Research Title
Non-invasive Diagnostics of Bone and Joint Infections in Children Using Cell-free DNA

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Summary
Osteoarticular infections (OAI)—infections of bones or joints—are serious illnesses that can lead to long-term disability in children if not diagnosed and treated quickly. New Zealand has higher rates of OAI than many other OECD countries, making rapid and reliable diagnosis especially important. This project aligns strongly with Te Niwha's mission to improve child health and strengthen Aotearoa's diagnostic capability by developing faster, less invasive tools for detecting OAI in children.

Why this research is needed
With improvements in molecular testing, *Kingella kingae* is now recognised as the leading cause of OAI in young children. However, it remains extremely difficult to diagnose. *K. kingae* is only briefly present in the bloodstream, meaning routine blood cultures often fail to detect it. More sensitive tests—such as joint aspirations, biopsies, or surgical sampling—are invasive and not always feasible for young children. Because of these challenges, up to one third of OAI cases in New Zealand have no identified pathogen, and children are treated on clinical suspicion alone.
A non-invasive test that can accurately identify *K. kingae* would be a major advance. At the same time, there has been limited local data on how *K. kingae* responds to commonly used antibiotics. This has important implications for treatment choices in Canterbury, where flucloxacillin was historically used as first-line therapy but is known internationally to be poorly active against *K. kingae*.

What the research set out to do
This project had two main aims:

1. **To develop a non-invasive diagnostic test** that detects fragments of bacterial cell-free DNA (cfDNA) in blood or urine using quantitative PCR (qPCR).
2. **To perform antimicrobial susceptibility testing** on *K. kingae* isolates from New Zealand and Australia to evaluate whether current treatment guidelines remain appropriate.

Developing a PCR assay for *K. kingae* using cfDNA
We designed a highly sensitive PCR assay capable of detecting cfDNA from *K. kingae* in urine, and cfDNA from both *K. kingae* and *Staphylococcus aureus* in blood plasma. Throughout my PhD, we recruited children with suspected OAI presenting to Christchurch Hospital and collected urine and blood samples. These clinical samples will be compared directly with current gold-standard diagnostic tests to assess real-world performance.
This work forms the foundation of a non-invasive diagnostic platform where *K. kingae* cfDNA can be captured from urine using magnetic-bead probes and then quantified through qPCR. The platform can also be adapted

in future to detect other childhood pathogens by designing new primers and probes, strengthening New Zealand's preparedness for future infectious disease challenges.

Antimicrobial susceptibility testing

In collaboration with Canterbury Health Laboratories and diagnostic laboratories in Auckland, Wellington, Dunedin, and South Australia Pathology, we assembled a collection of *K. kingae* isolates from across Australasia. These isolates were tested against a panel of commonly used antibiotics.

Our findings showed that *K. kingae* isolates were consistently susceptible to several antibiotic classes for which clinical breakpoints have been established. Importantly, they demonstrated low inhibitory concentrations for first-generation cephalosporins such as cefazolin but limited susceptibility to flucloxacillin—consistent with international evidence. These results directly support the recent shift in Canterbury from flucloxacillin to first-generation cephalosporins as first-line treatment for suspected OAI.

Key findings and potential benefits

This project provides the first local data showing that traditional empiric antibiotic guidelines may not fully cover *K. kingae*, reinforcing the need for cefazolin-based treatment. Aligning practice with this evidence will improve recovery times, reduce complications, and avoid unnecessary use of broad-spectrum antibiotics.

On the diagnostic side, the development of a sensitive PCR assay and early cfDNA capture system represents a major step toward a non-invasive test for OAI. Such a test could reduce the need for surgery, shorten hospital stays, and provide faster answers for whānau and clinicians. It will also support more equitable care by enabling accurate diagnosis in centres without paediatric surgical capability, benefiting tamariki across both urban and rural Aotearoa.

Collaborations and partnerships

This project was strengthened by close collaboration with Canterbury Health Laboratories, South Australia Pathology, laboratories in Auckland, Wellington, and Dunedin, and academic partners at the University of Otago Christchurch. These partnerships provided access to clinical isolates, technical expertise, and ongoing support for assay development.

Next steps

The next phase is a multi-centre clinical pilot study to validate this non-invasive cfDNA assay in children with suspected OAI across New Zealand hospitals. Alongside this, we will continue expanding the antimicrobial susceptibility database to support national guideline updates.

Ultimately, this research aims to deliver a rapid, accurate, non-invasive diagnostic test that can be implemented across the country—improving outcomes for tamariki, supporting equity of access, and reducing the burden of osteoarticular infections in Aotearoa.