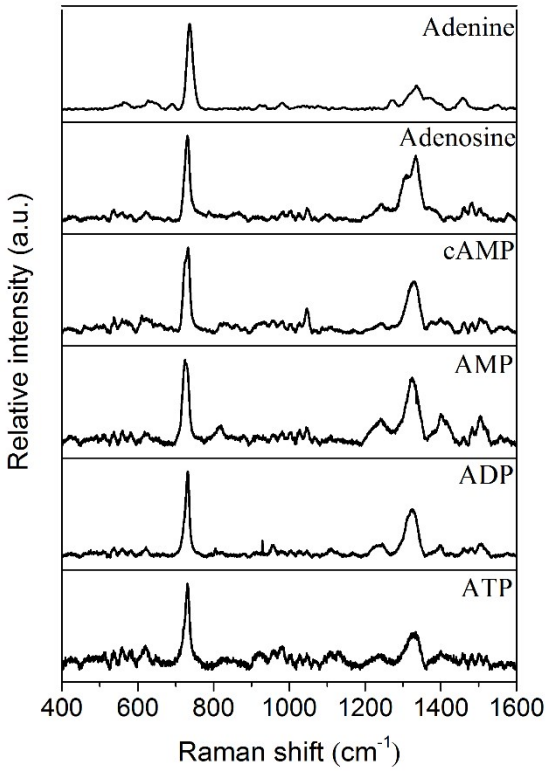
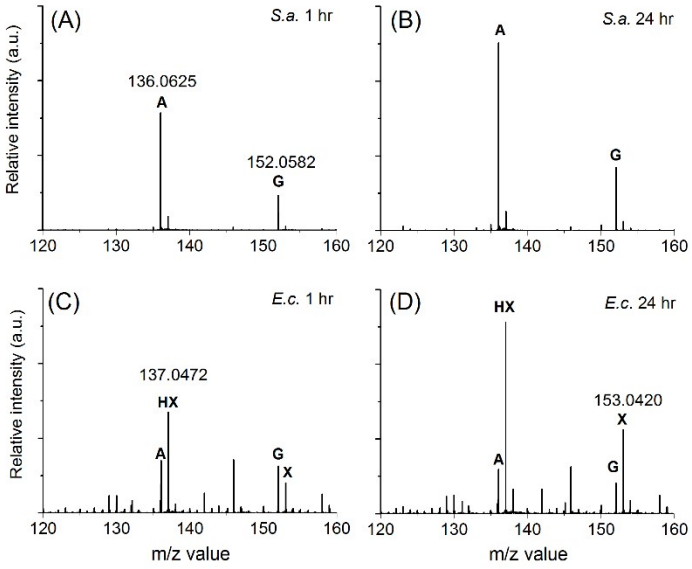
Supporting Information

Bacteria	*S. aureus*			*E. coli*		
Duration (hr)	0~1	1~6	6~24	0~1	1~6	6~24
Adenine	3.7×10^{6}	7.0×10^{5}	1.1×10^{5}	8.1×10^{4}	2.8×10^{5}	-4.5×10^{4}
Guanine	2.1×10^{6}	4.3×10^{5}	8.2×10^{4}	5.1×10^{5}	1.7×10^{5}	2.1×10^{4}
Hypoxanthine				5.4×10^{5}	1.9×10^{5}	1.8×10^{5}
Xanthine				4.8×10^{5}	4.4×10^{5}	2.6×10^{5}

Supporting Table S1. The average releasing rates (molecule·CFU^{-1}·hr^{-1}) of purine derivative molecules released from *S. aureus* and *E. coli* after different duration of water incubation (0~1 hr., 1~ 6 hrs., and 6~24 hrs.) The quantification of each molecule released by a single bacterium in the specific time point is achieved by comparing its mass signal with that of their corresponding internal or external standards in the MS analysis, and it is performed with four separate samples of each bacterial strain (shown in Figure 2). The average releasing rate of each molecule in different duration is then determined with the average numbers of the molecule released from four samples during such duration of water incubation.

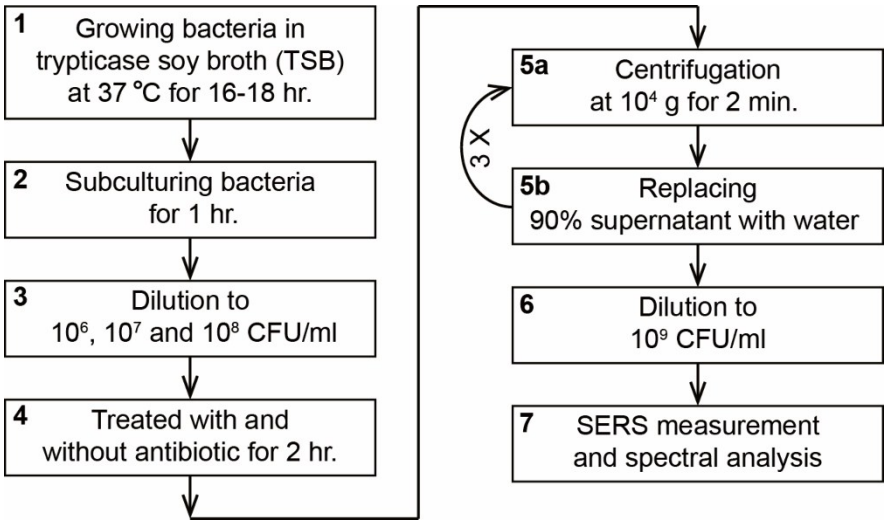


Supporting Figure S1. SERS spectra of adenine, adenosine, cyclic adenosine monophosphate (cAMP), adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). Six adenine derivatives share very similar SERS spectra with each other. 10^{-4} M solutions of six adenine derivatives were prepared by powder dissolved in deionized water, and the spectra were acquired after 2μl of each solution dried on the SERS-active substrate. All the spectra shown here are de-baselined and normalized.

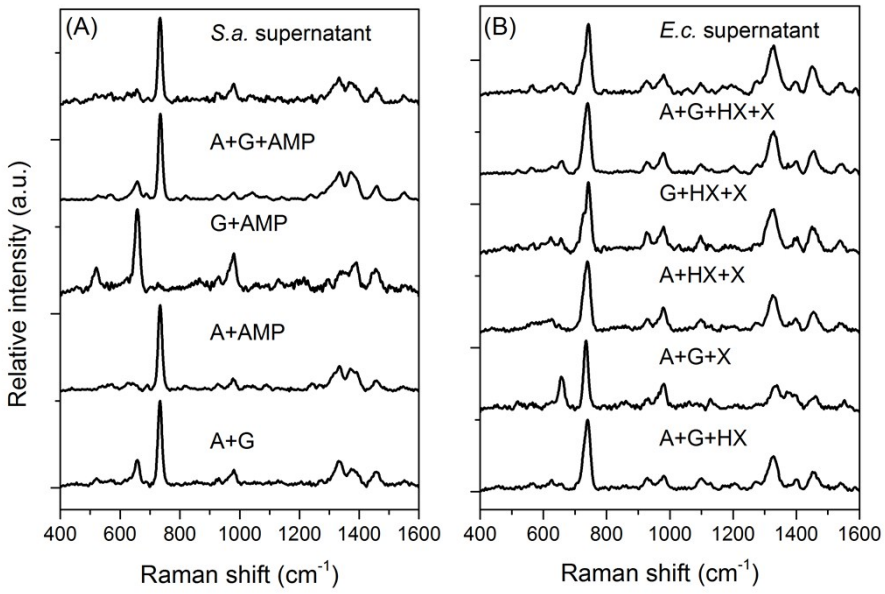


Supporting Figure S2. Positive-ion electrospray ionization mass (ESI-MS) spectra of supernatants of *S. aureus* (A and B) and *E. coli* (C and D) after incubation in water. The incubation times in water are (A and C) 1 hr. and (B and D) 24 hrs. A, G, HX and X mark the MS peaks of singly charged adenine, guanine, hypoxanthine and xanthine, respectively.

For *S. aureus*, the ions with most prominent intensities are located at *m/z* = 136.06 (retention time, RT = 1.2 min.), 152.05 (RT = 1.3 min.) and *m/z* = 348.07 (RT = 1.3 min.), respectively. Their *m/z* ratios match those of protonated singly charged adenine (molecular weight, MW = 135.13 g/mol), guanine (MW = 151.13 g/mol), and AMP (MW = 347.22 g/mol), respectively. For *E. coli*, the major species detected are hypoxanthine (*m/z* = 137.04; RT = 1.6 min.; MW = 136.11 g/mol), xanthine (*m/z* = 153.0421; RT = 1.7 min.; MW = 152.11 g/mol), adenine and guanine.



Supporting Figure S3. Flow chart of antibiotic susceptibility test (AST) and minimum inhibitory concentration (MIC) determination from Liu *et al*.

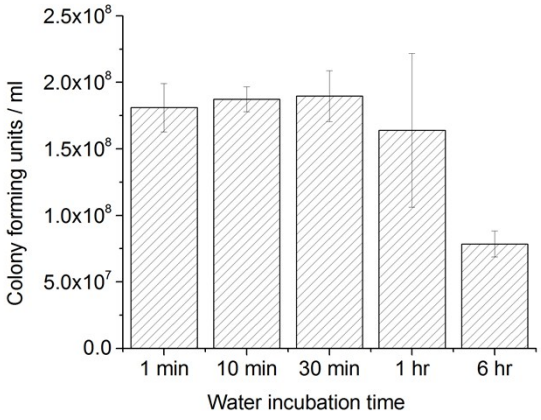


Supporting Figure S4. Changes in the simulated spectra of (A) *S. aureus* and (B) *E. coli* supernatants after the removal of one type of molecule from the original nucleobase mixtures, whose adenine, hypoxanthine, guanine, xanthine, and AMP concentrations were determined by UPLC/ESI-MS analysis of the corresponding bacterial supernatants. From top to bottom: (A) *S. aureus* supernatant after 1 hr. of water incubation, A+G+AMP (original), G+AMP, A+AMP, and A+G; (B) *E.coli* supernatant after 1 hr. of water incubation, A+G+HX+X (original), G+HX+X, A +HX+X, A+G+X, and A+G+HX. (The maximal peaks in all the spectra are normalized to one.)

For *S. aureus*, the most prominent peak at 733 (660) cm^{-1} disappeared when adenine (guanine) was removed from the mixture. In contrast, the removal of AMP from the mixture had little effects on the spectra, which is in agreement with the low concentration of AMP in bacterial supernatant. Therefore, we can infer that the SERS spectrum of *S. aureus* is primarily contributed by adenine and guanine together, with a very minor contribution from AMP.

For *E. coli*, the spectrum remained essentially unchanged when adenine was removed from the

nucleobase mixture because of its low concentration compared to other nucleobases. In the simulated spectra without guanine or xanthine, the peak at 660 cm^{-1} decreased, indicating that both molecules contribute to this specific peak in *E. coli* supernatant. When hypoxanthine was removed from the mixture, a significant 733 cm^{-1} peak replaced the 742 cm^{-1} peak, suggesting that the significant 742 cm^{-1} peak in the *E. coli* SERS spectrum was primarily contributed by hypoxanthine.



Supporting Figure S5. Viability of bacteria after different time of incubation in pure water. Enumeration of the culturable *S. aureus* cells after 1 min., 10 min., 30 min., 1 hr. and 6 hrs. of water incubation was performed with plate counting.

For the time-dependent quantification experiments, the bacteria were washed and incubated in water with a filter centrifugal device (see experimental section). This sample preparation procedure ensures no loss of bacterial cells during the washing steps and water incubation because all of the cells were left on the filter. Furthermore, the viability of these cells after stressful water incubation was examined with plate counting. Bacteria cultured to the same optical density value were washed and incubated in water for different durations, and the incubated cells were then re-cultured on agar plates to examine their culturability. The colony forming units (CFU) showed no significant changes in the initial period (1 min. to 1 hr.) while there was a slightly decrease in 1 hr., which was within the experimental error. Therefore, for the initial period, the number of bacterial cells remained essentially unchanged while the amounts of nucleobase metabolites kept increasing as the time progressed. For example, the numbers of adenine and guanine in the *S. aureus* supernatant increased about ten-fold and five-fold in 1 hr, respectively (see Figure 2), which means that the nucleobase metabolites were

released by live cells rather than from ruptured dead cells. To be noted is that the number of viable cells in 6 hrs. was reduced by one-half, indicating that the prolonged water incubation indeed turned the bacteria nonviable.