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RESEARCH PAPER

Department of Biomedicine

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OBJECTIVES AND HYPOTHESIS

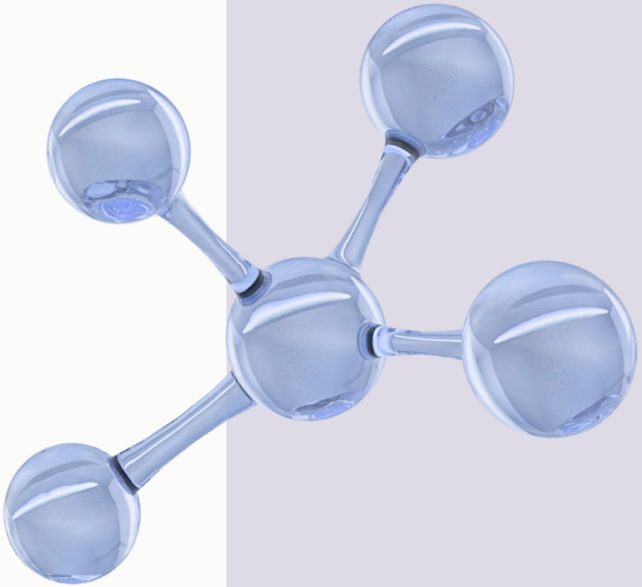
Hypothesis
Changes in Prevotella levels in the human microbiome can affect autoimmune disease development, and using the right antibiotics can help prevent and treat specific autoimmune conditions.

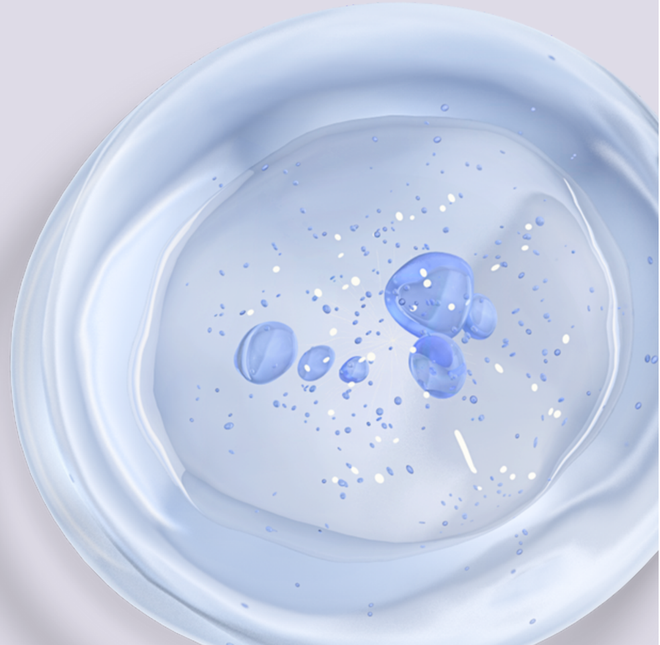
Objective 1.
Analyze the research methodologies used to test the efficiency of different antibiotical agents against Prevotella bacteria, investigate potential treatments for autoimmune progression.

Objective 2.
Create a list of the most efficient antibiotics against bacteria (Prevotella) that might cause specific types of autoimmune diseases.

Introduction

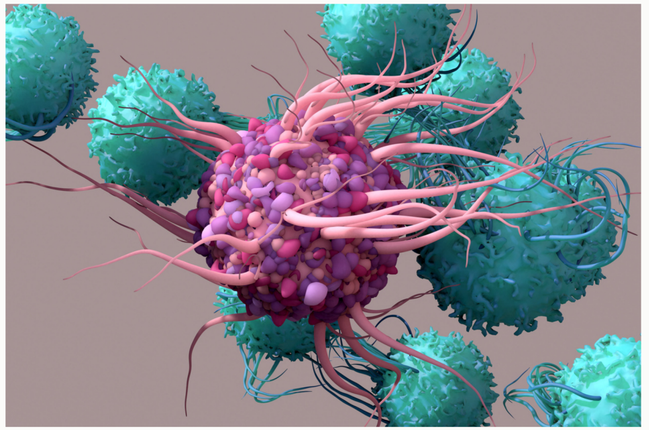
- The strength of our bodies relies on the trillions of microorganisms within us, with about 100 trillion microbes outnumbering human cells 10 to 1.
- Approximately 70%-80% of the human microbiome and immune system are located in the gut. Changes in gut microbiota, such as an increase in Prevotella bacteria, may be linked to autoimmune diseases.
- Since the early 1980s, autoimmune disease incidence has risen significantly, and recent research is exploring how immune system imbalances might contribute to these conditions.





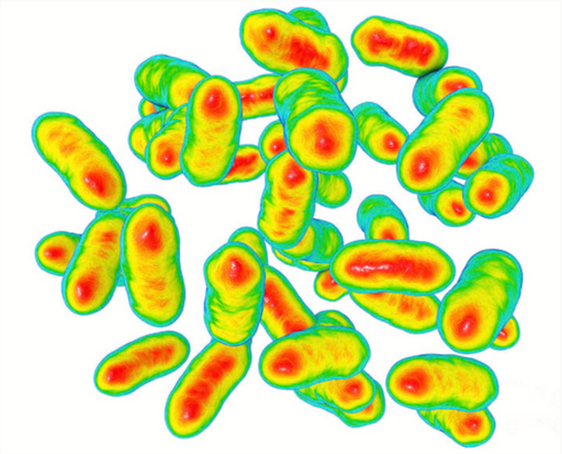
Background information

1 An autoimmune illness progresses when the immune system mistakenly attacks healthy, functional body elements as if they were foreign organisms. The exact causes are uncertain, varying from genetic to environmental factors.



Autoimmune diseases are triggered when the immune system malfunctions and attacks the body's own structures.

2 Prevotella species, including Prevotella copri, are common Gram-negative rods in the human gut. P. copri is controversially associated with rheumatoid arthritis, hypertension, and chronic gut inflammation.



Prevotella copri species

Introduction to Methodology

- Prevotella bacteria are **Gram-negative**, making them more resistant to some antibiotics and complicating treatment. My research will focus on **Prevotella copri**, a common species in the gut microbiome.

- The research methodology involves **Antimicrobial Susceptibility Testing (AST)** using the Disk diffusion method to identify the most effective antibiotics against Prevotella copri aiding in their use for treating autoimmune illnesses.

- In-vitro antimicrobial susceptibility testing (AST) predicts a bacterial pathogen's in-vivo response. Results from disc diffusion tests are categorized as resistant, susceptible, or intermediate. Choosing breakpoints is subjective, based on data analysis and expert judgment.

The in-vitro AST requirements

- ***Isolation***

Bacteria subjected to AST must be isolated in pure culture from the submitted sample.

- ***Storage***

Important bacterial isolates and others should be stored for future analysis via lyophilization or cryogenic preservation at −70°C to −80°C.

- ***Reference methods***

Standard reference methods should be used to ensure consistent and accurate identification of bacteria to the genus and/or species level.

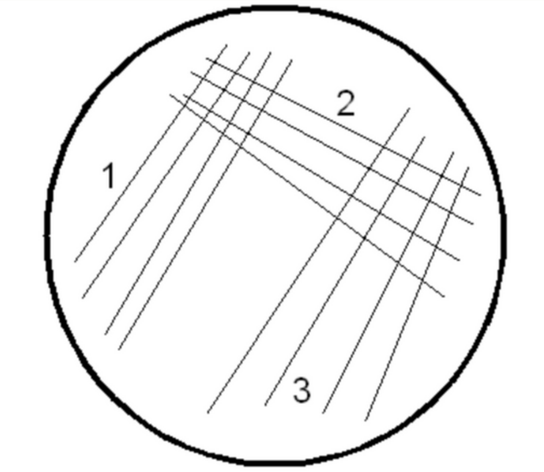
In my research I tested 5 antibiotics:

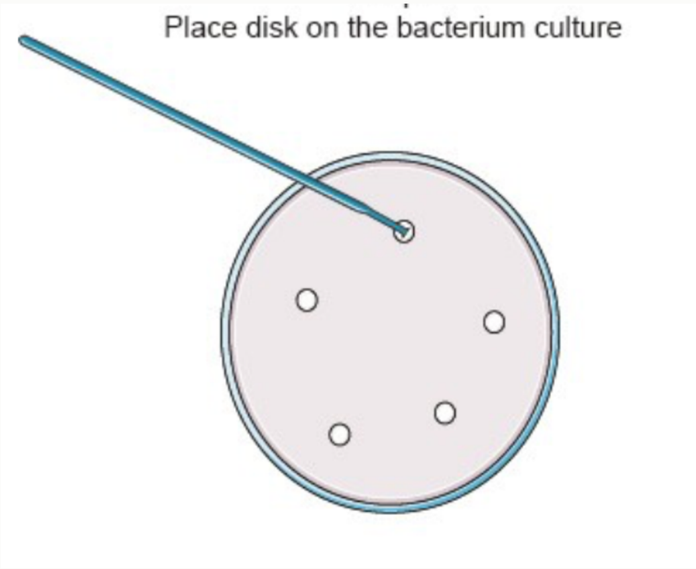
1. Amoxicillin
2.Ceftazidime
3.Metronidazole
4.Tetracycline
5. Piperacillin

The in-vitro AST proccess

Streaking

- Step 1. Using an inoculating loop or sterile needle, streak Prevotella samples onto the upper one-fourth of an agar plate with overlapping strokes. Use separate plates for each antibiotic, or divide a plate to test two tissues. Label the plate.





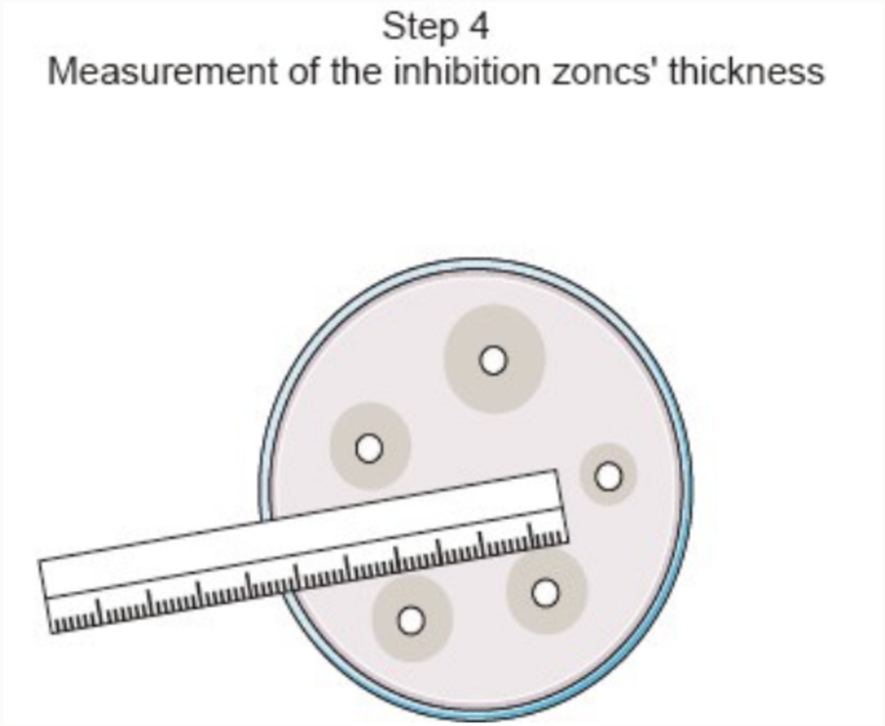
Placing antibiotics

- Step 2. After drying the plate for 1-3 minutes, use sterile forceps or a disk dispenser to place antibiotic disks evenly on the agar, avoiding edges. Press each disk gently to ensure contact.

The in-vitro AST

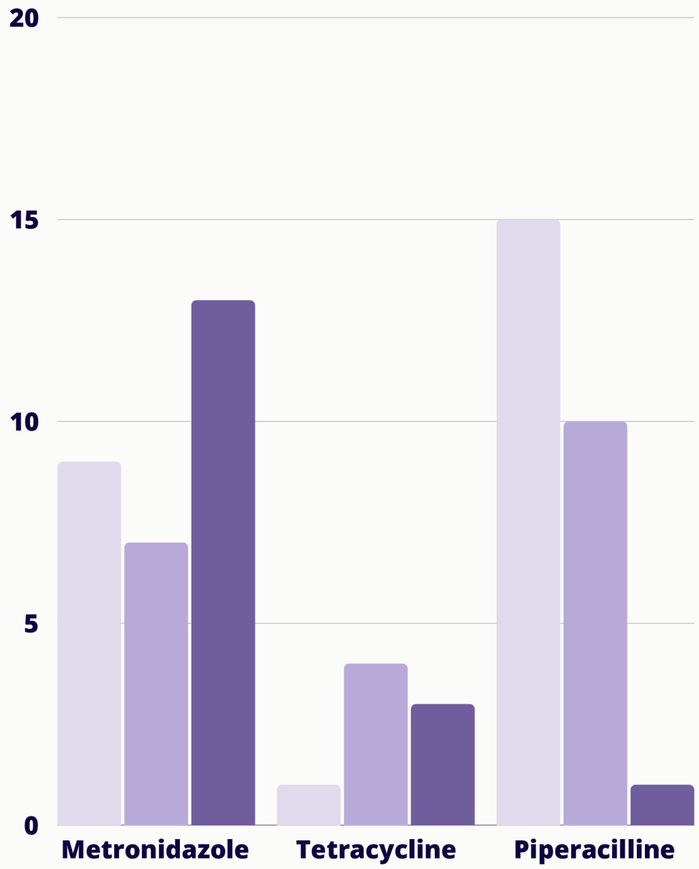
Incubation

Step 3. Place all samples into an incubator and set a temperature for 35-36 degrees by cesium. Incubate samples for 16-18 hours.

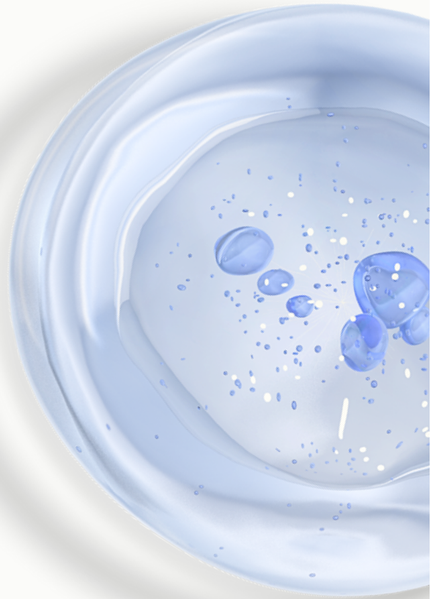


Measuring

Step 4. After incubation, measure and record the inhibition zones' diameters in millimeters using a ruler



Data Analysis



The results of the AST (Antibiotic Susceptibility Test) are going to be shown in the following tables

Antimicrobial agent		MIC/ mg/L	
Killing potential of an agent, %	range	50%	90%
Amoxicillin	<0.016 to >256	6	>256
Ceftazidime	0.094 to >256	6	>256
Metronidazole	<0.016 to >256	0.125	0.38
Tetracycline	<0.016 to >256	12	128
Piperacillin	<0.016-4	0.023	1,5

Table 1. The table includes the range of Minimum Inhibitory Concentrations (MIC), as well as the MIC values at the 50th percentile (50%) and 90th percentile (90%).

Antimicrobial agent		Percentage of isolates with indicated susceptibility	
	Susceptible	Intermediate	Resistant
Amoxicillin	NA	NA	NA
Ceftazidime	NA	NA	NA
Metronidazole	96%	0%	4%
Tetracycline	36%	0%	49%
Piperacillin	100%	0%	0%

Table 2. This table indicates the percentage of isolates that were susceptible, intermediate, or resistant to each agent.

RESULTS

Metronidazole has high efficiency:
96% of isolates are susceptible to this agent and only 4% of them are resistant.
The 50% MIC value is 0.125 which is very low. It indicates high efficiency in low concentrations of Metronidazole.

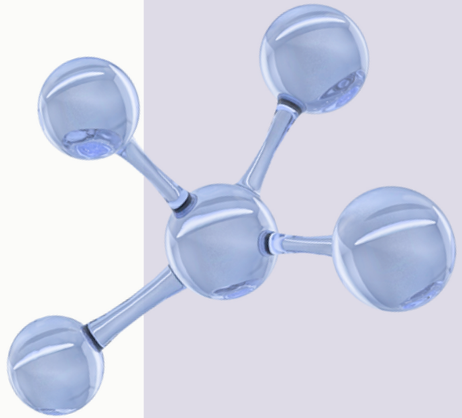
Tetracycline has a medium efficiency, and might not be the best option to address Prevotella:
With the susceptibility rate of only 36%, 49% of isolates remain resistant to this antimicrobial agent.

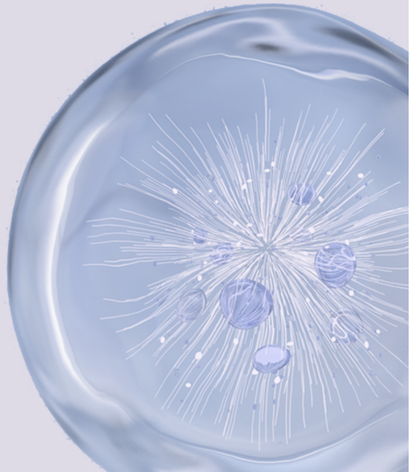
Piperacillin has the highest efficiency/killing capacity:
100% of Prevotella bacterias are susceptible to this antibiotic and the 50% MIC value is 0.023.

Efficiency *Amoxicillin* and *Ceftazidime* is low: these agents do not affect Prevotella bacterias.
The 90% MIC value of both antibiotics is >256 which is high for destroying bacterias. It is less likely to address Prevotellas.

Discussion

AST results show that both Metronidazole and Piperacillin effectively destroy Prevotella species, with Piperacillin achieving 100% bacterial destruction. However, since Prevotella bacteria play a role in immune system function, completely eliminating them could be harmful. Metronidazole, with a 50% MIC value of 0.125 compared to Piperacillin’s 0.023, allows some bacteria to survive, helping maintain gut microbiome balance. Given that Metronidazole is effective against 96% of Prevotella species and supports balanced microbiome, it is a more prudent choice for preserving gut health.





Conclusion

In conclusion, this research fulfilled its aim by studying methods to test antibiotic efficiency against Prevotella bacteria and exploring treatments for autoimmune diseases.

Readers gained insights into how gut microbiome changes affect autoimmune disease development and identified effective antibiotics for specific conditions. The hypothesis linking Prevotella levels to autoimmune diseases was confirmed, and the best antimicrobial agent for these bacteria was identified.

CITATIONS

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