

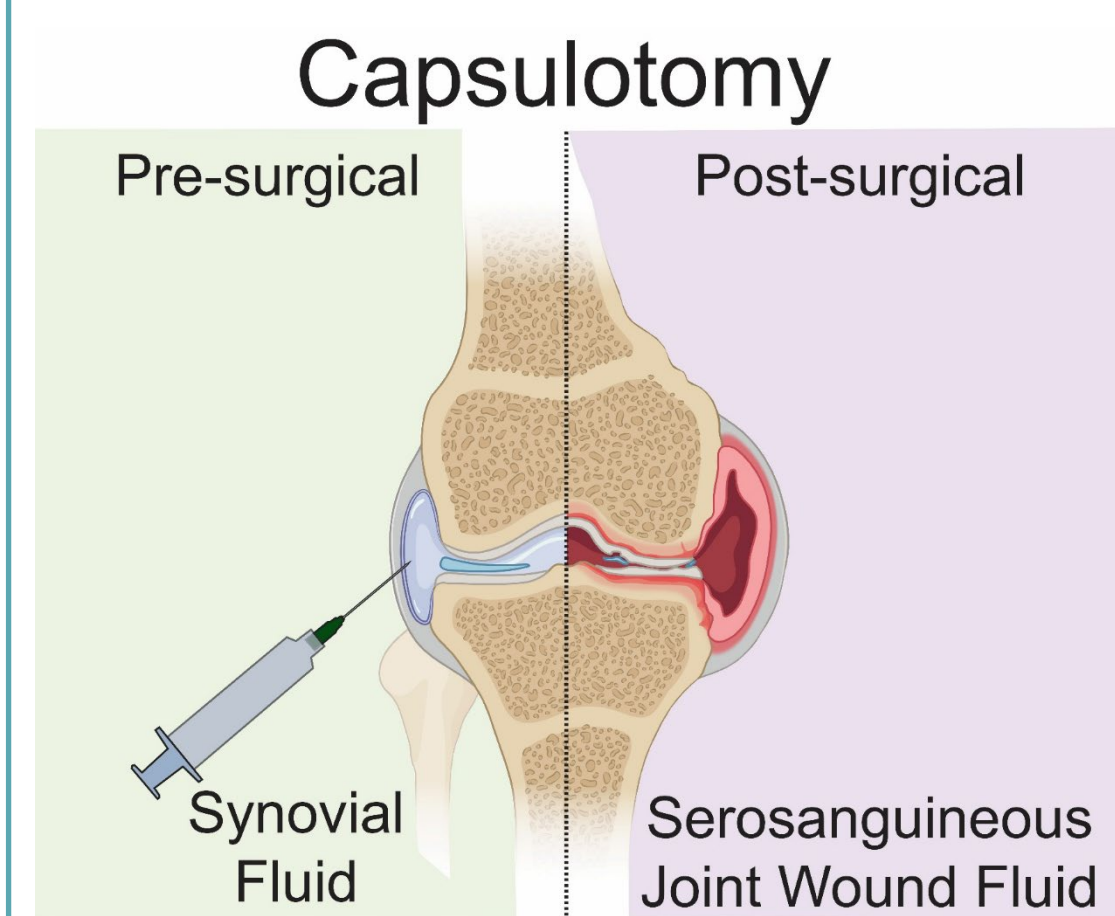
EP018: Synovial fluid alters *S. aureus* metabolism and antibiotic tolerance and is alkaline

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Question ➤ What is the effect of synovial fluid composition, including pH, on *S. aureus* metabolism and antibiotic tolerance?

INTRODUCTION



- Prophylaxis for preventing periprosthetic joint infection (PJI) ultimately relies on antibiotic activity in the joint space for success.
 - Antibiotics activity is sensitive to pH.
 - Cephalosporins (cefazolin/Ancef): most active in acidic pH
 - Aminoglycosides (gentamicin): most active in alkaline pH
 - Vancomycin: minimal pH sensitivity
- We asked the effect of the synovial fluid environment, as well as its pH on bacterial phenotype.

METHODS

Methicillin-sensitive *S. aureus* ATCC25923 (MSSA), or methicillin-resistant *S. aureus* (MRSA) USA300 LAC were cultured in human synovial fluid (hSynF, IRB approved collection), human serum (hSer), 50% hSer, 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 ($\lambda=600\text{nm}$); (2) Accessory gene regulatory (*agr*) activity was measured by luminescence using MRSA P3 LUX[1]. Planktonic MSSA cultures (10^7 colony forming units (CFU)/ml) were treated with 15 $\mu\text{g}/\text{mL}$ amikacin (AMK) or 5 $\mu\text{g}/\text{mL}$ vancomycin (VAN) for 24 h. Growth and antibiotic activity was 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 frozen synovial fluid is >7.4

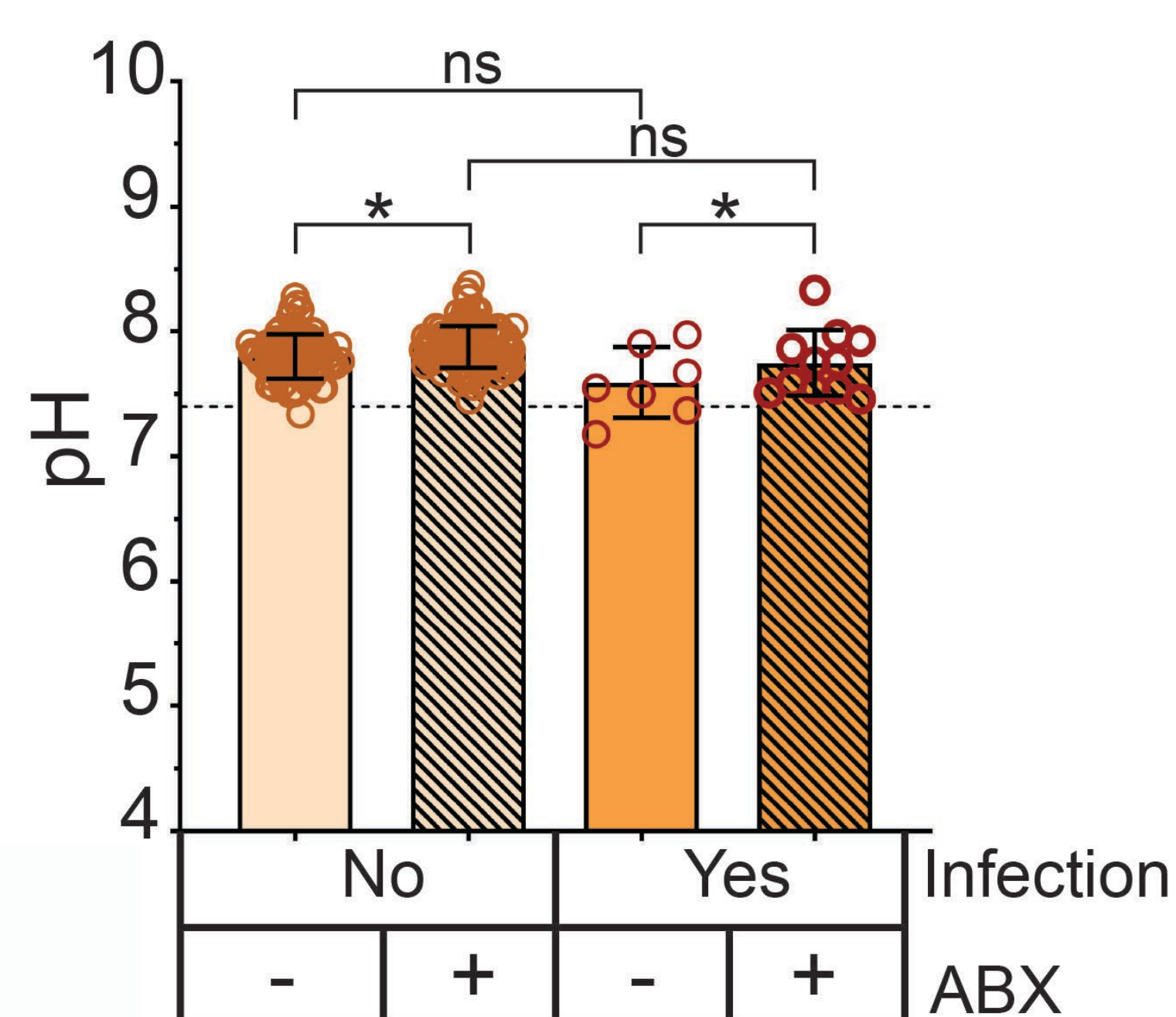


Figure 1: pH of frozen human synovial fluid from office aspirations or peri-surgically was frozen, thawed and after pH determination, sorted into groups based on infection status and antibiotic status. In all samples, antibiotic presence (predominately cefazolin) did not alter pH. While infected samples appeared to have a pH closer to that of the physiological environment, no statistical difference in pH was measured between uninfected and infected samples.

(n=85, 105, 5, 14. ns=not significant; **p<0.01; Kruskal-Wallis test)

Figure 2: Effect of bacterial proliferation on pH

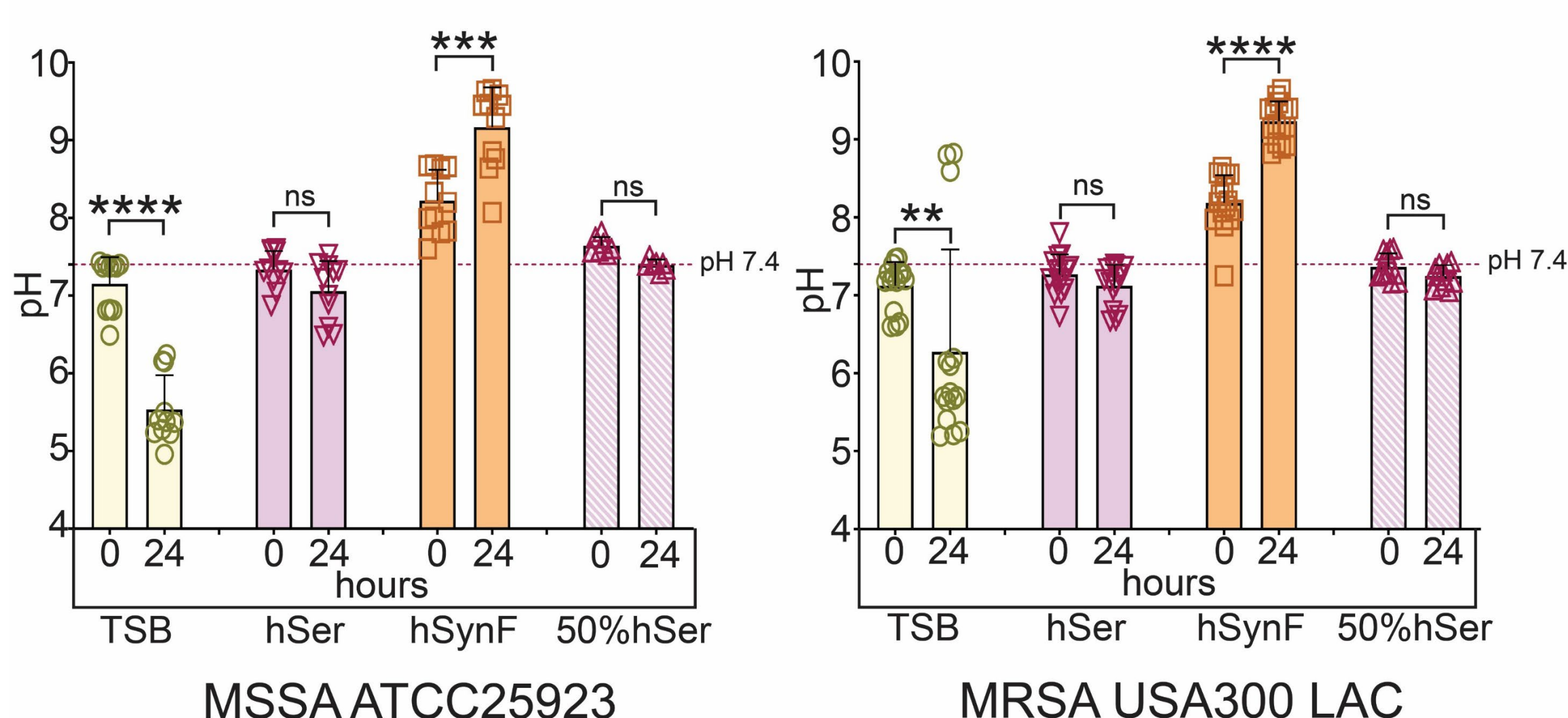


Figure 2: Methicillin-sensitive (MSSA) or -resistant (MRSA) *S. aureus* were cultured in trypticase soy broth (TSB), human serum (hSer), human synovial fluid (hSynF) or 50% hSer and pH measured after 24 h. Bacterial growth acidified the TSB, had no effect on hSer or 50% hSer, whereas the pH of hSynF increased.

MSSA ATCC 25923 (left, n=11 for TSB, hSer, hSynF; n=7 for oJWF, 50% hSer) or MRSA USA300 LAC (right, n=15 for TSB, hSer, hSynF; n=11 for oJWF, 50% hSer). Statistics: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns=not significant using Mann-Whitney test.

Figure 3: Synovial fluid suppresses bacterial growth and *agr* P3 LUX expression

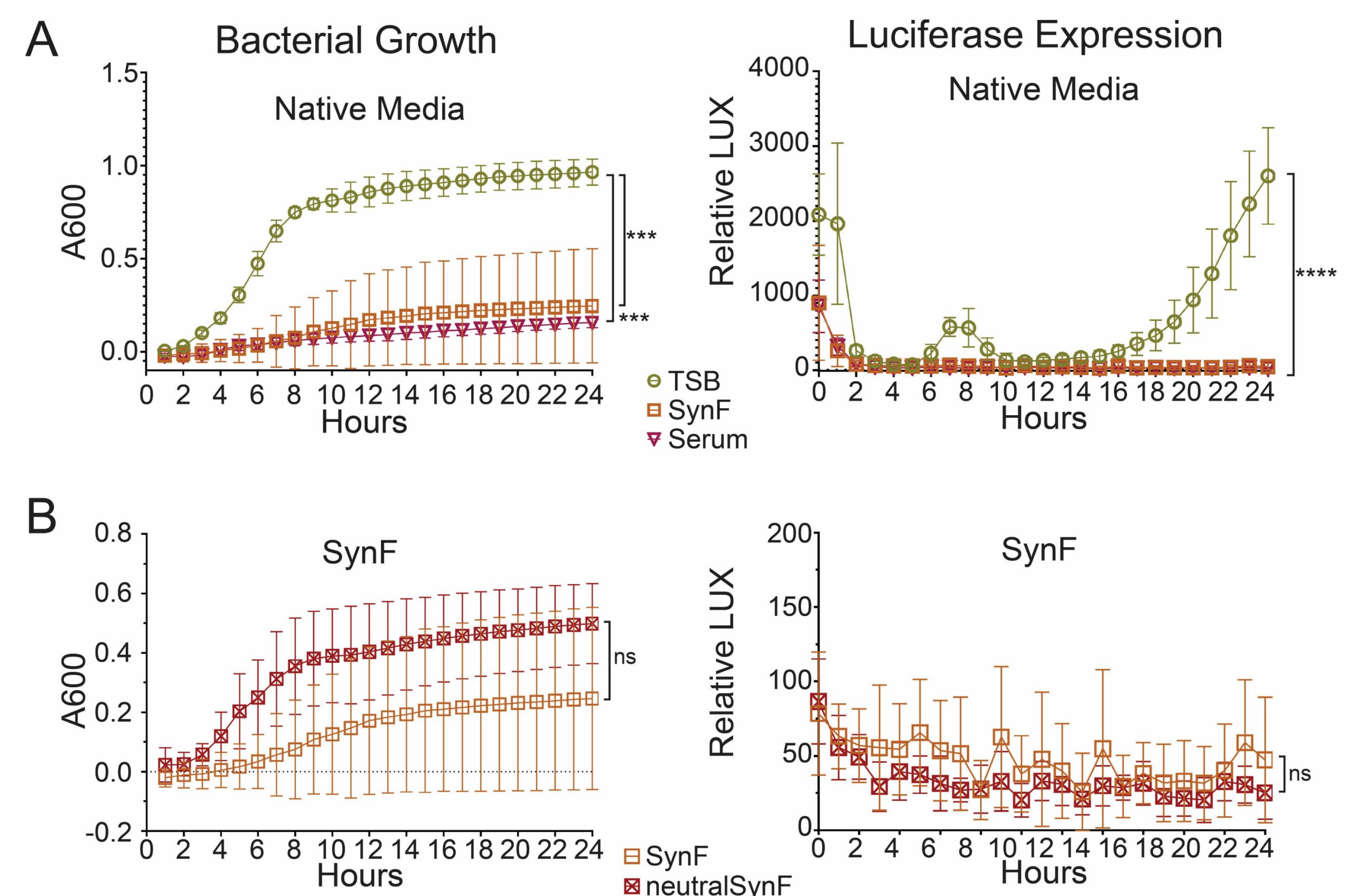


Figure 3: MRSA USA300 LAC P3-lux (gift, M. Otto (NIAID, NIH)) showed attenuated growth in SynF and serum compared to TSB (A, left). Neutralization of SynF showed no significant effect on bacterial growth (B, left). *agr* P3 expression reflects MRSA virulence. *agr* P3 lux expression is greatest in TSB but SyF and serum are at background levels (A, right). Neutralization of SynF had no effect on P3 expression.

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

Figure 4: Amikacin is more effective than vancomycin in native synovial fluid.

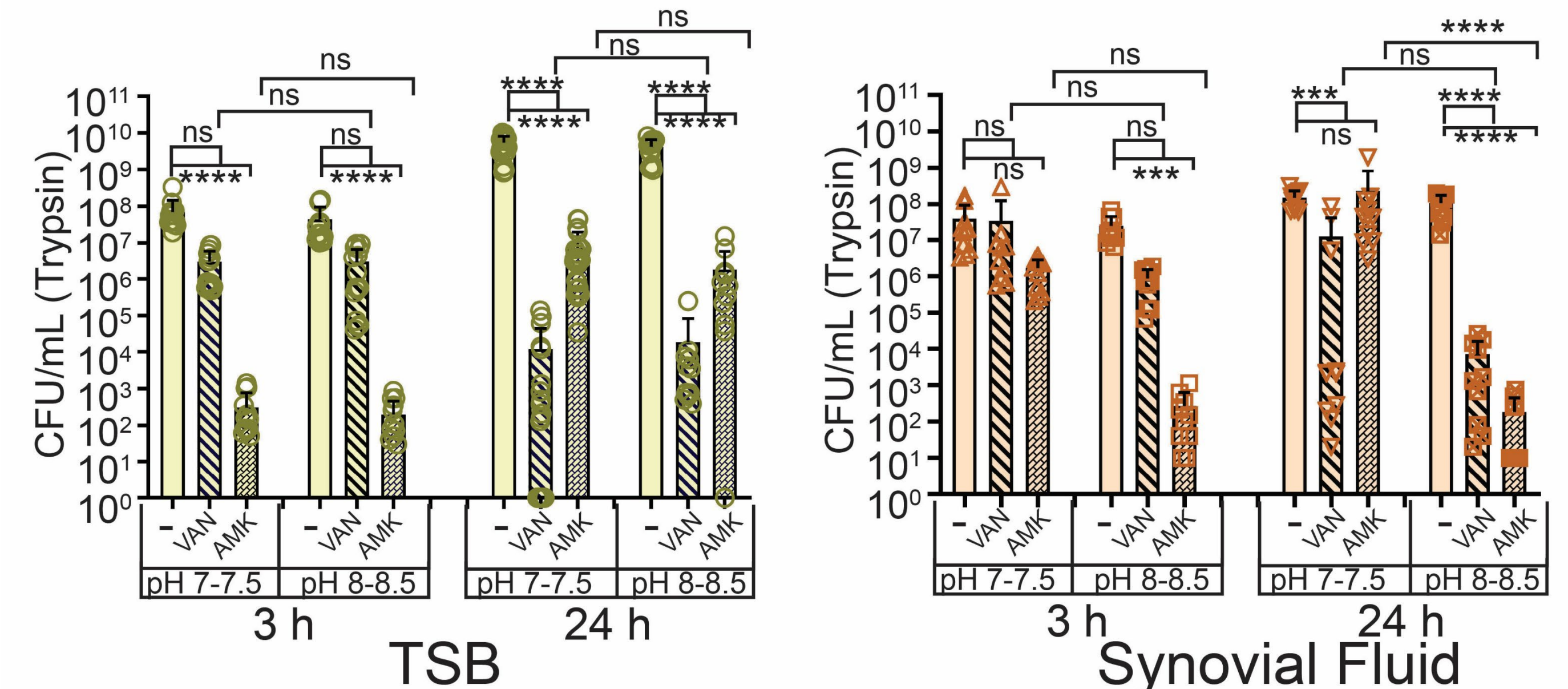


Figure 4: Amikacin (AMK), an aminoglycoside, shows increased activity in alkaline pH. Vancomycin (VAN) is a non-pH dependent, time-dependent peptide antibiotic. Bacteria were treated with 5 $\mu\text{g}/\text{mL}$ VAN or 15 $\mu\text{g}/\text{mL}$ AMK in TSB or SynF at neutral and basic pH's for 3 and 24 hrs. In TSB, AMK achieved greatest killing at 3 hrs; by 24 h, less activity was measured, independent of pH; VAN activity increased with time. In SynF, AMK showed sustained activity at 3 and 24 hrs in the native, alkaline environment of SynF; activity was equivalent to VAN at 24 h.

(n=11(TSB); 10-11 (SynF)). Statistics: Kruskal-Wallis tests, ns=not significant, ***p<0.001; ****p<0.0001.

CONCLUSIONS

- SynF pH remains alkaline, even in the presence of bacterial growth, whereas serum pH remains stable.
- SynF suppresses bacterial growth and *agr* P3 expression, independent of a pH reflective of blood or native SynF.
- AMK shows enhanced activity (vs. VAN) in native SynF.
- We suggest that aminoglycosides, which retain efficacy across a broader pH range, warrant greater consideration for PJI prophylaxis.**

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