The Inhibitory Effect of Tinian's Donni' Såli Extract

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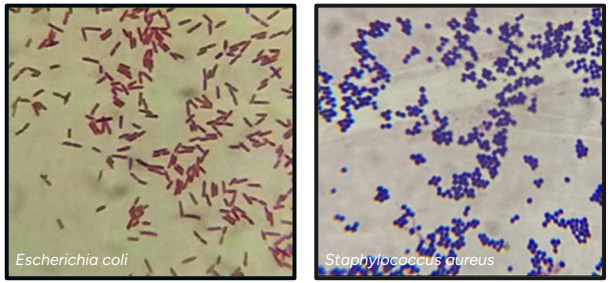


Gram's Staining of Bacterial Isolates. (a) Depicts the Gram Negative..., n.d.

What is Donni’ Såli?

- Cultivar of *Capsicum frutescens*.
- Measures roughly 2 centimeters in length.
- 50,000-100,000 Scoville Heat Unit.
- Used in local cuisine in the Northern Mariana Islands and Guam.

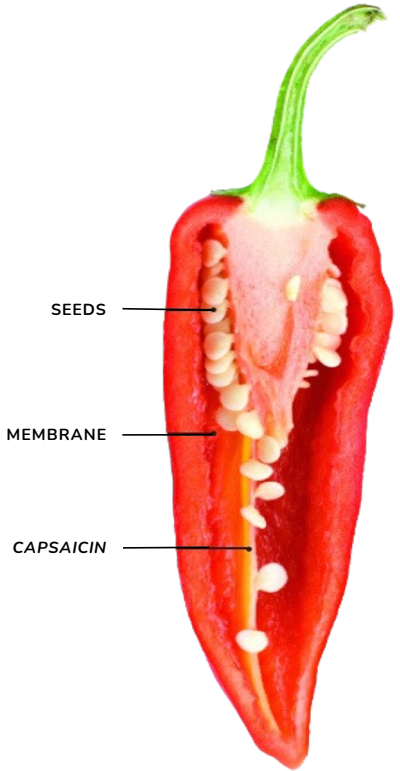
Donni’ plant

Såli or Micronesian Starling

Buehler, 2018
Bradley, 2010

Capsaicin in Hot Peppers

- Capsaicin is the compound responsible for the heat in hot peppers.
- In humans, it activates pain receptors across the body.
- Birds lack these receptors, so they can consume peppers and disperse seeds through droppings.
- Research indicates capsaicin exhibits antimicrobial properties.



Azlan, 2022
Gleason, n.d.

Antimicrobial Resistance (AMR)

AMR occurs when bacteria, viruses, fungi, and parasites no longer respond to antimicrobial medicines.

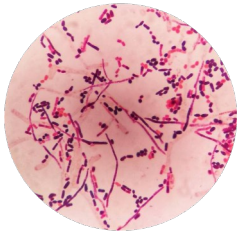
- Certain bacteria possess antibiotic resistance genes that can be transferred between bacteria through horizontal gene transfer
- Antibiotic misuse/overuse without proper medical guidance will give the bacteria an opportunity to develop resistance.

World Health Organization, 2023

AMR Snapshot

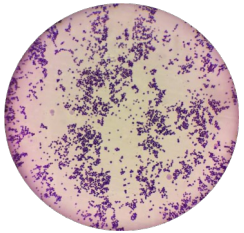
"Estimated 39 million deaths globally from AMR by 2050"

In 2019 alone, *E.coli* was linked to 950,000 deaths, *S. aureus* was associated with approximately 1.1 million deaths.



Escherichia coli

Gram-negative anaerobe commonly found in the gut, raw foods, and natural waters; some strains cause foodborne illness and urinary tract infections.



Staphylococcus aureus

Gram-positive aerobe found in the skin microbiome; some strains cause serious infections such as abscesses and sepsis. Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to most standard antibiotics.

Ikuta et al., 2022

Hypothesis

- Research shows that capsaicin contains antimicrobial properties.
- Will Tinian donni' extract inhibit the growth of *E. coli* and *S. aureus*?
- **We hypothesize that the Tinian donni' extract will inhibit the growth of bacteria at higher concentration levels.**

Methods: Donni’ Extract

1. Dehydrate donni’ for 4 days at 135°F
2. Blend & sift for fine powder
3. 1g donni’ powder : 6 millimeter (mL) methanol
4. Macerate for 48 hours
5. Collect extract using paper filter/syringe
6. Dilute extract with sterilized water to create 25%, 50%, and 75% solutions

Calculations were based on $\mathbf{C_1V_1 = C_2V_2}$
(C_1 = stock concentration, V_1 = volume of extract required; C_2 = desired concentration, V_2 = final volume)

Extract volumes were calculated at 30mL (25%), 60mL (50%), 90mL (75%)

Sterile water was added based on the difference $\mathbf{V_2 - V_1}$: 90mL (25%), 60mL (50%), 30mL (75%)

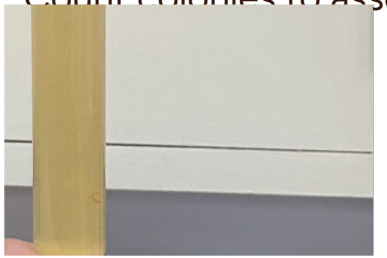
Dehydrated donni’

Grinding donni’

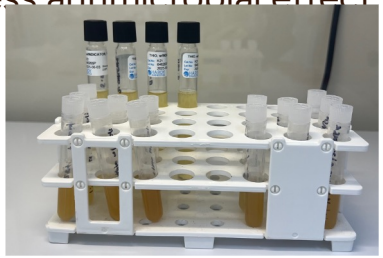
Donni’ extract

Methods: Antimicrobial Analysis

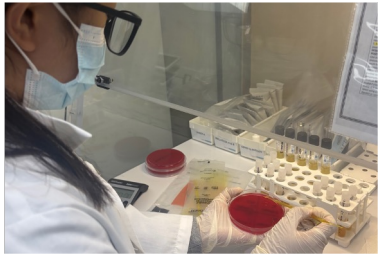
1. Inoculate bacteria from Trypticase Soy Agar slant to thioglycolate broth (thio); incubate 24 hours
2. Mix thio-broth cultures with donni' extract (25%, 50%, 75%) at 1:0.5 and 1:1 ratios; incubate 24 hours
    a. 1:0.5 (1 mL of thio with bacteria + 0.5 mL of donni' extract)
    b. 1:1 (1 mL of thio with bacteria + 1 mL of donni' extract)
3. Streak treated cultures onto blood agar plate, untreated bacterial culture as control; incubate 24 hours
4. Count colonies to assess antimicrobial effect



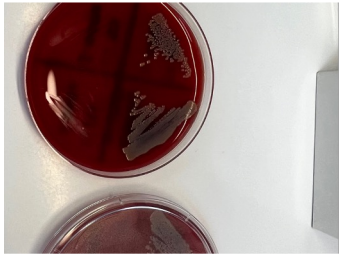
Broth with bacteria



Tubes of broth with bacteria + extract

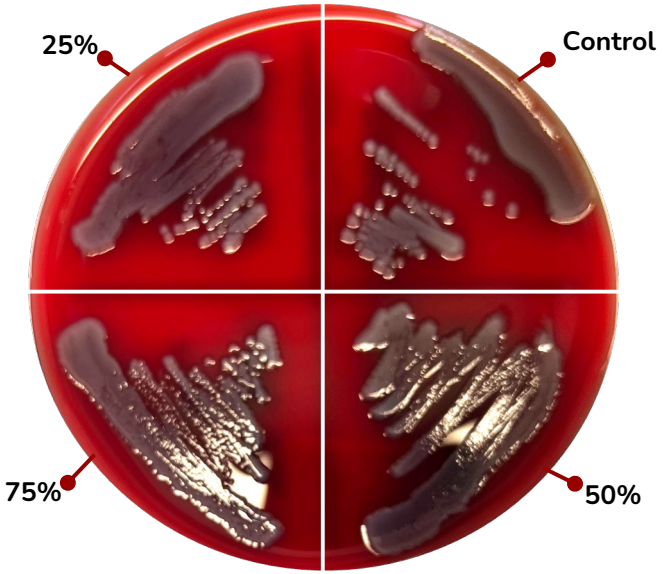


Streaking on blood agar plates

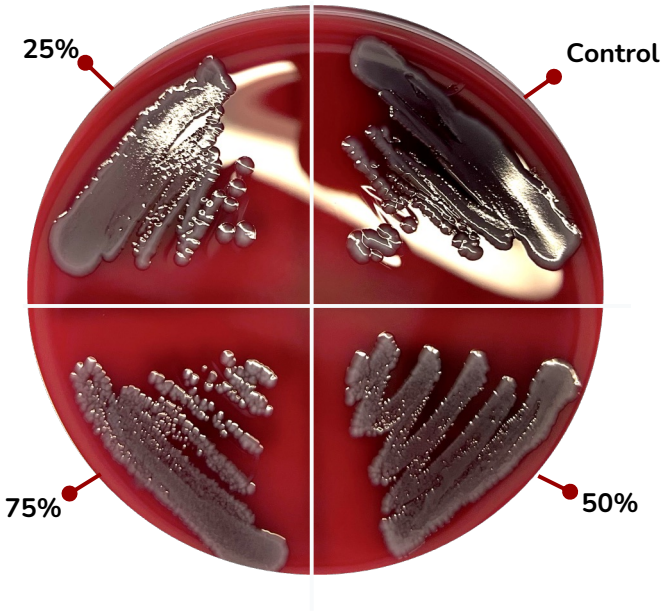


Results

Results: *E. coli*

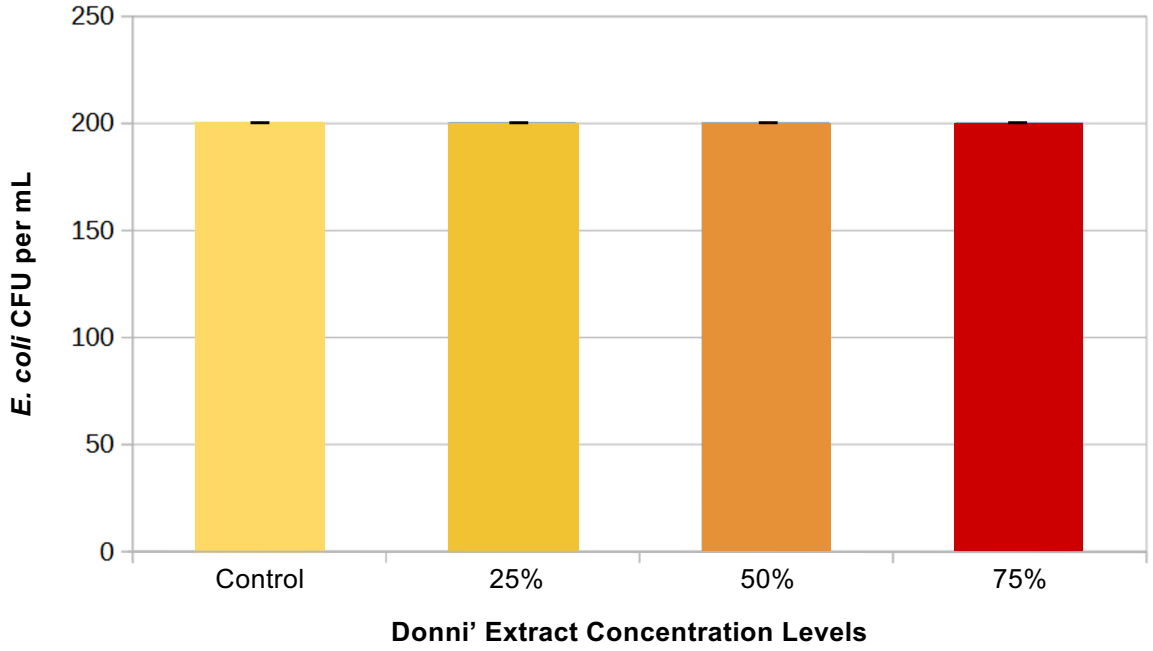


1:0.5



1:1

Results: *E. coli* 1:0.5



n=3

Colony-Forming Unit Computation

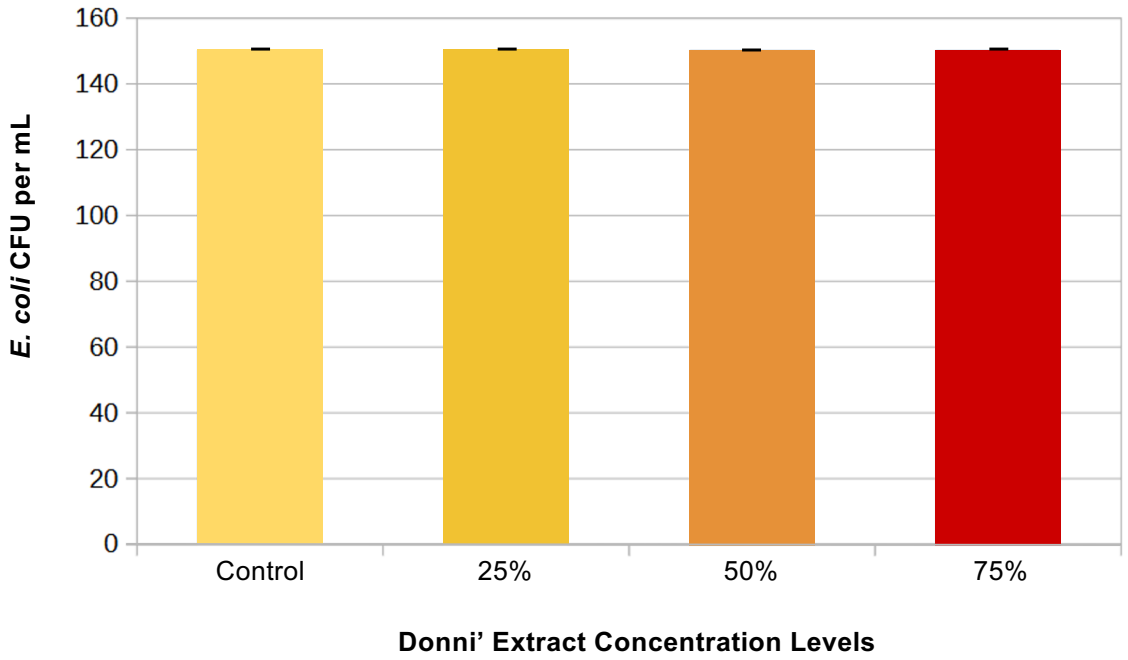
$$\frac{(\text{number of colonies} \times \text{dilution factor})}{\text{volume of culture}}$$

Colony Count= TNTC (~300) ± 0

CFU per mL calculated as:

- Control - 200 ± 0
- 25% - 200 ± 0
- 50% - 200 ± 0
- 75% - 200 ± 0

Results: *E. coli* 1:1



n=3

Colony-Forming Unit Computation

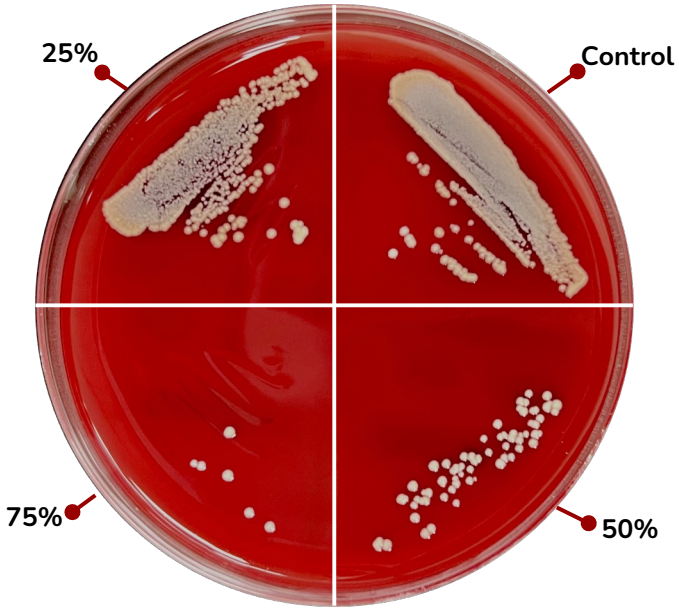
$$\frac{(\text{number of colonies} \times \text{dilution factor})}{\text{volume of culture}}$$

Colony Count= TNTC (~300) ± 0

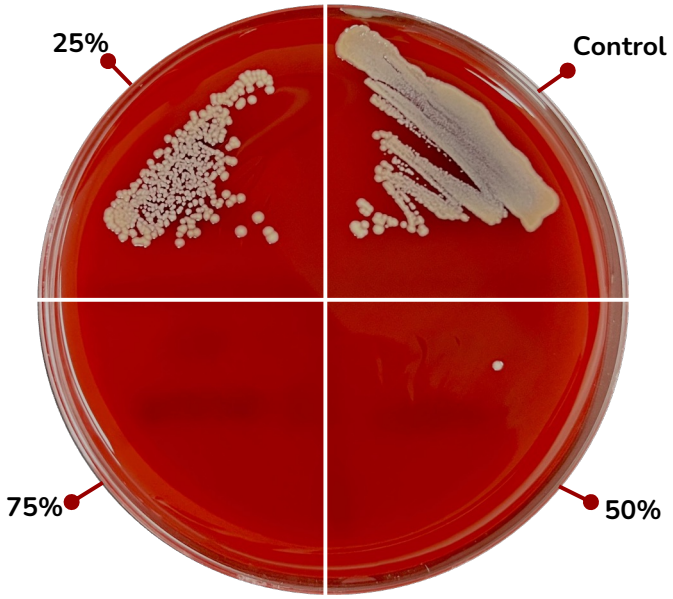
CFU per mL calculated as:

- Control - 150 ± 0
- 25% - 150 ± 0
- 50% - 150 ± 0
- 75% - 150 ± 0

Results: *S. aureus*

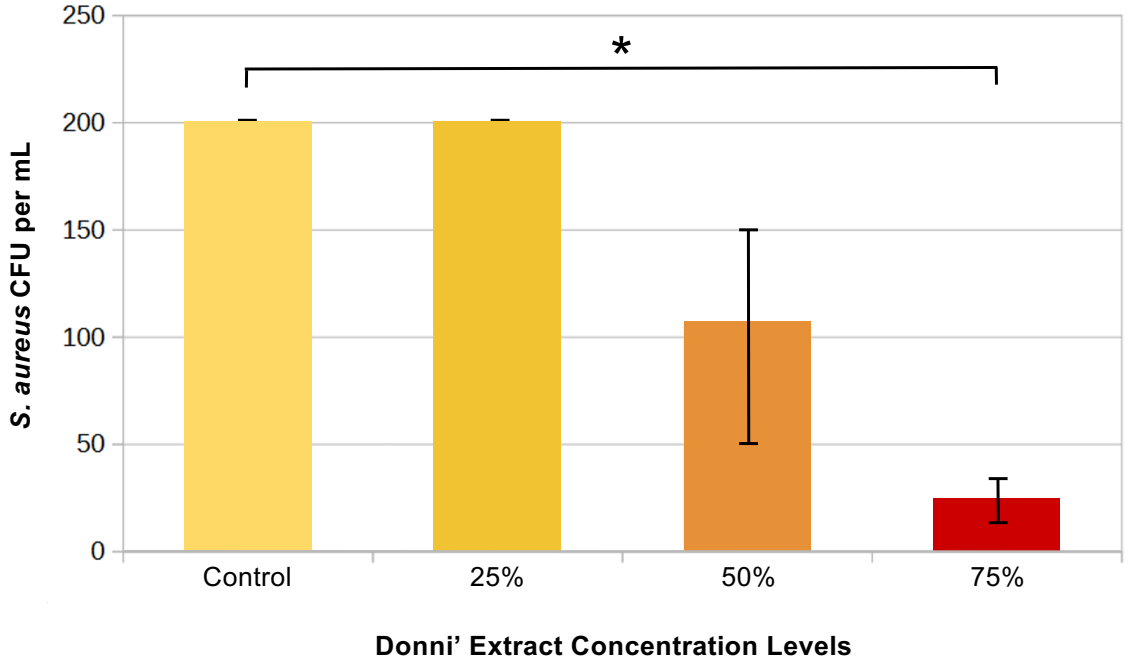


1:0.5



1:1

Results: *S. aureus* 1:0.5



One-way ANOVA with post-hoc Tukey HSD
* $p < 0.05$
n=3

Colony-Forming Unit Computation

$$\frac{(\text{number of colonies} \times \text{dilution factor})}{\text{volume of culture}}$$

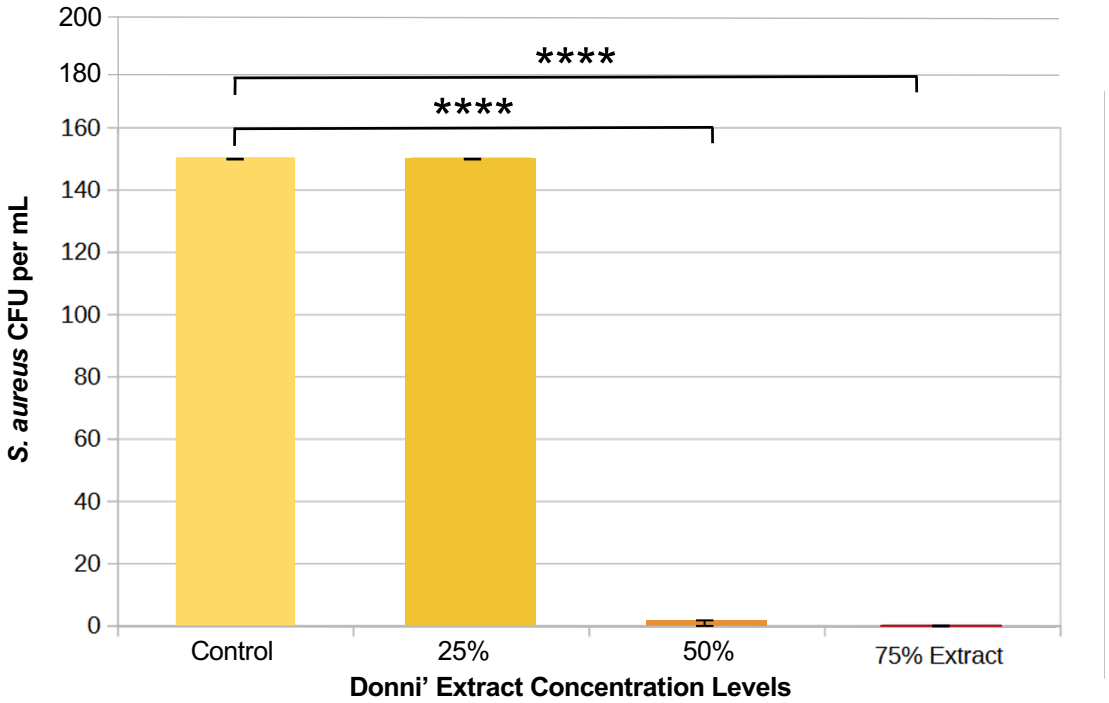
Colony count:

- Control - TNTC (~300) ± 0
- 25% - TNTC (~300) ± 0
- 50% - 159.67 ± 72.49
- 75% - 36 ± 15.01

CFU per mL calculated as:

- Control - 200 ± 0
- 25% - 200 ± 0
- 50% - 106.45 ± 48.32
- 75% - 24 ± 10.01

Results: *S. aureus* 1:1



One-way ANOVA with post-hoc Tukey HSD
**** $p < 0.00001$
n=3

Colony-Forming Unit Computation

$$\frac{\text{(number of colonies} \times \text{dilution factor)}}{\text{volume of culture}}$$

Colony count:

- Control - TNTC (~300) ± 0
- 25% - TNTC (~300) ± 0
- 50% - 2.33 ± 1.33
- 75% - 0 ± 0

CFU per mL calculated as:

- Control - 150 ± 0
- 25% - 150 ± 0
- 50% - 1.17 ± 0.67
- 75% - 0 ± 0

Conclusion

- Donni' extract **does not** inhibit the growth of *E. coli* at 25%, 50%, and 75% extract concentration levels and at 1:0.5 and 1:1 ratios
- Donni' extract **does** inhibit the growth of *S. aureus* at 50% and 75% extract concentration levels at both ratios but observed strongest and complete inhibition at 75% concentration levels at a 1:1 ratio

Implications:

- Suggests donni' extract has antimicrobial activity with *S. aureus* but not *E. coli*
- May contribute to the development of natural alternatives to conventional antibiotics.

Future Directions

- Evaluate safety and toxicity profile of capsaicin specifically against infections caused by *S. aureus*
- Explore higher donni' extract ratios against *E. coli*
- Test donni' extract against other gram-positive and gram-negative bacteria

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