the exception of ST1047, all other sequence types also harboured a chloramphenicol resistance gene (*catB7*). Moreover, the *parR* and *parS* genes, and the Resistance-Nodulation-Division (RND) efflux pump genes (*MexAB-OprM*, *MexCD-OprJ*, *MexCD-OprN*, *MexEF-OprN*, *MexGHI-opmD*, *MexJK-OpmH*, *MexPQ-OpmE*, *MexXY-OprM*, *MuxABC-OpmB*, *TriABC-OpmH*) were also detected in all the sequence types. *ParR* and *ParS* encode a two

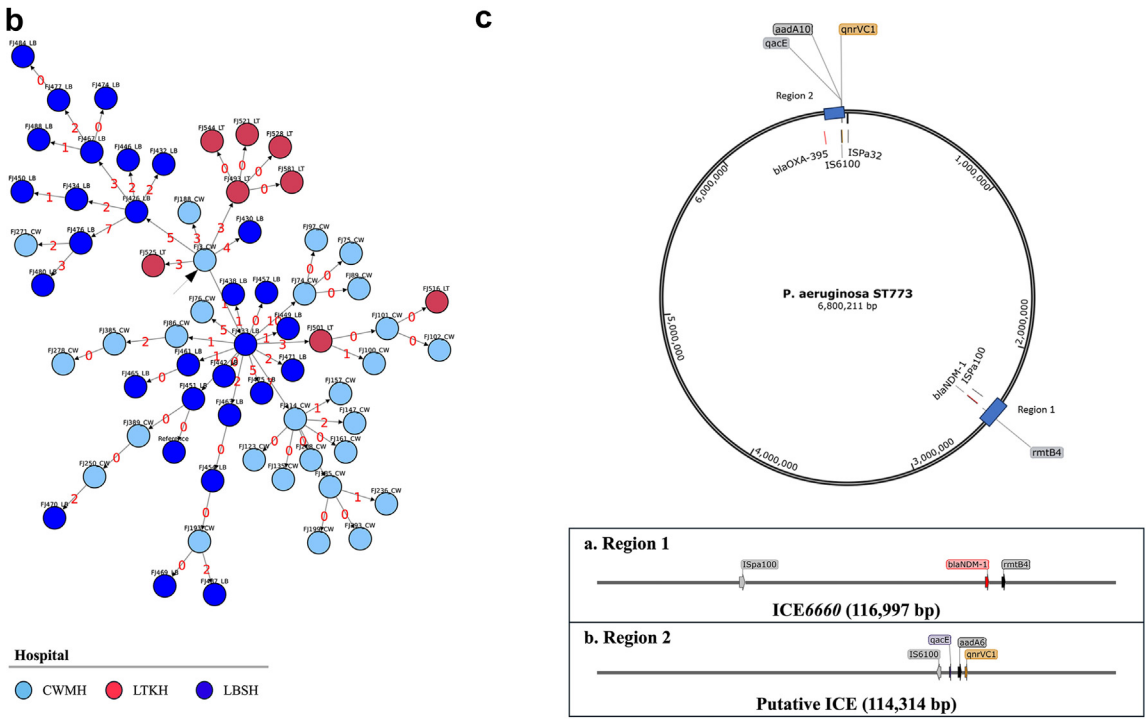

Fig. 2: (continued)

component regulatory system that controls expression of the *MexEF-OprN* and *MexXY-OprM* RND efflux pump systems, the carpabenem selective porin *OprD*, and the lipopolysaccharide modification operon *arnB-CADTEF-ugd*, conferring induced or constitutive resistance to multiple classes of antimicrobials including cephalosporins, cephamycins, monobactams, carbapenems, macrolides, aminoglycosides, fluoroquinolones, tetracyclines, phenicols, and the polymyxins.[58]

In the ST773 isolates, distinct features included the presence of the metallo β-lactamase $bla_{\text{NDM-1}}$, β-lactamase $bla_{\text{OXA-395}}$, aminoglycoside resistance genes *aadA10*, and *rmtB4*, the quinolone resistance gene *qnrVC1*, the sulfonamide resistance gene *sul1*, and the tetracycline resistance gene *tet(G)*. Additionally, the T83I mutation in *gyrA*,[59] associated with resistance to fluoroquinolones, and the quaternary ammonium compound resistance gene *qacE*,[60] which confers resistance to hospital disinfectants such as benzalkonium, benzethonium, chlorhexidine, and cetylpyridinium chloride, were detected.

All CR*Pa* ST664 isolates harboured intrinsic β-lactamase $bla_{\text{OXA-50}}$,[61] $bla_{\text{OXA-1}}$, and $bla_{\text{OXA-4}}$, conferring resistance to penicillins and carbapenems. In addition, *aac(6')-Ib*), *crpP* and *tetG* genes conferring resistance to aminoglycoside, fluoroquinolones, and tetracyclines respectively were also detected.

CR*Pa* ST654 isolates were characterised by β-lactamase $bla_{\text{OXA-396}}$,[62] and extended-spectrum β-lactamase (ESBL) $bla_{\text{VEB-1}}$, conferring resistance to carbapenems, cephalosporins and penicillins. Additionally, they harboured aminoglycoside resistance genes *ant(2)-Ia* and *aph(3)-Via*, the T83I mutation in *gyrA*, and the disinfectant resistance gene *qacE*. CR*Pa* ST9 harboured intrinsic β-lactamase $bla_{\text{OXA-486}}$ gene, while CR*Pa* ST1047 harboured $bla_{\text{OXA-488}}$ gene.

Mobile genetic elements

In the representative CR*Pa* ST773 isolate, we identified three types of transposable elements: ISPa*32*, ISPa*100* and IS*6100*, located in two distinct genomic regions (region 1 and 2) (Fig. 2c). Specifically, region 1 (2,349,629–2,349,704 bp) comprised an integrative and conjugative element (ICE) that contained ISPa*100* (part of the IS*5* family) and $bla_{\text{NDM-1}}$, *rmtB4*, and *sul1* genes (a). This ICE was highly similar (99.98%) to the ICE*6660* found in *P. aeruginosa* ST773 (NCBI accession CP041945.1) collected from the United States of America, originating from a patient previously treated in India.[63] In contrast, region 2 (6,685,067–6,799,380 bp) comprised a putative ICE and contained IS*6100* (an IS*6* element), ISPa*32*, and *qacE*, *aadA6* and *qnrVC1* genes (b).

Carbapenem resistant *E. coli*

Phylogenetic analysis

Of the 46 CR*Ec* isolates, 44 (95.7%) belonged to ST410 (Fig. 3a) and the remaining 2 (4.3%) to ST167. All ST410 displayed O8:H9 serotype and were closely

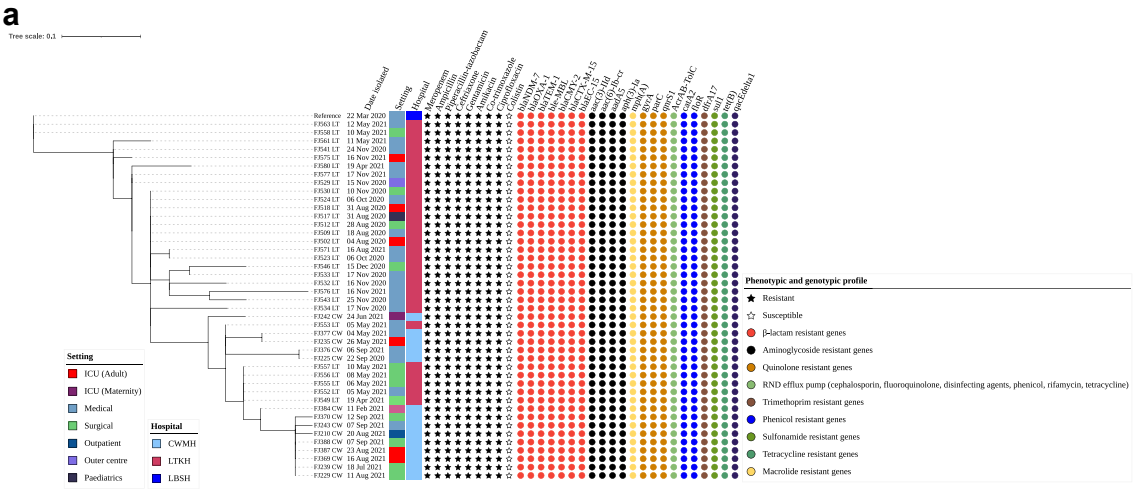

Fig. 3: Phylogenetic analysis, transmission networks, and mobile genetic elements of carbapenem resistant *Escherichia coli* ST410 isolated in Fiji (2020–2021). a) Core genome SNP phylogeny of carbapenem resistant *Escherichia coli* ST410 isolated in Fiji (2020–2021). Phylogenetic tree inferred from core genome SNPs of 44 CR*Ec* ST410 isolates. The core genome was generated by aligning at least 95% of the available sequence data for all isolates. The core SNP density, representing the average number of SNPs per base pair in the core genome, was 53 SNPs across 5,121,089 bp in the reference genome. The reference genome used was CR*Ec* ST410 (FJ431_LB), isolated from LBSH in 2020. The tree was rooted using the earliest isolate (reference). Isolation dates, patient admission locations, and AMR phenotypic and genotypic profiles are annotated on the tree. The scale bar on the tree represents the frequency of mutations per site, providing a measure of genetic distance between isolates. b) Putative transmission tree of CR*Ec* ST410 in CWMH, LTKH and LBSH, 2020–2021. A transmission tree shows inter-hospital transmission directions of CR*Ec* ST410 based on sample SNP distance and collection timeline. Numbers in red signify the SNP distance. Transmission tree generated and visualised using GraphSNP.[52] The black arrow represents the earliest positive isolate. c) Plasmids harbouring insertion sequence (IS) and AMR genes in CR*Ec* ST410. i) The CR*Ec* ST410 representative isolate carried an IncX3 plasmid that contained bla_{NDM-7} and multiple insertion elements. An IS5 element was detected upstream of the bla_{NDM-7} in the IncX3 plasmid. ii) IncFII carried multiple AMR genes and IS. Plasmids were visualised using SnapGene (v6.2.1).[44] AMR abbreviation: *aph*, aminoglycoside phosphotransferase; *aadA*, aminoglycoside adenylyltransferase; *aac*, aminoglycoside acetyltransferase; bla_{NDM}, New Delhi metallo-beta-lactamase; bla_{OXA}, OXA-type beta-lactamase; bla_{TEM}, temoneira beta-lactamase; bla_{CMY}, cephamycinase beta-lactamase; bla_{CTX-M}, cefotaximase-munich extended-spectrum beta-lactamase; bla_{Ec}, beta-lactamase *Escherichia coli*; *ble-MBL*, bleomycin resistance gene; *cat* chloramphenicol acetyltransferase; *gyr*, DNA gyrase; *dfrA*, dihydrofolate reductase; *florR*, florfenicol ressitance gene; *mph*, macrolide phosphotransferase; *par*, Type IV topoisomerase; *qnrS1*, quinolone resistance S1; *acrAB-tolC*, multidrug efflux pump; *qacE*, quaternary ammonium compounds; RND, resistance nodulation-division; *sul*, sulfonamide resistance gene; *tet*, tetracycline resistance gene.

related with a median (range) difference of 5 (0–14) SNPs using representative ST410 (FJ431_LB) as a reference genome (Appendix Fig. 6a). Of the 44 ST410 isolates, 29 (65.9%) were from LTKH, 14 (31.8%) from CWMH and one (2.3%) from LBSH. In LTKH, of the 29 ST410 isolates, three (10.3%) were from adult ICU and 25 (86.2%) from other inpatient wards, including 16 (55.2%) from medical, eight (27.6%) from surgical, and one (3.4%) from paediatric wards; one (3.4%) was from an outer centre. Within CWMH, four (28.6%) of the 14 CR*Ec* ST410 isolates were from ICU including three (21.4%) from adult and one (7.1%) from MICU. Nine (64.3%) isolates were from three other inpatient settings including four (28.6%) each from medical and surgical, and one (7.1%) from maternity ward. In addition, one (7.1%) isolate was collected from outpatient services. The putative transmission networks delineating the connections among all isolates belonging to the CR*Ec* ST410 clone within the three healthcare facilities are shown in Fig. 3b. The two CR*Ec* ST167 isolates, isolated from the medical and surgical units at CWMH, exhibited complete genomic identity with no SNP differences (Appendix Fig. 6b).

Detection and distribution of resistance genes

Both CR*Ec* ST410 and CR*Ec* ST167 harboured a metallo β-lactamase gene, with bla_{NDM-7} in ST410 and bla_{NDM-5} in ST167, conferring resistance to a range of β-lactam antimicrobials, including cephalosporins and carbapenems. The extended-spectrum β-lactamase (ESBL) gene $bla_{CTX-M-15}$ and the broad spectrum class A β-lactamase gene bla_{TEM-1} were also carried by all isolates of both STs. Moreover, both STs also harboured an *ampC* cephalosporinase gene with bla_{CMY-2} in ST410 and bla_{CMY-42} in ST167. All CR*Ec* ST410 isolates harboured the bla_{OXA-1} gene, while CR*Ec* ST167 isolates did not. Mutations in *gyrA* (D87N, S83L) and *parC* (S80I), conferring high-level fluoroquinolone resistance, were detected in both STs. Additionally, ST167 isolates also had an additional mutation in *parC* (E84G). All CR*Ec* ST410 isolates carried the fluoroquinolone resistance genes *aac(6')Ib-cr* and *qnrS1*, while CR*Ec* ST167 isolates did not. The sulfonamide

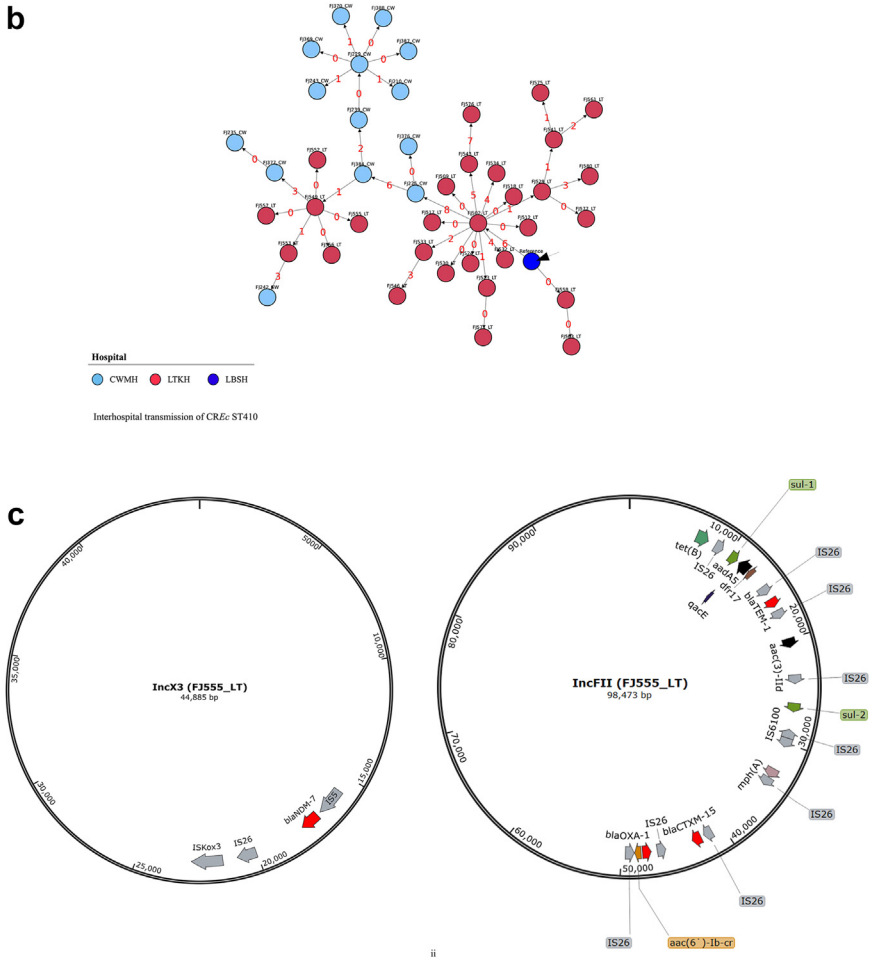

Fig. 3: (continued)

resistance gene *sul1* was also present in both the STs. Both STs harboured aminoglycoside resistance genes, with *aph(3)-IId*, and *aph(6)-Id* in all ST410 and *aadA* and *rmtB* in ST167. Both STs harboured aminoglycoside resistance genes, with *aph(3)-IId*, and *aph(6)-Id* in all ST410 isolates, *aph(3)-Ib* in 43 (98%) ST410 isolates, and *aadA* and *rmtB* in all ST167 isolates. CR*Ec* ST167 isolates harboured the macrolide resistance gene *mph(A)*. Isolates of both STs also harboured trimethoprim resistance genes, with *dfrA17* in ST410 and *dfrA12* in ST167. All CR*Ec* ST410 isolates carried chloramphenicol resistance genes *flo(R)* and *catA2*. All CR*Ec* ST167 isolates harboured the macrolide resistance gene *mph(A)*. Additionally, the efflux pump gene *acrAB-tolC* and the disinfectant resistance gene *qacEdelta1* were present in all isolates of both STs, indicating a broad potential for multidrug resistance.

Mobile genetic elements

In the representative genome of CR*Ec* ST410 (FJ555_LT), four distinct plasmid replicons were identified, including IncX3, IncC, Col (BS512), and IncF. The IncF replicon was further subtyped into IncFIA, IncFIB, and IncFII, all of which all belong to the pMLST type F1:A1:B49. Of note, the IncX3 plasmid exhibited a high degree of similarity (100% coverage and identity) to a plasmid from a CR*Ec* ST448 strain isolated in Kuwait in 2012[64] (NCBI accession: KX214669.1) and contained bla_{NDM-7}, IS*5*, IS*26*, and ISKox*3* (Fig. 3ci). Moreover, the IncFII plasmid, with a high degree of similarity (100% coverage, 99.99% identity) to IncFII plamid carried by a CR*Ec* ST410 strain isolated in France in 2013 (NCBI accession: LR595692.1), contained a plethora of AMR genes including bla_{OXA-1}, $bla_{CTX-M-15}$, bla_{TEM-1}, *aadA5*, *aac(3)-IId*, *aph(3)-Ib*, *mph(A)*, *qacEdelta1*, *sul1*, *dfrA17*, *tetB*, and *aac(6)Ib-cr*, along with multiple copies of IS*26* (Fig. 3cii). The CR*Ec* ST410 isolate also possessed various relaxase types (MOBF, MOBH, and MOBP), essential for plasmid conjugation. For CR*Ec* ST167, an IncFIA type plasmid carrying bla_{NDM-5}, *qacEdelta1*, *sul1*, *aadA1*, *dfrA17* (Appendix Fig. 7) was also present.

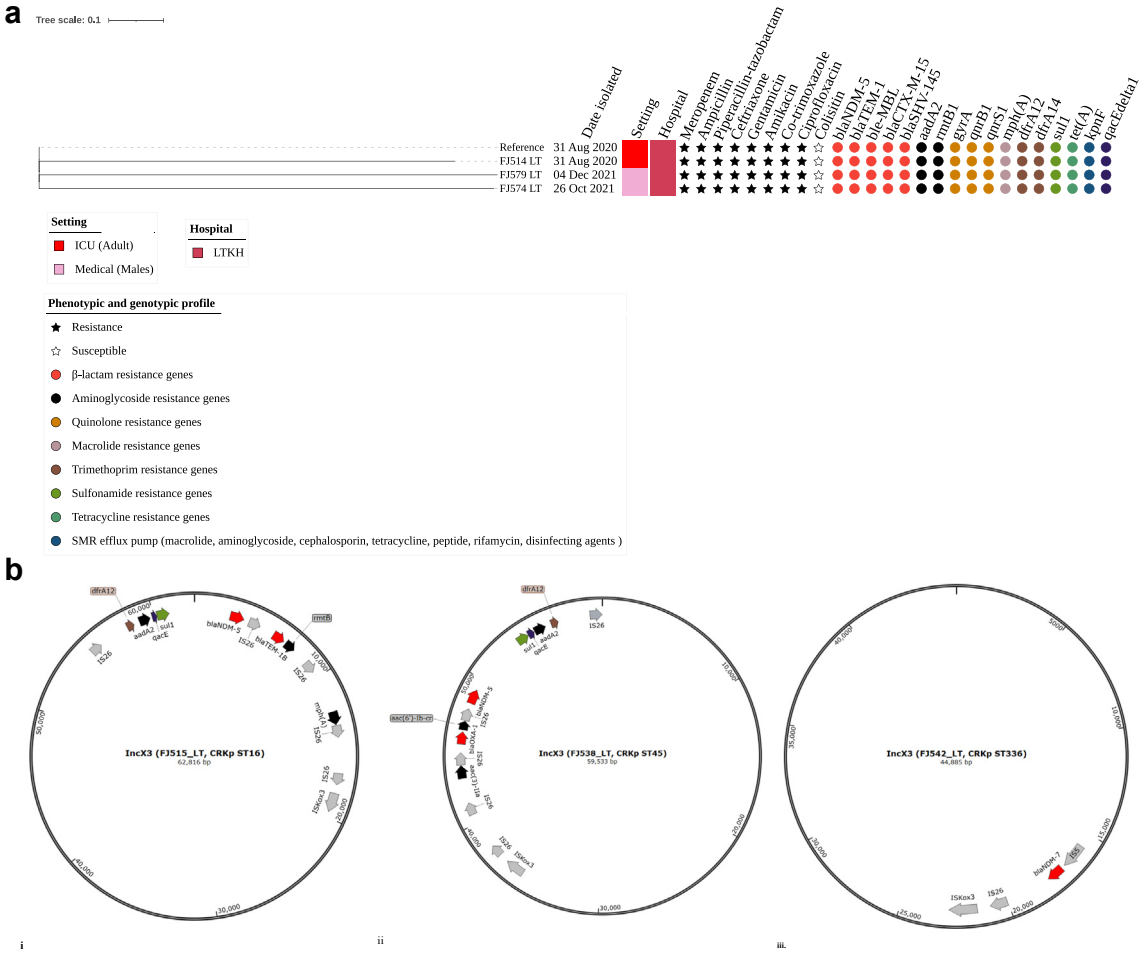

Fig. 4: Phylogenetic analysis and mobile genetic elements of carbapenem resistant *Klebsiella pneumoniae* isolates from Fiji (2020–2021). a) Core genome SNP phylogeny of carbapenem resistant *Klebsiella pneumoniae* ST16 isolates from Fiji (2020–2021). Phylogenetic tree inferred from core genome SNPs of four CR*Kp* ST16 isolates. The core genome was generated by aligning at least 97% of the available sequence data for all isolates. The core SNP density, representing the average number of SNPs per base pair in the core genome, was 3 SNPs across 5,758,482 bp in the reference genome. The reference genome used was CR*Kp* ST16 (FJ515_LT), isolated from LTKH in 2020. Sequence types, isolation dates, patient admission locations, and AMR phenotypic and genotypic profiles are annotated on the tree. The scale bar on the tree represents the frequency of mutations per site, providing a measure of genetic distance between isolates. b) IncX3 plasmids in CR*Kp* ST16, CR*Kp* ST45, and CR*Kp* ST336: mapping of prominent IS and AMR genes. IncX3 plasmids from representative (i) CR*Kp* ST16 (FJ515_LT), (ii) CR*Kp* ST45 (FJ538_LT), and (iii) CR*Kp* ST336 (FJ542_LT). The IncX3 plasmid detected in CR*Kp* ST336 (FJ542_LT) exhibited a 100% similarity to the plasmid in CR*Ec* ST410 (FJ555_LT), when analysed using BLAST database comparsion[42] (Appendix Fig. 8). Plasmids were visualised using SnapGene (v6.2.1).[44] AMR abbreviation: *aadA*, aminoglycoside adenylyltransferase; bla_{NDM}, New Delhi metallo-beta-lactamase; bla_{TEM}, temoneira beta-lactamase; bla_{CTX-M}, cefotaximase-munich extended-spectrum beta-lactamase; bla_{SHV}, sulfhydryl variable beta-lactamase; *ble-MBL*, bleomycin resistance gene; *gyr*, DNA gyrase; *dfrA*, dihydrofolate reductase; *kpnF, K. pneumoniae* efflux pump; *mph*, macrolide phosphotransferase; *qnr, quinolone resistance; qacE, quaternary ammonium compounds; rmtB*, 16S rRNA methyltransferase; *SMR*, small multidrug resistance; *sul*, sulfonamide resistance gene; *tet*, tetracycline resistance gene.

Carbapenem resistant *K. pneumoniae*

Phylogenetic analysis

All six CR*Kp* isolates were from LTKH. Of these, four (66.7%) belonged to ST16, and one (16.7%) each to ST45 and ST336. Two (50.0%) of the ST16 were isolated from patients admitted to the adult ICU and the remaining two (50.0%) from medical inpatient wards (Fig. 4a). The ST16 isolates were closely related, with a median (range) difference of 0 (0–1) SNPs using representative CR*Kp* ST16 (FJ515_LT) as the reference genome. The CR*Kp* ST45 and ST336 were isolated from the adult ICU and a medical inpatient ward, respectively.

Detection and distribution of resistance genes

Both the CR*Kp* ST16 and ST45 isolates contained bla_{NDM-5}, while CR*Kp* ST336 contained bla_{NDM-7}, all

mediating resistance to carbapenems. The beta-lactamases bla_{TEM-1} and $bla_{CTX-M-15}$ were found in all CR*Kp* isolates, but the *ampC* cephalosporinase gene bla_{CMY-2} was exclusive to ST336. CR*Kp* ST16 also harboured *ampC* bla_{DHA-6}. CR*Kp* ST16 carried the *aadA2* aminoglycoside resistance gene, while ST336 and ST45 both carried *aac(3)IIa*, and *aac(6)-Ib-cr*. Mutations of *gyrA* (D87N, S83L) and *parC* (S80I) were detected in ST336, while ST16 had mutations in *gyrA* (S83F, D87N), mediating resistance to fluoroquinolones. All CR*Kp* also carried the *qacEdelta1* gene, conferring resistance to common hospital disinfectants such as benzalkonium, benzethonium, chlorhexidine, and cetylpyridinium chloride.

Mobile genetic elements

The representative genome of CR*Kp* ST16 (FJ515_LT) and CR*Kp* ST45 (FJ538_LT) contained multiple plasmids, including IncX3, IncFIA, IncFIB and ColKP3. Of note, in both strains, the bla_{NDM-5} gene was carried by IncX3 plasmids, which contained a plethora of insertion sequences (IS) and AMR genes (Fig. 4b). While both IncX3 plasmids also contained *sul1* (sulfamethoxazole resistance), *dfrA12* (trimethoprim resistance), *aadA2* (spectinomycin and streptomycin resistance), and *qacE* (chlorhexidine and benzylkonium chloride resistance), they differed from each other in structure and gene content. The ST16 IncX3 plasmid contained bla_{TEM-1}, *rmtB* (aminoglycoside resistance), and *mph(A)* genes (macrolide resistance), while the ST45 IncX3 plasmid contained bla_{OXA-1}, *aac(3)-IIa*, and *aac(6')-Ib-cr* genes (aminoglycoside resistance).

The CR*Kp* ST336 isolate (FJ542_LT) also harboured an IncX3 plasmid, but this plasmid contained bla_{NDM-7} gene, a feature similarly observed in the CR*Ec* ST410 representative isolate (FJ555_LT). The positions of all IS elements and the bla_{NDM-7} gene were consistent across the plasmids from both species (Appendix Fig. 8).

Interhospital transfers of patients with CROs

In our analysis of prospective cases, we observed multiple instances of inter-hospital transfers involving clonally related CRO cases. Four cases (FJ454_LB, FJ467_LB, FJ476_LB, FJ487_LB) with CR*Pa* ST773 were transferred from LBSH to CMWH. One of these cases (FJ467_LB) was also co-infected with CR*Ab* ST2 clone 2. Notably, this represents the sole co-infection case we observed in the study. Another case (FJ493_LT), also infected with CR*Pa* ST773, was transferred from LTKH to an outer health centre. Two cases (FJ57_CW and FJ29_CW) with CR*Ab* ST2 clone 2 originating from CWMH were transferred to LBSH and to an outer centre outside LTKH respectively. Another LBSH patient (FJ483_LB) infected with CR*Ab* ST2 clone 2, was transferred to the CWMH's ICU and subsequently returned to LBSH two months later. Yet another LTKH case (FJ503_LT) with CR*Ab* ST2 clone 2, was transferred to a different outer health centre. One LBSH patient (FJ382_CW) was transferred to CWMH's ICU, where the patient acquired a CR*Ab* ST2 clone 3 infection before being moved back to LBSH. Interestingly, the first instance of CR*Ab* ST2 clone 3 at LBSH was recorded two months following this transfer. A case (FJ370_CW) infected with CR*Ec* ST410 at CWMH was transferred to LBSH. In contrast, a patient (FJ354_CW) from LTKH carrying CR*Ab* ST25 was relocated to the paediatric ward at CWMH. Notably, all instances of CR*Kp* ST16, ST336, and ST45 infections were solely identified among the medical inpatients and ICU patients at LTKH, with no recorded transfers for these cases. A visual representation of all these hospital transfers is provided in Fig. 5.

Discussion

Our study reveals evidence of the transmission of multiple clones of CR*Ab*, CR*Pa*, CR*Ec*, and CR*Kp* within and between hospitals in Fiji. We observed multiple previously unrecognised, nosocomial outbreaks of three distinct clones of CR*Ab* ST2 and one clone each of CR*Pa* ST773, CR*Ec* ST410, and CR*Kp* ST16 in the three major hospitals in Fiji. These outbreaks were primarily associated with both urinary and intravascular catheter-associated infections, respiratory tract infections including intubated patients in ICU, surgical site infections, and blood stream infections. Significantly, these infections were most common in high-risk hospital settings, such as ICUs and other inpatient wards, particularly in medical and surgical wards, with occasional cases in maternity and paediatric units and some from the outpatients and communities, indicating the widespread nature of this health threat. Of note, CR*Ab* ST2 and CR*Pa* ST773 have been present in the CWMH since at least 2016 and 2019, respectively, suggesting that they have become endemic in the CWMH setting. Moreover, the proportion of carbapenem-resistant *A. baumannii*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* isolates, surpassing those reported in other Pacific island countries like the Cook Islands, Kiribati, Samoa, and Tonga, further exacerbates the situation.[17,65] Given the complexity of these multiple clonal outbreaks, it is essential to implement immediate and multifaceted infection control measures, alongside early detection and monitoring of prevalent clonal types, and to formulate targeted interventions.

The detection and distribution of AMR genes and mobile genetic elements, including plasmids, in CROs provide valuable insights into the mechanisms driving resistance and the potential for dissemination within healthcare facilities in Fiji. Of note, all the isolates, regardless of species, were resistant to all antimicrobials tested including meropenem, except for colistin; resistance was associated with carbapanemase genes

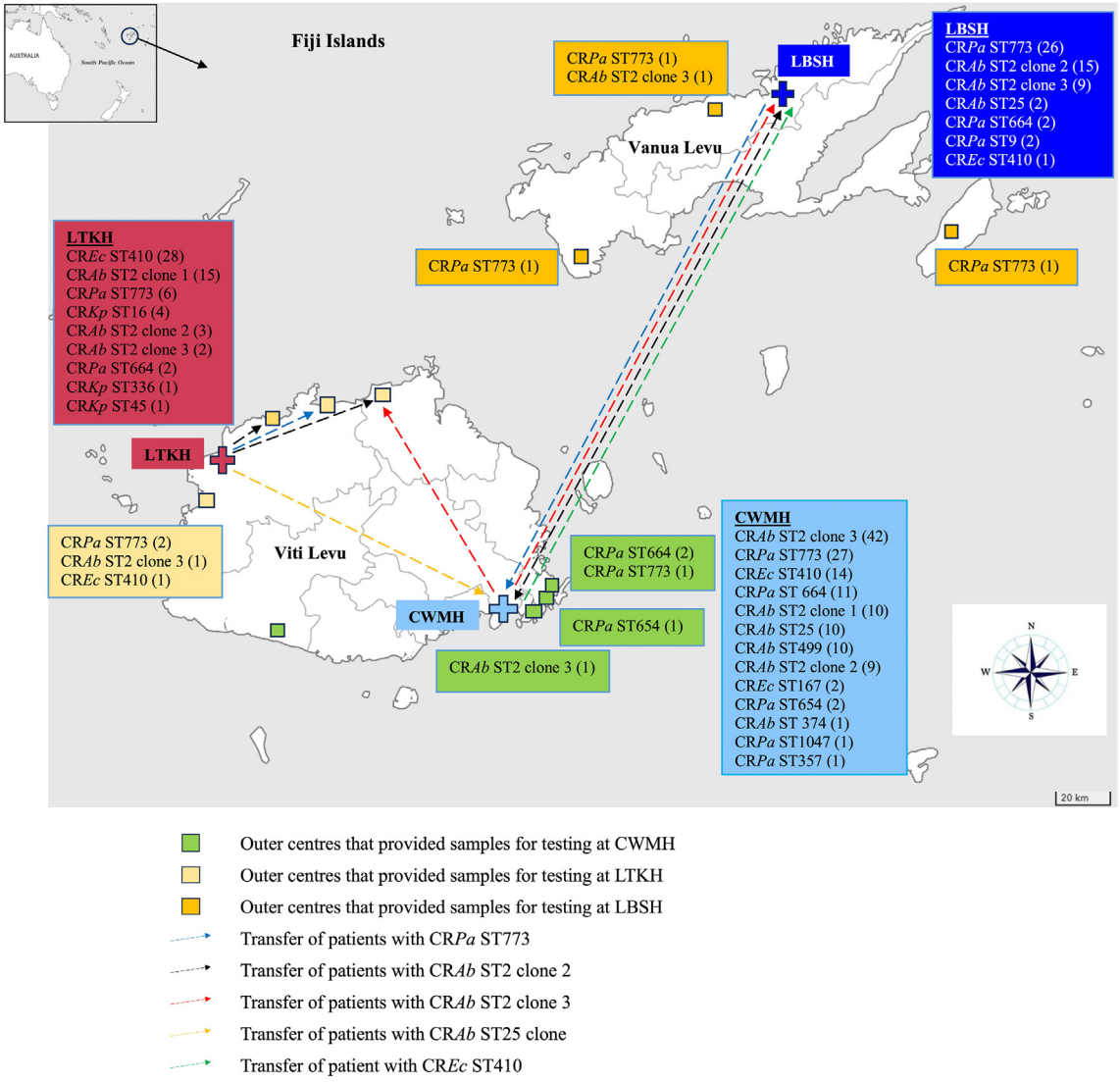

Fig. 5: Geographical distribution and transfer of patients with CROs across health facilities in Fiji, 2020–2021. A total of 273 CRO strains were present across the three major hospitals and outer centers in Fiji. Arrows denote the transfer of patients across various hospitals and outer centers. Total number of CROs of each ST are shown in parentheses. Map created with National Geographic MapMaker.

(bla_{NDM-1}, bla_{NDM-5}, bla_{NDM-7}, bla_{OXA-23}), other AMR genes, and efflux pumps. One notable finding was the detection of the metallo β-lactamase gene bla_{NDM-1} within the chromosome of the representative genome of CR*Pa* ST773. This bla_{NDM-1} gene was located within the ICE*6660* element, widely recognised for its role in mobilising AMR genes.[63,66] Notably, this ICE*6660* was highly similar to the ICE*6660* found in a CR*Pa* ST773 isolate from a patient in the United States with a history of medical treatment in India,[63] underscoring the potential transnational movement of such resistant strains. Considering Fiji's medical ties with India,[67,68] together with genetic linkages of CR*Ab* ST2 strains identified in both countries as indicated in our recent study,[16] it raises concerns and suggests that India could potentially be the original source of CR*Pa* ST773 carrying bla_{NDM-1} in Fiji. However, the genetic similarities of Fiji's endemic CR*Ab* ST2 strains to those identified in other Oceania countries,[16] suggests that Fiji's role is not only as a recipient but also as a potential transmission hub of CROs across the Pacific. Despite the lack of carbapenemase genes in other CR*Pa* strains (ST354, ST664), the presence of RND efflux pumps (*MexAB-OprM* and *MexXY-OprM*) together with intrinsic bla_{OXA-50}, may have contributed to carbapenem resistance.[69,70]

Conjugative plasmids, including IncX3 and IncFII, have been highlighted as key agents in the dissemination of AMR genes among Enterobacterales.[71] Interestingly,

both the CR*Ec* ST410 and CR*Kp* ST336 representative isolates shared the same IncX3 plasmid carrying bla_{NDM-7}, suggesting possible horizontal gene transfer between the two.[7,8] Moreover, the presence of an extended spectrum β-lactamase ($bla_{CTX-M-15}$), aminoglycoside (*aac(6)Ib-cr*, *aadA1*) and disinfectant (*qacEdelta1*) resistance genes, together with multiple transposable elements (IS*26*), in an IncFII plasmid further exacerbates the risk of dissemination of other AMR and disinfectant resistance genes from CR*Ec* ST410. All CR*Ab* ST2 clone 1 and 2 isolates harboured bla_{OXA-23}, while clone 3 co-produced bla_{OXA-23} and bla_{NDM-1}, mediating high level carbapenem resistance. Notably, the CR*Ab* ST2 clone 1 and 3 representative isolates contained two copies of the composite transposon Tn*2006*, carrying bla_{OXA-23} flanked by IS*Aba1* elements, while clone 2 contained a single copy. This was consistent with our previous findings of the CR*Ab* ST2 isolates obtained from CWMH in 2019.[16] This composite transposon has been associated with the increased dissemination of the bla_{OXA-23} carbapenemase gene in *Acinetobacter baumanni*.[72,73] The presence of the IS*Aba125* element upstream of bla_{NDM-1} in the representative isolate of CR*Ab* ST2 clone 3 also points towards increased mobilisation potential of this metallo β-lactamase.[74,75] Collectively, these findings underscore the considerable potential for the dissemination of carbapenemase and other AMR genes to other bacterial pathogens in Fiji.

Our study not only demonstrated that the majority of patients acquired CRO infections during their hospital stays, but also showed probable community onset CRO infections. The identification of a high proportion of outbreak clones of CR*Ab* ST2 (clones 1–3), CR*Pa* ST773, and CR*Ec* ST410 primarily in hospital inpatient and ICU settings suggests that these hospital settings may act as reservoirs of these outbreak clones. Future studies should investigate potential environmental sources and reservoirs within hospitals. However, the presence of similar clones in community cases highlights the likelihood of CRO spread beyond hospital settings. The presence of clustered cases with the same CRO clone within three different hospitals suggests that these outbreaks predominantly resulted from transmission occurring both within and between healthcare facilities. This intra- and inter-hospital spread highlights the complex network of transmission routes in Fiji's healthcare settings. Furthermore, the likelihood of undetected transmission, facilitated by the transfer of patients colonised with CROs between these hospitals, outer peripheral centres, and the broader community, notably raises concerns about the spread of these clones. In particular, patients discharged from hospitals to the community represent a potential critical means for extending the reach of these outbreak clones beyond healthcare facilities into the wider community. This suggests substantial challenges in the form of inadequate AMR tracking and reporting systems, interfacility communication (especially during patient transfers), and inconsistent infection control practices within Fiji's healthcare system. Of note, all CR*Kp* strains were exclusively detected at the LTKH. The presence of the IncX3 plasmid, which carries the bla_{NDM-7} gene, in both CR*Kp* ST336 and the representative CR*Ec* ST410 isolate implies that there might have been transfer of this transferable plasmid between the two clones within LTKH. However, other potential sources of acquisition cannot be excluded. Moreover, an IncX3 plasmid containing bla_{NDM-5} gene was also found in CR*Ec* ST16. Ours is the first study to report IncX3 plasmids carrying bla_{NDM-5} and bla_{NDM-7} genes in *E. coli* and *K. pneumoniae* in the Pacific Islands. Given that only 26% of CR*Ec* and 10% of CR*Kp* were available for WGS, combined with the low rates of testing for meropenem susceptibility, it would not be surprising if there were additional outbreak clones of Enterobacterales circulating in Fiji. Furthermore, the identification of patients colonised with carbapenem resistant Enterobacterales in Fiji health facilities and peripheral centres intensifies the risk of community transmission, especially given that these CROs harbour mobile genetic elements and plasmids that may potentiate the spread of carbapenemase genes to other Enterobacterales species and strains. Moreover, detection of multiple other strains of CR*Ab*, CR*Ec*, and CR*Kp* suggests that there have been multiple previous introductions of CROs into Fiji, although acquisition of carbapenem resistance in Fiji cannot be excluded.

Managing CRO infections in clinical settings poses a substantial challenge, particularly due to the limited therapeutic options available.[76,77] In Fiji, the current approach to combat these resistant organisms involves a stringent process controlling meropenem and colistin use.[78] Despite this, we identified a troubling trend in the management of CROs across Fiji's major hospitals.[16,18,19] Specifically, we discovered continued inappropriate use of meropenem, a finding consistent with what we observed at the CWMH in 2019.[16] Furthermore, few patients were treated with colistin. This signifies the urgent necessity for improved communication and coordination among healthcare providers, particularly between the microbiology laboratories, infection prevention and control (IPC) teams, pharmacists, and prescribing doctors. Access to potential alternative treatment options to colistin, such as aztreonam plus ceftazidime-avibactam,[79,80] plazomicin,[81] and eravacycline[82,83] should be urgently investigated. Interestingly, the presence of ESBL bla_{PER-7} in CR*Ab* ST2 clone 2 raises significant concerns due to its role in contributing to resistance against cefiderocol,[84] a recently introduced siderophore cephalosporin for treating CRO infections.[85,86]

Strict adherence to IPC measures, such as careful management of catheters[87–89] and ventilators,[90,91] stringent wound management,[92] and rigorous sepsis control procedures,[93] combined with consistent hand hygiene

practices,[94] use of effective disinfectants,[95] and thorough sanitisation of hospital environments and medical equipment[93,94] is crucial in managing nosocomial infections.[96–98] During the COVID-19 pandemic, stringent IPC measures and restrictions on travel were globally implemented.[99] However, despite these heightened measures and movement restrictions within and between Fiji's three major hospitals, a substantial number of CRO nosocomial infections were still observed over the period of the study. Of note, the presence of the quaternary ammonium compound resistance gene *qacE*, which confers increased resistance to commonly used hospital disinfectants such as benzalkonium, benzethonium, chlorhexidine, and cetylpyridinium chloride, in CR*Pa* ST773, CR*Ab* ST2 (clones 1 and 3), and all CR*Ec* and CR*Kp* isolates, further complicates control efforts and underscores the resilience of these organisms.[60,100]

Patients with AMR infections have higher case fatality ratio than those infected with susucpetible organims.[1,18] Fiji's 30-day case fatality ratio for those with CR*Ab* infection (26.7%) was higher than China (16.7%)[101] but lower than USA (32.2%).[102] As for CR*Pa*, it (18.9%) was higher than combined data from Australia and Singapore (11%), and China (7%),[77] but lower than South and Central America (27%).[77] Additionally, for carbapenem resistant Enterobacterales, Fiji's case fatality ratio (25.0%) was consistent with that of the 10 Southern European countries (23.8%)[103] but lower than China (65.4%).[104] The scarcity of available effective antimicrobial treatment options and the underutilisation of colistin likely contributed to the poor outcomes.[19,102] Further studies, however, will be required to investigate the direct casual pathway of death in patients with CRO infections; it is not possible to attribute death to CRO infection based upon the data available in this study.

We identify a number of limitations with study. First, the availability of isolates from only 30% of patients with CRO infections and the non-inclusion of screening isolates (which were not available as screening for asymptomatic colonisation was not performed) over the study period may potentially underestimate the population diversity of CRO strains in Fiji and overlook other potential reservoirs. Second, the absence of data on bed days, inconsistencies in total admission data, and the lack of information on the population at risk restrict our ability to investigate the incidence of CRO infections. Third, the COVID-19 pandemic increased demands on resources, changed clinical practices, and influenced patient use of study healthcare facilities that may have reduced the collection and storage CRO isolates for analysis. Fourth, meropenem susceptibility testing was restricted to isolates resistant to other antimicrobials or on the basis of specific medical consultant requests. Moreover, the use of CLSI clinical breakpoints may have missed organisms with low carbapenemase activity, such as OXA-48-like carbapenemase-producing Enterobacterales. This may have led to underestimation of meropenem resistance incidence and overestimation of associated mortality in our study. Fifth, our use of isolates based on availability, rather than randomised sampling may have introduced bias in our results. Sixth, the lack of available data on the usual place of residence for patients with community-onset CRO infection limited our ability to investigate the geographic distribution associated with these infections. Seventh, our study was unable to evaluate the full causal pathway to death among decedents, so we cannot be confident of the relative contribution of antimicrobial resistance, underlying causes, and co-morbidities on vital outcomes associated with antimicrobial-resistant infections. Finally, putative transmission networks were based on core genome SNPs and date of isolation of available isolates, and as such may not represent complete transmission pathways.

In conclusion, our study underscores the challenges posed by CROs in Fiji's major hospitals, characterized by the spread of multiple CRO clones within and between healthcare facilities over many years. The identification of specific clonal nosocomial outbreaks of CR*Pa*, CR*Ab*, CR*Ec*, and CR*Kp*, along with associated high case fatality ratios, indicates a growing concern for public health. Additionally, the presence of community-onset CRO infections, particularly with CR*Pa*, CR*Ec*, and CR*Kp*, suggests potential spread beyond hospital settings into the broader community. These findings raise important questions about the sources and transmission modes of CROs in non-hospital environment, including the role of asymptomatic carriers, environmental reservoirs, and possible cross-transmission between healthcare settings and the community. Compounding these issues, the COVID-19 pandemic's overwhelming impact on Fiji's healthcare infrastructure during the study period likely reduced the efficacy of IPC protocols,[105] inadvertently aiding the continued spread of these CRO pathogens. The study also identified frequent inappropriate use of meropenem as a target for intervention, including enhanced hospital interdepartmental communication, more rigours enforcement policies, continuous monitoring and evaluation processes, and the integration and use of electronic decision support tools[106] within the existing patient information systems. These discrepancies collectively underscore the need for IPC strategies specifically tailored to combat distinct challenges posed by CROs. Moreover, our findings suggest the need for strategic reassessment of IPC measures, emphasising the importance of addressing the unique challenges posed by CROs, alongside other hospital-acquired pathogens. Establishing coordinated AMR surveillance across all healthcare facilities in Fiji is imperative. This entails integrating passive surveillance techniques, utilising available data, and implementing targeted surveillance initiatives to collect data and isolates specifically for AMR surveillance. Moreover, the

establishment of a reference laboratory equipped with cost-effective and sustainable sequencing platforms, such as those from Oxford Nanopore Technology,[107] would benefit Fiji. This reference laboratory would play a pivotal role in establishing genome surveillance, seamlessly integrating with epidemiological data to enhance AMR monitoring and management. Ultimately, addressing the complex challenges posed by CROs requires a multifaceted approach that encompasses enhanced IPC strategies, targeted interventions, and coordinated AMR surveillance efforts. By implementing these measures, Fiji can more effectively combat the spread of CROs and safeguard public health across healthcare settings and the broader community.

Contributors

Conceptualization, S.C.B., J.A.C., J.E.U.; writing – original draft preparation, S.C.B.; writing, review and editing, S.C.B., S.M., V.S., S.S., J.L., S.S.S., S.V.D., L.V.B., S.K., A.L., D.W., Y.S., J.A.C., J.E.U.; collection of samples from CWMH, S.M., S.V.D., J.M.; collection of data from CWMH, S.M., S.C.S., S.V.D., L.V.B., N.V., S.K.; collection of samples from LTKH, V.S., J.L., collection of data from LTKH, V.S., J.L., L.V.B., Y.S.; collection of sample from LBSH, S.S., V.S.; collection of data from LBSH, S.S., V.S., A.L.; interpretation of data from CWMH, LTKH, and LBSH, S.C.B., D.W., J.A.C., J.E.C.; funding acquisition, S.C.B., D.W., J.A.C., J.E.C. Every author (except N.V. – deceased) involved in this study has given their consent to the version of the publication that is set to be published.

Data sharing statement

Access to the whole genome sequencing data from this study is available via the Sequence Read Archive (SRA) at NCBI, under the BioProject accession number PRJNA1096343.

Declaration of interests

The authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanwpc.2024.101095.

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