Search and Contain: Impact of an integrated genomic and epidemiological surveillance and response program for control of carbapenemase-producing *Enterobacterales*.

Courtney R. Lane (M Phil(App Epi))[1, 2], Judith Brett (BN)[3], Mark Schultz (PhD)[1, 2], Claire L. Gorrie (PhD)[1, 2], Kerrie Stevens (BAppSci)[1], Donna R. M. Cameron (BN)[1,4], Siobhan St George (M Phil(App Epi))[1], Annaliese van Diemen (FAFPHM)[4], Marion Easton (MPH)[4], Rhonda L. Stuart (FRACP)[5], Michelle Sait (PhD)[1], Anton Y. Peleg (PhD)[6,10], Andrew J. Stewardson (PhD)[6], Allen C. Cheng (PhD)[6,11], Denis W. Spelman (MBBS)[6,12], Mary Jo Waters (FRCPA)[7], Susan A. Ballard (PhD)[1], Norelle L. Sherry (MBBS)[1,2,8], Deborah A. Williamson (PhD)[1], Finn Romanes (FAFPHM)[4], Brett Sutton (FAFPHM)[4], Jason C Kwong (PhD)[2,8], Torsten Seemann (PhD)[2], Anders Goncalves da Silva (PhD)[1,2], Nicola Stephens (PhD)[2,4,9], Benjamin P. Howden (MD)[1,2,8]

[1] Microbiological Diagnostic Unit Public Health Laboratory and [2] Department of Microbiology & Immunology, The University of Melbourne at the Peter Doherty Institute for Infection & Immunity (Lvl 1, 792 Elizabeth St, Melbourne, Victoria, Australia, 3000).

[3] VICNISS Healthcare Associated Infection Surveillance Coordinating Centre, at the Peter Doherty Institute for Infection & Immunity (Lvl 2, 792 Elizabeth St, Melbourne, Victoria, Australia, 3000).

[4] Department of Health and Human Services, Victoria, Australia (Lvl 14, 50 Lonsdale St, Melbourne, Victoria, Australia, 3000).

[5] Monash Infectious Diseases, Monash Health, Monash Medical Centre (Lvl 3, 246 Clayton Rd, Clayton, Victoria, Australia, 3168).

[6] Department of Infectious Diseases, Alfred Hospital, and Central Clinical School, Monash University (Level 2, 85 Commercial Road, Melbourne, Victoria, Australia, 3004).

[7] Department of Microbiology, St Vincent's Hospital Melbourne (41 Victoria Parade, Fitzroy, Victoria, Australia, 3065).

[8] Department of Infectious Diseases, Austin Health (Lvl 7 Harold Stokes Building, Austin Health, Heidelberg, Victoria, Australia, 3084).

[9] University of Tasmania (Medical Science Precinct, ABC Building, Private Bag 34, Hobart, Tasmania, Australia, 7000).

[10] Infection and Immunity Program, Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University (19 Innovation Walk, Clayton, Victoria, Australia, 3800).

[11] School of Public Health and Preventive Medicine, Monash University (553 St Kilda Rd, Melbourne, Victoria, Australia, 3004).

[12] Department of Microbiology, The Alfred Hospital. (55 Commercial Road, Melbourne, Victoria, Australia, 3004).

Corresponding author:

Prof Benjamin P. Howden

Email: bhowden@unimelb.edu.au

Phone: +61 3 8344 5701

Microbiological Diagnostic Unit Public Health Laboratory

Doherty Institute for Infection & Immunity

792 Elizabeth Street, Melbourne, Victoria, Australia 3000

Summary: A state-wide multi-modal intervention for the control of Carbapenemase-producing *Enterobacterales* was associated with a significant increase in case ascertainment, with no rise in clinical infections. Timely prospective epidemiological and genomic surveillance identified numerous small local transmission networks permitting rapid response.

Abstract

Background: Multi-resistant organisms (MROs) pose a critical threat to public health. Population-based programs for control of MROs such as Carbapenemase-producing *Enterobacterales* (CPE) have emerged and evaluation is needed. We assess the feasibility and impact of a state-wide CPE surveillance and response program deployed in December 2015 across Victoria, Australia (population 6.5 million).

Methods: A prospective multi-modal intervention including active screening, carrier isolation, centralised case investigation and comparative pathogen genomics was implemented. We analyze trend in CPE incidence and clinical presentation, risk factors and local transmission over the program's first three years (January 2016 to December 2018).

Results: CPE case ascertainment increased over the study period to 1.42 cases/100,000 population, linked to increased screening without a concomitant rise in active clinical infections (0.45-0.60 infections/100,000 population, p=0.640). KPC-2 infection decreased from 0.29 infections/100,000 population prior to intervention to 0.03 infections/100,000 population in 2018 (p=0.003). Comprehensive case investigation identified putative overseas community acquisition. Median time between isolate referral and initial genomic and epidemiological assessment for local transmission was 11 days (IQR 9-14). Prospective surveillance identified numerous small transmission networks (median 2, range 1-19 cases), predominantly IMP and KPC, with median pairwise distance of 8 (IQR 4-13) single nucleotide polymorphisms; low diversity between clusters of

the same sequence type suggested genomic cluster definitions alone are insufficient for targeted response.

Conclusions: We demonstrate the value of centralised CPE control programs to increase case ascertainment, resolve risk factors and identify putative local transmission through prospective genomic and epidemiological surveillance; methodologies are transferable to low-prevalence settings and MROs globally.

Keywords: Antimicrobial resistance, Public Health Surveillance, Carbapenemase producing *Enterobacterales*, infection control, genomics

Introduction

Antimicrobial resistance (AMR) is a major threat to public health and patient safety. Carbapenemase-producing *Enterobacterales* (CPE) are considered one of the most serious classes of multi-resistant organisms (MRO), requiring immediate and aggressive public health action (1). Attributable mortality for invasive infections ranges from 29-75%, but improvement has not been observed despite development of novel therapies (2-6). Most concerningly, CPE have considerable epidemic potential, and have been responsible for numerous large clonal hospital outbreaks (7,8).

In Australia, CPE have rarely been identified and are usually associated with patients receiving overseas medical care in endemic countries, except for IMP-4 which has established low-level endemicity in some states (9,10). Limited outbreaks of CPE within healthcare facilities have also been described (10-13). Between 2012 and 2014 an outbreak of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacterales* occurred in Victoria (population 6.5 million) affecting multiple healthcare settings and facilities (14). A state-wide outbreak investigation coordinated by the Victorian Government Department of Health and Human Services (DHHS) identified multiple transmission networks involving inter- and intra-facility spread and determined that centralized collation of genomic and epidemiological data were needed to identify areas of CPE transmission due to the long colonization periods, asymptomatic transmission, and complex patient movements often experienced by patients with CPE (14).

In December 2015, the Victorian Guideline on Carbapenemase Producing *Enterobacteriaceae* for Health Services (the guideline) was released, implementing a comprehensive prospective genomic and epidemiological surveillance and response program for the control of CPE (15). Here we describe the epidemiology of CPE in Victoria and assess the feasibility and effect of the first three years of the program.

Methods

The intervention

The guideline implemented a prospective, multi-modal population-based intervention for control of CPE (Supplementary methods). Standardized active screening and carrier isolation were required in all Victorian health services accepting overnight patient admissions (Supplementary Box 1). All Victorian diagnostic laboratories servicing both inpatient and outpatient providers referred suspected CPE isolates to the state reference laboratory for confirmatory testing, whole genome sequencing (WGS) and investigation (Supplementary methods, Supplementary Box 2, Supplementary Figure 1). Epidemiological data was collected for all persons from whom an isolate with a confirmed carbapenemase gene originated, regardless of phenotypic susceptibility or clinical presentation (16). Combined phylogenomic and epidemiological outbreak investigations to identify putative local transmission were conducted prospectively and iteratively where two or more patients with CPE isolates of the same species, multi locus sequence type (ST), and carbapenemase gene were identified, and at least one isolate was from a person not suspected to have acquired CPE overseas (Supplementary methods, Supplementary Table 1). A transmission risk area (TRA) was declared where local transmission was thought to represent a risk to other persons present in the location for a defined timeframe, necessitating increased screening and other infection control actions (Supplementary Table 2).

Assessment of program impact

All confirmed CPE cases (a distinct carbapenemase gene, species and/or ST combination in a given person) identified between January 2016 and December 2018 were included in analyses, performed using Stata/SE 13.1 (StataCorp, College Station, Texas) or R <v3.5.1> (17). To assess effects of the program rates of CPE identification and active clinical infection were calculated using population estimates for the respective time-period and trends assessed using negative binomial or Poisson regression (18). Associations between carbapenemase genes, risk factors and clinical data were measured using a comparison of proportions ($\chi 2$ and Fisher exact tests). $P < .05$ was considered statistically significant.

Results

Occurrence of CPE remained low over the study period with 402 cases identified in 362 people. CPE was considered a colonizing organism in 60% (n=242/402) of cases (Supplementary materials, Supplementary Table 3). Identified carbapenemase genes were diverse and varied by species (Supplementary materials, Supplementary Table 4, Supplementary Figure 2).

Risk factors for CPE acquisition

Risk factors differ by carbapenemase gene

Overall, 95% (n=379/399) of cases reported hospital admission in the previous 12 months, and 63% (n=231/369) overseas travel in the past four years. Of these, 188 are suspected to have acquired their CPE overseas, representing 47% (n=188/402) of all cases.

Risk factors differed significantly between gene groups (Figure 1), with overseas acquisition suspected in a higher proportion of NDM (n=111/114, 93%), both NDM and OXA-48-like (n=6/7, 86%), OXA-48-like (n=60/75, 80%), and KPC (n=11/46, 24%), when compared to IMP cases (n=0/142, 0%; $p<0.001$, Fisher's Exact). Region of travel differed by carbapenemase gene group (Figure 1).

KPC (n=32/46, 70%), IMP (n=84/142, 59%), and OXA-48-like (n=4/75, 5%) cases were identified as part of defined local transmission networks based on genomic and epidemiological data (Figure 1). A source of acquisition was unable to be identified in 22% (n=89/402) of cases, indicating potential unrecognized risk factors, colonized persons, and/or horizontal transfer of carbapenemase genes; however this ranged from only 3% (n=3/114) of NDM to 41% (n=58/142) of IMP cases.

Non-healthcare related acquisition of NDM-producing E. coli in travelers returning from South and South-east Asia

Overseas hospitalization is a known risk factor for CPE acquisition and a criterion for screening upon admission to a Victorian hospital. Of the 188 cases with suspected overseas acquisition 132 (72%, 5 unknown) reported hospital admission while overseas, and an additional 28 had attended an emergency department, medical, or dental clinic. The remaining 23 cases represent suspected overseas acquisition in the absence of healthcare contact. The majority (18/23 cases, 78%) involved NDM-producing *E. coli*, commonly NDM-5 (n=14); 22 of the 23 cases travelled to south-east or south and central Asia, most commonly India (n=14) (Supplementary Table 5).

Cases with suspected non-healthcare related acquisition overseas were significantly less likely to have been identified through screening (35%, n=8/23) than other CPE cases (57%, n=217/379, p=0.035), and most notably those with overseas hospitalization (73%, n=117/160, p<0.001).

Local acquisition of CPE in Victoria

Centralized combined genomic and epidemiological surveillance allows identification of small transmission networks involving cases temporally and geographically dispersed at CPE identification

A major aim of the program is early identification of putative local transmission. To enable timely action, time between isolate referral and initial assessment for local transmission was monitored, with a median delay of 11 days (IQR 9-14) during the study period. Following process improvements median delay decreased from 15 days (IQR 9-34) in 2016 to 10 days (IQR 9-13) in 2018.

Amongst the 53% (n=214/402) of cases where overseas acquisition was not suspected, routine prospective surveillance uncovered 28 clusters of genomic and epidemiologically linked cases, most commonly involving IMP-4-positive organisms (Figure 2). Identified transmission networks involved a median of 2 cases (range 1-19) during the study period.

Previous studies have indicated that local transmission is suspected where isolates from two patients are within ~23 single nucleotide polymorphisms (SNPs) of each other (7,19). In our data, 97% (2296/2366) of inter-cluster pairwise isolate comparisons were below this threshold when a cluster was defined using both genomic and epidemiological data (median 8, IQR 4-13 SNPs) (Figure 2). However, where multiple clusters have been identified within a single species and ST , considerable overlap was observed between intra- and inter-cluster pairwise SNP comparisons (Figure 3), suggesting use of a genomic cluster definition alone may merge epidemiologically dispersed cases, reducing power to identify risk factors, and geographical or temporal focus of transmission.

Twenty-eight location and time specific TRAs were identified across 18 of the 28 clusters, allowing targeted intervention and screening. One TRA was in a residential aged care facility, with the remaining in single hospital wards. Organisms involved in TRAs varied by health service, with 13/18 (72%) IMP-4 TRAs, 6/8 (75%) KPC-2 TRAs and 2/2 (100%) OXA-232 TRAs each occurring within different single health services. Inter-facility collation and the combined use of genomic and epidemiological data was crucial in identifying transmission where patients were temporally and geographically dispersed on identification, including six TRAs involving patients identified with CPE post-discharge, in different health services or the community. Similarly, in fourteen TRAs more than 30 days elapsed between identification of CPE in the first and last cases involved in the transmission. The case study below further highlights the value of centralized intra-facility surveillance methods.

Case study: Outbreak investigation leading to the declaration of TRAs in two hospitals

In July 2018, a patient with OXA-232 positive *Klebsiella pneumoniae* ST 2096 was identified without a history of hospitalization in a high burden country (Patient 3). Two cases of this gene, species and ST combination had been seen previously, from Patients 1 and 2, both reporting prior hospitalization in India (Supplementary Table 6). An outbreak investigation was undertaken and updated on identification of Patients 4 and 5, including a phylogenetic analysis of isolates from all patients (Figure 4A). Bed movement data were obtained from any hospital where multiple cases had attended and plotted against the phylogenetic tree (Figure 4B).

Analysed together, centralized genomic and epidemiological surveillance enabled:

(i) identification of an outbreak of phylogenetically related CPE obtained from Patients 2-5 over a six-month period;

(ii) inclusion of Patient 5 in the outbreak, despite admission to a different health service at the time of CPE identification;

(iii) exclusion of Patient 1 from the outbreak;

(iv) identification of Patient 2 as the likely index case for this outbreak despite no known contact with other patients, suggestive of unrecognized intermediary transmission; and

(v) identification of putative transmissions in two different hospitals, despite identification of CPE in Patient 5 six months after likely transmission; resulting in the declaration of TRAs in two healthcare facilities.

Multiple local transmission patterns observed, including sustained propagated outbreaks and suspected environmental acquisition

Among identified local outbreaks, multiple apparent patterns of local transmission were observed. Most notably, transmission of KPC producing *K. pneumoniae* ST 258 persisted across all three years, causing small periodic outbreaks in facility B (Figure 2). All clusters of KPC-2 producing *K. pneumoniae* ST 258 observed were derived from outbreak clones first observed in 2012, with current clusters thought to represent epidemiological and genomic diversification over time and not separate importations (14). A similar pattern has been observed within IMP-4 producing *E. cloacae* ST 93 and 114 clusters, in contrast with discrete apparently time-limited transmissions, exemplified by IMP-4 producing *K. oxytoca* of novel ST (Figure 2). While plasmid transmission was not directly examined in local transmission investigations, 50/73 (68%) case of a ST not associated with local clonal dissemination contained IMP-4, indicating limited plasmid transmission of other carbapenemases.

Following identification of environmental CPE contamination in some facilities, Version 2 of the guideline incorporated suspected acquisition from an environmental source within the definition of a

TRA. Four such TRAs were observed, all involving sink drains contaminated with IMP-4 positive *S. marcescens* within intensive care units across two health services.

Effects of comprehensive CPE surveillance

Targeting patients present at time and location of suspected transmission increases screening yield

TRA designation enables communication of the time and place of CPE transmission risk to other health services, with persons admitted to the TRA location during the designated period requiring isolation and screening on admission to any healthcare facility (Supplementary Table 2). Version 2 of the guideline increased follow-up of exposed patients, including a requirement to send letters informing patients of their CPE exposure (20). Subsequently, the proportion of screened cases linked to a TRA increased 11-fold to 0.9% (n=21/2394, p=0.002, Fisher's Exact).

Intensified screening increases case ascertainment, clinical infections stabilize

To assess effect of the intervention, rates of CPE identification and active clinical infection were examined. Incidence of CPE increased 13%/half-year (95% CI 6%-19%, p<0.001) (Figure 5A) to 1.42 cases/100,000 population in the second half of 2018. Despite this, both the rate and number of cases identified as active clinical infections remained steady since 2017 (IRR 0.94, 95%CI 0.73-1.21, p=0.640)(Figure 5B), indicating the observed increase in rate of CPE cases is likely due to increased screening and case ascertainment. By the second half of 2018, 36% (n=33/92) of cases were identified through infection control interventions following case or transmission identification, such as contact screening (Figure 5B). This coincided with a steady decrease in cases where source of acquisition could not be determined, from 30% (n=18/61) in second-half 2016 to 15% (n=14/78) in second-half 2018 (χ^2, p=0.033) (Figure 5C), indicating increased ascertainment and resolution of transmission networks.

No change in the population rate of KPC occurred over the surveillance period (IRR 1.00, 95% CI 0.85-1.19, p=0.968). However, active clinical infections with KPC-2 producing *Enterobacterales* decreased significantly following the implementation of the program from a high of 0.29

infections/100,000 population in 2014 to 0.03 infections/100,000 population in 2018 (p=0.003). A non-significant decrease in total KPC-2 cases was observed over the same period (p=0.554) (Figure 5D).

Discussion

We have described the findings, feasibility and impact of one of the first centralized comprehensive and systematic phylogenomic and epidemiological surveillance and response programs for a primarily healthcare-associated MRO globally (21, 22). The aim of this program was to rapidly detect and control transmission of CPE in a low-prevalence setting. Implementation was possible due to an existing public health genomics program, enabling analysis to be completed in a timeframe applicable for infection control action; and through embedded cooperation between public health authorities, diagnostic laboratories, and healthcare facilities to implement responses. While resourcing and capacity for genomic surveillance, centralized case investigation and patient screening differ between settings, we share this information to demonstrate the value of a state-wide centralized 'search and contain' intervention for a low-prevalence MRO (23).

Historically, Victoria has had limited central coordination regarding MROs, and outbreaks were managed within individual institutions. The alarming occurrence of a disseminated inter-facility KPC outbreak necessitated action by public health authorities (14)and resulted in this comprehensive surveillance and response program. We have demonstrated the success of the program through increased case ascertainment with a steady rate of active clinical infections, a reduced number of cases where source of acquisition could not be determined, an increased proportion of cases identified through infection control interventions, the identification of small transmission networks enabling early response, and through the significant decrease in KPC-2 clinical infections observed following program implementation.

As consistently demonstrated in retrospective outbreak studies, and in our presented case study, the increased pathogen discrimination provided by genomic analysis enhances assessment of alternate

transmission hypotheses generated by epidemiological investigation (14, 22, 25-26). Our results support this principle, demonstrating that CPE colonization can result in patients involved in a transmission event being temporally and/or geographically dispersed upon CPE identification, and in considerable intra-host genomic diversity; making genomic or epidemiological data alone insufficient to define transmission events. When used prospectively, this process has enabled us to identify the temporal and geographical focus of transmission networks early, when case numbers remain small, allowing targeted intervention and reducing the resources required for intense epidemiological data collection (19).

While the importation of CPE, particularly from south and central Asia, and the predominance of IMP carbapenemase in local transmissions within Australia were anticipated (9-10,12-13,24), our results differed from those previously reported in several ways. Firstly, intensive case investigation identified travel to south and south-east Asia without health-care contact as an emerging risk factor for NDM-producing *E. coli*; IMP transmission, thought to be endemic in Australia's eastern states (24), and initially debated for inclusion in the program, affected only a small number of facilities; and intensive infection control actions have resulted in a higher proportion of CPE cases identified through screening, and a lower occurrence of serious infections, than reported elsewhere (25).

There are several limitations to the current program. The system is resource intensive and sustainability may be threatened by accumulating case numbers, rising global incidence of CPE, and broadening risk factors for acquisition - all increasing the risk of CPE introduction and resources required for screening and isolation of at-risk patients (27-29). While other comprehensive interventions have shown success in reducing incidence in outbreak or high burden settings, rapid transmission detection and intervention may help maintain low prevalence, and costs must be assessed against the escalating economic burden of CRE infection demonstrated internationally (30,31). Program evaluation, including cost effectiveness analysis is underway. Screening all patients with travel to high-burden countries without healthcare contact, which we have identified as a risk factor for CPE acquisition, is infeasible, yet such patients may represent an ongoing threat of CPE

introduction to our health services. We are encouraged by our results demonstrating increased case ascertainment through screening and low levels of active clinical infection; however, we may not identify all subsequent clinical infection in patients with known CPE colonization. Research into pathogen and host factors influencing CPE colonization in diverse, non-outbreak settings is needed to focus resources for screening and isolation towards those at higher risk of ongoing carriage, transmission, and within limits of duration of colonization (29,32-33).

Case ascertainment and sensitivity to detect transmission is strongly influenced by screening practices, which may differ between settings and facilities. While we have attempted to standardize screening of at-risk persons, transmissions may not be identified due to unrecognized colonized patients, difficulty in screening exposed contacts post-discharge or when presenting to another healthcare facility, and in identifying transmission in community settings. Further, we are currently unable to routinely determine transmissions which result from transfer of resistance elements between species and clones; this is under investigation for future action, however the low proportion of cases where source was unable to be determined, suggests this is not a dominant feature of CPE transmission in our setting. Finally, TRAs enable focused intervention but may be difficult to determine where large case numbers within a cluster result in multiple temporal and geographical overlaps between cases. In facilities or settings with higher CPE incidence, targeted investigation and control measures may not be feasible; protocols to identify and manage transmission in such settings are needed. Further research into infection control interventions effective against persistent propagated outbreaks and environmental reservoirs is also required.

In an era when AMR threatens the stability of the health system globally, significant effort and resources are justified if they can reduce the burden of AMR, especially the highest-risk pathogens such as CPE. We have described the successful implementation of a comprehensive, centralized program, and some indicators of success. Further work is required to define the economic value of the intervention, and to further refine enhanced screening activities at the hospital level, enabled by the comprehensive surveillance data collected. The methodologies used are transferable to other low-prevalence settings and MROs globally a will be expanded to other emerging AMR threats, such as *Candida auris*.

NOTES

Author contributions

FR, BS, BPH, AvD, NS, ME, CRL, JB, DRMC, and JCK conceived, designed and led the intervention. BPH, NS, CRL, AvD, and ME designed the study. KS, NLS, BPH, MSa, SAB, and DAW led the microbiological testing and interpretation. MSc, AGdS, TS and CLG led the bioinformatics analysis. JB and DRMC undertook data collection and advised on infection control processes and interpretation. RLS, AYP, AS, ACC, DWS & MJW led and advised on health service actions. CRL and SSG analysed the data. CRL wrote the first draft of the manuscript. All authors reviewed the manuscript, approved and agreed to submit the final version of the manuscript.

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Competing interests

A.P. reports an investigator-initiated research grant from MSD, outside the submitted work. All other authors declare no competing interests.

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Figure Captions

Figure 1: Risk factors for Carbapenemase-producing *Enterobacterales* acquisition, by carbapenemase gene group, Victoria, Australia 2016-2018.

[a] Unknown sex, n=1

[b] Includes suspected transmission from a returned traveler

Figure 2: Temporal and genomic variation amongst Carbapenemase-producing *Enterobacterales* local transmission clusters, Victoria, Australia 2016-2018.

Note: N reported in table includes only human cases identified during the study period, however clusters may include environmental isolates or pre-surveillance cases. Duplicate isolates are excluded from both epidemic curves and intra-cluster pairwise SNP distance boxplots, with environmental samples from the same ward in the same week considered duplicates. For further information see Supplementary materials.

[a] Intra-cluster pairwise SNP distance boxplot include human or environmental isolate(s) collected prior to the start of the study period.

[b] TRA declared where patient is suspected to have acquired CPE from an environmental source. Collection of environmental isolate(s) must precede exposure of the patient(s) to the health service for TRA declaration to occur.

[c] Outlying point at 72 SNPs not displayed for scale.

[d] Data not available, see Supplementary Table 1.

Figure 3: Inter- and intra-cluster genomic variation amongst Carbapenemase-producing *Enterobacterales* local transmission clusters, Victoria, Australia 2016-2018.

Note: Analyzes include human or environmental isolate(s) of the relevant species and/or sequence type collected prior to the start of the study period as context isolates. Designated clusters must contain at least one human isolate collected during the study period (2016-2018). All non-clustered comparisons with ≤23 pairwise SNP distance involve only context isolates.

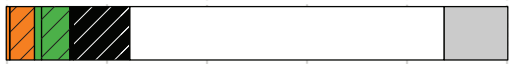
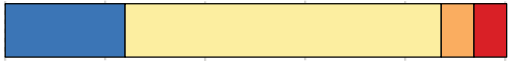
Duplicate isolates are excluded, with environmental samples from the same ward in the same week considered duplicates. For further information see Supplementary materials.

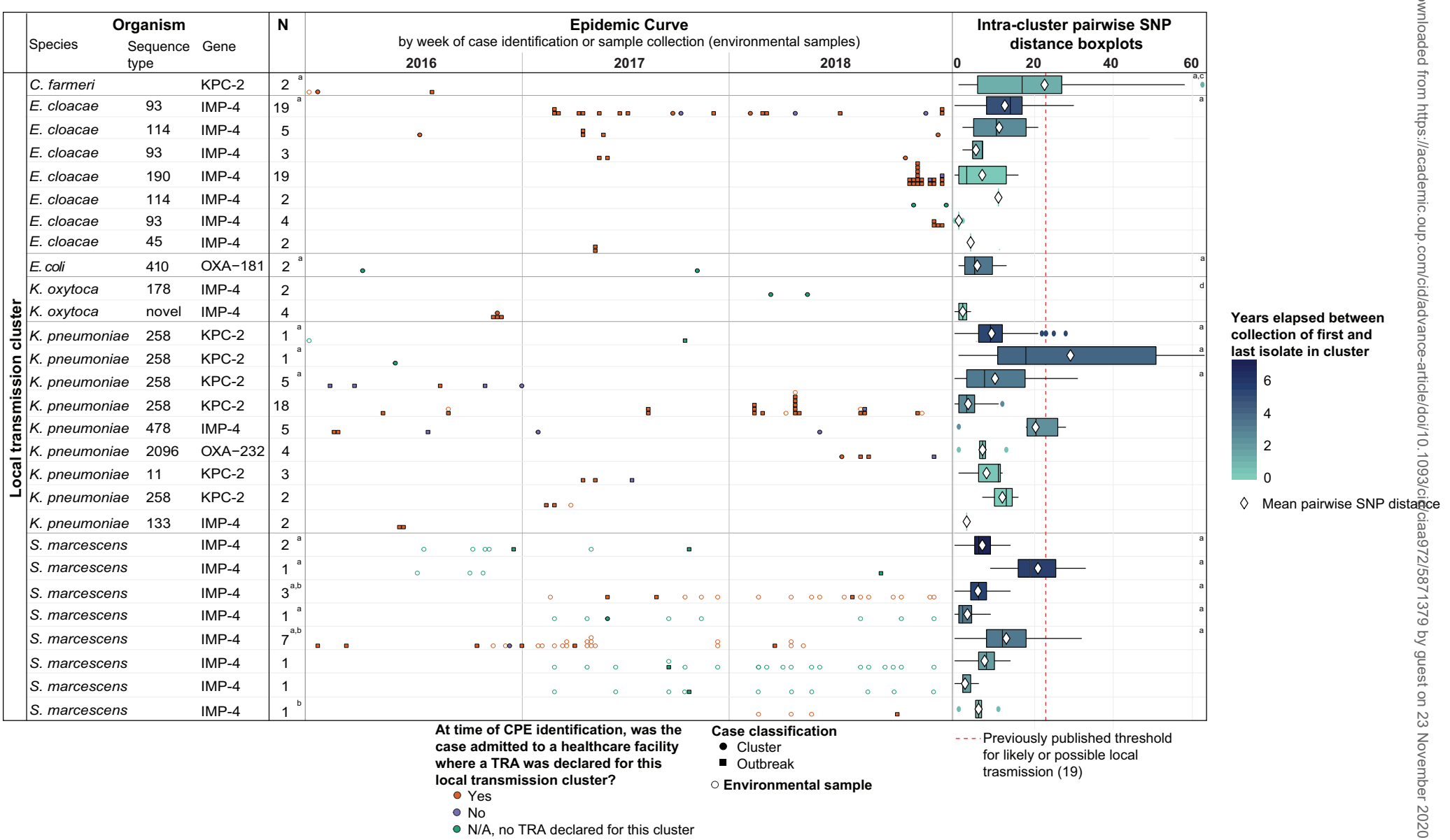
Figure 4: Case study, outbreak investigation of OXA-232 positive *Klebsiella pneumoniae* sequence type 2096, Victoria, Australia 2016-2018.

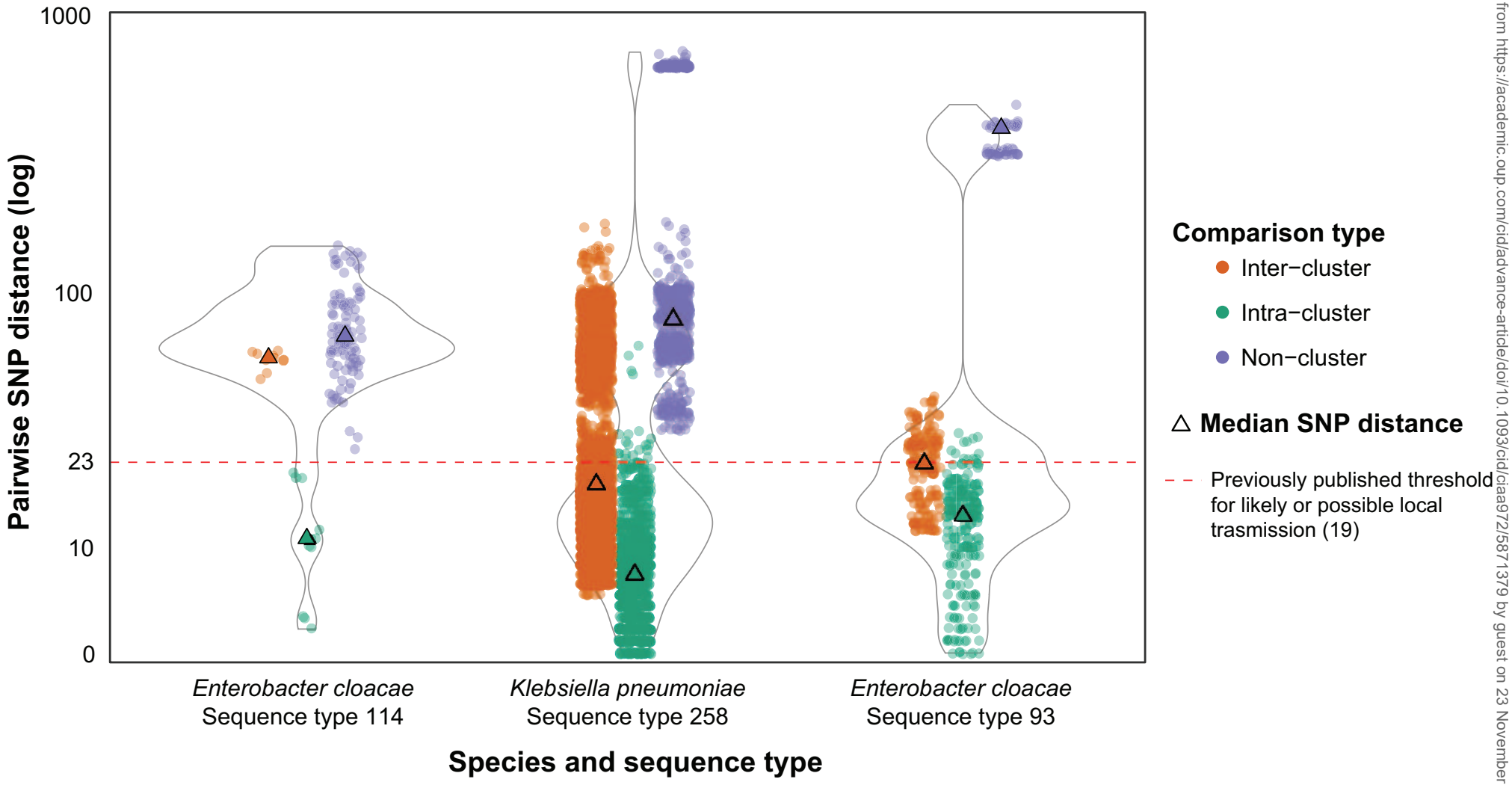
Panel A: Phylogenetic tree of *K. pneumoniae* ST2096 isolates colored by patient number and annotated with date of collection. Branch support values >99 shown. **Panel B:** Trace graph including hospital and ward of admissions for the corresponding patient. Admission data not displayed for duplicate isolates from the same patient.

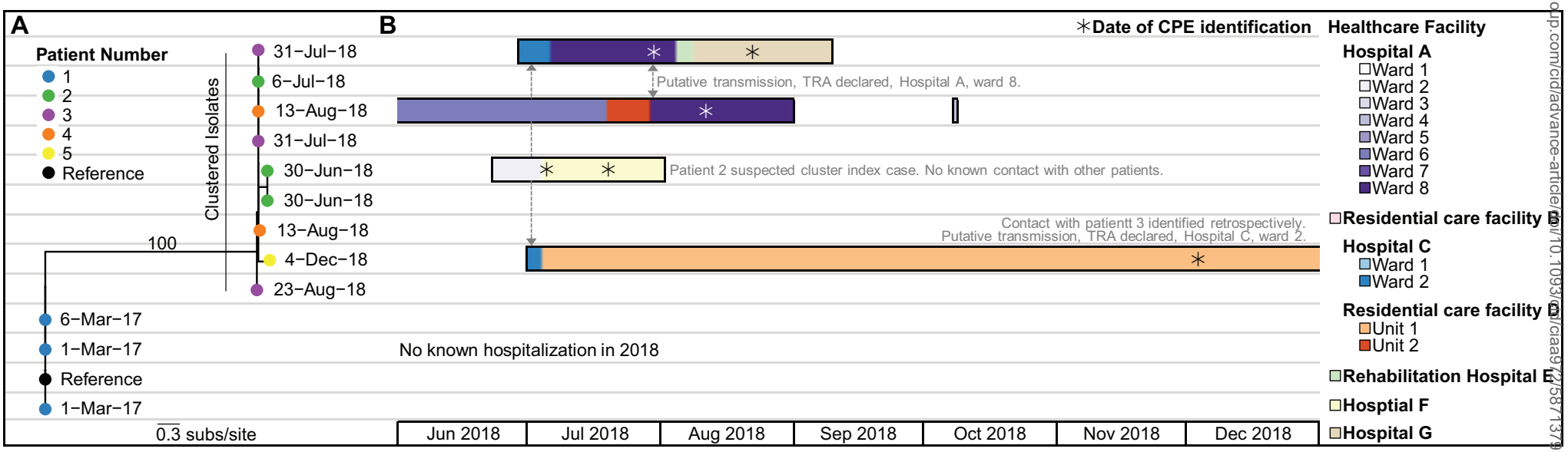
Figure 5: Epidemic curve and population rate of rates of Carbapenemase producing *Enterobacterales* (CPE) cases, by clinical and epidemiological characteristics, Victoria, Australia.

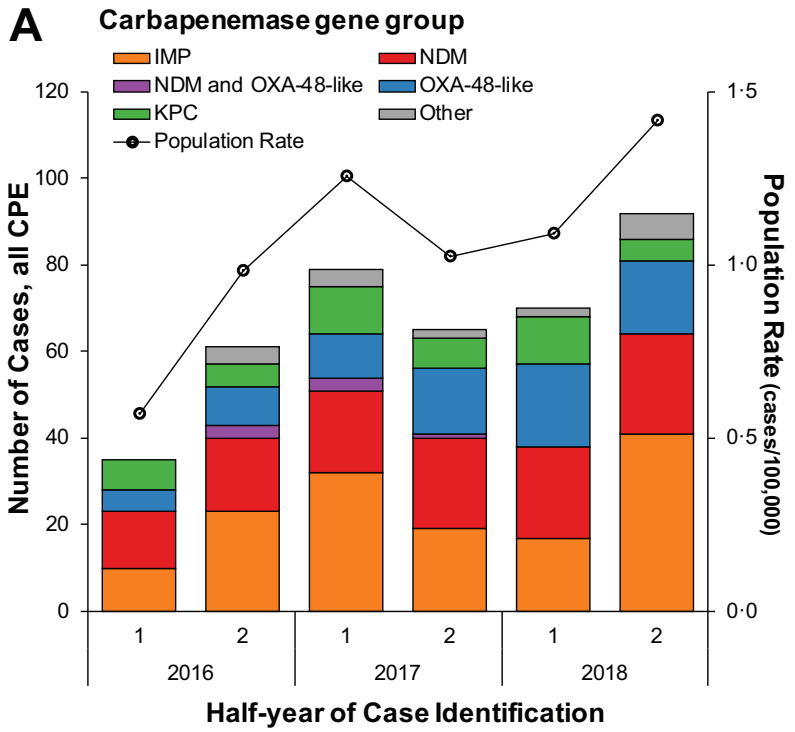
Panel A: Number of cases by carbapenemase gene group and population rate for all cases by half-year, 2016-2018. **Panel B:** Number of cases by reason for specimen collection and population rate for clinically indicated cases by half-year, 2016-2018. **Panel C:** Number of cases by suspected source of CPE acquisition, and proportion of total cases where a source of acquisition could not be determined by half-year, 2016-2018. Local outbreak categorization includes patients with genomic and epidemiological links, overseas includes patients with household contact with returned travelers. **Panel D:** Number of cases by clinical significance and population rate of KPC-2 producing *Enterobacterales* pre- and post-intervention, by year, 2012-2018.
Note: *Includes suspected transmission from returned traveler

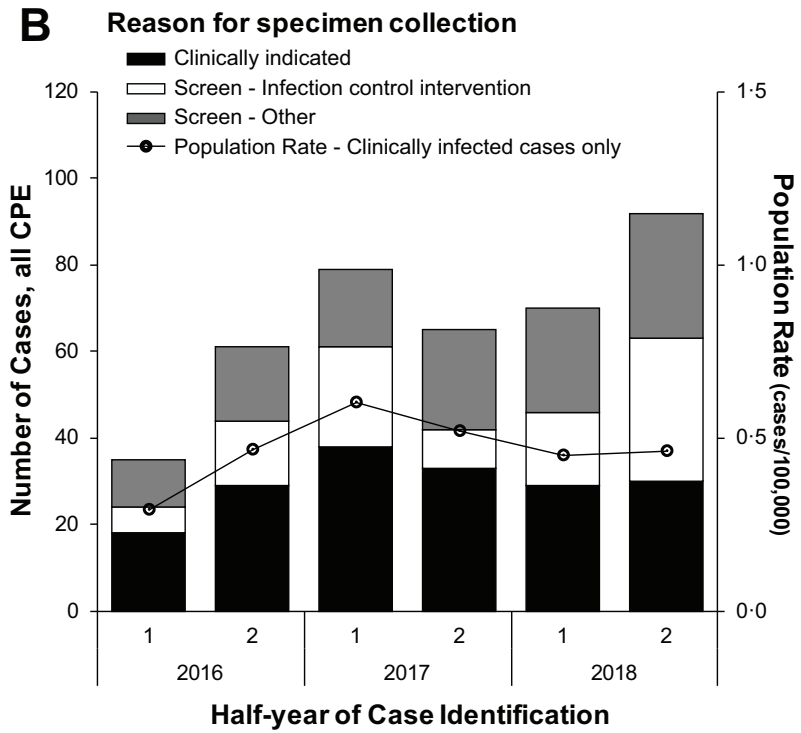
Carbapenemase gene group	N cases	Suspected source of acquisition	Travel, previous four years	N male (%)	Median Age (IQR)
IMP	142			85 (60%)	65 (46 - 77)
NDM	114			69 (61%)	60 (39 - 69)
OXA−48−like	75			38 (51%)[a]	63 (36 - 73)
KPC	46			27 (59%)	70 (59 - 78)
NDM, OXA−48−like	7			4 (57%)	69 (58 - 77)
Other	18			7 (39%)	58 (43 - 70)

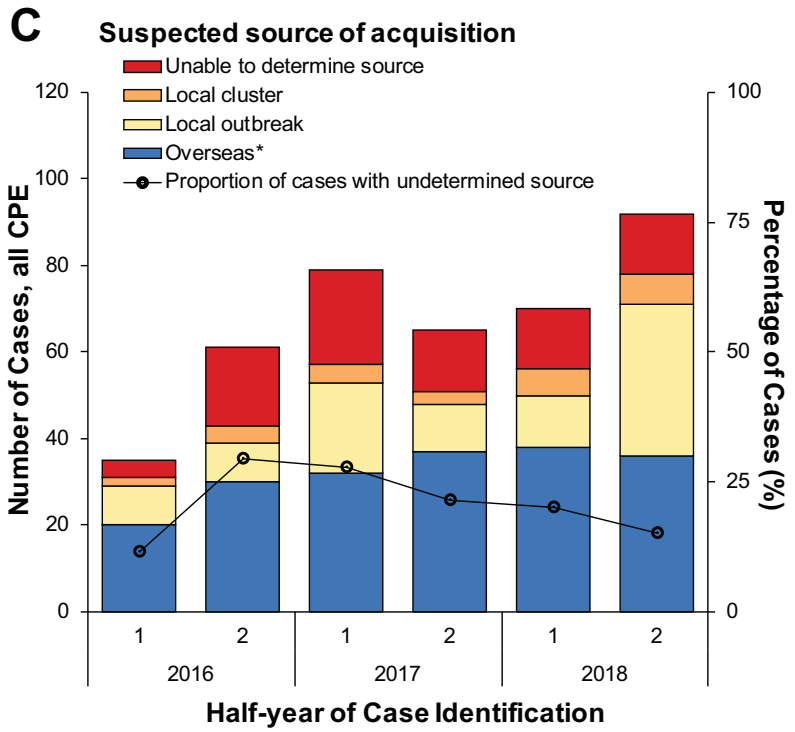


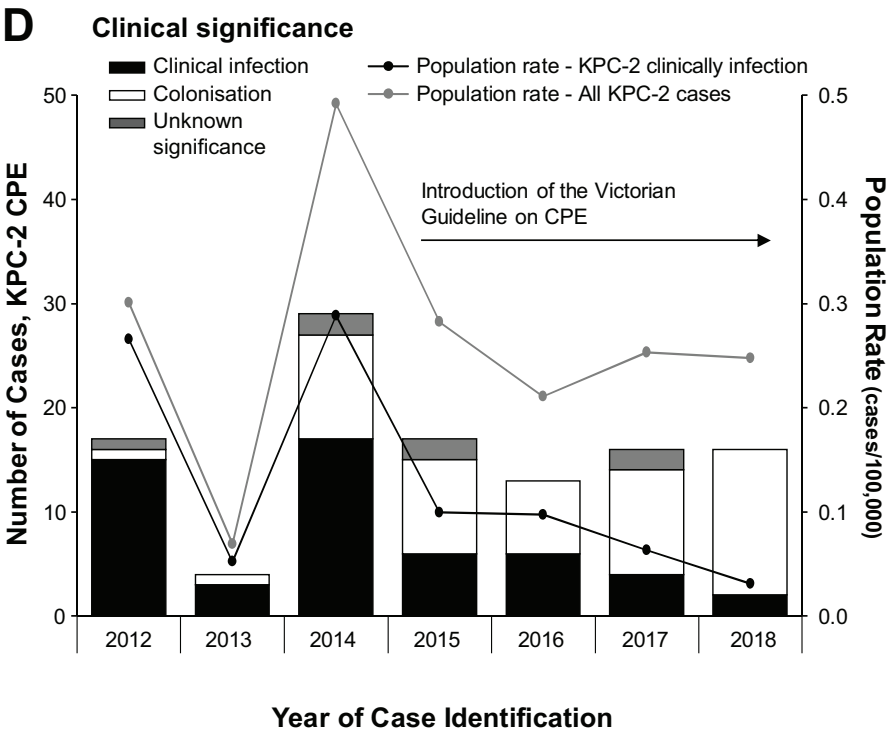












Author/s:
Lane, CR; Brett, J; Schultz, M; Gorrie, CL; Stevens, K; Cameron, DRM; St George, S; van Diemen, A; Easton, M; Stuart, RL; Sait, M; Peleg, AY; Stewardson, AJ; Cheng, AC; Spelman, DW; Waters, MJ; Ballard, SA; Sherry, NL; Williamson, DA; Romanes, F; Sutton, B; Kwong, JC; Seemann, T; Goncalves da Silva, A; Stephens, N; Howden, BP

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