The 40-kDa protein of *Lumbricus rubellus* eradicates methicillin-resistant *Staphylococcus aureus* in an long-term nasal carriage model

La proteína de 40 kDa de Lumbricus rubellus *erradica* Staphylococcus aureus *resistente a la meticilina en un modelo de portador nasal a largo plazo*

Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) plays a pivotal role in the epidemiology and pathogenesis of MRSA infection.[1] Although mupirocin has emerged as the agent of choice for elimination of *S. aureus* nasal carriage,[3] several studies have identified high rates of MRSA resistant to mupirocin.[3,4] Therefore, the development of eradication therapy for MRSA nasal carriage is needed.

Previously, it has been demonstrated that the 40-kDa fetidin of the earthworm *Eisenia fetida* suppressed the growth of *Bacillus megaterium*.[5] Moreover, the extract of *Lumbricus rubellus,* another species of earthworm, displays more potent antibacterial activity *in vitro* against *S. aureus* than *E. fetida*.[6] Hence, this study aimed to investigate the antibacterial activity of the 40-kDa protein of *L. rubellus* in a model of long-term MRSA nasal carriage. Besides, the innate immune response, secretory IgA (sIgA) levels, was also evaluated.

L. rubellus was obtained from a local store in Malang, Indonesia. Total proteins of *L. rubellus* were extracted and characterized by SDS-PAGE. The 40-kDa protein band of *L. rubellus* was cut and eluted, yielding a concentration of 3550 mg/L.[7–10] The bacterial isolate, MRSA, was provided by the Department of Clinical Microbiology, Faculty of Medicine, Brawijaya University. For *in vivo* experiments, thirty female Balb/c mice (8–12 weeks of age) were randomly divided into six groups (5 mice/group), such as negative control, positive control, and infected mice treated with mupirocin and three different doses of the 40-kDa protein of *L. rubellus.* The study was approved by the ethics committee of Brawijaya University (Ref. No. 26-KE).

The model of long-term MRSA nasal carriage was performed as previously described.[2] Briefly, mice were injected with hydrocortisone subcutaneously (100 mg/kg/day at days 0 and 4), followed by intranasal infections of MRSA (10 μL containing 3×10^4 CFU) on days 5, 7, 9, 30, 32 and 34. On day 35, nasal swab specimens were inoculated on CHROMagar to confirm the nasal carriage of MRSA. The control treatment group was administered with 2% mupirocin (Bactroban, 125 mg diluted in 240 mL saline[11]), while the treatment groups were treated with the 40-kDa protein of *L. rubellus* (diluted in saline at a concentration of 1:10, 1:50, and 1:100 from the total protein obtained previously) in a volume of 10 μL to the respective nares for 5 days starting from day 36

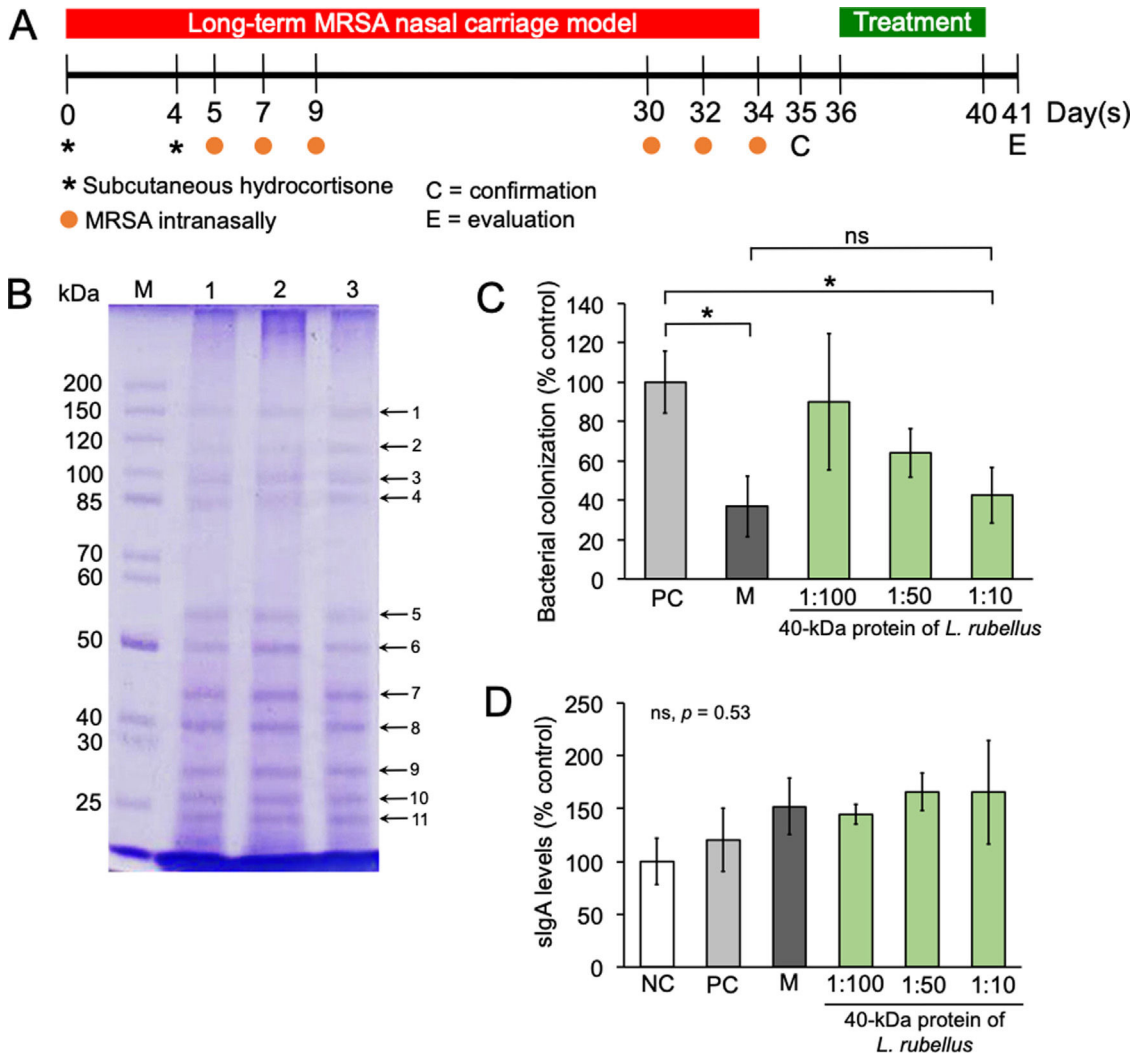

Fig. 1. Effect of the 40-kDa protein of *Lumbricus rubellus* in the long-term MRSA nasal carriage model. (A) Experimental timeline; (B) Protein profile of *L. rubellus*; M, marker; lane 1–3, replication 1–3; (C, D) MRSA colonization and the level of sIgA in the nasal mucosa after treatment with mupirocin or 40-kDa protein of *L. rubellus*, respectively. NC, negative control; PC, positive control; M, mupirocin; ns, not significant. (*) Indicates a significant difference between the two groups indicated in the graphs ($p < 0.05$).

(Fig. 1A). Negative controls were treated with saline only. The nasal swabs were conducted 24 h after the last administration of mupirocin or 40-kDa protein of *L. rubellus*. Swabs were then cultured in phenol red broth at 37 °C. The next day, 10 μL of broth at a concentration of 10^{-1} CFU/mL were inoculated on CHROMagar, incubated at 37 °C for 24 h, and then bacterial numbers were counted. No colonization of MRSA was observed in negative controls.

For quantification of sIgA levels, mice were sacrificed. Nasal mucosa was scrapped, diluted in 5 mL PBS containing a protease inhibitor cocktail (25 μg/mL) and centrifuged at 12,000 rpm (4 °C) for 15 min. Supernatants were purified with 40% ammonium sulfate. Suspensions were then diluted in 1 mL PBS and used for ELISA of sIgA.[12] All experiments were done in duplicate, resulting in similar findings. The numbers of bacterial colonization and sIgA levels were analyzed by one-way ANOVA, followed by Tukey's post hoc test using StatPlus. Significant differences were accepted when $p < 0.05$.

We identified eleven conserved protein bands from *L. rubellus* (Fig. 1B). Among them, low molecular protein bands (50, 40, 27, 25, and 22-kDa) displayed high concentration profiles. Mupirocin and a high dose of the 40-kDa protein of *L. rubellus* significantly inhibited MRSA colonization ($p = 0.03$ and $p = 0.04$, respectively) in the nasal mucosa of mice (Fig. 1C), thereby implying that the 40-kDa protein of *L. rubellus* was able to eradicate MRSA colonization as effectively as mupirocin. No significant antibacterial activity was observed from the other protein bands (data not shown). Although we failed to show that neither mupirocin nor the 40-kDa protein of *L. rubellus* were able to stimulate sIgA secretion ($p = 0.35$) (Fig. 1D), both groups tended to have higher levels of sIgA, suggesting that the level of sIgA would initially be beneficial to protect the nasal mucosa from MRSA invasion. Altogether, the 40-kDa protein of *L. rubellus* exhibits potent activity anti-MRSA *in vivo*.

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None to declare.

Conflict of interest

None to declare.

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Pneumococcal osteomyelitis of the rib in a vaccinated infant: An exceptional case☆

Osteomielitis costal por neumococo en lactante vacunado, un caso excepcional

Osteomyelitis mainly affects the long bones of the lower limbs. Rib osteomyelitis is rare (<1%). In the ribs *Staphylococcus aureus* is the cause of the majority of cases, followed by *Mycobacterium tuberculosis*.[1] There are three cases in the literature caused by microorganisms of the genus Streptococcus, and only one by *Streptococcus pneumoniae*.[2]

This was a six-month-old infant who came to the emergency department with a swelling in his left rib cage, with no history of trauma, with a low-grade fever of 37.5 °C 48 h previously, although apyrexial at the time of the hospital visit. No relevant family or personal history. No allergies. Vaccination schedule up to date, including three doses of 13 V pneumococcal vaccine (first dose batch G64205, second G75546), the third dose of pneumococcal vaccine had been administered ten days before (batch G92049). No previous infectious symptoms except for an isolated fever peak of 38.6 °C without other symptoms 24 h after the pneumococcal vac-

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