Beneficial bacteria such as *Lactobacillus* spp. may exhibit promising therapeutic potential due to their inhibitory effects against antibiotic-resistant microorganisms. The present study aimed to evaluate the potency of *Lactobacillus helveticus* C2 isolated from kefir against multidrug-resistant *Klebsiella pneumoniae*. The ability of *L. helveticus* C2 to inhibit MDR *K. pneumoniae* adhesion to intestinal enterocytes was observed *in vitro*. Intestinal colonization by MDR *K. pneumoniae* in the presence of *L. helveticus* C2 was further evaluated using a mice model. *L. helveticus* C2 exhibited strong antibacterial activity against MDR *K. pneumoniae*. Direct antagonism between *L. helveticus* C2 and MDR *K. pneumoniae* cells could not be ascertained *in vitro*. However, the adhesion of MDR *K. pneumoniae* to the small intestinal enterocytes of mice was inhibited by pili and outer membrane protein of *L. helveticus* C2. Moreover, *L. helveticus* C2
demonstrated inhibitory activity against MDR *K. pneumoniae* colonization by reducing the number of MDR *K. pneumoniae* colonies in BALB/c mice as induced by pellets and cell-free supernatant of *L. helveticus* C2. *L. helveticus* C2 is a probiotic strain of significant therapeutic or preventive potential due to its strong antibacterial activity, as well as the ability to inhibit the adhesion and colonization of MDR *K. pneumoniae*.

**Key words**
Antibacterial, Anti-adhesion, Anti-colonization, MDR *Klebsiella pneumoniae*, *Lactobacillus helveticus* C2

**Introduction**

In the last decades, probiotics have garnered intensive public attention due to their presumable beneficial health effects.\(^1\) Many studies have shown that fermented milk may have some role in cancer prevention, modify inflammatory bowel disease history, improve protein and fat digestion and vitamin synthesis, and augment in toxin protection and detoxification processes.\(^2-5\) *Lactobacilli* have been identified as a group of bacteria that are useful as probiotics. Moreover, *Lactobacilli* may potentially inhibit the growth of pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*.\(^6\) Several studies have demonstrated that *Lactobacilli* spp. can be used in pathogenic bacteria eradication through inhibition of the latter’s adhesion and colonization leading to prevention of biofilm development.\(^7,8\) This activity refers to Gram-negative bacteria that cause major infectious diseases such as urinary tract infections, pneumonia, intra-abdominal infections, and pyogenic liver abscesses.\(^9\) *Klebsiella pneumoniae* is a normal inhabitant in the mouth of healthy people.\(^10\) However, in a weak immune condition, it can migrate and colonize the digestive tract and can also cause pneumonia.\(^9,11\) The significance of *K. pneumoniae* infections has become more urgent with the discovery of multi-drug resistant (MDR) *K. pneumoniae*. Furthermore, the antibiotic resistance spectrum of MDR *K. pneumoniae* is expanding. MDR *K. pneumoniae* is a strong producer of biofilms, and thick biofilms that surround the cell become a barrier to the absorption of antibiotics.\(^11\)

Raras *et al.*\(^12\) successfully isolated *L. helveticus* C2 from milk kefir. *L. helveticus* C2 demonstrated the capacity to inhibit the adhesion and colonization of MDR *K. pneumoniae* in vitro. The present study screened *L. helveticus* C2 as a promising probiotic strain against MDR pathogens. The antibacterial activity of *L. helveticus* C2 against MDR *K. pneumoniae* was determined. Furthermore, the potential inhibiting effect of *L. helveticus* C2 against the adhesion of MDR *K. pneumoniae* in vitro was evaluated and the *in vivo* colonization in BALB/c mice was explored.

**Material and method**

**Bacterial Strains and Culture Conditions**

Bacterial strains of MDR *K. pneumoniae* and *L. helveticus* C2 used in this research were obtained from previous panel by Raras *et al.*\(^12\) MDR *K. pneumoniae* was cultivated in Luria-Bertani (LB) broth (Millipore, Catalog number: 28713-500G-F) and incubated at 37°C for 18 h under anaerobic conditions. *L. helveticus* C2 was cultivated in MRS broth (Millipore, Catalog number: 1106600500) media and incubated at 37°C for 24 h under aerobic conditions. Working stocks of cultures
were maintained in 20% (volume fraction) glycerol suspension frozen at -80°C.

**Antibacterial Activity Assay**

**Dual agar overlay**

Antibacterial activity of *L. helveticus* C2 pellet was determined against MDR *K. pneumoniae* bacteria using dual agar overlay.¹³ *L. helveticus* C2 pellet was inoculated in spots (∼ 6 mm diameter) on MRS agar (Millipore, Catalog number: P8074) plates and incubated at 37°C for 24 h in anaerobic conditions. The plates were overlaid with 15 ml of Muller-Hinton agar (Millipore, Catalog number: 70191-500G) containing 10⁷ cfu/mL MDR *K. pneumoniae*. After 24 h of aerobic incubation at 37°C, the diameter of inhibition zone was measured.

**Agar well diffusion**

Antibacterial activity of cell-free supernatant (CFS) and neutralized CFS (NCFS) of *L. helveticus* C2 was determined against MDR *K. pneumoniae* bacteria using agar-well diffusion.¹⁴ CFS of *L. helveticus* C2 was obtained by centrifugation at 4032 g for 15 min and filtration of overnight MRS broth inoculated with *L. helveticus* C2 isolates and incubated at 37°C for 24 h anaerobically. The pH of the supernatant was neutralized to prepare NCFS of *L. helveticus* C2. CFS or NCFS of *L. helveticus* C2 (100 µL) was inoculated on Muller-Hinton agar containing MDR *K. pneumoniae* (10⁷ CFU/mL) and incubated at 37°C for 24 h, and the diameter of inhibition zone was measured.

**In vitro assessment of anti-adhesive activity of Lactobacillus helveticus C2 isolate against MDR Klebsiella pneumoniae**

**Isolation of BALB/c mice enterocytes**

Isolation of enterocytes was carried out according to Weiser method as described by Nagayama and coll.¹⁵ Intestinal tissue of mice was cut into 5-cm pieces. For each piece, the lumen intestine was opened using the transverse cutting method. Intestinal contents were cleaned by using PBS (pH 7.4) containing 1 mm dithiothreitol. Subsequently, they were incorporated into the solution containing 1.5 mM KCl, 9.6 mM NaCl, 2.7 mM Na-citrat, 8 and 5 mM KH₂PO₄, 6 mM Na₂HPO₄ (pH 7.3). Next, they were incubated at 37°C and shaken slowly. The cloudy part of the suspension was collected with a sterile pipette and placed in sterile tubes. The concentration of enterocytes in the suspension was adjusted to 10⁶/mL. The enterocytes were finally kept at 4°C until they were used for adhesion tests.¹⁵

**Anti-adhesive activity of Lactobacillus helveticus C2**

The suspension (100 µL) of *L. helveticus* C2 at a concentration of 10⁶ CFU/mL and the suspension of MDR *K. pneumoniae* at a concentration of 10⁶ CFU/mL were mixed with 100 µL of enterocytes (10⁸ CFU/mL). The mixture was then incubated in an incubator shaker at 252 g and 37°C for 30 min. Cells were collected by centrifugation at 1008 g for 2 min. Next, the precipitate was collected for Gram staining. The precipitate was observed under microscope at 1000× magnification to obtain the cell type and adhesion index.¹⁵

**Inhibition test of Pili protein and outer membrane protein (OMP) of Lactobacillus helveticus C2 cells against the adhesion of MDR Klebsiella pneumoniae on BALB/c mice enterocytes**

Isolation of Pili and OMP was carried out according to the method of Ehara with modification by the use of Pili cutter designed by Sumarno.¹⁶,²⁶ The doses of pili protein and OMP of *L. helveticus* C2 were prepared with 0 mg (control), 25 mg, 50 mg, 100 mg, 200 mg, 200 mg, 400 mg, and 800 mg of the proteins in 300 µL PBS in Eppendorf tubes. In each Eppendorf tube, the enterocyte suspension (300 µL) at a concentration of 10⁸/mL was added and shaken gently in the water bath at 37°C for 30 min. Next, the bacterial suspension of MDR *K. pneumoniae* (300 µL, 10⁷/mL) was added to each mixture. The mixture was incubated in an incubator shaker at 252 g at 37°C for 30 min. The precipitate was taken, smeared on a glass object, and stained with Gram stain. The preparations were observed under a microscope at 1000× magnification, and the average number of bacteria attached to the enterocytes was calculated. Adhesion/ activity index was calculated by the number of bacteria attached to the apparatus, the latter equal on average to 100 enterocytes.¹⁶

**In vivo assessment of anticolonization activity in mice intestinal tissue**

Intestinal MDR *K. pneumoniae* colonisation assay was performed with 8-week-old male BALB/c mice. The mice were treated with 5 g/L streptomycin in drinking water. They were intragastrically inoculated with 200 µL of MDR *K. pneumoniae* suspension (3x10⁸ cfu/mL). After 1 day and subsequently every day for 5 days, they were intragastrically fed with *L. helveticus* C2.
(3×10^8 cfu/mL), supernatant of \textit{L. helveticus} C2, and LB as control in a total volume of 200 µl. Faeces were collected daily, homogenized in 1 mL of saline, and serial dilutions were plated on selective media to measure the total number of MDR \textit{K. pneumoniae} as cfu/g faeces.

To analyze the bacterial mucosa-associated burden, the mice were euthanized by chloroform on day 6, and their colons were taken and washed with saline. Samples (1 cm length) of the distal colon were collected, weighed, and homogenized in 1 ml of potassium phosphate buffer (pH 6) to determine the number of MDR \textit{K. pneumoniae} adhered to the mucosa. Results are expressed as cfu/g of tissue.

### Results

#### Antibacterial Activity of \textit{Lactobacillus helveticus} C2 Against Bacteria MDR \textit{Klebsiella pneumoniae}

The isolate of \textit{L. helveticus} C2 demonstrated a robust antibacterial activity (Figure 1) against MDR \textit{K. pneumoniae}, as indicated by the diameter of the clear zone formed. The diameter of the clear zone formed after gentamicin treatment was 44 mm. Treatment with \textit{L. helveticus} C2 pellets and \textit{L. helveticus} C2 pellets with neutralized pH, resulted in clear zones with diameters of 41 mm and 40 mm, respectively. Finally, treatment with CFS and nCFS of \textit{L. helveticus} C2 resulted in clear zone diameters of 44 and 42 mm, respectively.

#### Assessment of antagonism between \textit{Lactobacillus helveticus} C2 and MDR \textit{Klebsiella pneumoniae} cells for adherence to Enterocytes

\textit{Lactobacillus helveticus} C2 as well as MDR \textit{K. pneumoniae} properly adhered to the enterocytes, covering the entire cell surface. However, when both bacteria were administered together, \textit{L. helveticus} C2 could not prevent the attachment of MDR \textit{K. pneumoniae} to the enterocytes. MDR \textit{K. pneumoniae}, which has smaller size compared to \textit{L. helveticus} C2, adhered to the enterocytes in the space that was not occupied by \textit{L. helveticus} C2 (Figure 2).

Antagonism between MDR \textit{K. pneumoniae} and \textit{L. helveticus} C2 for adherence to mice enterocytes could not be ascertained with Gram staining. However, different concentrations of \textit{L. helveticus} C2 cells failed to cover the surface of epithelial cells. \textit{L. helveticus} C2 did not antagonize MDR \textit{K. pneumoniae} due to molecular size difference, \textit{L. helveticus} C2 being 10 times longer than MDR \textit{K. pneumoniae}. Increasing the volume of \textit{L. helveticus} C2 bacterial cells to 50 µL, 100 µL, and 200 µL was not able to inhibit the adherence of MDR \textit{K. pneumoniae} (Figure 2).

#### Influence of Pili and OMP of \textit{Lactobacillus helveticus} C2 on the Adherence of MDR \textit{Klebsiella pneumoniae} to Enterocytes

Since we were not able to demonstrate the existence of antagonism between the two bacteria, we sought to determine whether pili and OMP \textit{L. helveticus} C2 played a role in the inhibition of MDR \textit{K. pneumoniae}. First, the pili protein of \textit{L. helveticus} C2 was purified and administered into enterocytes exhibiting significant ability of inhibiting the bacterial adhesion of MDR \textit{K. pneumoniae}. In the group without treatment, enterocytes were surrounded by MDR \textit{K. pneumoniae} bacteria (Figure 3A). The administration of 25 µg pili reduced the number of MDR \textit{K. pneumoniae} when purified. Pili protein was added prior to the inoculation of MDR \textit{K. pneumoniae}, and the number of bacteria attached to enterocytes, as expressed by adhesion index value, was significantly reduced. The addition of 25 mg pili protein resulted in 30 % reduction of the adhesion index (Figure 3B). The administration of higher doses of pili apparently significantly (p = 0.000) reduced the number of MDR \textit{K. pneumoniae} attached to enterocytes. The higher the concentration of protein pili, 50 mg (Figure 3C), 100 mg, 200 mg, 400 mg and (Figure 3D–F), the smaller the adhesion index (Pearson correlation r = -0.983, p = 0.000). With 800 mg pili of \textit{L. helveticus} C2, 90% inhibition was achieved (Figure 3G).

Regarding OMP of \textit{L. helveticus} C2, when added to enterocytes that had been previously inoculated with MDR \textit{K. pneumoniae}: In the control treatment, MDR \textit{K. pneumoniae} adhered to the enterocytes (Figure 4A). However, upon OMP administration, the numbers of MDR \textit{K. pneumoniae} that adhered to the cells significantly reduced (p = 0.000) (Figure 4B-G). The higher the doses of OMP, the fewer the numbers of attached MDR \textit{K. pneumoniae} as determined by Pearson correlation analysis (r = -0.998, p = 0.000).

#### Influence of \textit{Lactobacillus helveticus} C2 pellets and CFS on intestinal colonization by MDR \textit{Klebsiella pneumoniae} Colonies in a Mice model

\textit{K. pneumoniae} is part of the normal flora that inhabits...
Figure 1
Antibacterial activity of *L. helveticus* C2 against MDR *K. pneumoniae* bacteria. Gentamicin, an antibiotic, as a control (A1; B1) and treatments with different forms of *L. helveticus* C2: pellets (A2), neutral pH pellets (A3), CFS (B2), and NCFS (B3).

Figure 2
Gram staining of intestinal enterocytes of BALB/c inoculated with MDR *K. pneumoniae* (10^8 cfu/mL) only (A) and with various concentrations of *L. helveticus* C2 (10^8 cfu/mL): 50 µL (B), 100 µL (C), and 200 µL. (1) BALB/c mice intestinal enterocytes; (2) *L. helveticus* C2 cells; and (3) MDR *K. pneumoniae* cells.

Figure 3
Gram staining of intestinal enterocytes of BALB/c inoculated with MDR *K. pneumoniae* only (A) and with various concentrations of Pili *L. helveticus* C2 protein: 25 mg (B), 50 mg (C), 100 mg (D), 200 mg (E), 400 mg (F), and 800 mg (G). (1) BALB/c mice intestinal enterocytes; (2) *L. helveticus* C2 cells; (3) MDR *K. pneumoniae* cells.
the human intestinal tract. We developed a mice model of MDR K. pneumoniae intestinal colonization and challenged it with L. helveticus C2, and the number of viable MDR K. pneumoniae cells was counted every day for 6 days. Mice faeces (without L. helveticus C2 administration) served as negative control.

The number of MDR K. pneumoniae cells was evaluated after challenging the mice with L. helveticus C2. The number of MDR K. pneumoniae in the control mice faeces was evaluated for 6 days, and a very dense colonization was observed after induction. The colonies’ number steadily rose until day 6. MDR K. pneumoniae in the faeces of mice without L. helveticus C2 administration showed high colonization on the first day (4.71×10^8 cfu/g faeces) and steadily increased, although not significantly (p = 3.371), on the sixth day.

As MDR K. pneumoniae was inoculated with L. helveticus C2, the number of colonies of MDR K. pneumoniae bacteria dropped drastically on day 6 (8.19×10^3 cfu/g faeces) (p=0.001). However, when CFS was administered daily, the colony number of MDR K. pneumoniae dropped significantly (p=0.003) from 4.70×10^8 cfu/g faeces to 8.07×10^6 cfu/g faeces in day 6 (Table 1).

Some values were 0, and hence, data transformation was carried out. The results of data transformation were then tested for normality and homogeneity, and we obtained data that were normally distributed, degree of freedom (df) = 4, n = 16, p > 0.05 and homogeneous, df1 = 3, df2 = 12, p > 0.05. One-way ANOVA statistical test found that there were significant differences in the number of MDR K. pneumoniae colonies, F = 109,857, df = 15, n = 16, p <0.05. Tukey’s statistical test was carried out as a follow-up test, and it showed that the treatment of the BALB/c mice in the positive control group was significantly different from other treatments (p = 0.000). However, the treatment of BALB/c mice with L. helveticus C2 pellets and the treatment of BALB/c mice with CFS L. helveticus C2 did not differ significantly (p = 0.228).

Discussion

K. pneumoniae is a major bacterial cause of pediatric mortality in Indonesia. A factor contributing to increased mortality is the development of multiple-antibiotic resistant strains of K. pneumoniae. In a previous study, we screened several Lactobacillus spp. from goat milk kefir that showed potency as anti-biofilm agents against MDR K. pneumoniae, and L. helveticus C2 exhibited the highest inhibitory effect during the biofilm development of MDR K. pneumoniae. In the present study, we further observed the potency of L. helveticus C2 as a therapeutic agent against MDR K. pneumoniae.
The antibacterial test showed that the pellets, CFS, and NCFS of *L. helveticus* C2 demonstrated a strong antibacterial activity against MDR *K. pneumoniae*. Lactic acid is a fermentation by-product of *Lactobacillus*. It can affect the permeability of Gram-negative bacterial cell membranes and facilitate the entry of other bactericidal compounds, thereby inducing the death of pathogenic bacteria. Maldonado et al. (2007) found that the supernatant of *L. fermentum* culture inhibited the growth of *K. pneumoniae*, whereas neutral supernatants had a weaker effect compared to whole cell cultures and acid supernatants, implying that the large amount of lactic acid directly produced by *Lactobacillus* has a significant effect on the inhibition of the proliferation of pathogenic bacteria.

Although our study was not specifically designed to identify the effective molecule involved in the antibiofilm effect of *Lactobacilli*, treatment of the *L. helveticus* C2 supernatant with Sodium hydroxide did not alter its anti-biofilm activity (data not shown), suggesting that molecules other than acids are involved. These molecules could be certain antimicrobial molecules, such as ethanol, fatty acids, hydrogen peroxide, and bacteriocin.

Other studies have shown that *Lactobacillus* spp. have the ability to inhibit several bacterial pathogens, including *Clostridium difficile*, *Escherichia coli*, *Streptococcus mutans* and *Staphylococcus aureus*.

*K. pneumoniae* is part of the normal intestinal flora and hence, we determined if *L. helveticus* C2 and MDR *K. pneumoniae* bacteria can antagonize for attachment to the mucus layer of small intestinal enterocytes of BALB/c mice. However, because the size of *L. helveticus* C2 (0.5–1.2 µM) is much larger than MDR *K. pneumoniae* (0.5 × 1.2 µM), MDR *K. pneumoniae* cells could easily stick to empty spaces not occupied by *L. helveticus* C2 cells.

One study has proposed that biofilm characteristics can be applied to a certain extent, to gut microbiota. The intestinal colonization by MDR *K. pneumoniae*, within the mucus layer, in a murine model was facilitated by the formation of biofilm. To investigate the activity of *L. helveticus* C2 against MDR *K. pneumoniae* in such a complex in vivo model, *L. helveticus* C2 was administered daily to mice after the colonization of their intestine by MDR *K. pneumoniae*. Surprisingly, the bacterial burden declined tremendously in the feces of experimental mice when compared to that of control mice. A similar effect was also observed when the CFS of *L. helveticus* C2 was administered, suggesting that live bacteria are more effective in the clearance of MDR *K. pneumoniae* colonization. Daily administration of *L. helveticus* C2 led to the modification of the colonisation kinetic rate of MDR *K. pneumoniae*. This hypothesis was supported by *in vitro* experiment where the adhesion of MDR *K. pneumoniae* to the small intestinal enterocytes of BALB/c mice was inhibited when the small intestinal enterocytes were coated with the pili protein and OMP of *L. helveticus* C2. Bacterial adhesion proteins are generally grouped into fimbrial/pili adhesins and OMP/afimbrial adhesins. Both of these adhesin proteins act as virulence factors through the adhesion and colonization process. The coating of the enterocytes with pili and OMP of *L. helveticus* C2 facilitated the adhesion process of *L. helveticus* C2, thereby inhibiting the attachment of MDR *K. pneumoniae*. Hence, it is speculated that the *in vitro* biocidal and antibiofilm activity of *L. helveticus* C2 disturbed the aggregation of cells with high adherence ability, leading to the destabilization of intestinal microbiota *in vivo* and the death of MDR *K. pneumoniae* in the gut.

### Table 1

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<thead>
<tr>
<th>Day</th>
<th>Number of MDR <em>K. pneumoniae</em> Colonies in <em>BALB-C</em> Mice Faeces</th>
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<tr>
<td></td>
<td>Number of MDR <em>K. pneumoniae</em> Colonies in MacConkey Agar Per 1cc Homogenate Faeces of <em>BALB-C</em> Mice</td>
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<tr>
<td>K (-)</td>
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The interaction of specific proteins on the surface of microbes (adhesion) with carbohydrate and glycoconjugate chains (receptors) on host cells is the initial stage of an infection.\textsuperscript{19,20} This study demonstrated that the coating of pili protein and OMP of \textit{L. helveticus} C2 can significantly (p < 0.05) reduce the amount of MDR \textit{K. pneumoniae} attached to the small intestinal enterocytes of BALB/c mice. The amount of MDR \textit{K. pneumoniae} negatively correlated with the concentrations of pili protein (r = -0.998; p < 0.05) and OMP (r = -0.983; p < 0.05) of \textit{L. helveticus} C2. The optimal dose of pili and OMP of \textit{L. helveticus} C2 protein for the maximal reduction of the adhesion index of MDR \textit{K. pneumoniae} was 800 µg.

When comparing the effect of pili and OMP of \textit{L. helveticus} C2 against the adhesion index of MDR \textit{K. pneumoniae}, \textit{L. helveticus} C2 pili protein had a stronger decreasing effect on the adhesion index value of MDR \textit{K. pneumoniae} than \textit{L. helveticus} C2 OMP. This can occur because most bacteria use two adhesion processes, the first step through pili (fimbriae) with loose bonds and then the second stage involves the surface protein or afimbrial adhesin which tightens the attachment.\textsuperscript{25} Thus, if the receptors on enterocytes have been fulfilled by the pili adhesin protein, the fimbrial adhesin \textit{K. pneumoniae} would not function properly and would not be easily replaced by afimbrial adhesin because this is only involved with the second stage. The situation is different if the enterocyte receptor is fulfilled by OMP; the fimbrial adhesin continues to function properly because it plays a role in the early stages of the adhesion process. This can also occur especially if the receptors of the two proteins are different.\textsuperscript{25,27}

Biosurfactants affect the interaction between bacterial cells through changes in the surface tension and cell wall load, and both factors are needed for attachment of bacterial cells to the substrate and between bacterial cells.\textsuperscript{28}

Although \textit{K. pneumoniae} belongs to the normal flora of the human digestive tract, it is also a common pathogen that can cause infection.\textsuperscript{29,30,31} The emergence of MDR \textit{K. pneumoniae} that is resistant to various antibiotics is a major medical concern due to the limited treatment options available and the significant morbidity and mortality caused by MDR \textit{K. pneumoniae} infection.\textsuperscript{32,33} Therefore, new strategies for the prevention and treatment of individuals infected with MDR \textit{K. pneumoniae} are needed.

In this study, evaluation of \textit{K. pneumoniae} colonies from faeces of BALB/c mice cultured on MacConkey media showed that BALB/c mice without any inoculation did not show any MDR \textit{K. pneumoniae} colonies from the first day to the sixth day of BALB/c mice faecal culture. This contradicts the report of Li \textit{et al.}\textsuperscript{34} which states that there are about 5% of \textit{Klebsiella pneumoniae} in the intestine and digestive tract as normal flora. No colony of MDR \textit{K. pneumoniae} was found from the first day until the sixth day of BALB/c mice faecal culture because the mice had been previously exposed to streptomycin for 48 h, thereby eradicating the normal flora of \textit{K. pneumoniae}. Meanwhile, a group of BALB/c mice, inoculated with MDR \textit{K. pneumoniae} but not treated with pellets and CFS of \textit{L. helveticus} C2, showed a high density of MDR \textit{K. pneumoniae} colonies on MacConkey agar media. These results indicate that MDR \textit{K. pneumoniae} bacteria can properly adhere and colonize the intestinal tract of BALB/c mice. MDR \textit{K. pneumoniae} directly attaches to the epithelial cells, and after occupying the site of primary infection, bacteria multiply and spread directly to the bloodstream through the tissues or lymphatic system. The process of infection allows bacteria to spread widely in the body and reach tissues conducive for multiplication.\textsuperscript{35}

\textit{Lactobacillus helveticus} C2 showed good adhesion and colonization inhibitory activity against MDR \textit{K. pneumoniae}. This inhibitory effect was demonstrated by a decrease in the number of MDR \textit{K. pneumoniae} colonies in BALB/c mice, which was induced by the pellets and CFS of \textit{L. helveticus} C2. \textit{Lactobacillus} may have a potential protective role against pathogens through different presumptive mechanisms including the production of antimicrobial compounds, inhibition of pathogenic bacterial adhesion to epithelial receptors, immune stimulation, and antagonism with pathogens for host cell binding sites.\textsuperscript{8,12}

Conclusion

\textit{Lactobacillus helveticus} C2 is a potential probiotic strain due to its strong antibacterial activity, as well as the ability to inhibit the adhesion and colonization of MDR \textit{K. pneumoniae}.

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Declaration of competing interest

The authors declare no conflict of interest.

Ethical approval

This research has been approved by the Animal Care and Use Committee, Brawijaya University (Reference No. 1071-KEP-UB).
Περίληψη

Ανταγωνιστική δραστικότητα του Lactobacillus helveticus C2 έναντι πολυανθεκτικής Klebsiella pneumoniae

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Λέξεις κλειδιά
αντιβιοτική δράση, ανασταλτική προσκόλληση, ανασταλτική αποικισμός, MDR Klebsiella pneumoniae, Lactobacillus helveticus C2
References


7. Atassi F, Servin AL. Individual and co-operative roles of lactic acid and hydrogen peroxide in the killing activity of enteric strain Lactobacillus johnsonii NCC933 and vaginal strain Lactobacillus gasseri KS120.1 against enteric, uropathogenic and vaginosis-associated pathogens. FEMS Microbiol Lett 2010; 304:29-38.


acid against Pseudomonas fluorescens and Methyl-


24. Favre-Bonté S, Licht TR, Forestier C, Krogfelt KA. Klebsiella pneumoniae capsule expression is necessary for colonization of large intestines of streptomycin-


