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Genomic epidemiology of methicillin-susceptible *Staphylococcus aureus* across colonisation and skin and soft tissue infection

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Summary *Objectives: Staphylococcus aureus* skin and soft tissue infection (Sa-SSTI) places a significant burden on healthcare systems. New Zealand has a high incidence of Sa-SSTI, and here most morbidity is caused by a polyclonal methicillin-susceptible (MSSA) bacterial population. However, MSSA also colonise asymptomatically the cornified epithelia of approximately 20% of the population, and their divide between commensalism and pathogenicity is poorly understood. We aimed to see whether MSSA are genetically differentiated across colonisation and SSTI; and given the close interactions between people and pets, whether strains isolated from pets differ from human strains.
Methods: We compared the genomes of contemporaneous colonisation and clinical MSSA isolates obtained in New Zealand from humans and pets.
Results: Core and accessory genome comparisons revealed a homogeneous bacterial population across colonisation, disease, humans, and pets. The rate of MSSA colonisation in dogs was comparatively low (5.4%).
Conclusions: In New Zealand, most Sa-SSTI morbidity is caused by a random sample of the colonising MSSA population, consistent with the opportunistic infection model rather than the paradigm distinguishing strains according to their pathogenicity. Thus, studies of the factors

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determining colonisation and immune-escape may be more beneficial than comparative virulence studies. Contact with house-hold pets may pose low zoonotic risk.

Introduction

Skin and soft tissue infection (SSTI) places a significant burden on healthcare systems due to its propensity to recur and complicate.[1] *Staphylococcus aureus* is the predominant pathogen isolated from SSTI, globally.[2] The incidence of *S. aureus* SSTI (Sa-SSTI) has increased significantly in many developed countries.[3,4] Variations in the rate of isolation of methicillin-resistant versus methicillin-susceptible *S. aureus* strains (MRSA; MSSA) have been observed between continents, with a higher rate of MRSA registered in North America than in Europe.[2] New Zealand has a high incidence of Sa-SSTI, and a recent national survey indicated that here, most morbidity is caused by a polyclonal MSSA population.[5]

S. aureus also colonises asymptomatically the cornified epithelia and is found in the nares of 20–30% of the population at any point in time.[6] Some observations indicate colonisation and disease-causing isolates are approximately equally distributed among the predominant lineages.[7,8] Nevertheless, although MSSA colonisation and endogenous infections are common, only a small proportion of colonised people develop illness, and this dual lifestyle as a commensal and a pathogen has puzzled researchers for decades.[9,10] On one side of the spectrum, the host-centred model of pathogenesis considers MSSA as opportunistic agents and attributes a central pathogenetic role to the variations or breaches in host defences.[11,12] This model predicts a homogeneous MSSA population across colonisation and disease. Alternatively, the microorganism-centred model distinguishes between strains according to their pathogenicity.[13] The conflict between these models remains unresolved mainly because population-based studies rarely compare the relative abundances of contemporaneous strains across colonisation and disease.

Colonised people are at increased risk of developing MSSA infection,[14] but the exogenous sources of Sa-SSTI are still elusive.[15] Modern society fosters close physical interactions between people and pets, and MSSA are isolated sporadically from a range of infection sites in dogs and cats,[16] and also from the nares and skin of asymptomatic dogs.[16,17] Recent studies showed that pets can be colonised by the same MRSA lineages causing infection in people,[18] but the relatedness of MSSA colonising pets and causing Sa-SSTI in humans is not known.

Comparative genomics enables an understanding of the differentiation of bacterial populations across ecological niches. Most recent studies comparing colonising and clinical *S. aureus* isolates concerned MRSA. Extrapolation of these data to MSSA is problematic as MRSA belong to a relatively small spectrum of lineages, whereas MSSA are highly diverse.[19] Hence, we performed a comparative genomics study of contemporaneous human and pet colonisation and clinical MSSA isolates obtained in New Zealand. Our study was motivated by two main questions. Firstly, we wanted to ascertain whether MSSA is differentiated across colonisation and SSTI, which is critical to the understanding of the pathogenesis of Sa-SSTI. Secondly, considering the potential role of pets as reservoir of zoonotic Sa-SSTI, we aimed to assess MSSA host-specificity.

Materials and methods

MSSA isolates

We used 85 nearly contemporaneous MSSA isolates obtained from four epidemiological niches: human nasal colonisation (n = 27); human clinical (n = 17); pet colonisation (n = 15); and pet clinical (n = 26) isolates. The human nasal isolates were obtained in 2014 from 77 consenting adults (>15 years old) admitted to Auckland City Hospital for acute or elective orthopaedic care, within 48 h of admission. Exclusion criteria included hospital admission within the last 6 months, or more than two admissions in the past 12 months; living in a long term care facility; having more than two long term conditions; taking more than two regular medications; receiving antibiotics; and suspected of having an orthopaedic infection. Sampling was performed by rotating a swab in each anterior nares ten times. The Northern B New Zealand Health and Disability Ethics Committee approved the sampling. Swabs were placed in transport media, plated onto Mannitol-Salt agar the same day and incubated for 48 h at 37 °C. Colonies resembling *S. aureus* were sub-cultured and identified by phenotypic tests and later confirmed by genotyping.

The human clinical isolates originated from a large collection of isolates obtained in 2014 from clinical microbiology laboratories throughout the country. The collection had been genotyped previously for a national survey.[5] For this study, 17 clinical isolates were selected at random among the predominant spa types identified in the national survey (and therefore, were tested by multilocus sequence typing and/or microarray). Fourteen of these isolates were recorded as originating from SSTI, one from an eye swab, one from an ear swab, and one from a urine specimen. Nine isolates were from patients with community-onset infections, seven from healthcare-associated infections, and one was unknown (definitions of healthcare-associated and community-onset infection are provided in Ref. 5).

The 26 clinical isolates from pets (dogs: 19; cats: 7) were isolated by two New Zealand animal diagnostic laboratory networks from a range of infection sites between June 2012–June 2013,[16] and between February and July 2016. The 15 colonisation isolates from pets (dogs: 13; cats: 2) were isolated from swabs taken from a cross sectional survey of 391 animals (colonisation prevalence: 3.8%; prevalence in dogs: 5.4%; in cats: 1.2%). Of these, 257 (148 dogs; 109 cats) were sampled in Auckland between June 2012 and June 2013 as previously described.[16] Additional