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Decade of Carbapenemase-Producing Enterobacterales in New Caledonia: Integrative Surveillance Though Genomic, Phenotypic and Clinical approaches

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Abstract

Carbapenemase-producing Enterobacterales (CPE) have been identified by the World Health Organization as global priority pathogens. The dissemination of these bacteria and outbreaks within healthcare facilities are of serious concern. This study investigated the transmission patterns and genetic characteristics of CPE isolated in New Caledonia from 2013 to 2022. The isolates were identified and characterized both phenotypically and whole-genome sequencing (WGS). In total 214 CPE were isolated: 199 non duplicate clinical isolates from 164 patients and 15 from hospital environmental surface. The most common genera in clinical samples were *Enterobacter* (34%) and *Klebsiella* (25%), with 194 isolates (98%) carrying IMP-type carbapenemase. WGS of 89 isolates revealed the dominance of the bla_{IMP-4} carbapenemase gene, found in 82 isolates. The bla_{IMP-4} was primarily predicted to be carried by IncL/M-type plasmid, found in 69% of the sequenced isolates. Our work revealed the circulation of 12 bacterial clusters with 61 strains involved in outbreaks or persistent over time. Genomic, phenotypic and clinical approaches identified 12 distinct outbreaks involving IMP producers. These results highlight the importance of studying plasmid transmission to better prevent silent spread of CPE. Ultimately, this study provides new guidelines for limiting the clinical spread of CPE in New Caledonia.

Introduction

The World Health Organization (WHO) has recognized antimicrobial resistance (AMR) as a major threat to global health [1]. Among mechanisms of antibiotic resistance, carbapenem resistance is particularly concerning, and carbapenemase-producing Enterobacterales (CPE) are categorized into critical groups to guide research and development and public health interventions into the 2024 WHO Bacterial Priority Pathogens List [2].

The advent and availability of genomic approaches have led to substantial advancements in comprehending antibiotic resistance genes dissemination [3,4]. ISO-certified genomics workflow for identification and surveillance of antimicrobial resistance are developed for clinical and public health microbiology [5]. Genomic data provide additional information, specifically on the identification of Antibiotic Resistance Genes (ARGs) and plasmids carrying these genes, complementing to the phenotypic approaches typically used in medical laboratories. In Enterobacterales, carbapenem resistance can be driven by the acquisition of carbapenemase-encoding genes, including Ambler class A serine β-lactamases (bla_{KPC}, bla_{GES}), metallo β-lactamases (bla_{NDM}, bla_{VIM}, bla_{IMP}) and carbapenem-hydrolysing class D β-lactamases ($bla_{OXA-48-like)}$ [6]. These genes are often carried on plasmids, which can be horizontally transmissible between Enterobacterales driving the rapid spread of resistance [7]. In addition, these plasmids often carry other resistance genes, making the bacteria extremely drug resistant and limiting treatment options.

New Caledonia (NC), a French archipelago located in the tropical southwest Pacific Ocean, is not spared by diffusion of CPE. Notably, since the implementation of CPE surveillance on the territory (2004, two major CPE outbreaks have been identified using epidemiological investigations in the Centre Hospitalier Territorial (CHT), the main hospital in NC: one in the Intensive Care Unit (ICU) in 2017 and another one in the Neonatal Intensive Care Unit (NICU) in 2018 [8]. Although NC's population is relatively isolated, there are links notably with Australia, the main site of many medical evacuations (Med Evac), and mainland France, where many population transfers occur. In this archipelago, IMP-type carbapenemase-producing isolates were the first CPE identified in 2013. Since then, the number of IMP-type carbapenemase) carriers increased from 2013 (4 isolates) to 2018 (33 isolates found) [8]. This carbapenemase, encoded here by the bla_{IMP-4} gene, is prevalent in the Asia-Pacific region [9–11] and has become particularly dominant in Australia [12–15].

In this study, we combined epidemiological and genomic analyses of all CPE cases detected in NC between July 2013 and December 2022 to provide a comprehensive overview of the local epidemiology of CPE. Clinical and epidemiological data, along with screening test results, were collected for each patient. Suspected outbreaks were first identified through epidemiological investigations: hygiene control investigations and routine microbial analyses. Then, whole-genome sequencing (WGS) was performed to identify ARGs, as well as associated plasmids and integrons, and to confirm suspected outbreaks.

Results

1. Patients’ characteristics and corresponding isolates

As shown in Table 1, out of the 164 patients, 57 (34%) of isolates came from clinical samples from patients with symptoms, whereas 107 (66%) where obtained from screening samples (contacts). Figure 1a presents this proportion along the years. Among the clinical samples, 14 (9%) were from blood cultures, 22 (13%) from urine samples, 5 (3%) from respiratory samples, and 16 (10%) from other sources, mainly abscesses. Ninety-nine patients (60%) were males and 65 (40%) were females. One hundred twenty-five patients (78%) were from the CHT and 30 (18%) from other external laboratories. Among the 125 patients from CHT, 51 were admitted in ICU (31%), 18 (11%) in the cohorting unit 7 (4%) in pediatrics, 14 (9%) in NICU, 11 in nephrology and 24 in other departments (Fig. 1b). Following a significant increase observed in 2017 and 2018 (Fig. 1a and 1b), CPE incidence between 2019 and 2022 was relatively stable (13.30 for 100,000 hospitalization-days and 12.63 for 100,000 hospitalization-days respectively). The clinical data, collected for 118 out of 125 patients admitted in CHT are summarized in Table 2. The median hospital stay before CPE isolation was 19.5 days [interquartile:10–38]. Fifty three percent of patients had been hospitalised for an infectious condition, 78% had been in the ICU, and 87% had received antibiotics prior CPE detection.

Table 1
Characteristics of samples from 164 patients

Characteristics		***No.(%)***
Sex	Male	99(60.3)
	Female	65(39.6)
Age	< 30	32(19.5)
	30–60	47(28.6)
	> 60	85(51.8)
Ward	ICU*	51(31.1)
	External laboratory	30(18.2)
	Other *	24(14.6)
	Cohorting unit*	18(10.9)
	NICU*	14(8.5)
	Nephrology*	11(6.7)
	Pediatrics*	7(4.2)
	CHN	5(3.0)
	CHS	4(2.4)
Origin	Fecal swab	107(65.2)
	Urines	22(13.4)
	Other	16(9.7)
	Blood	14(8.5)
	Respiratory	5(3.0)
Ward suspicion	Yes	72(43.9)
	No	97(56.1)
Spread events	2017-ICU	17(23.6)
	2018-NICU	10(13.8)
	2018-ICU	6(8.3)
	2018-Pediatrics	6(8.3)
	Other clusters in ICU	17(23.6)
	Other clusters outside of ICU	16(22.2)

Services marked with a star (*) are associated with CHT. Services without a star are from other hospitals (CHN, CHS) or external laboratories.

Table 2
Characteristics of the 118 clinical data recovered hospitalized at the CHT

		No(%)
Lenght of stay (days)		
	< 7	21(17,80)
	7–30	60(50,85)
	> 30	37(31,36)
Medical history		
	Surgery	58(49.1)
	None	39(33.0)
	Diabetes	33(27.9)
	Chronic obstructive Pulmonary Disease	14(11.8)
	Tumor	13(11.0)
	Med Evac	13(11.0)
	Travel	10(8.4)
	Chemotherapy	5(4.2)
ICU		
	Yes	92(77.9)
	No	26(22.0)
Lenght of stay in ICU (days)		
	< 7	25(27.1)
	7–30	52(56.5)
	> 30	15(16.3)
Risk factor		
	Antibiotics	103(87.2)
	Catheter	79(66.9)
	Mechanical ventilation	62(52.5)
	Multi-drug resistant bacteria carrier	38(32.0)
	None	9(7.6)
Death		
	Yes	20(16.9)
	No	98(83.0)

2. Phenotypic and genomic profiles of CPE isolates

A total of 199 CPE strains were collected from the 164 patients between July 2013 and December 2022. The predominant genera identified using MALDI-TOF were *Enterobacter* (34%), *Klebsiella* (25%), *Citrobacter* (18%), *Escherichia* (12%) and *Serratia* (8%; Fig. 1c). Among these isolates, 194 strains (98%) were identified as possessing an IMP-type carbapenemase (Fig. 1d).

Additionally, we included 15 IMP-carrying isolates from hospital environmental surfaces, belonging to the genera *Enterobacter* (n = 7), *Serratia* (n = 7) and *Klebsiella* (n = 1). These isolates were collected during three suspected large outbreaks (2017 in ICU, 2018 in both NICU, and in Pediatrics), and two suspected small outbreaks (2020 in Nephrology 1 and 2). AST was conducted on 130 CPE isolates (119 from patients and 11 from hospital environmental surface) with clinical isolates results detailed in Fig. 2. All clinical isolates were resistant to the tested third generation cephalosporins (3GCs; cefotaxime, ceftazidime) except for one *Citrobacter* (CLIN139, isolated from a blood culture) and one *Klebsiella* producing an OXA-48 carbapenemase (CLIN189, isolated from a cutaneous wound). Notably, 25/119 isolates (21%) showed susceptibility to at least one carbapenem (ertapenem or imipenem). In addition, 57 out of 119 clinical isolates (48%) were susceptible to ciprofloxacin and 71/119 (60%) were susceptible to trimethoprim-sulfamethoxazole. Susceptibility to the piperacillin-tazobactam combination was evaluated in 112 clinical CPE isolates, with 27 isolates (25%) showing susceptibility. This finding was confirmed for all 14 isolates tested using the Etest®. Notably, all clinical *Serratia* isolates tested were susceptible to piperacillin-tazobactam (n = 11).

WGS performed on the selection of 89 representative CPE isolates throughout the study period allowed us to precisely identify 19 species using TYGS (Supplementary Table 1). The resistance gene annotation revealed the dominance of the IMP-4 producers (82/89). For two old isolates (CLIN02 and CLIN04) and CLIN61, the bla_{IMP-4} was not identified in our sequencing, but these strains had been confirmed using immunochromatographic assays during routine analysis Moreover, across all isolates, bla_{IMP-4} gene was consistently associated with a class 1 integron structure (Supplementary Table 1). This class 1 integron (with bla_{IMP-4} carbapenemase-encoding gene) was predicted mainly on an IncL/M type plasmid associated with the AA002 MOB_typer primary cluster (61/89 isolates, 69%) (Table 3, Figs. 3 and 4a). Interestingly, this IncL/M plasmid replicon was also found in 6 isolates in which the bla_{IMP-4} gene was predicted on other plasmids. This plasmid was identified in isolates from the following genera: *Enterobacter* (n = 29), *Serratia* (n = 18), *Klebsiella* (n = 15), *Escherichia* (n = 4), *Citrobacter* (n = 3). Additionally, 10 of these bla_{IMP-4}/IncL/M CPE were isolated from the hospital environmental surface (Table 3, Fig. 3). Other ARGs such as bla_{TEM-1B}, *aac(3)IId, catB3, mph(A), qnrB* and *sul1* were predicted to co-occur on this plasmid (Fig. 4a). The bla_{IMP-4} carbapenemase gene was also predicted for 9 CPE to be carried on an IncC type plasmid (AA860 MOB_typer primary cluster) found in *Citrobacter* (n = 1), *Enterobacte*r (n = 4) and *Klebsiella* (n = 4) with two CPE isolated from the hospital environmental surface (Table 3, Fig. 3). The same ARGs described above were predicted on this plasmid for these CPE (Fig. 4a). In addition, according to MOB_type prediction, 3 CPE harboured the bla_{IMP-4} gene on an IncHI2A-type plasmid (AA739 MOB_typer primary cluster) (Table 3, Fig. 3). Finally, bla_{NDM-1} was detected in two *Escherichia coli* isolates (CLIN01 and CLIN116) predicted to be carried on a IncC-type plasmid (AA860 MOB_typer primary cluster) within a class 1 resistance integron structure and in two *Providencia rettgerii* isolates (CLIN111 and CLIN114) on an undefined incompatibility plasmid (AA855 MOB_typer primary cluster) also within a class 1 resistance integron structure. Notably, in CLIN114 isolate, bla_{IMP-4} and bla_{NDM-1} were predicted to be collocated on the same IncC type plasmid (AA860 MOB_typer primary cluster).

Table 3
Characteristics of plasmids found in sequenced isolated

MOB_typer primary cluster	Plasmid Type	predicted_mobility	Carbapenemase predicted	Genera	Sample	Implicated in suspected spread event
AA002 (n = 68)	IncL/M	conjugative except non mobilizable for CLIN05 ; CLIN102 ; CLIN32 ; CLIN61 ; CLIN95	*bla*$_{IMP-4}$ (n = 61)	*Citrobacter* (n = 3) *Enterobacter* (n = 29) *Escherichia* (n = 3) *Klebsiella* (n = 15) *Serratia* (n = 18)	Infectious sample (n = 23) Screening sample (n = 35) Hospital Environmental sample (n = 10)	2017-ICU (n = 10) 2018-Digestive Surgery (n = 1) 2018-NICU (n = 11) 2018-Pediatrics (n = 10) 2018-ICU (n = 6) 2019-ICU 1 (n = 2) 2019-ICU 3 (n = 2) 2020-Nephrology1 (n = 2) 2020-Nephrology2 (n = 1) 2021-Cohorting unit (n = 1)
AA855 (n = 2)	Rep_cluster_1506: CLIN114 Rep_cluster_1220: CLIN11	conjugative: CLIN 114 non mobilizable: CLIN 111	*bla*$_{IMP-4}$ (n = 1) *bla*$_{NDM-1}$ (n = 2)	*Providencia* (n = 2)	Screening sample (n = 2)	2022-ICU (n = 2)
AA627 (n = 7)	IncC	mobilizable	*bla*$_{IMP-4}$ (n = 1)	*Citrobacter* (n = 7)	Infectious sample (n = 3) Screening sample (n = 4)	2021-Cohorting unit (n = 3)
AA275 (n = 3)	IncFIB, IncFII, rep_cluster_2183	conjugative	*bla*$_{IMP-4}$(n = 3)	*Klebsiella* (n = 3)	Infectious sample (n = 2) Screening sample (n = 1)	2017-ICU (n = 2) 2019-ICU (n = 1)

MOB_typer primary cluster	Plasmid Type	predicted_mobility	Carbapenemase predicted	Genera	Sample	Implicated in suspected spread event
AA860 (n = 11)	IncC	conjugative	*bla*$_{IMP-4}$ (n = 9) *bla*$_{NDM-1}$ (n = 2)	*Citrobacter* (n = 1) *Enterobacter* (n = 4) *Escherichia* (n = 2) *Klebsiella* (n = 4)	Infectious sample (n = 5) Screening sample (n = 4) Hospital Environmental sample (n = 2)	2018-Digestive Surgery (n = 1) 2019-ICU 1 (n = 2) 2019-ICU 3 (n = 1) 2020-Nephrology 1 (n = 1) 2020-Nephrology 2 (n = 1)
AA739 (n = 9)	IncHI2A	conjugative	*bla*$_{IMP-4}$ (n = 3)	*Enterobacter* (n = 8) *Escherichia* (n = 1)	Infectious sample (n = 3) Screening sample (n = 4) Hospital Environmental sample (n = 2)	2018-Pediatrics (n = 2)
AB130 (n = 5)	Col(VCM04)	conjugative	*bla*$_{IMP-4}$ (n = 3)	*Citrobacter* (n = 5)	Infectious sample (n = 2) Screening sample (n = 3)	2021-Cohorting unit (n = 2)

The relationships of the sequenced isolates are detailed in Fig. 3 and core genome SNP analysis are reported in Supplementary Table 2. Clonal relationships between strains (core genome SNP threshold < 21) are reported in several STs (ST98, ST254, ST104, ST125, ST536) on in each *Serratia* groups. Unfortunately, not all strains within each ST have SNPs below 21. Using plasmid content and AST profiles (considering the random errors of the VITEK and clonal relationships based on WGS, we considered identical ASTs if they differed by no more than one result) and hygiene investigations (link between patients), (Fig. 4) we were able to regroup strains between clusters. We identified 12 clusters (core genome SNP threshold < 50) encompassing 64 CPE isolates (72%; Supplementary Table 2). Among them, the three main clusters were associated with the genera *Serratia* and *Enterobacter*. The *Serratia* cluster group III (mean SNPs: 16.75, max: 33, min: 1) identified as the recently described *S. sarumanii* [16] associated with also IncL/M type plasmid. The latter group includes 10 CPE isolates from outbreak at the NICU in 2018. The *Enterobacter* ST104 cluster (mean SNPs: 23.86; max: 33; min: 11), identified as *E. hoffmanii* according to the latest nomenclature [17], includes 9 resistant strains harbouring the IncL/M type plasmid, isolated during two outbreaks at the ICU in 2017 and 2018. The *Citrobacter* ST98 cluster (mean SNPs: 19.16; max: 32; min: 1); identified as *Citrobacter freundii* with however several plasmids (IncL/M, IncC, Col (VCM04)) and found at several time and ward during the study.

3. CPE clustering

Based on hygiene investigations, 4 large outbreaks (n ≥ 6) and 13 small outbreaks (n < 6) were initially suspected over the study period. The 13 small outbreaks between 2018 and 2022 involved 2 to 5 CPE isolates each. Six of these episodes occurred in the ICU, and two in the Nephrology ward, where CPE were also isolated from the hospital environmental surface (Table 3). The remaining episodes occurred sporadically across other wards. Taken together, WGS, AST and epidemiological investigations confirmed the 4 large outbreaks identified on epidemiological grounds and 5 of the 13 small outbreaks (Fig. 5). Two suspected small outbreaks were not confirmed and six could not be investigated because the isolates were not preserved. In addition, 1

isolate initially labelled as isolated cases (CLIN104) was assigned to one small outbreak (Cohorting unit 2021). These analyses also uncovered 3 small outbreaks that had not been previously suspected (Fig. 5). Interestingly, *E. hoffmanii* ST104 was found in the two large outbreaks in ICU (Figs. 4 and 5) with one strain isolated from the hospital environmental surface in 2017. Moreover, IncL/M plasmid type was implicated in the four large outbreaks (Fig. 4). For example, during the large outbreak in Pediatrics in 2018, four genera (*Citrobacter, Enterobacter, Klebsiella* and *Serratia*) are implicated harbouring IncL/M type plasmid predicted as carrier of bla_{IMP-4} (Fig. 5).

Discussion

Since the initial detection of the first CPE in NC (*Klebsiella pneumoniae* IMP on a catheter in a patient admitted to the CHT's pneumology unit) in July 2013, the number of patients carrying CPE has increased, peaking in 2018 with outbreaks observed in the ICU and the NICU [8]. This increase is also observed in Asia Pacific region [18]. However, since these spread events, we observed a stabilisation in the situation and even a reduction in the number of cases. This situation can be partially explained by the COVID-19 pandemic with a zero-covid strategy applied in New Caledonia with a rapid border closure in New Caledonia, reinforcement of hygiene measures and reduction of patients number in the hospital [19]. All the measures put in place within the hospital, such as the introduction of a cohorting unit, systematic screening of patients before and after MedEvac from Australia (2015), and automated email alerts when the admission of an identified CPE carrier occurs (2017), could limit a potential increase of CPE in New Caledonia [8]. Although the incidence has stabilised over the last four years in NC, the risk of CPE spreading should not be overlooked, hence the need for increased surveillance to prevent new outbreaks. Indeed, our findings indicate that increase of CPE was associated with spread events involving multiple strains, as well as the circulation of dominant carbapenemase-encoding plasmids among various species and genera.

In the Western Pacific region, implementing pathogen genomics surveillance is an evidence [20]. Regarding the circulation of several strains, in NC we identified through WGS the persistence of *K. pneumoniae* ST16, also reported in Fiji [21] which is globally recognized as a CPE sublineage with critical public health implications [22]. Additionally, we isolated *Citrobacter freundii* ST98 between 2014 and 2021. This ST is also known to include carbapenemase producers and to be present in several countries [23]. In NC, the first isolates of these two lineages were detected in samples from external laboratories before outbreaks at the CHT (ICU 2017; Cohorting unit 2021). We might hypothesize that these STs were circulating within the community prior to their identification at the hospital. Even if we were as exhaustive as possible (the CHT is the reference centre for CPE in New Caledonia), very few community strains were collected. It would therefore be interesting to extend this study outside hospitals to get a more accurate picture of the circulation of CPE in NC.

In addition to the characterization major STs, the WGS approach enabled to establish connections between several outbreaks. In our study a threshold at 50 core genome SNPs, was proposed to discriminate hospital outbreak. This is in agreement with the current literature where thresholds range from 21 [24] to 80 core genome SNPs [25] to discriminate hospital outbreaks depending on the studied bacterial genus.

We found that *E. hoffmanii* ST104 and related strains were implicated in ICU outbreaks in 2017 and 2018. The presence of an isolate in the hospital environmental surface (CLIN37) suggests a single outbreak. *E. hoffmanii* ST254 were implicated in outbreaks in ICU and Pediatrics in 2018 suggesting link between these two events. Interestingly, this ST was previously reported to be implicated in the widespread of transmission of bla_{IMP-4} in Australia [26]. In the collection, our results showed that the carbapenemase-encoding genes are primarily associated with an IncL/M type flanking structure. This replicon was implicated in four large and small outbreaks and isolated cases highlighting its significance in New Caledonia. The putative IncL/M plasmid reconstructed with MOB-suite software was predicted to be conjugative and found in 5 different bacterial genera in our collection, emphasizing its notable diffusion potential [27]. It is therefore essential to enhance monitoring and control of this plasmid, as limiting its spread will help to prevent further CPE outbreaks in NC. Although our short read sequencing data do not allow us to determine whether the bla_{IMP-4} gene was localized on a particular plasmid when multiple plasmid structures are found within the same strain, our results showed that in all cases the bla_{IMP-4} gene was located on a class 1 resistance integron. This structure is also widely recognised in Australia for harbouring the bla_{IMP-4} gene [28]. Some CPE contain several entities of the

plasmids described in Table 3, which are likely to harbour the *bla*$_{IMP-4}$ gene. However, short-read sequencing often results in the integron being found in small contigs, making it difficult to pinpoint the exact *bla*$_{IMP-4}$-carrying plasmid. Long reads sequencing is planned to study the genetic environment around these integrons and to confirm the position of the ARGs on a given plasmid. Our results highlight the value of studying plasmids, particularly during dissemination episodes involving different strains but the same plasmid (Pediatry – 2018).

Long reads sequencing is currently being implemented at the CHT. Given the results of genomics in this work (dominance of IncL/M) and the speed with which results can be obtained (few hours in Madueno et al., (2024)[29]), its implementation appears to be essential to limit the spread of CPE in New Caledonia, particularly if an outbreak is suspected. With plasmids, this time could be further reduced, for example by displaying plasmid profiles on an electrophoresis gel before sequencing. This plasmid gel migration technique could be implemented within the hospital to provide additional, less precise but more timely information for rapid decision-making. This method may demonstrate intra- and interspecies horizontal transmission and thus help hygiene operational teams. Also, once the plasmids have been circularised, RFLP (restriction fragment length polymorphism) approaches combined with separation by gel electrophoresis or plasmid-specific PCR could be used in the future.

We conducted a retrospective epidemiologic and genomic study, confirming the dominance of IMP-type carbapenemase in NC. This carbapenemase, encoded here by the *bla*$_{IMP-4}$ gene, is prevalent in the Asia-Pacific region [9–11] and has become particularly dominant in Australia [12–15]. Our study revealed that in over 68% of sequenced isolates, *bla*$_{IMP-4}$ was predicted to be carried on an IncL/M type plasmid (AA002 MOB_typer primary cluster). This plasmid type has also been identified in Australian outbreaks described in Sydney [14,28,30]. Interestingly, using MOB-typer prediction, the nearest IncL/M type plasmid (AA002 MOB_typer primary cluster) plasmids obtained using mash distance are reported in strains isolated from The Alfred Hospital in Sydney[14]. This hospital is particularly associated with medical evacuations of patients from NC. Of the 13 patients who tested positive upon return from these medical evacuations, seven isolates were sequenced. Six of these were found to carry on a IncL/M plasmid, and two of them were linked to local outbreaks (ICU-2017 and Pediatrics-2018). Several clones, such as *C. freundii* ST98 with AA860 MOB_typer primary cluster[31], *K. pneumoniae* ST20 with AA02 MOB_typer primary cluster [14], with AA739 MOB_typer primary cluster or AA860 MOB_typer primary cluster [5] have been previously reported in Australia. These findings suggest potential epidemiological links between Australia and NC as already reported for Vancomycin resistant *Enteroccocus* [8] or *Corynebacterium diphtheriae* [32]. In addition, one patient repatriated from Australia was identified with an *Enterobacter xiangfangensis* (CLIN71; formerly named *E. hormaechei* subsp. *xiangfangensis*) with an IncHI2A type plasmid (AA739 MOB_typer primary cluster), which has been previously reported in this country [17,33]. Although our WGS panel included a wide variety of bacterial strains, those belonging to the genus *Enterobacter* were predominant. The *bla*$_{IMP-4}$ gene is particularly prevalent in this genus in Australia [28,34]. Notably, *E. xiangfangensis*, the main species in our dataset, has been involved in an outbreak [33]. In contrast, *Enterobacter chengduensis* isolates are rarely described as carbapenemase producer in the literature. Apart from CLIN07 (ST414), the only identified *bla*$_{IMP-4}$ carriers have also been reported in neighbouring Australia [35]. Further genomic analysis and collaboration are essential to confirm the microbial and antibiotic resistance connections between our two territories, but these suspected links with Australia at various points underscore the importance of screening patients returning from medical evacuations.

The data presented in this study underscores the importance of using screening agar to effectively detect CPE strains carrying the *bla*$_{IMP-4}$ gene. Goire *et al*. (2016) demonstrated in Australia that screening media such as Brilliance™ CRE Agar are insufficiently sensitive for detecting *bla*$_{IMP-4}$, unlike other CPE types [15]. For *bla*$_{IMP-4}$ endemic situation, the authors recommended combining ESBL screening media with the CarbaNP test and in-house *bla*$_{IMP-4}$ real-time PCR. Here, we also confirmed the low level of resistance to carbapenems in strains carrying the *bla*$_{IMP-4}$ gene [9,33], which are however well captured when using ESBL agar plate. Our study reported a 15% fatality in infected patients, lower than the rates reported in the literature [36,37]. This low fatality rate might be attributed to the limited number of associated resistances, with retained susceptibility to cotrimoxazole (59%) and fluoroquinolones (47%). Additionally, the low level of resistance to carbapenems conferred by *bla*$_{IMP-4}$ allowed the use of certain carbapenems in combination with cotrimoxazole or fluoroquinolones [38,39].

The introduction of the cohorting unit to isolate patient seems beneficial in NC, as the number of CPE cases has stabilised, and no further outbreaks have occurred in CHT since its establishment. However, our WGS data are more concerning, as we detected *Citrobacter freundii* ST98 in several patients associated with this unit, raising questions about the efficacy of the cohorting system [40]. Many clones detected from patients were also found in environmental isolates, suggesting an important role of the hospital environment in their spread and persistence. In our study, most of *Serratia* isolates originated from the hospital environment, a finding consistent with previous reports highlighting the relevance of environmental reservoirs in ICU [41] or NICU [42] in the endemicity of opportunistic pathogens such as *S. marcescens*. Based on these results and litterarure[43], a more in-depth analysis of the hospital environmental surface and notably cohorting unit environment should be considered.

To summarise, we integrated phenotypic, genomic and epidemiological analyses to investigate the transmission pathways of CPE in NC. Our findings revealed that multiple clones were responsible for outbreaks in ICU and NICU and identified an IncL/M plasmid as probable vehicle for the dissemination of bla_{IMP-4} in NC since 2013. These results highlight the importance of studying plasmid transmission to better prevent the spread of CPE and support the concept that AMR is a silent pandemic.

Methods

We conducted a retrospective study to analyse all CPE isolates collected in NC from July 2013 (first isolate detected) to December 2022. NC's healthcare infrastructure includes three public hospitals and one private clinic for patients' hospitalization. These facilities operate under the authority of the Department of Health and Social Affairs (DASS) of New Caledonia Government, which has decision-making powers in health-related matters, a feature that is consistent with the administrative structure in France. Study was conducted in the CHT, the main and reference hospital of the archipelago. It comprises 645 beds, 12 operating rooms, medical units, surgery units and intensive care units. The number of hospitalizations approximates 40,000 a year. Moreover, CHT laboratory is the reference laboratory for CPE in NC, where all suspected CPE isolates are sent for expertise. In order to prevent spread of CPE, patients with a CPE at the CHT are transferred in a cohorting unit by a dedicated medical team[8].

1. Workflow for CPE isolation at CHT

All patients admitted to the ICU, and those returning from a foreign hospital, or known previous carriers of CPE were systematically screened for CPE carriage. When a CPE is isolated in a patient, all contacts within the department were also screened. CPE screening involves using a faecal swab culture on ChromID® ESBL (bioMérieux, Marcy-l'Étoile, France), with results available 24 to 48 h after incubation at 37°C. All colony morphologies are identified using MALDI-TOF mass spectrometry (Microflex LT-MS, Bruker Daltonics). Subsequently, Enterobacterales were plated on Mueller-Hinton agar with different antibiotic discs: ertapenem (10µg), temocillin (30µg) and ceftolozane/tazobactam (30/10µg) inspired by the 2022 European Committee on Antimicrobial Susceptibility Testing [44]. Confirmation was performed using NG-CARBA-5® immunochromatographic assays (NG Biotech Laboratories, Guipry, France), which detects the major carbapenemase types (VIM, IMP, NDM, KPC, and OXA-48-like) [45,46]. Antimicrobial agent Susceptibility Testing (AST) was systematically conducted for clinical strains on VITEK-2® system (bioMérieux, Marcy l'Etoile, France) with AST N-234 or N-372 cards. Finally, carbapenem minimum inhibitory concentrations (MICs) can be confirmed using the Etest® method (bioMérieux, Marcy l'Etoile, France).

This approach was applied to all samples collected during the study and retrospectively to all CPE isolates available in our collection. The study included CPE strains from both carrier and infected patients, irrespective of the type of specimen collected, as well as environmental isolates (recovered from table surface and sink in the lavatory in patient rooms, or healthcare trolley) identified during a suspected outbreak for comparison. Furthermore, to ensure the accuracy and reliability of the results, all ASTs were interpreted using the EUCAST guidelines as updated in 2022 [44].

2. Collection of clinical data

We systematically recorded the origin of each sample, specifying the hospital ward or name of the external laboratory, and categorized the sample types into five mains groups: rectal swab, blood culture, urine, respiratory, and other. For all patients hospitalized at CHT, we collected clinical data relevant to the study and recognized as risk factors for CPE acquisition in other

studies [47]. Finally, we recorded whether the isolate was likely associated with a spread event. Suspected outbreaks were characterised by the incidental discovery leading to the detection of a secondary case, or the detection of several CPE in the same department at short period (less than one month). In this study, an outbreak was considered as a large outbreak if there were at least five secondary cases, otherwise, if there are fewer than 5 secondary cases, we considered this event as a small outbreak.

3. Whole-genome sequencing

A batch of 64 CPE (corresponding to first isolates in NC or suspected of belonging to outbreaks) isolated from 2013 to 2019 was submitted to WGS by the French National Reference Center of Antimicrobial Resistance using Pasteur P2M core facility. Sequencing was conducted using a NextSeq500 instrument (Illumina, San Diego, CA; 2 × 150-nucleotide paired-end protocol). Additionally, WGS was performed on 25 CPE (in order to complete the study period until 2022) collected between 2020 and 2022 by the Beijing Genomics Institute (BGI) Tech Solutions Hong Kong Co., Limited. Isolates were inoculated in Luria Broth medium and incubated overnight at 37°C. Genomic DNA was extracted from 1.5 ml of LB culture using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. DNA purity was determined using Nanodrop (ThermoFisher Scientific, Waltham, MA, USA) and agarose gel deposition. Paired-end libraries were prepared with an insert size of approximately 300 bp and sequenced on DNB-SEQ PE150 according to the supplier's protocol.

a. Genome assembly and quality assessment

Paired-end reads were *de novo* assembled using the fq2dna workflow V21.06, which also includes various steps such as trimming, error correction, contaminant removal, and polishing. (https://gitlab.pasteur.fr/GIPhy/fq2dna). For completeness and contamination analysis, CheckM (V1.1.3, taxonomy_wf at genus level) and QUAST version 5.2.0 [48]. were employed.

***b. In-silico* species identification and genomic screening**

Multi-locus sequence typing (MLST) was conducted using MLST software V2.19.0 (https://github.com/tseemann/mlst) and associated profiles provided by PubMLST [49] or BIGSdb-Pasteur (*Klebsiella pneumoniae* species complex). To evaluate ARGs, Abricate software V1.0.1 was employed (https://github.com/tseemann/abricate; --minid 70 --mincov 90 --db resfinder) [50]. The prediction of genomic location of ARGs, plasmid identification and typing were performed using MOB-suite V3.0.1 [27]. Furthermore, the identification of integrons carrying ARGs was conducted using IntegronFinder V2.0.2 [51].

c. Phylogeny and relationship between isolates

The initial phylogenetic relationships between isolates from the different compartments and sample sites were examined using the k-mer-based tool JolyTree V2.1 [52]. The associated tree was midpoint-rooted and annotated with iTOL V6 [53]. Species identification was achieved for each assembly through the utilisation of a digital DNA:DNA hybridization approach (dDDH cut-off ≥ 70.0% *formula* d_4) as facilitated by the Type Strain Genome Server (TYGS) [54]. Regarding *Enterobacter* genus, an additional analysis was performed using the approach and taxonomy proposed by Wu *et al.* with FastANI V1.33 (ANI cut-off ≥ 96.0%) [17]. Due to the significant genetic diversity of the sample and the need for precise comparison within clinical isolates, we conducted core genome-based phylogenetic analyses on isolates of the same sequence type (ST) with more than two assemblies. For each ST group, a good-quality reference genome was identified by using the online tool SimilarGenomeFinder against all public genomes in the BV-BRC resource center (V3.30.19a;) [55,56]. Mapping was performed using Snippy V4.6.0 against these selected references. The resulting core-SNP alignment was used to infer maximum-likelihood phylogenetic trees for each ST group using IQ-tree V2.1.4 [57], and recombination elements were removed with ClonalFrameML V1.12 [58]. Then, specific SNP differences matrixes were generated with snp-dists V0.8.2. As proposed by David *et al.*, (2019)[59], we used 21 core genome SNP as threshold for discrimination of clonal diffusion.

Declarations

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Author contributions

A.B. and J.C. designed the study, L.C., G.D, P.S. and J.C. delivered clinical data, A.B., M.K. and T.O. performed the microbiology experiments, S.B., R.A.B. and L.D performed the WGS experiments, A.B., F.P. and M.P. performed the bioinformatics analyses, P.S performed statistical analyses, A.B., S.B., R.A.B., L.D., F.V and J.C. purchased the funds, A.B., L.C and J.C. wrote the original manuscript, A.B.,G.D., A.Bi., T.R., R.A.B., L.D., S.B., P.S., M.P., F.V., C.G and J.C revised the manuscript. Every authors read and approved the final manuscript.

Data availibility

Access to the assembly data from this study is available on GenBank, under the BioProject ID PRJNA1146064.

Competing interests

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

Ethical approval

This work was approved by the CHT ethics committee (Avis N°3 2024).

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Figures

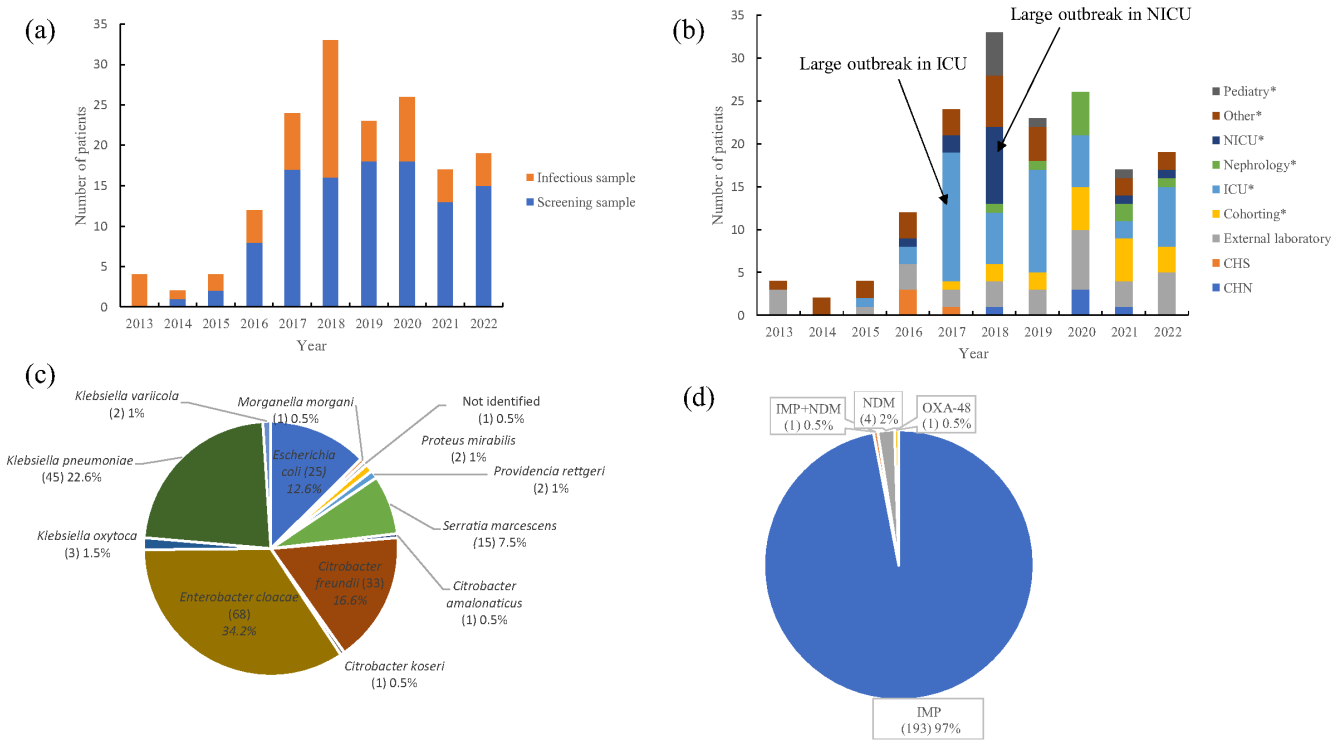


Fig. 1: **Number of CPE carriers and infected patients (n=164) during time according to the nature of the sample (a) or hospitalization ward (with two large outbreaks described by Colot *et al.*, (8) (b) and MALDI-TOF identification (c) and carbapenemase type (d) of the 199 CPE.** Services marked with a star (*) are associated with CHT. Services without a star are from other hospitals (CHN, CHS) or external laboratories.

Figure 1

See image above for figure legend.

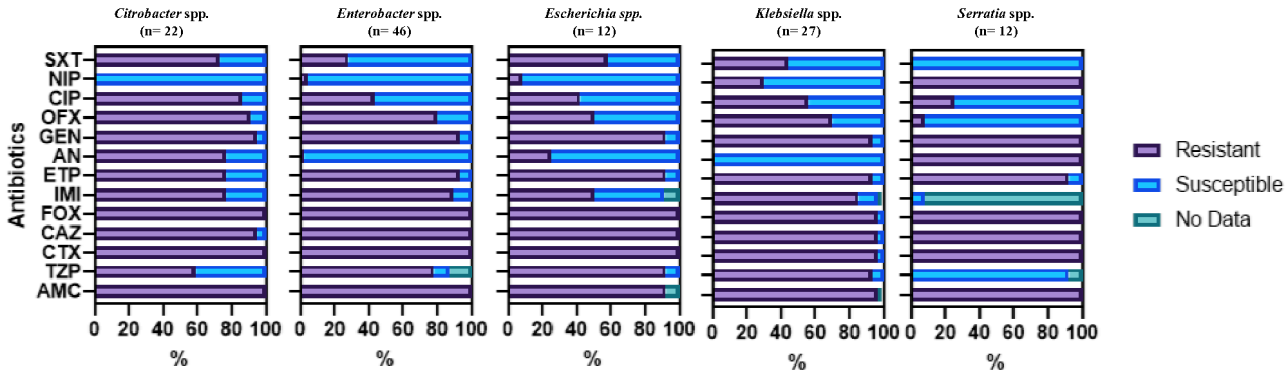


Fig. 2: **Antimicrobial agent susceptibility (AST) profiles of the 119 clinical CPE isolates according to each genus based on MALDI-TOF identification**. AMC: amoxicilin/clavulanic acid; TZP: piperacillin/tazobactam; CTX: cefotaxime; CAZ: ceftazidime; FOX: cefoxitin; IMI: imipenem; ETP: ertapenem; AN: amikacin, GEN: gentamicin; OFX: ofloxacin; CIP: ciprofloxacin; NIP: nitrofurantoin; SXT: trimethoprim/sulfamethoxazole.

Figure 2

See image above for figure legend.

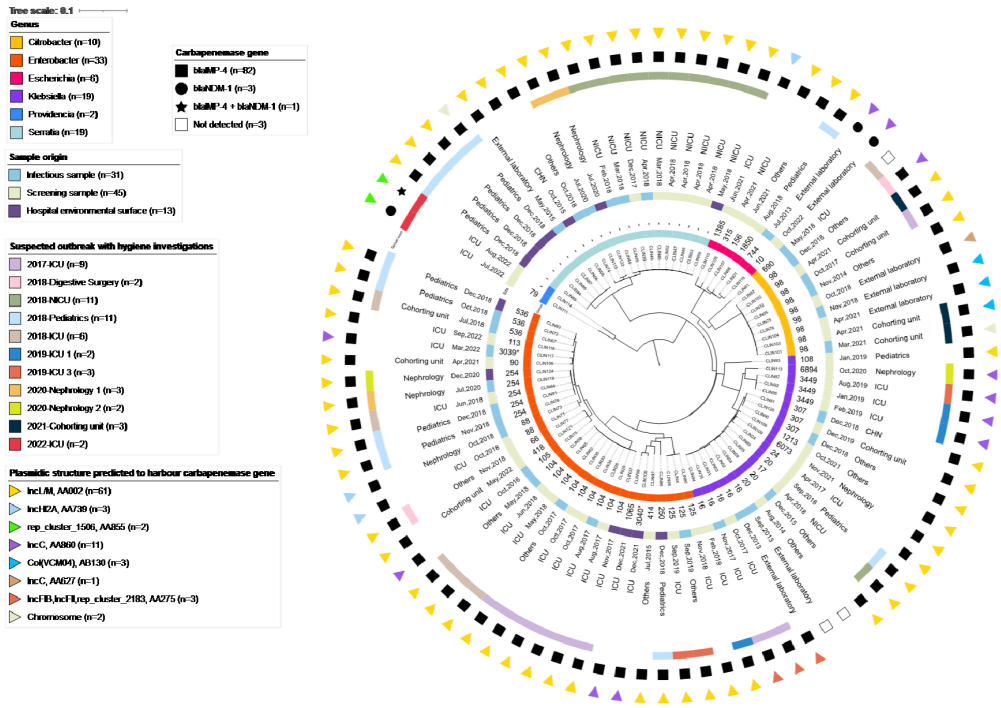


Fig. 3: **Phylogenetic tree of the 89 sequenced carbapenemase-producing Enterobacterales.** The tree was constructed using JolyTree, rooted at the midpoint and metadata were added with iTOL. For the circles, the legend is read from the centre circle (genus) to the outermost circle (outbreak suspected). The plasmid structure associated with the carbapenemase genes was predicted using MOB_Typer. Carbapenemase gene was predicted on a chromosome replicon for 3 isolates. No MLST pattern is available for *Serratia* spp., and other new ST profiles are indicated by an asterisk.

Figure 3

See image above for figure legend.

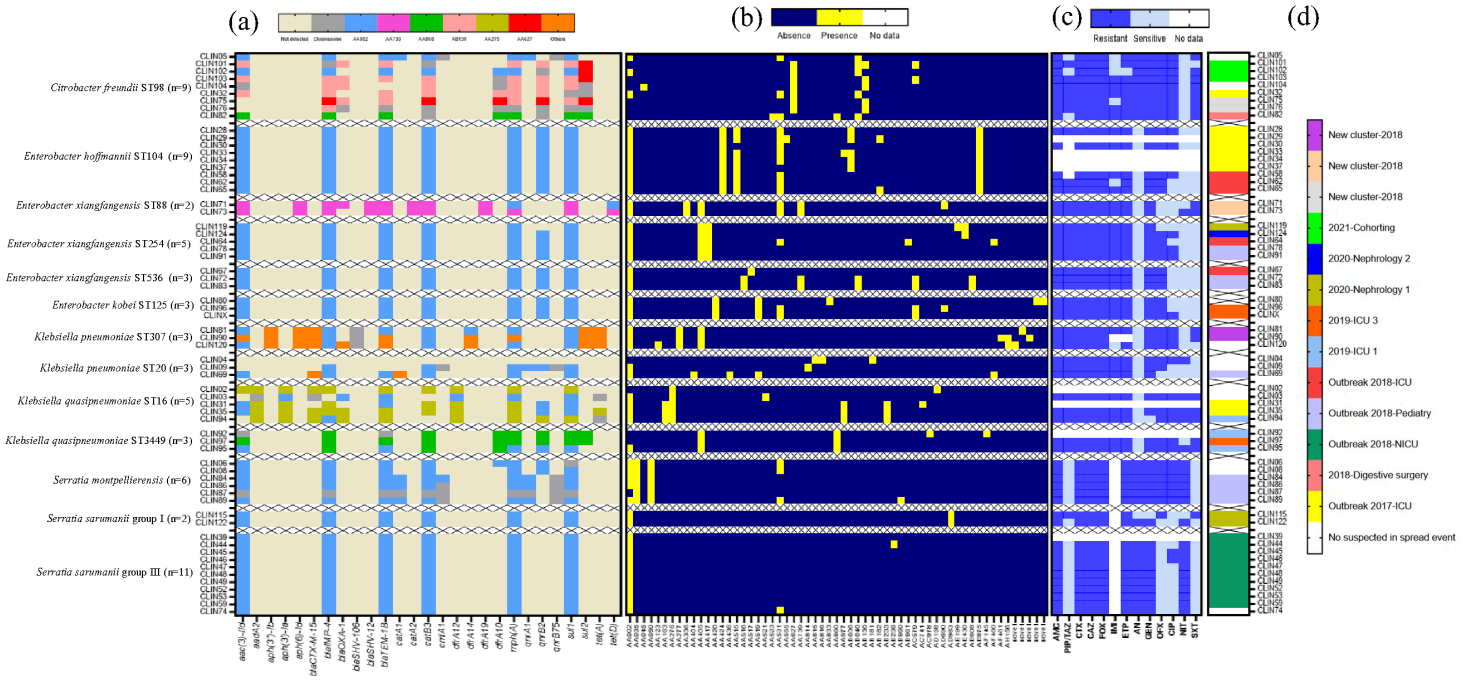


Fig. 4: **Predicted genomic localisation of antibiotic resistance genes (a), occurrence of plasmidic structure using MOB_Typer (b), antimicrobial agent susceptibility profil (c) and suspected outbreak based on hygiene investigations (d).** AMC: amoxicilin/clavulanic acid; TZP: piperacillin/tazobactam; CTX: cefotaxime; CAZ: ceftazidime; FOX: cefoxitin; IMI: imipenem; ETP: ertapenem; AN: amikacin, GEN: gentamicin; OFX: ofloxacin; CIP: ciprofloxacin; NIP: nitrofurantoin; SXT: trimethoprim/sulfamethoxazole

Figure 4

See image above for figure legend.

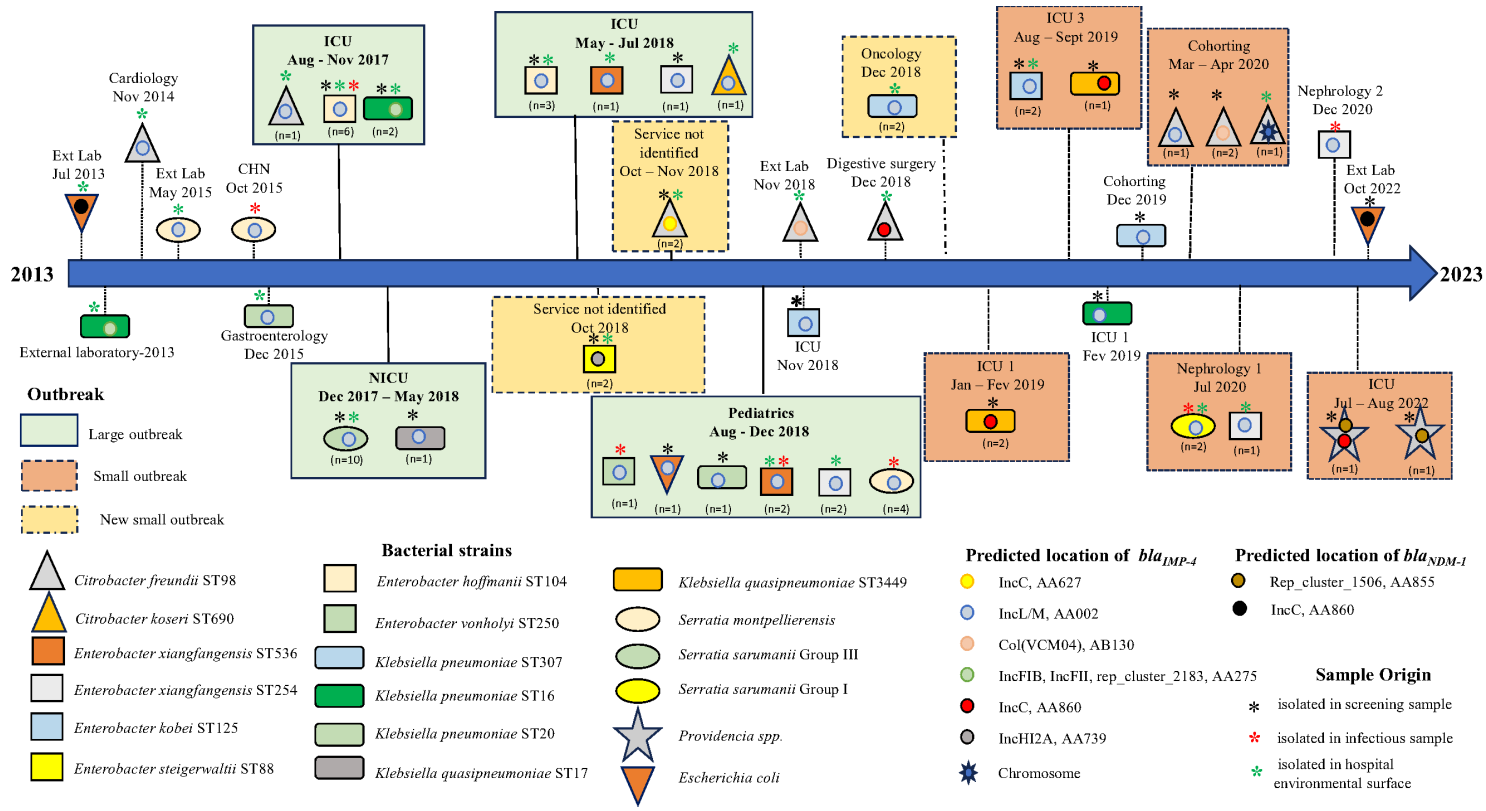


Fig. 5: CPE implicated in spread events or persistant during time and plasmids which harboured carbapenemase genes associated between 2013 and 2022 in New Caledonia. CPE found in isolated patients are represented without a box.

Figure 5

See image above for figure legend.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS2.docx