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High prevalence of multiple drug resistant staphylococci observed in macaque-populated locations in Brunei Darussalam

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Abstract. The spread of antibiotic resistant bacteria is a growing problem worldwide. Staphylococci bacteria is recognized as a genus of bacteria often closely related to human and animal hosts. Macaques are one type of non-human primate host that could potentially spread antibiotic resistant bacteria in an environment in close proximity to humans. Bacteria isolated from locations in Brunei Darussalam with and without the presence of long-tailed macaques revealed the existence of multiple drug resistant staphylococci bacteria with a higher prevalence observed in locations with a presence of long-tailed macaques. These findings have important zoonotic implications on infectious disease control and surveillance in public spaces frequented by macaques.

INTRODUCTION

Human impact on the environment and encroachment into wildlife's natural habitat is one of the causes of emerging infectious diseases (PearceDuvet, 2006). Highly mobile animals such as birds, bats and monkeys present a greater risk of zoonotic transmission because of the ease with which they can move into areas of human habitation. Brunei Darussalam is a small tropical country located on the world's third largest island, Borneo (Figure 1). The mammalian population between Brunei Darussalam and the rest of Borneo is similar (Gumert *et al.*, 2011). The long-tailed macaques (*Macaca fascicularis*, also known as the Crab-eating macaque) are an active daytime monkey commonly found in some public locations in Brunei Darussalam (MacLeod, 2014). Mammalian skin (including the anterior nares) is one of the major habitats of staphylococci and although still understudied, staphylococci bacteria can be found on non-human primates, including macaques (Van Den Berg *et al.*, 2011). The transfer of antibiotic resistant genes between other staphylococci species in a particular environment can contribute to the emergence of multiple drug resistant (MDR) staphylococci bacteria in the community (Gootz, 2010). Environments where humans are in close proximity to non-human primates could be a high risk source of antibiotic resistant staphylococci bacteria, where drug resistant genes may be freely transferred across bacterial species to create what is known as the "bacterial resistome" effect (Perry & Wright, 2014). Indeed, close contact of companion animals and humans permits the active interspecies dissemination of staphylococci bacteria carrying several

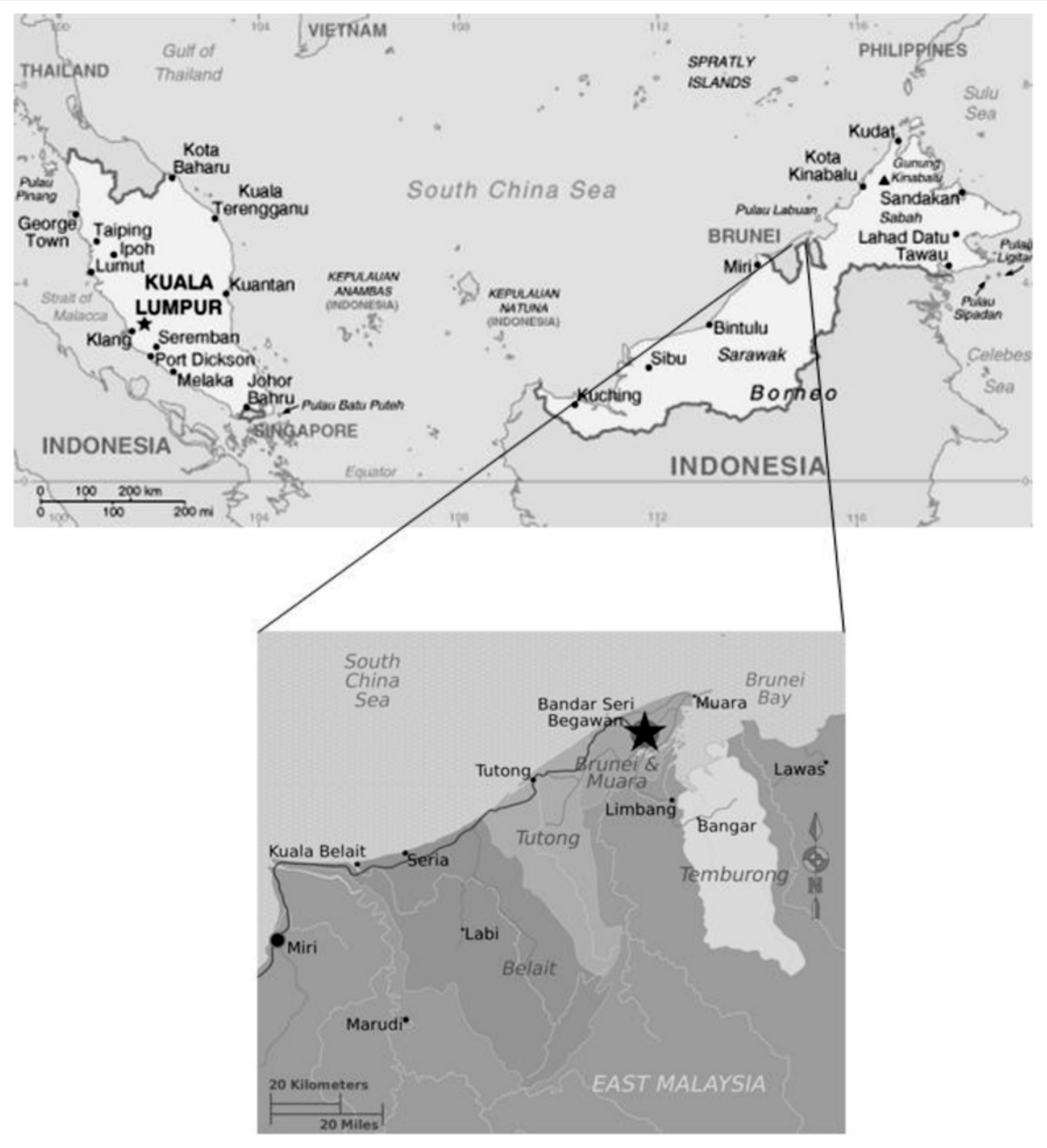

Figure 1. Map of Brunei. Adopted from The Department of Economic Planning and Development, Prime Minister's Office, Brunei Darussalam.

antibiotic resistance genes (Drougka *et al.*, 2016). However, the prevalence of staphylococci in non-human primate-populated environments proximal to humans is not well studied. Using long-tailed macaque as a model non-human primate, this preliminary study aims to investigate the possible association between the prevalence of MDR staphylococci and the presence of long-tailed macaque in places frequented by the general public in Brunei Darussalam.

METHODS

Sample collection

Sampling was carried out on potential human contact points in two locations (Location A (which has macaque presence) and Location B (which has no macaque presence)). In the Brunei-Muara district over a period of 1 week, avoiding rainy days as previously reported (Chong *et al.*, 2016). Surface swabs were collected from various sites including public exercise equipment, children playgrounds, hand rails and public benches. The swab sites chosen were areas where a high frequency of humans and, for one location, where macaques activity were frequently observed, based on several months of on-site observation. Dry sterile cotton swabs (Omnicell, Mountain View, CA, USA) were used to collect samples from the parks. A total of 12 A total of 102 surface samples (approximately 4 cm^2 area) were collected in both locations. Swab sampling, storage and handling were done as previously reported (Chong *et al.*, 2016).

Staphylococci bacteria identification and antibiotic testing

Staphylococci bacteria, culturing identification and antibiotic testing were done as previously reported (Chong *et al.*, 2016). Mannitol Salt Agar (MSA, Oxoid, Basingstoke, UK) selective medium was used to presumptively select for staphylococci bacteria. Staphylococci bacteria were positively identified by matrix -assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Staphylococci strains were screened for resistance to antibiotics by agar disk diffusion using Mastring M13 and Mastring M48 according to manufacturer's instructions (Mast Diagnostics, Merseyside, UK) and CLSI antimicrobial susceptibility testing standards.

Statistical analysis

At each of the two parks, the frequency of staphylococci being resistant to three or more antibiotics was calculated. A Fisher's exact test was used to determine if location was independent of the distribution of staphylococci bacteria resistance to three or more antibiotics, and subsequently for resistance to four or five or more antibiotics. Statistical significance was set at a value of $P<0.05$, and all statistical analyses were performed using IBM SPSS Version 21.

RESULTS

Even though exact population densities of long-tailed macaques are not known in Location A, previous studies observed a large macaque presence in such location (MacLeod, 2014). We previously identified 36 staphylococci isolates out of the 102 environmental isolates collected by MALDI-TOF analysis (Chong *et al.*, 2016). 26 of these staphylococci isolates were found to be antibiotic resistant (Figure 2). Although no standardized definitions for MDR have been agreed upon yet by the medical community, the majority define MDR as 'resistant to three or more antimicrobial classes' (Magiorakos *et al.*, 2012). A total of 20 staphylococci isolates were found to be resistant to three or more antibiotics at both locations (12 out of 12 at Location A and 8 out of 14 at Location B, Figure 3). There were significantly more isolates resistant to three or more antibiotics at Location A than Location B ($P=0.017$). Additionally, a total of 18 staphylococci isolates were identified as resistant to four or more antibiotics at both locations, with Park A having significantly more than Park B (12 out of 12 at Location A and 6 out of 14 at Location B, $P=0.002$, Figure 3). These results indicate there appears to be significantly more MDR staphylococci bacteria in areas with a presence of long-tailed macaques.

DISCUSSION

We previously reported the first-ever evidence of drug-resistant staphylococci in the non-hospital environment in Brunei Darussalam (Chong *et al.*, 2016). This short study indicates an environment populated with long-tailed macaques and have a high degree of human-animal interaction, could be potential reservoirs for MDR staphylococci bacteria transmitted via skin-to-surface contact. However,

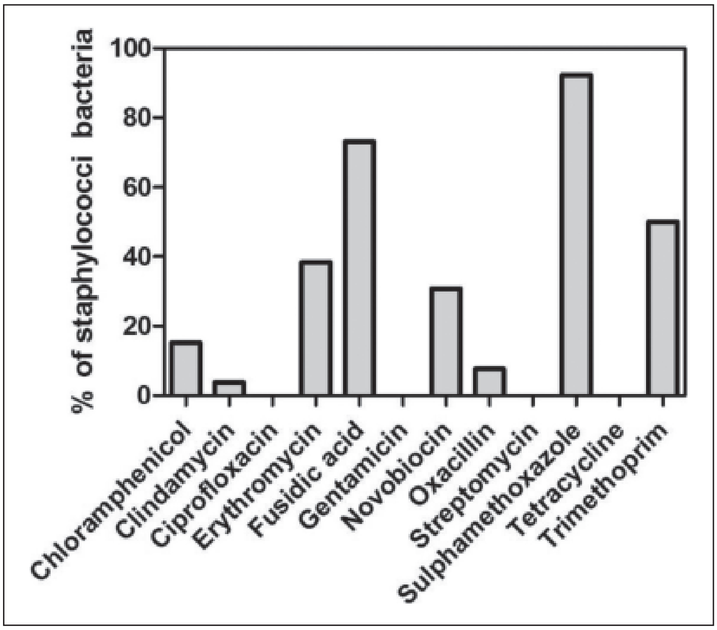

Figure 2. Percentage of staphylococci isolates resistant to each tested antibiotic.

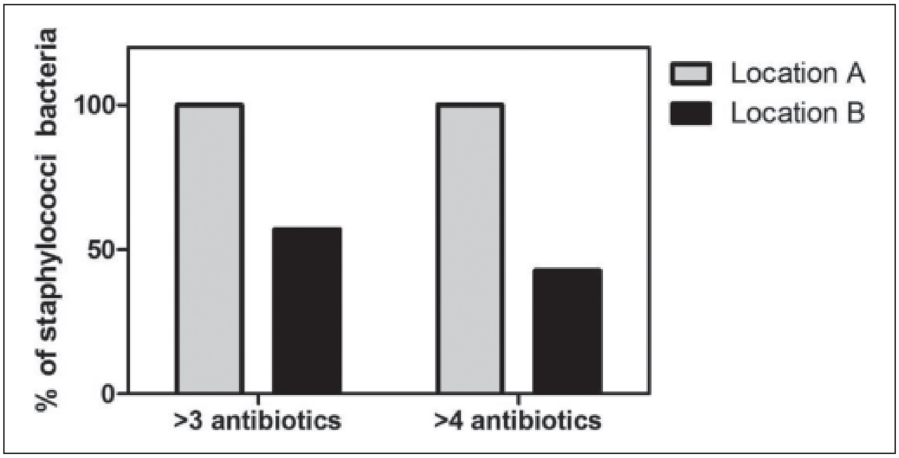

Figure 3. Distribution of staphylococci bacteria resistant to antibiotics.

given that Location A is frequented by the general public, the origin of staphylococci could possibly be human, and not animal, hence questioning the original source of these bacteria. Another limitation is that our study can only determine the prevalence of MDR staphylococci at a single time point. Future studies should take surface swab samples over several time points within a set period (e.g. weeks or months) to definitively determine the presence of MDR staphylococci in these locations. In conclusion, our short study suggest that a large presence of long-tailed macaques could be associated with a higher occurrence of MDR staphylococci bacteria. Though preliminary, these findings also suggest the possibility that long-tailed macaques could act as hosts for transferring antibiotic resistant staphylococci to humans. These findings have important implications on the future control, monitoring and surveillance of potentially pathogenic antibiotic resistant bacteria in public spaces.

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Conflict of interest

The authors declare that there is no conflict of interest.

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