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Isolation of Kytococcus schroeteri from the brown rat Rattus norvegicus

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Rodents such as *Rattus rattus* and *Rattus norvegicus* have adapted to living close to human settlements[1]. This presents a public health challenge as these rodents are considered as pests that can transmit a number of zoonotic diseases to humans[1,2]. A rodent surveillance programme was initiated between the years 2014 and 2015, focusing on rodents captured at wet markets where there is abundant food and considerable human traffic. The present study was conducted in several wet markets in Kuala Terengganu, Malaysia (5.333 3 °N, 103.150 0 °E).

Rodents were trapped alive using collapsible metal cage traps. After euthanization of the captured rodents (n=58), selected organs (kidney, liver, lung and spleen) were aseptically harvested and homogenized. The tissue suspensions were inoculated onto Columbia agar supplemented with 5% sheep blood. The agar plates were incubated at 37 °C under ambient condition for 24-48 h. Plates with poly-microbial growth were sub-cultured until pure colonies were obtained. Bacteria from the pure colonies were Gram stained and those with atypical visual morphological characteristics were subjected to 16S rDNA sequencing[2]. Isolate TRE153902 originating from the lung tissues of a female Rattus norvegicus grew small (1.0-1.5 mm in diameter), Gram-positive, mucoid, non-hemolytic, yellow-pigmented colonies after 48 h incubation (Figure 1A). Analysis of the 16S rDNA sequences resulted in the identification of Kytococcus (K.) schroeteri (accession no. LR812102). The ability to hydrolyse Tween 80[3], further affirmed the isolate as K. schroeteri TRE153902. Inspection of the bacteria cellular structures via transmission electron microscopy revealed spherical cells in tetrads (Figure 1B), typical of the Kytococcus genus[4]. Assessment of the antimicrobial susceptibility using the minimum inhibitory concentration interpretive standards for Staphylococcus sp.[5] found that the isolate was resistant to cefuroxime, ciprofloxacin and clindamycin, oxacillin and penicillin. K. schroeteri TRE153902 was however, sensitive to co-trimoxazole, erythromycin, gentamicin, rifampicin and vancomycin.

While *K. schroeteri* is usually found on the human skin as harmless commensal^[6,7], the originally described isolates were obtained from a 34-year-old woman with endocarditis^[3]. Since then, there were numerous reports implicating *K. schroeteri* as the causative agent

causing infections in humans[3,4,6-10]. No reports of infection in animals however, has been reported. Isolation of the bacteria from lung tissues of a wild brown rat suggests infection of animals. It is not likely that the isolation was due to cross contamination as this was prevented by using adequate personal protective equipment and the washing of surgical tools in between surgical procedures. Furthermore, we would expect more than one K. schroeteri isolate in the event of cross contamination[2]. It was possible that K. schroeteri has been largely misidentified and dismissed as a contaminant in many clinical cases[6,7,9]. This was compounded by the lack of reliable automated identification systems in the clinical microbiology laboratories and the difficulty in the identification of Kytococcus sp. using conventional biochemical assays[6,9]. In this study, accurate identification was achieved using 16S rDNA sequencing, complemented by Tween 80 hydrolysis. It was demonstrated that sequence-based identification remains the most practical and reliable method for the identification of emerging zoonotic bacterial pathogens[2,7].

Review of the current literature showed that reports of *K*. *schroeteri* in humans were predominantly associated with immunocompromised patients and those with silicone graft, prosthetic valve or shunt implantations[6–9]. Additionally, the isolation of *K*. *schroeteri* from the bronchoalveolar lavage fluid in a patient with pneumonia strongly implicated this pathogen as the cause of respiratory infection[10]. The finding here that *K*. *schroeteri* was recovered from the rat's lung tissues, raised the possibility that

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Figure 1. Images of *Kytococcus schroeteri* TRE153902 isolated from the lung tissues of a female *Rattus norvegicus*. (A) Culture of *Kytococcus schroeteri* TRE153902 on Columbia agar supplemented with 5% sheep blood after 48 h incubation. Scale bar=5 mm. (B) Transmission electron microscopy imaging of *Kytococcus schroeteri* TRE153902. Scale bar=500 nm.

the bacterium has a predilection towards the mammalian respiratory system. The strong expression of the adhesion-associated genes possibly aided the anchoring of bacteria onto respiratory tissues in the case of *K. schroeteri*-related pneumonia[10]. It is also possible that the strong adhesion trait of *K. schroeteri* supported its attachment onto medical implants such as prosthetic valves[7].

Even though clinicians are divided in deciding the best antimicrobial treatment for *K. schroeteri* human infections, they all agree that this species is intrinsically resistant to penicillin and oxacillin[3,7–10]. While it appears that *K. schroeteri* TRE153902 is sensitive to erythromycin, others have reported a resistant phenotype[3]. The *K. schroeteri* TRE153902 unlike other previously reported clinical isolates, was found resistant to cefuroxime[9] and ciprofloxacin[4]. This suggests that treatment of infection with *K. schroeteri* TRE153902 has to be guided by the antimicrobial susceptibility test results, though gentamicin and rifampicin would be effective in general[3,4,7–10].

The finding of *K. schroeteri* in a common brown rat highlights the potential of zoonotic spillover to humans. Further studies are required to determine the prevalence of *K. schroeteri* in the rodent population. This is especially important since emerging bacterial pathogens may be harboring resistant and virulent genes that are harmful to humans and easily transferable to other bacteria species.

This study received approval from the University of Malaya Institutional Animal Care and Use Committee (Reference no. ISB/31/01/2013/SNMZ (R)) and did not involve any endangered or protected animal species.

Conflict of interest statement

The authors declare that there are no competing interests.

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Authors' contributions

S.K.L., N.A.A.C.M.S., K.K.T., N.S.A. and S.N.A.N performed the experiments. S.N.M.Z. managed the field collection of rodents. S.K.L. wrote the manuscript together with S.A., who obtained funding for the study.

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