

EP019: Post-operative joint wound fluid is alkaline and suppresses *S. aureus* metabolism and virulence

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Objective ➤ Does joint wound fluid’s alkalinity alter *Staphylococcus aureus* growth and antibiotic tolerance?

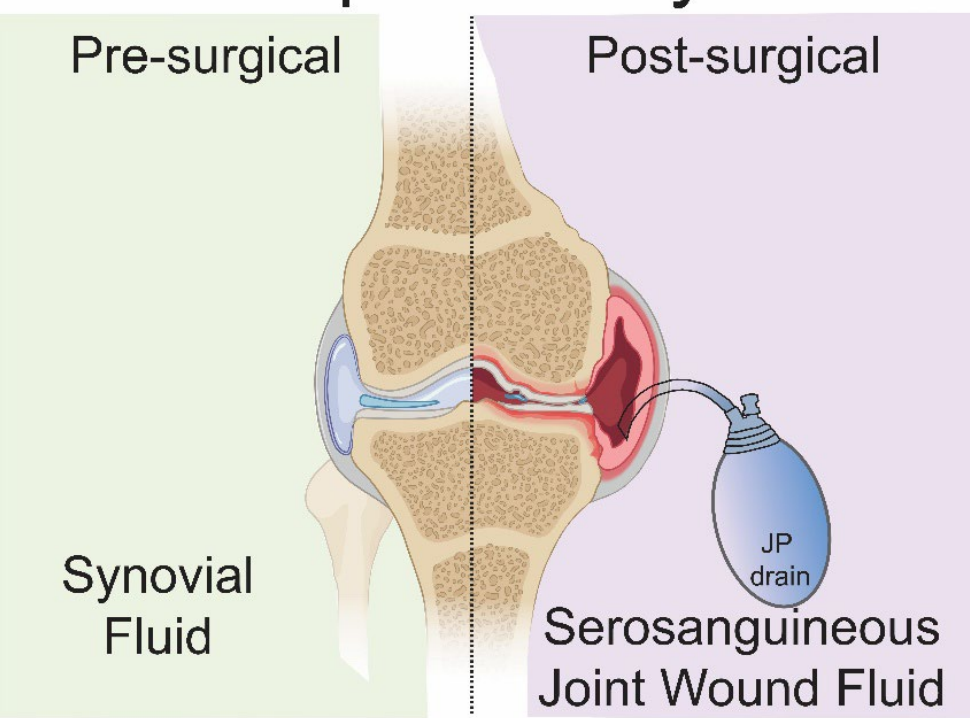
Capsulotomy

Pre-surgical

Post-surgical

Synovial Fluid

Serosanguineous Joint Wound Fluid



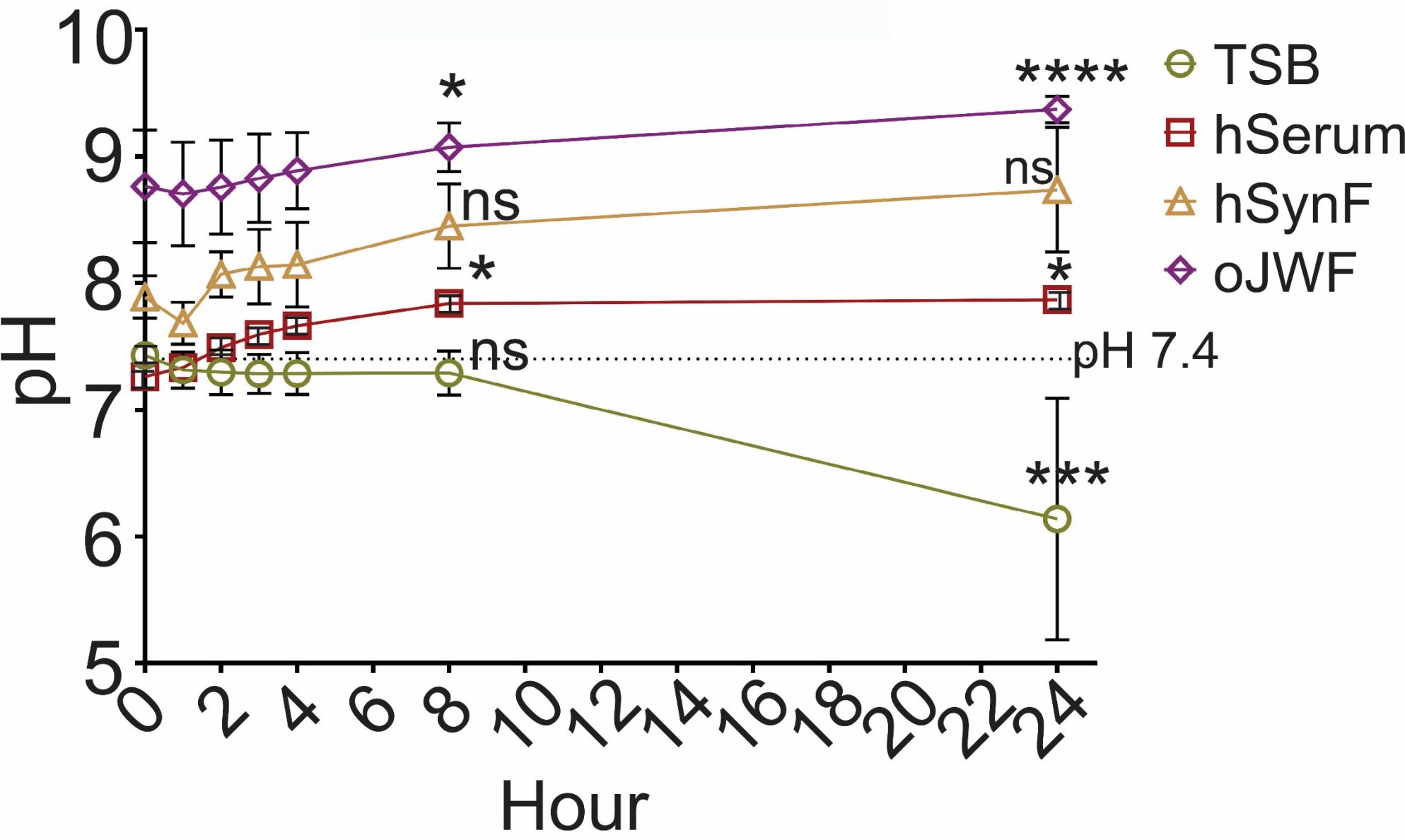
- During surgery, the joint space is lavaged, removing the majority of resident synovial fluid.
- Post-surgery, the joint space is rapidly occupied by a serosanguineous fluid (joint wound fluid) that lacks the proteoglycan-rich (e.g. hyaluronic acid, lubricin), viscous environment of synovial fluid.
- We evaluated the effects of joint wound fluid and its alkaline pH on Staphylococcus aureus metabolism and antibiotic tolerance to better tailor peri-operative antibiotic prophylaxis*

METHODS

Methicillin-sensitive *S. aureus* ATCC25923 (MSSA) or methicillin-resistant *S. aureus* (MRSA) USA300 LAC were cultured in ovine joint wound fluid (oJWF, from Jackson-Pratt drain, IACUC approved), human serum (hSerum), human synovial fluid (hSynF), or trypticase soy broth (TSB). pH was adjusted with HCl, NaOH, or PBS/bicarbonate buffers and measured with a calibrated pH meter (Mettler Toledo SevenCompact™ Conductivity Meter). Using hourly recordings in a Tecan Infinite plate reader, (1) MRSA growth was measured spectrophotometrically ($\lambda=600\text{nm}$); and (2) Accessory gene regulatory (*agr*) activity was measured by luminescence using MRSA P3 LUX. Adhesion was measured at 24h to a PLA rod; planktonic MSSA cultures were treated with 15 $\mu\text{g/mL}$ amikacin (AMK) or 5 $\mu\text{g/mL}$ vancomycin (VAN) for 24h (both 10^7 colony forming units (CFU)/ml). Growth and antibiotic activity were measured by direct counting, with or without trypsin dispersion. Student’s t-test, ANOVA, or Kruskal-Wallis tests were used.

RESULTS

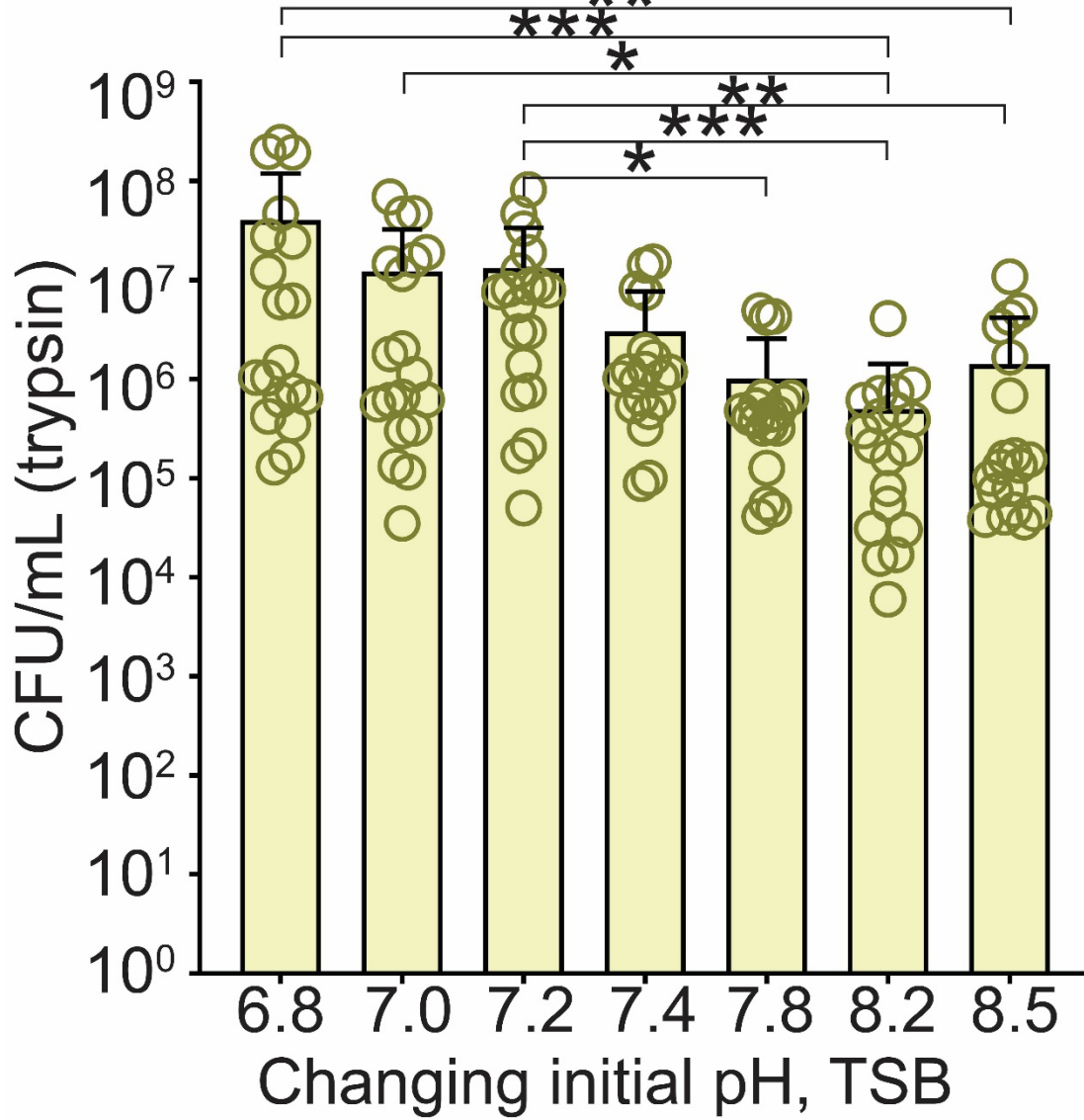
Figure 1: pH of common bacterial media in sterile conditions



- Ovine joint wound fluid (oJWF) initially exhibited a pH>8.0
- oJWF pH increased with time to a maximum of ~pH 9
- Human synovial fluid (hSynF) was also alkaline over time.
- Human serum (hSerum) pH was pH 7.4 -7.6 during incubation.
- Trypticase soy broth (TSB) pH decreased from neutral to acidic (~pH 6.2) during sterile incubation.

TSB (n=8): preferred medium for *S. aureus* growth; hSerum (n=4); hSynF (n=4); oJWF (n=12); oJWF. ns=not significant, *p<0.05; ***p<0.001; ****p<0.0001, compared to t+0 reading of fluid by Mann-Whitney test.

Figure 2: Bacterial adhesion is pH dependent



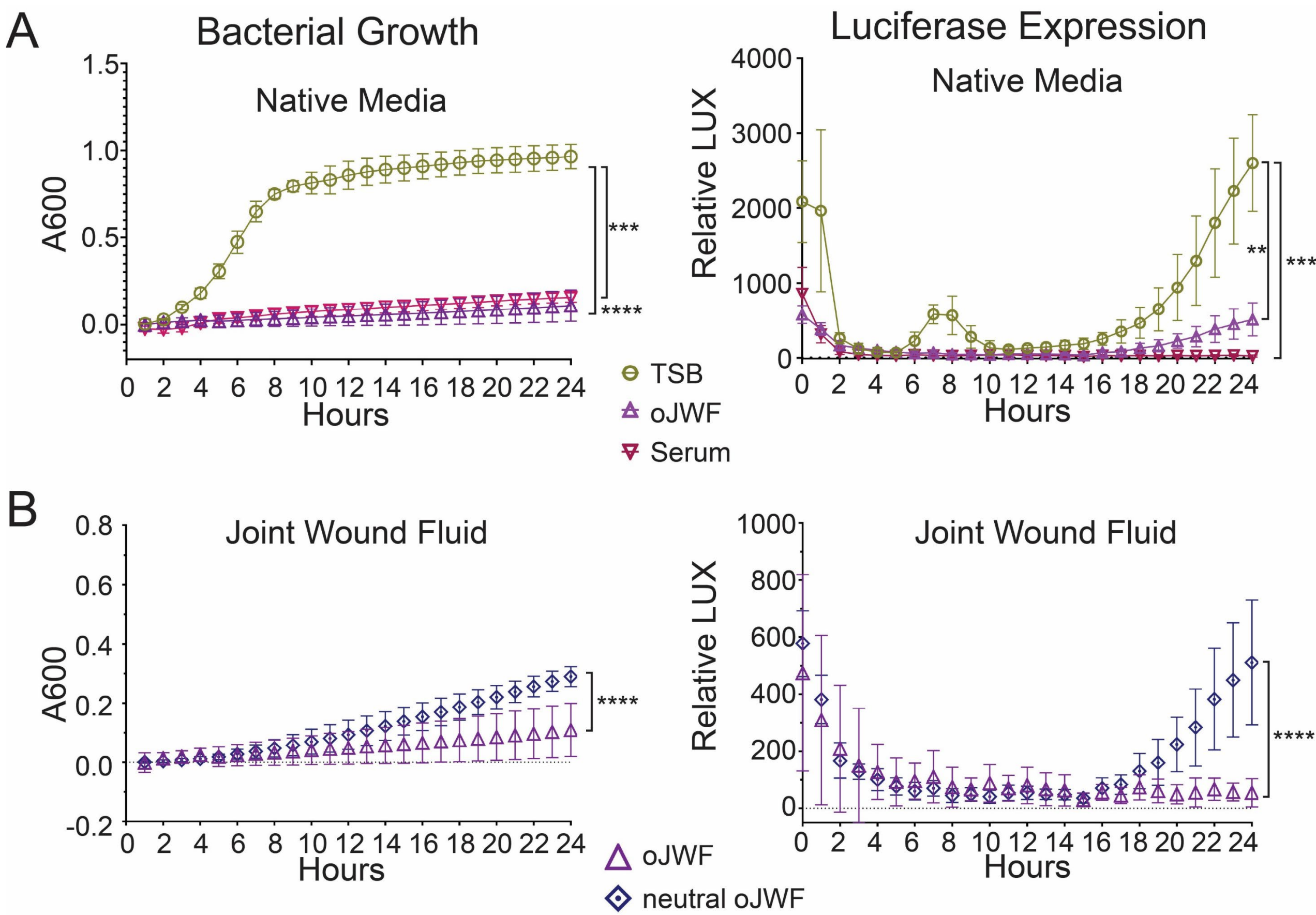
- S. aureus* ATCC25923 cultured with a polylactic acid rod for 24 hrs and adherent bacteria suspended with trypsin (CFU(trypsin)).
- Maximal adhesion was measured at neutral pH’s.
- Above pH 7.8, no further decrease in adherence was measured.

Effect of pH on *S. aureus* adhesion (24 h) to rods as a function of initial pH using TSB (n=19 per condition). Statistical differences determined by Kruskal Wallis test for multiple comparisons; nonsignificant comparisons are not shown.

ACKNOWLEDGEMENTS

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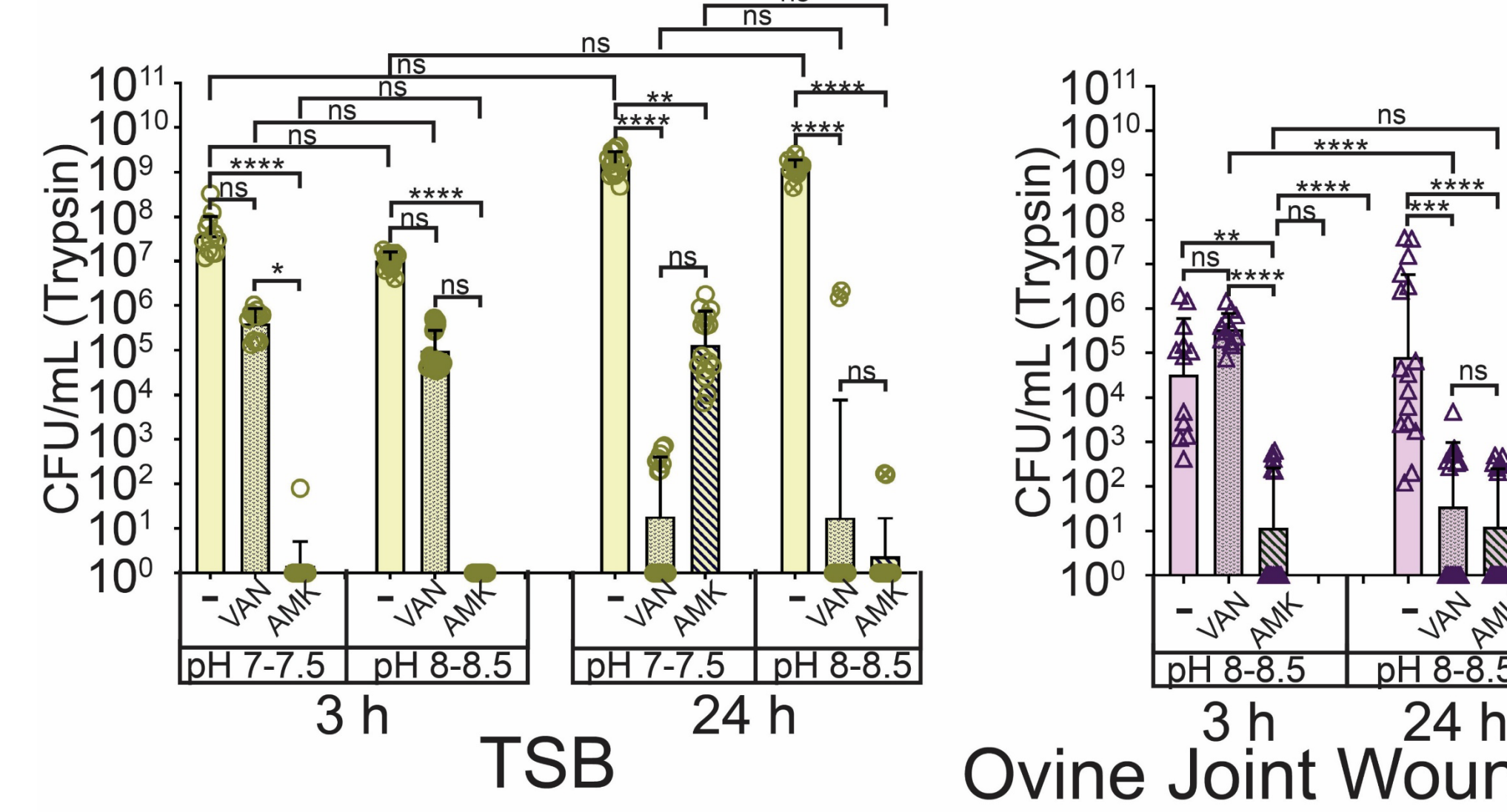
Figure 3: Physiological fluids and alkaline pH suppress bacterial growth and *agr* P3 LUX expression



- MRSA USA300 LAC P3-lux (gift, M. Otto (NIAID, NIH)) showed lower growth in oJWF and serum compared to TSB (A, left).
- Neutralization of oJWF increased growth (B, left)
- agr* P3 expression reflects MRSA virulence.
- In native media, P3 lux expression is greatest in TSB but measurable in oJWF; serum is at background levels (A, right).
- Neutralization of oJWF markedly increased P3 expression.

Statistics (all panels): n=12 determinations/condition/time; significance at 24 h, ns=not significant; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. No significant differences between oJWF, and serum were calculated in Panel A for either growth or luminescence

Figure 4: Amikacin is more effective than vancomycin in alkaline conditions at 3 hrs.



Amikacin (AMK), an aminoglycoside, showed increased activity in alkaline pH. Vancomycin (VAN) is a non-pH dependent, time-dependent peptide antibiotic, which showed:

- Bacteria treated with 5 $\mu\text{g/mL}$ VAN or 15 $\mu\text{g/mL}$ AMK in TSB at neutral and basic pH’s; ovine joint wound fluid at native pH (≥ 8.0) for 3 and 24 h.
- In TSB, AMK achieved greatest killing at 3 hrs; by 24 h, less activity at neutral pH’s.
- In oJWF, AMK showed greatest activity at 3 hrs and equivalence with VAN at 24 h.

(n=9-16, TSB; =12-16, oJWF). Statistics: Kruskal-Wallis tests, ns=not significant, *p<0.05; ** p<0.01; ***p<0.001; ****p<0.0001.

CONCLUSIONS

- oJWF decreased *S. aureus* growth and *agr*-P3 expression.
- VAN shows time-dependent killing of bacteria in oJWF.
- The aminoglycoside AMK shows rapid bacterial killing in oJWF.
- We suggest that aminoglycosides, which retain efficacy across a broader pH range, warrant greater consideration for local PJI treatment, especially when required for longer than 24 h.*