Tropical Biomedicine 33(3): 589–593 (2016)

Research Note

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

Loong, S.K.[a], Johari, J.[a], Che Mat Seri, N.A.A.[a], AbdulRazak, O.[b], Douadi, B.[b], Ahmad Nasrah, S.N.[c], Mohd Zain, S.N.[b] and AbuBakar, S.[a,c*]

[a]Tropical Infectious Diseases Research & Education Centre, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

[b]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

[c]Department of Medical Microbiology, Faculty of Medicine, 50603 Kuala Lumpur, Malaysia

[*]Corresponding author e-mail: sazaly@um.edu.my

Received 4 December 2015; received in revised form 17 December 2015; accepted 20 December 2015

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 37ºC 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 37ºC 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

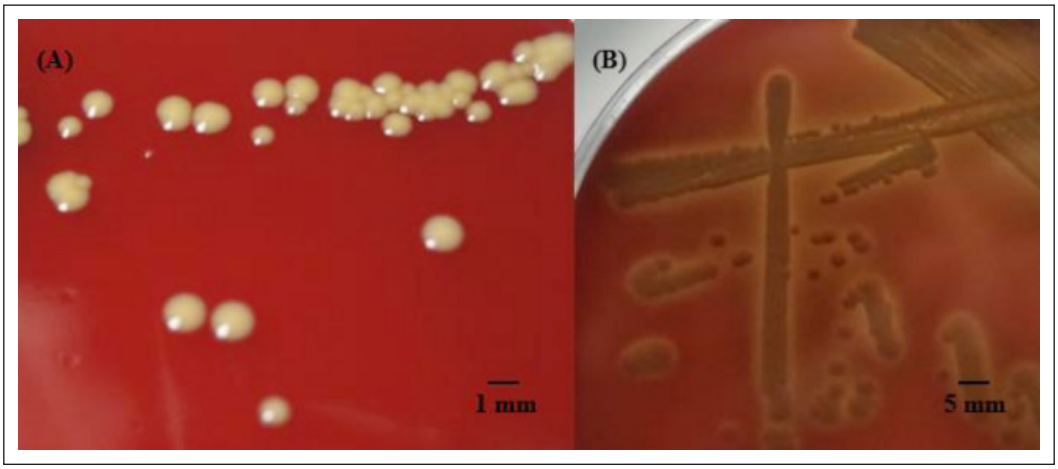

Figure 1. (A) Phenotypic appearance of *K. marina* TRE150902 showing yellow colonies. (B) -hemolytic characteristic of *K. marina* TRE150902 on Columbia agar supplemented with 5 % sheep blood after 48 h incubation.

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

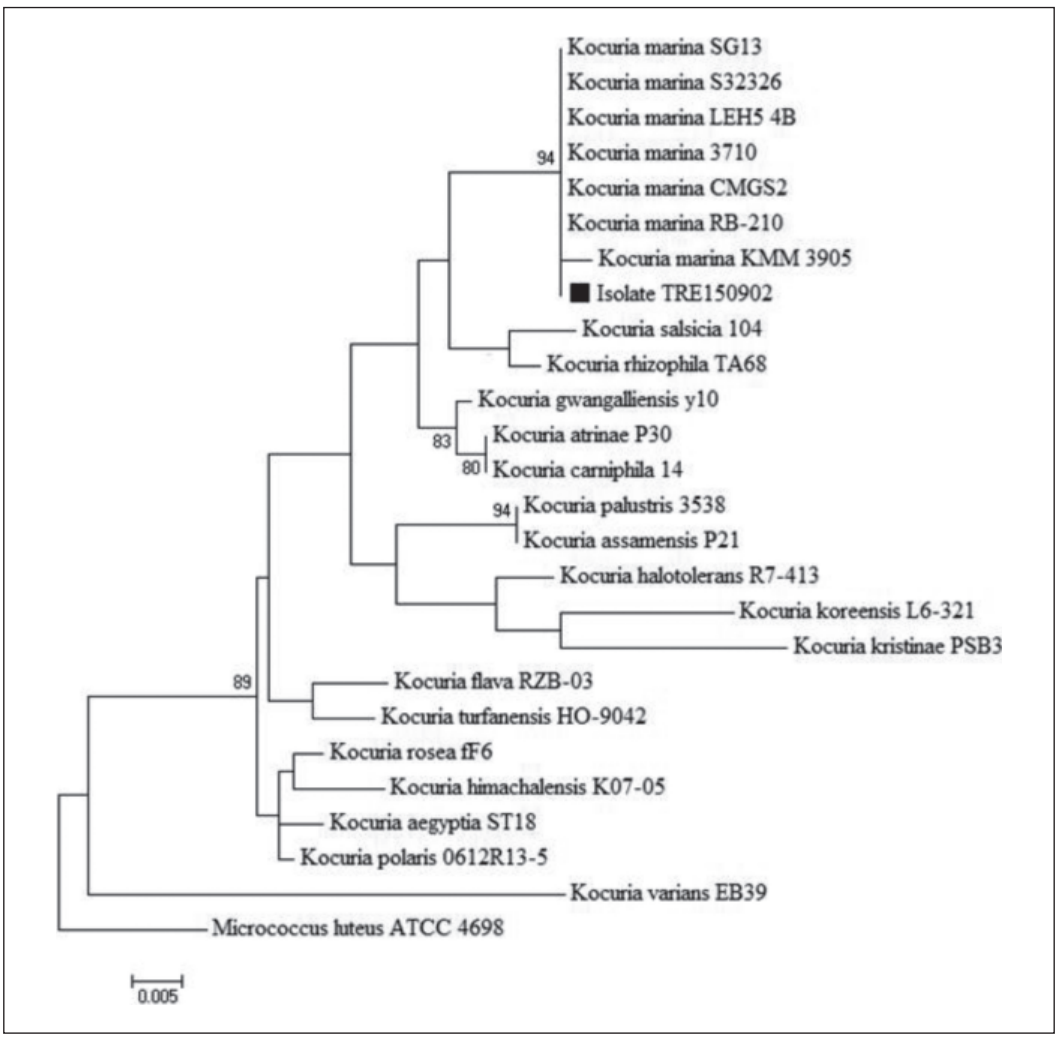

Figure 2. Maximum likelihood phylogenetic tree of selected *Kocuria* species partial 16S rDNA gene. Isolate TRE150902 was indicated by a solid-black square symbol. Isolate/strain names are indicated after the respective *Kocuria* species. *Micrococcus luteus* was used as an outgroup. Number at nodes indicate bootstrap values (%) for 1 000 replicates. Scale bar indicates nucleotide substitutions per site.

Table 1. Antibiotic susceptibilities of *K. marina* TRE150902

Antibiotic	Sensitivity testing results by disc diffusion assay[#] on isolate TRE150902
Amikacin	S (20 mm)
Cefoxitin	S (30 mm)
Chloramphenicol	S (18 mm)
Ciprofloxacin	S (22 mm)
Clindamycin	S (35 mm)
Gentamicin	S (23 mm)
Penicillin	S (42 mm)
Rifampin	S (36 mm)
Teicoplanin	S (25 mm)
Trimethoprim/sulfamethoxazole	S (30 mm)

[#] Zone diameter values are in parentheses. S, sensitive.

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).

Acknowledgements. This study was supported in parts by the University of Malaya Research Grant (RP016C-14AFR), Research Officer Grant Scheme (BR007-2015) and the High Impact Research Grant Scheme (E000013-20001).

REFERENCES

Alias, S.N., Sahimin, N., Edah, M.A. & Mohd-Zain, S.N. (2014). Epidemiology of blood parasitic infections in the urban rat population in peninsular Malaysia. *Tropical Biomedicine* **31**: 230-240.

Anang, D.M., Rusul, G., Radu, S., Bakar, J. & Beuchat, L.R. (2006). Inhibitory effect of oxalic acid on bacterial spoilage of raw chilled chicken. *Journal of Food Protection* **69**: 1913-1919.

Benacer, D., Mohd Zain, S.N., Amran, F., Galloway, R.L. & Thong, K.L. (2013). Isolation and molecular characterization of *Leptospira interrogans* and *Leptospira borgpetersenii* isolates from the urban rat populations of Kuala Lumpur, Malaysia. *American Journal of Tropical Medicine and Hygiene* **88**: 704-709.

Brändle, G., L'Huillier, A.G., Wagner, N., Gervaix, A., Wildhaber, B.E. & Lacroix, L. (2014). First report of *Kocuria marina* spontaneous peritonitis in a child. *BMC Infectious Diseases* **14**: 719.

Carretto, E. & Barbarini, D. (2010). *Kocuria*. In: Molecular detection of foodborne pathogens. Liu, D. (editor). Florida: CRC Press. pp. 201-205.

Clinical and Laboratory Standards Institute. (2014). Performance standards for antimicrobial susceptibility testing. *24th informational supplement M100-S24*. Wayne, PA.

Jalal, K.C.A., Najiah, N., Fathiyah, M., Kamaruzzaman, Y., Omar, M.N., Nurul Amin, S.M. & Jaswir, I. (2009). Bacterial pollution in molluscs arch clam, *Orbicularia orbiculata* and blood cockle, *Anadara granosa* of Pahang estuary, Malaysia. *Journal of Biological Sciences* **9**: 841-850.

Kim, S.B., Nedashkovskaya, O.I., Mikhailov, V.V., Han, S.K., Kim, K.O., Rhee, M.S. & Bae, K.S. (2004). *Kocuria marina* sp. nov., a novel actinobacterium isolated from marine sediment. *International Journal of Systematic and Evolutionary Microbiology* **54**: 1617-1620.

Lai, C.C., Wang, J.Y., Lin, S.H., Tan, C.K., Wang, C.Y., Liao, C.H., Chou, C.H., Huang, Y.T., Lin, H.I. & Hsueh, P.R. (2011). Catheter-related bacteraemia and infective endocarditis caused by *Kocuria* species. *Clinical Microbiology and Infection* **17**: 190-192.

Lee, J.Y., Kim, S.H., Jeong, H.S., Oh, S.H., Kim, H.R., Kim, Y.H., Lee, J.N., Kook, J.K., Kho, W.G., Bae, I.K. & Shin, J.H. (2009). Two cases of peritonitis caused by *Kocuria marina* in patients undergoing continuous ambulatory peritoneal dialysis. *Journal of Clinical Microbiology* **47**: 3376-3378.

Misbah, S., Hassan, H., Yusof, M.Y., Hanifah, Y.A. & AbuBakar, S. (2005). Genomic species identification of *Acinetobacter* of clinical isolates by 16S rDNA sequencing. *Singapore Medical Journal* **46**: 461-464.

Moissenet, D., Becker, K., Mérens, A., Ferroni, A., Dubern, B. & Vu-Thein, H. (2012). Persistent bloodstream infection with *Kocuria rhizophila* related to a damaged central catheter. *Journal of Clinical Microbiology* **50**: 1495-1498.

Molloy, E.M., Cotter, P.D., Hill, C., Mitchell, D.A. & Ross, R.P. (2011). Streptolysin S-like virulence factors: the continuing saga. *Nature Reviews Microbiology* **9**: 670-681.

Purty, S., Saranathan, R., Prashanth, K., Narayanan, K., Asir, J., Sheela Devi, C. & Kumar Amarnath, S. (2013). The expanding spectrum of human infections caused by *Kocuria* species: a case report and literature review. *Emerging Microbes & Infections* **2**: e71.

Safarin, Y., Donio, M.B., Velmurugan, S., Michaelbabu, M. & Citarasu, T. (2014). *Kocuria marina* BS-15 a biosurfectant producing halophilic bacteria isolated from solar salt works in India. *Saudi Journal of Biological Sciences* **21**: 511-519.

Savini, V., Catavitello, C., Masciarelli, G., Astolfi, D., Balbinot, A., Bianco, A., Febbo, D., D'Amario, C. & D'Antonio, D. (2010). Drug sensitivity and clinical impact of members of the genus *Kocuria*. *Journal of Medical Microbiology* **59**: 1395-1402.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731-2739.