Research Note

Isolation and Identification of an Emerging Pathogen, Kocuria marina, from Rattus rattus diardii

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Abstract. Members of the genus Kocuria are commonly found in the environment and they are also commensals of the mammalian skin and oropharynx mucosa. Human infections, although rare, are increasingly being reported recently suggesting that this genus has mostly been overlooked or misidentified. Its transmission route however, is still not known. We report here the isolation and identification of a Kocuria marina isolate from the lung of a wild urban rat (Rattus rattus diardii) caught at a wet market. The isolate was susceptible to most of the commonly used antibiotics. The finding suggests a possibility that rats could be a vector for K. marina.

The genus Kocuria is part of the Micrococcaceae family which currently consists of 18 species (Purty et al., 2013). Members of this genus are frequently found in the environment and they are also established commensals of the mammalian skin and the oropharynx mucosa (Savini et al., 2010; Purty et al., 2013; Brändle et al., 2014). Certain Kocuria species (spp.) are associated with improving the flavor and quality of fermented meats in the food industry (Carretto & Barbarini, 2010). Reports of clinical cases are rare. However, an increasing number of human infections have recently surfaced (Purty et al., 2013; Brändle et al., 2014), suggesting that this genus could have been overlooked as specimen contaminants (Carretto & Barbarini, 2010; Lai et al., 2011) and it could also have been misidentified using the classical phenotypic identification systems (Lee et al., 2009; Savini et al., 2010; Lai et al., 2011). Although Kocuria spp. are found in various environmental niches (Savini et al., 2010; Purty et al., 2013), to the best of our knowledge, there has been no report of Kocuria marina isolation from animals. Here, we report the isolation and identification of a K. marina isolate from the lung of a wild urban rat in Malaysia. This finding highlights rats as the possible vector for transmission of K. marina.

Rat trappings were conducted around the vicinity of several wet markets in Kuala Terengganu, the capital city of Terengganu on the east coast of Peninsular Malaysia (5.3333° N, 103.1500° E) for a period of one week in April 2015. All rats were trapped alive, have their age, sex and species determined, and morphometric measurements recorded prior to humane euthanization according to previously published methods (Alias et al., 2014).
Selected organs and body fluids (liver, kidney, blood and urine) were harvested and inoculated into the modified semisolid Ellinghausen-McCullough-Johnson-Harris (EMJH) medium for the isolation of Leptospira spp. (Benacer et al., 2013). Rat lungs however, were sterilely homogenized using the Precellys 24 tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) and inoculated onto Columbia agar supplemented with 5% sheep blood followed by 24 – 48 h incubation at 37°C under aerobic condition. The current study and its protocols received approval from the University of Malaya Research Ethics Committee (reference no. ISB/31/01/2013/SNMZ(R)).

Cultured bacteria isolates were subjected to Gram staining and had their morphological characteristics recorded. Sequencing of the partial 16S rDNA gene was performed to identify the species of bacteria isolated according to published protocols (Misbah et al., 2005). The DNA sequences were then compared with those on the GenBank public database using the BLAST program. The phylogenetic relationship between the Malaysian K. marina isolate and other Kocuria spp. was inferred by the construction of a maximum likelihood phylogenetic tree using MEGA version 5 (Tamura et al., 2011).

Antibiotic disk diffusion assay was performed on Mueller-Hinton agar incubated for 18 h at 37°C using amikacin (30 µg), cefoxitin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), gentamicin (10 µg), penicillin (10 U), rifampin (5 µg), teicoplanin (30 µg) and trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg) (BBL Sensi-Disc, Becton, Dickinson and Company, New Jersey, USA). Interpretation of susceptibility, i.e. susceptible, intermediate-resistant or resistant to the selected antibiotics was performed according to the Clinical and Laboratory Standards Institute breakpoints for Staphylococcus spp. (Clinical and Laboratory Standards Institute, 2014), since there are no breakpoints available for Kocuria spp. The reference strain, S. aureus ATCC 25923 was used as control for the disk diffusion assay.

A total of 58 rats were captured and screened. However, only one isolate TRE150902, from the lung of an adult male Rattus rattus diardii grew small (0.5 – 1.0 mm in diameter), mucoid, yellow, non-hemolytic, Gram positive coccoid clusters after 24 h incubation (Figure 1A). The colonies grew to 1.5 - 2.0 mm in diameter and exhibited β-hemolysis after 48 h incubation (Figure 1B). The lung specimen from which isolate TRE150902 was isolated, also grew mixed cultures of Staphylococcus spp. and

![Figure 1](image.png)

Figure 1. (A) Phenotypic appearance of K. marina TRE150902 showing yellow colonies. (B) β-hemolytic characteristic of K. marina TRE150902 on Columbia agar supplemented with 5% sheep blood after 48 h incubation.
Streptococcus spp. Comparison of the 16S rDNA sequence of isolate TRE150902 (accession no. LN871827) revealed 99.7% sequence similarity to K. marina strain RB-210 (Figure 2), hence the isolate was identified as K. marina. Results of the disk diffusion assay revealed that isolate TRE150902 was susceptible to amikacin, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, gentamicin, penicillin, rifampin, teicoplanin and trimethoprim/sulfamethoxazole (Table 1).

The isolation of K. marina from the lung specimen of a wild urban rat was unexpected. After reviewing the literature, we did not find any description of K. marina isolation from rats and this could well be the first report. It was not likely that this microorganism was a common environmental contaminant as we would expect more than one cultured isolate and also from other rat tissues if it was. Although at this point, we could not rule out if K. marina existed as a commensal of the oropharynx translocated to the rat’s lung. K. marina has been isolated from marine sediment in the East Siberian Sea (Kim et al., 2004) and K. varians from sea clams in Pahang, Malaysia (Jalal et al., 2009). The
association with marine environment substantiated the suggestion of K. marina transmission through seafood consumption in a case of spontaneous peritonitis (Brändle et al., 2014). It is possible that this rat may have contracted K. marina TRE150902 through ingestion of fresh seafood products sold in the market or from aerosol inhalation into the nasopharynx. Analysis of the 16S rDNA sequence obtained, identified isolate TRE150902 as K. marina and this method remains the most accurate method of identifying species within the Kocuria genus (Lee et al., 2009; Carretto & Barbarini, 2010; Savini et al., 2010; Purty et al., 2013; Brändle et al., 2014).

The antibiotic susceptibility profile of isolate TRE150902 is consistent with earlier published reports on the in vitro drug sensitivity of K. marina to antibiotics (Kim et al., 2004; Lee et al., 2009; Savini et al., 2010). However, isolate TRE150902 was sensitive to penicillin and trimethoprim/sulfamethoxazole, unlike the clinical isolates reported in Taiwan (Lai et al., 2011) and Korea (Lee et al., 2009). This suggests that the bacteria is largely still susceptible to most of the common antibiotics reflecting its origin from an antibiotic-free environment. We observed that the K. marina TRE150902 displayed β-hemolytic phenotype only after 48 h incubation, similar to K. marina isolated from solar salt works in India (Safarin et al., 2014). This phenotype seemed inconsistent with the earlier report of its non-hemolytic property (Lee et al., 2009). Perhaps the incubation condition is the difference between the two results. The ability to hemolyse red blood cells is most likely due to the production of streptolysin-like toxins similar to those found in group A Streptococcus (Molloy et al., 2011). These toxins were observed to have roles in causing tissue injury, destruction of neutrophils and bacteria translocation into deeper tissues (Molloy et al., 2011), which could explain the translocation of isolate TRE150902 into the rat lung.

The potential transmission of K. marina through seafood ingestion is worrying, even though currently there is a lack of evidence regarding the ability of the K. marina to induce foodborne diseases in humans (Carretto & Barbarini, 2010). The closely related K. rhizophila, shown previously to cause spoilage of raw chicken meat (Anang et al., 2006) and persistently infect a 3-year old girl with Hirschsprung's disease (Moissenet et al., 2012) highlighted an association of the bacteria with environmental origin and human disease. The discovery of K. marina from rat magnifies the zoonotic potential of this species and its potential to cause human infections, especially considering the involvement of K. marina in hospital patients (Lee et al., 2009; Lai et al., 2011; Brändle et al., 2014) and the affinity of Kocuria species for medical devices such as catheters (Savini et al., 2010).

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REFERENCES


