Hidden reservoirs of hospital-associated infections

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***Klebsiella pneumoniae* (*Kp*) is a Gram-negative bacterium that is ubiquitous in the environment and is of increasing concern in public health. *Kp* can be carried asymptomatically as a commensal organism and can cause opportunistic infections in susceptible individuals; this is further complicated by an increasing incidence of multi-drug-resistant (MDR) strains. Given *Kp* can be carried asymptomatically, and can cause infections, it is possible that asymptomatic carriage acts as a reservoir for infection. Our recent work in Melbourne confirms this is often true. Individuals who tested positive for carriage of *Kp*, on admission to ICU, were over five times more likely to develop an infection during their hospital stay, compared to non-carriers. Whole genome sequence analyses revealed extensive diversity amongst the *Kp* infection-causing strains. These results indicate the majority of opportunistic infections are caused by patients' own microbiome strains that are already present on ICU admission. As such, screening of individuals on admission may enable clinicians to identify who is most at risk of developing infections during their hospital stay, and who is harboring drug-resistant strains that could transmit to others.**

During a one-year cohort study conducted at the Alfred hospital intensive care unit (ICU) in Melbourne, 498 patients were screened for gut carriage of *Kp* shortly following admission and were monitored for *Kp* infection[1]. The frequency of gut carriage was 10% overall, but only 6% amongst those who had no recent contact with healthcare prior to being admitted to ICU. Fifty ICU patients (1.85% of admissions) had one or more infections attributed to *Kp*, and 29 of these individuals were also screened for carriage. Bacterial isolates underwent whole genome sequencing (WGS) and comparative analyses were conducted, using strict thresholds of genomic similarity to identify potential transmission and to explore whether carriage strains matched those causing infections. Ten patients (34% of infection patients who were screened) were carrying *Kp* strains that matched their infecting strain (<0.0005% DNA sequence divergence); in six of these cases, the infection occurred at least two days after carriage was detected. Additionally, six patients developed infections with strains that were near-identical to strains previously detected in other ICU patients, consistent with intra-hospital transmission (Figure 1).

Not all infections could be attributed to prior carriage or transmission, mainly because not all ICU patients were screened for carriage (screening required informed consent from the patient or a close relative, which was not always obtainable). So for many patients it is not possible to tell whether they were carrying their infecting strain on ICU admission. However, the WGS analysis showed that these individuals were typically infected with unique strains; not closely related to strains elsewhere in the hospital. Hence it is likely that these unexplained infections were caused by the patients' own strains than new strains that they acquired in the hospital.

This study suggests that routine screening for *Kp* could identify patients at high risk of developing infections during their hospital stay. Some hospitals already have routine screening procedures of patients in place for carriage of various MDR bacteria, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci*, or carbapenem-resistant Enterobacteriaceae; some have reported a corresponding reduction in hospital-associated infections[2–5]. During the one-year period of this study, *Kp* infections were diagnosed by the clinical microbiology laboratory in 320 patients across Alfred Health (*unpublished observations*). Of these, 11% were caused by strains carrying extended spectrum beta-lactamase (EBSL) genes that confer resistance to third generation cephalosporins (*unpublished observations*), and a small number of these were additionally resistant to carbapenems (CP-R) (*unpublished observations*), which are typically the last line drug for treatment of Gram-negative bacterial pathogens[6]. Importantly, these genotypically ESBL and CP-R isolates displayed corresponding resistant phenotypes, when they underwent antimicrobial susceptibility profiling (*unpublished observations*). Whilst MDR infections are still in the minority, they are the most difficult to treat, having been estimated to cost billions of dollars in additional

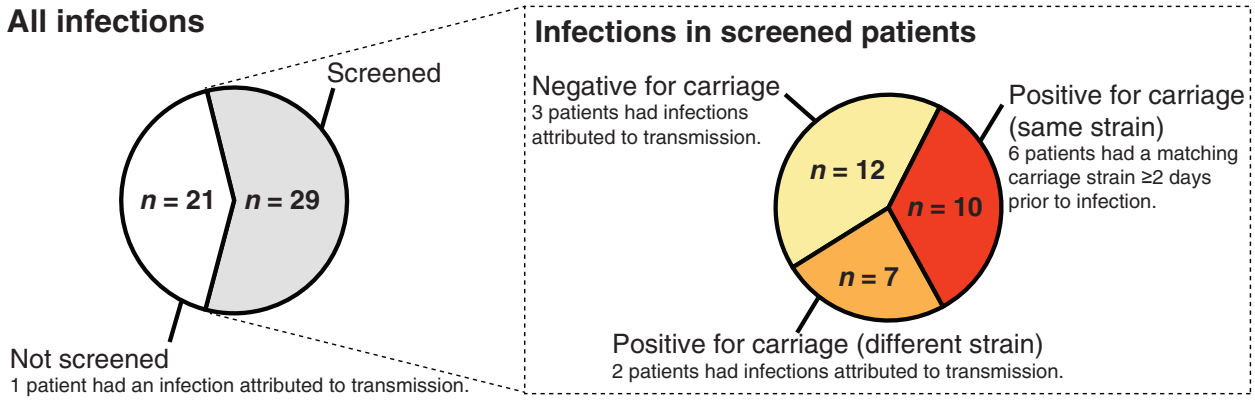

Figure 1. Infection numbers and attribution of infecting strain to carriage or transmission among individuals admitted to the ICU. Among the 50 ICU individuals with infections, six had infections that were attributed to transmission within the hospital. Ten patients had carriage and infection strains that were extremely closely related, including six patients for which the carriage strain was identified prior to infection. (Note: One infection isolate and two carriage isolates were excluded after genome sequencing and quality control.)

treatment costs and hospital stays, as well as thousands of deaths globally every year that would have been preventable had the infections not proved resistant to treatment[7–9]. Hence a screening strategy that looks for gut carriage of any MDR Gram-negative bacteria, which would detect *Kp* but also other common causes of hospital infections, could be of greatest clinical utility.

Important questions remain about how such information should be used. From an infection control standpoint, additional isolation measures could be taken to prevent transmission of MDR strains to other patients or healthcare workers. From a patient management standpoint, the drug susceptibility profile of known carriage strains could be used to guide the choice of antimicrobials used for therapy or prophylaxis[2,10]. First, this would help to avoid drugs that could select for overgrowth of resistant strains in the gut that could subsequently cause difficult-to-treat infections. Second, should an infection arise, foreknowledge of the likely strains and antimicrobial susceptibility profiles could be used to supplement empirical treatment protocols while awaiting laboratory confirmation of the susceptibility profile of the infecting organism.

The study demonstrates the value of WGS for fine-scale investigation of carriage-infection relationships, by clearly elucidating relationships between strains that cannot be distinguished by traditional laboratory typing techniques, e.g. multi-locus sequence typing or PFGE. As such, it is recommended that WGS approaches be implemented when investigating potential sources of infection, including for routine infection control as well as further research studies. Coupled with routine screening for carriage of MDR organisms, WGS could enable not just the identification of closely related strains but could also reveal the presence of mobile genes associated with antimicrobial resistance. Such genes can be readily transferred between species, hence knowledge of their presence could inform empirical treatment choices for Gram-negative infections generally and not just the isolated MDR organism. The WGS approach, with its continually developing sequencing and analyses platforms, is rapidly becoming more affordable and increasingly easy implement. Use of this technology in conjunction with, or in place of, traditional techniques could lead to much needed decreases in the numbers of hospital infections, particularly for difficult-to-treat MDR infections, leading to long term benefits both reducing the strain on the public health system and budget, but also for bettering patient outcomes.

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Biography

Claire Gorrie is a PhD student at The University of Melbourne. Her research combines whole genome sequencing, bacterial genomics, and epidemiology to investigate carriage-infection dynamics of *Klebsiella pneumoniae*.

Whole genome sequencing as a novel approach for characterising *Neisseria meningitidis* in Australia

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Neisseria meningitidis **(meningococcus) is the causative agent of invasive meningococcal disease that manifests as life-threatening septicaemia and/or meningitis. This review provides a brief overview of the prevention of the disease and also highlights the importance of whole genome sequencing (WGS) in detecting outbreaks of meningococci in Australia. The use of WGS in identifying the emergence of a penicillin-resistant cluster of meningococci is Western Australia is used as an example for advocating the implementation of WGS on the routine surveillance in Australia.**

Invasive meningococcal disease

Neisseria meningitidis, also known as the meningococcus (plural: meningococci), is a Gram-negative diplococcus that asymptomatically colonises the nasopharynx of approximately 10–30% of the adult population[1]. Occasionally, the bacterium crosses the epithelial layer and invades the host, causing invasive meningococcal disease (IMD) if the correct immune response is not elicited. IMD generally manifests as septicaemia and/or meningitis. Incidence of IMD follows a bimodal distribution with the first peak occurring in infants, due to lack of a mature immune system, and the second peak occurring in teenage years and early adulthood when the rate of transmission is highest due to lifestyle factors[2]. The onset of the disease is rapid and death may occur within 24 hours if the recommended antibiotics are not administered. Although IMD is rare, the mortality rate is approximately 10% and approximately 15% of survivors suffer from permanent sequelae such as limb loss and neurological disability[3,4]. Invasive strains of *N. meningitidis* express a polysaccharide capsule, which is used to classify the bacteria phenotypically into one of 12 serogroups – A, B, C, E, G, I, K, L, W, X, Y and Z[5]. IMD is predominantly caused by six serogroups (A, B, C, W, X and Y) and the distribution of these serogroups varies geographically and temporally[6,7].

Prevention of IMD

IMD can be prevented through vaccination. Although there is no vaccine available against the most recent disease-causing serogroup X, conjugate polysaccharide vaccines targeting the capsule have been used to control endemic IMD caused by serogroups A, C, W and Y for the past decades[8]. However, the majority of IMD cases in developed countries, including Australia, has been caused by meningococcal serogroup B (MenB)[9]. Vaccine development against MenB has been hampered as the capsule polysaccharide elicits autoantibodies in humans. As of now, two multi-component vaccines against serogroup B disease have been developed, which target specific sub-capsular surface antigens expressed by *N. meningitidis*. These vaccines are Bexsero® (also known as 4CMenB)[10] and Trumenba® (also known as rLP2086)[11]. Although both MenB vaccines have been licenced for use in the United States and the United Kingdom, Bexsero® is the only MenB vaccine available in Australia. Since the majority of cases (>65%) in Australia were caused by MenB post the implementation of the meningococcal C conjugate vaccine on the national immunisation programme (NIP) in 2003, inclusion of the Bexsero® vaccine on the NIP has been proposed to protect the Australian population from MenB infections. However, this focus has now shifted as there has been a switch in the predominant meningococcal serogroup