Investigating Commensal-Dependent Penicillin Resistance in Group A *Streptococcus*

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BACKGROUND

- Antimicrobial resistance (AMR) is predicted to kill 10 million people per year by 2050
- *In vitro* susceptibility tests are confounded when bacteria utilise their environment to bypass antibiotics(1–3)
- Antibiotic cross-protection is well reported between pathogens, especially for secreted β-lactamases(4-7)
- Despite *S. pyogenes* being universally susceptible to penicillin *in vitro*, treatment failures occurs in ~10-20% of Streptococcal pharyngitis cases(8)

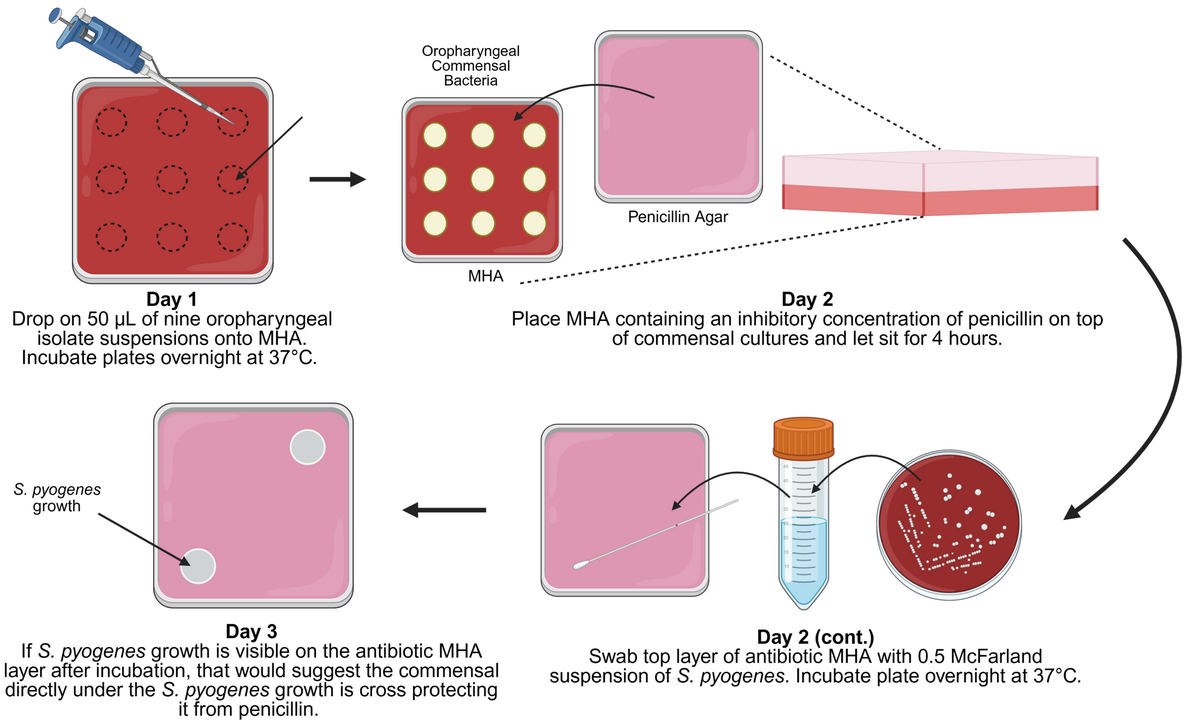
Key Question: Could β-lactamase-producing commensals be responsible for penicillin treatment failure?

AIMS

Aim 1. Identify oropharyngeal commensal bacteria that rescue *S. pyogenes* from penicillin.

Aim 2. Determine if the rescuing oropharyngeal commensal isolates are rescuing via a secreted β-lactamase or by other means

SCREENING COMMENSAL ISOLATES FOR PENICILLIN CROSS PROTECTION (Method 1)



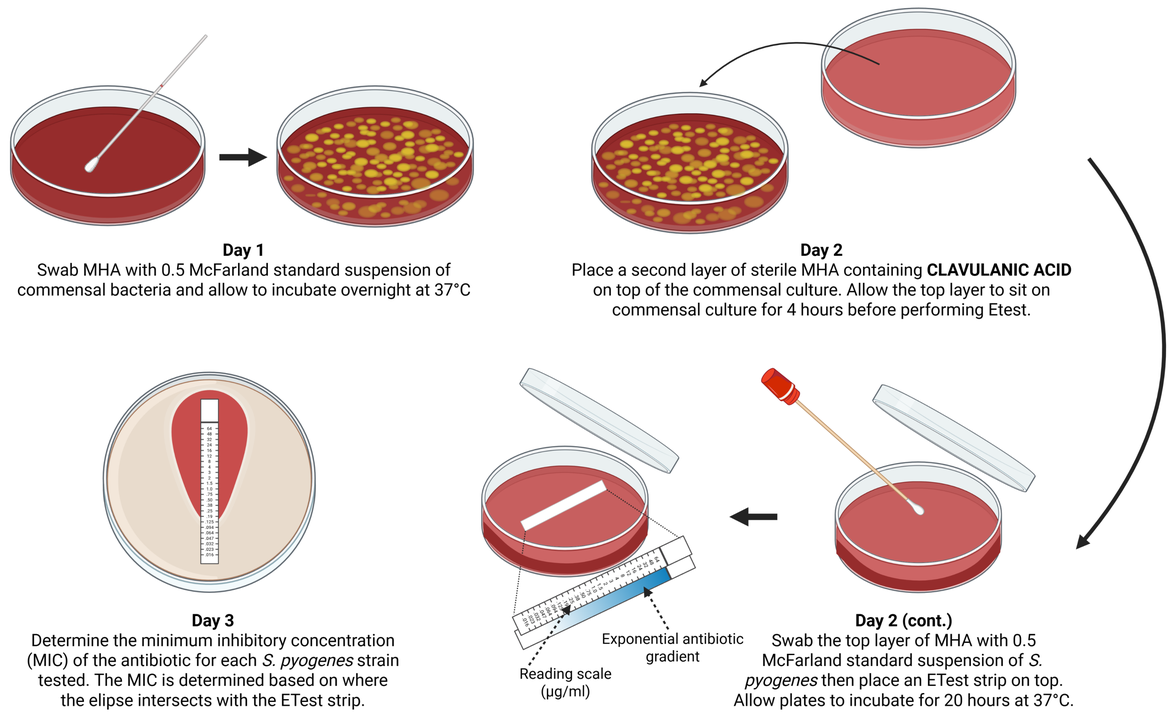
Day 1
Drop on 50 µL of nine oropharyngeal isolate suspensions onto MHA. Incubate plates overnight at 37°C.

Day 2
Place MHA containing an inhibitory concentration of penicillin on top of commensal cultures and let sit for 4 hours.

Day 2 (cont.)
Swab top layer of antibiotic MHA with 0.5 McFarland suspension of *S. pyogenes*. Incubate plate overnight at 37°C.

Day 3
If *S. pyogenes* growth is visible on the antibiotic MHA layer after incubation, that would suggest the commensal directly under the *S. pyogenes* growth is cross protecting it from penicillin.

COMMENSAL UNDERLAY PENICILLIN + CLAVULANIC ACID ETEST (Method 2)



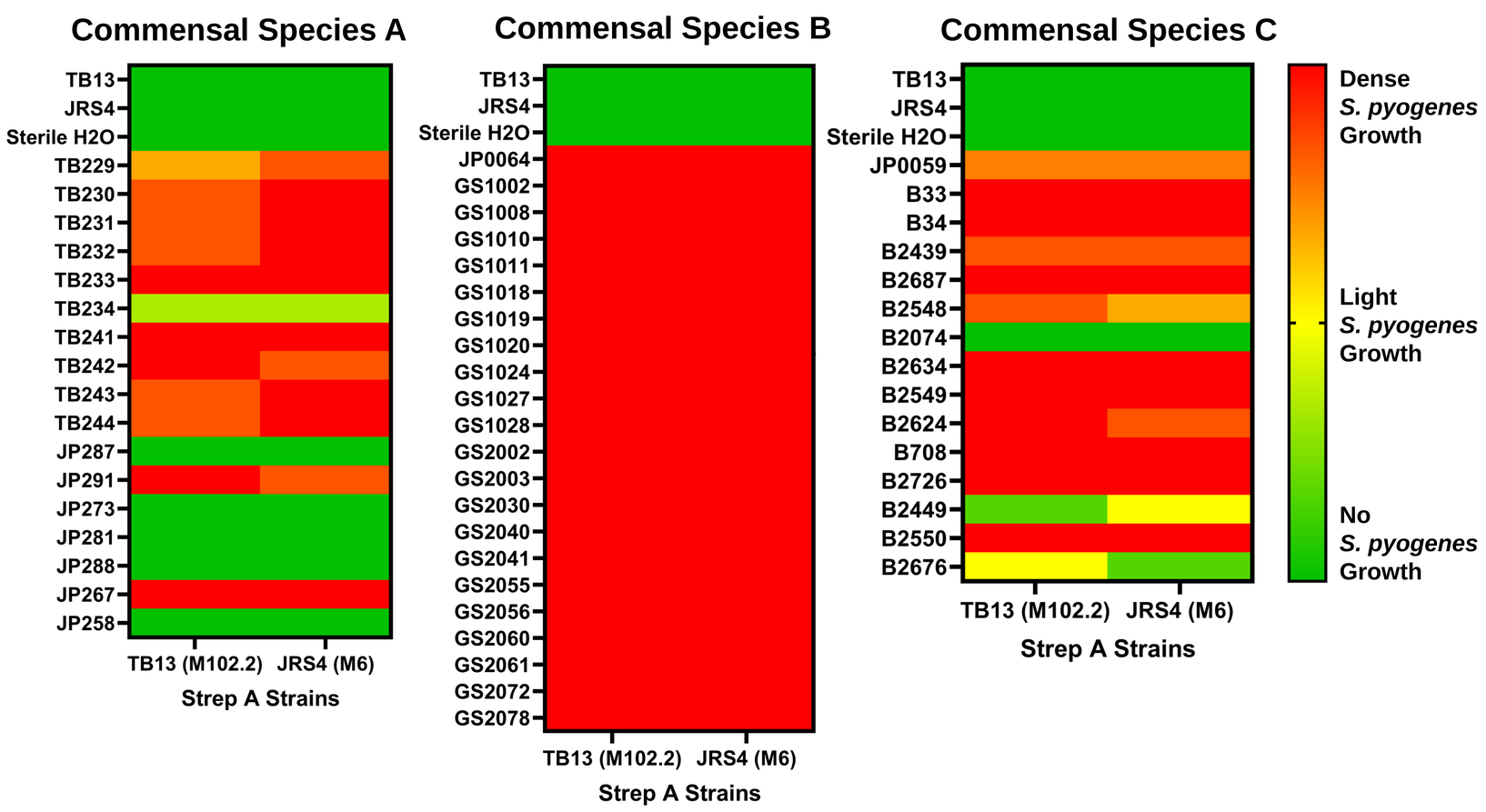
Day 1
Swab MHA with 0.5 McFarland standard suspension of commensal bacteria and allow to incubate overnight at 37°C

Day 2
Place a second layer of sterile MHA containing **CLAVULANIC ACID** on top of the commensal culture. Allow the top layer to sit on commensal culture for 4 hours before performing Etest.

Day 2 (cont.)
Swab the top layer of MHA with 0.5 McFarland standard suspension of *S. pyogenes* then place an ETest strip on top. Allow plates to incubate for 20 hours at 37°C.

Day 3
Determine the minimum inhibitory concentration (MIC) of the antibiotic for each *S. pyogenes* strain tested. The MIC is determined based on where the elipse intersects with the ETest strip.

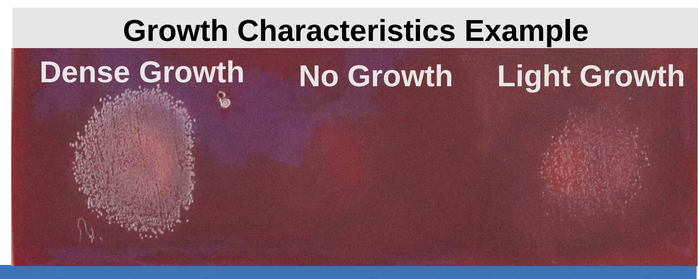
THREE SPECIES OF OROPHARYNGEAL COMMENSALS CAN RESCUE *S. PYOGENES* FROM PENICILLIN



Results 1: Using method 1, it was found that the majority, if not all, of the commensal isolates from each species could rescue *S. pyogenes* from a normally inhibitory concentration of penicillin.

Earlier investigations involved screening 60 oropharyngeal isolates for their ability to rescue *S. pyogenes* from penicillin in a broth microdilution assay using commensal supernatants. This screen identified three commensal species that could rescue *S. pyogenes* from penicillin, which are the three species investigated in this work.

Results are the average of three biological replicates.



OROPHARYNGEAL COMMENSAL SPECIES CAN RESCUE *S. PYOGENES* VIA A SECRETED β-LACTAMASE AND OTHER MEANS

Results 2: Using method 2, *S. pyogenes* is penicillin sensitive in the presence of the same rescuing isolates from commensal species A and B. However, *S. pyogenes* remains penicillin resistant in the presence of most commensal species C isolates. This suggests that commensal species A and B are rescuing via a secreted β-lactamase, but commensal species C is rescuing *S. pyogenes* from penicillin by other means.

Notes
- MIC: Minimum Inhibitory Concentration
- ND: Not done due to contamination or top layer falling off

Table 1. Penicillin MIC for TB13 and JRS4 in the presence of commensal species A isolates and clavulanic acid

Underlay Culture	Penicillin MIC (µg/mL)	
	TB13 (M102.2)	JRS4 (M6)
TB13 (M102.2)	0.008	0.004
JRS4 (M6)	0.006	0.006
Commensal Species A		
TB229	0.012	ND
TB230	0.012	0.012
TB231	0.012	0.016
TB232	0.016	0.016
TB233	0.016	ND
TB241	0.016	0.012
TB242	0.023	0.016
TB243	0.023	ND
TB244	0.016	0.016
JP291	0.023	0.016
JP267	0.016	0.016

Table 2. Penicillin MIC for TB13 and JRS4 in the presence of commensal species B isolates and clavulanic acid

Underlay Culture	Penicillin MIC (µg/mL)	
	TB13 (M102.2)	JRS4 (M6)
TB13 (M102.2)	0.006	0.004
JRS4 (M6)	0.006	0.004
Commensal Species B		
JP0064	0.023	0.016
GS1002	0.023	0.012
GS1008	0.016	0.008
GS1010	0.016	0.008
GS1011	0.023	0.012
GS1018	0.016	0.016
GS1019	0.032	0.012
GS1020	0.023	0.016
GS1024	0.016	0.012
GS1027	0.023	0.012

Table 3. Penicillin MIC for TB13 and JRS4 in the presence of commensal species C isolates and clavulanic acid

Underlay Culture	Penicillin MIC (µg/mL)	
	TB13 (M102.2)	JRS4 (M6)
TB13 (M102.2)	0.012	0.004
JRS4 (M6)	0.008	0.004
Commensal Species C		
JP0059	0.023	0.023
B33	32	32
B34	0.25	0.25
B2439	1.5	32
B2687/3687	0.023	0.008
B2548	32	0.032
B2634	0.094	0.38
B2624/2674	32	ND
B708	32	0.25
B2726	0.047	0.047
B2550	32	0.032

EUCAST Clinical Breakpoint for Penicillin G: S ≤0.03, R >0.03 — Penicillin Sensitive — Penicillin Resistant

CONCLUSIONS

- This work has identified that oropharyngeal commensal bacteria can affect penicillin susceptibility.
- Results may explain antibiotic treatment failure, despite susceptible results in ASTs.
- Future work will involve testing oropharyngeal swabs in this optimised assay to look for other commensal bacteria that can rescue *S. pyogenes* from penicillin and clavulanic acid.

ACKNOWLEDGEMENTS AND REFERENCES

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