Electronic Supplementary Material (ESI) for Soft Matter.

Observations on phenomenological changes in *Klebsiella* Pneumoniae under fluidic stresses

Siddhant Jain[1], Anmol Singh[2], Nivedita Tiwari[1], Aparna Naik[1], Ritika Chatterjee[2], Dipshikha Chakravortty[2,3*], Saptarshi Basu[1*]

[1]Department of Mechanical Engineering, Indian Institute of Science, Bengaluru 560012

[2]Department of Microbiology and Cell Biology, Indian Institute of Science, Bengaluru 560012

[3]School of Biology, Indian Institute of Science Education and Research, Thiruvananthapuram, India

SUPPLEMENTARY DATA

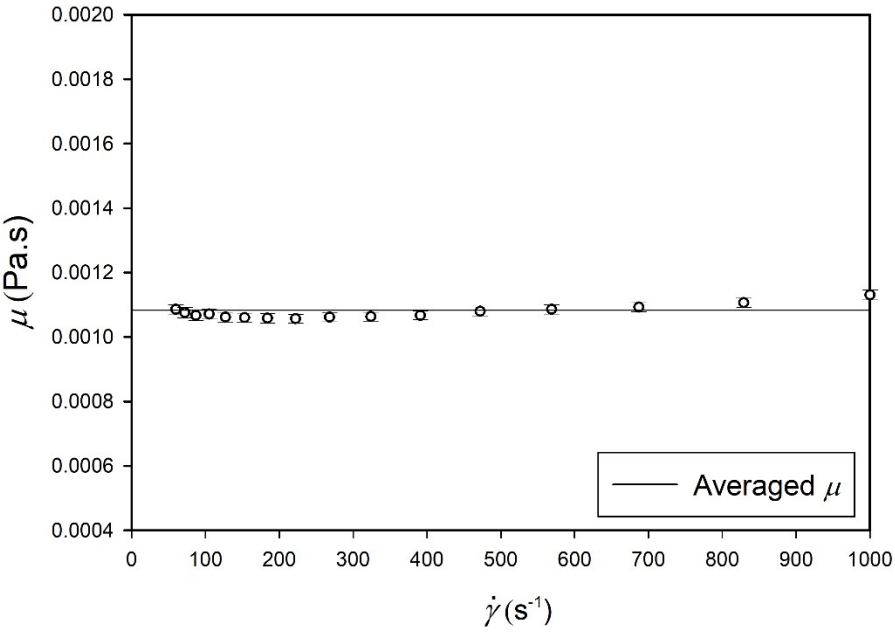

Figure S1: $Viscosity$ (μ) vs shear rate ($\dot{\gamma}$) curve for bacterial solution shows a constant viscosity value with increase in the shear rate. The graph plotted is a result of three experimental runs and the error bars depicts the SD values.

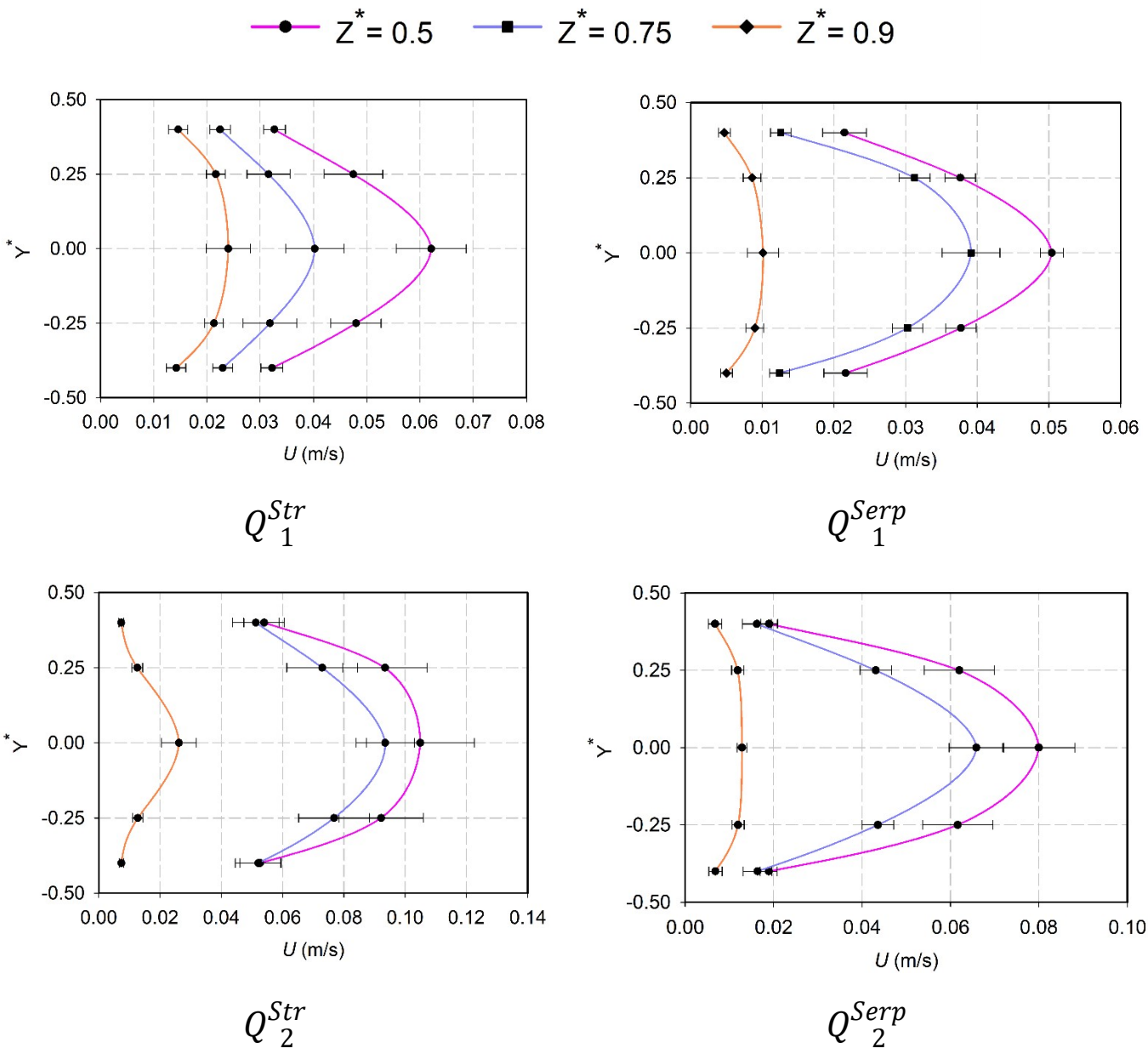

Figure S2: The velocity profiles obtained experimentally at different planes are shown for different stressing conditions. Here, $Y^* = Y/W$ represents various points in the XY plane where the measurement has been made and $Z^* = Z/H$ represents the XY planes in the Z direction. The error bar denotes the SD.

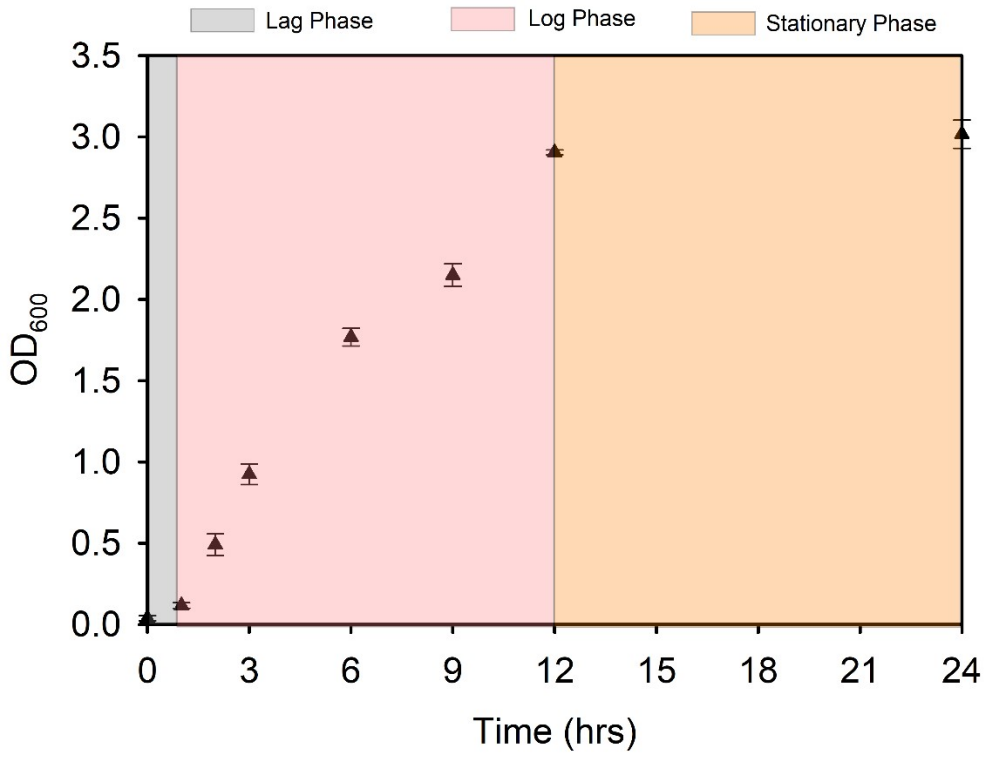

Figure S3: Optical density (OD at 600 nm) vs time (hrs) for the bacterial sample used in the present study. The three phases of bacterial growth are shown for 24 hrs. All the experiments conducted during this work were carried out in the stationary phase of the bacterial sample. The error bars depict the SD.

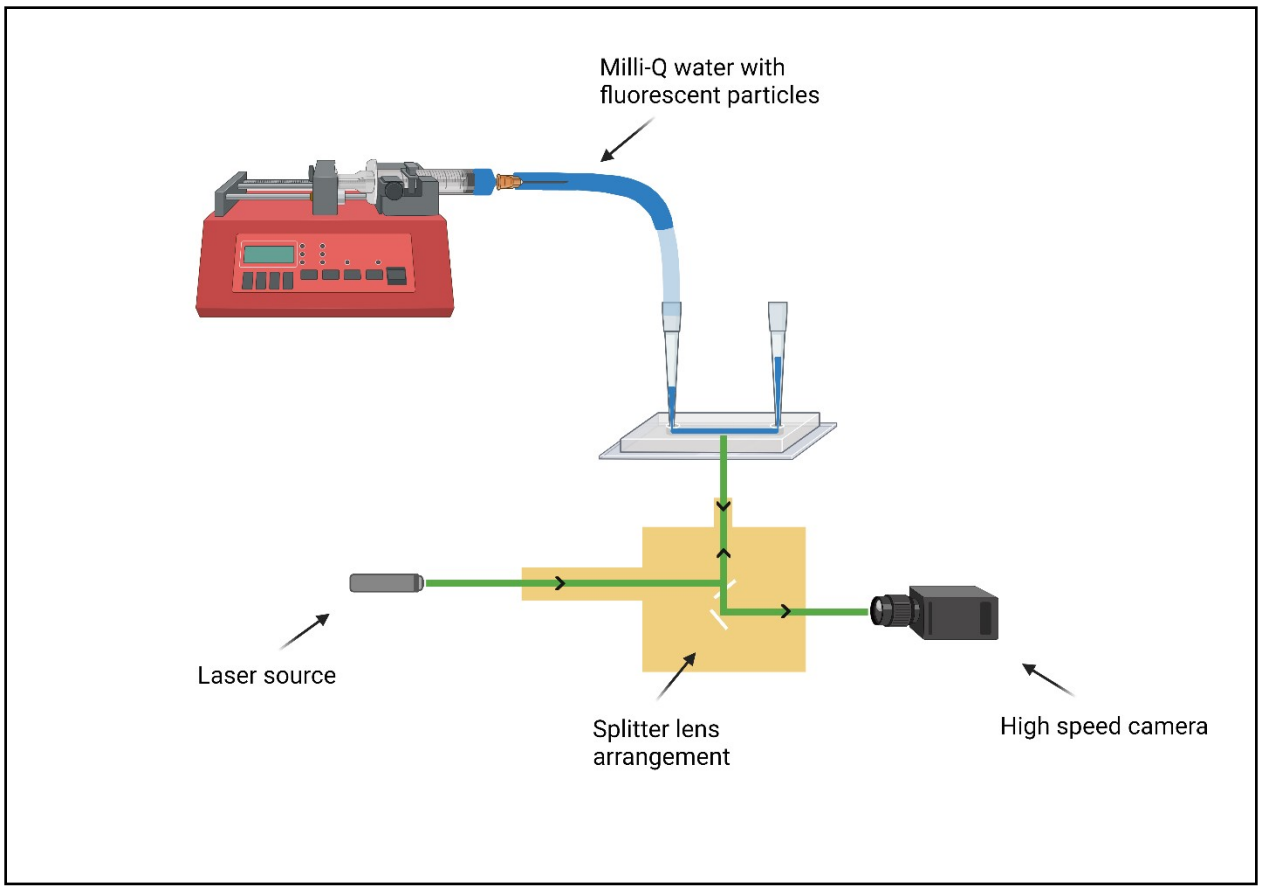

Figure S4: The experimental setup for measuring the velocity profiles inside the microchannel. A splitter lens arrangement is employed to selectively return the fluorescent signals from the fluorescent particles on getting excited by laser light (532nm). The images are captured using high speed camera at 10000 to 12000 Hz. The plots obtained are shown in figure S2.

Phagocytosis of bacterial cell

Figure S5 shows the percent phagocytosis of the stressed and unstressed bacterial sample. Two experimental sets were carried out keeping the geometry constant and varying the flow rates. We see that the stressed bacteria are much more prone to phagocytosis by RAW 264.7 cells implying restriction in infection. The values are found to be more for maximum stress cases (Q_2) suggesting the dependence of phagocytosis on the flow stresses. This difference is significant in straight channel compared to serpentine channels. Similar trends have been previously reported by our group for *Salmonella Typhimurium* subjected to mechanical stresses (1).

Relation between phagocytosis and proliferation

Phagocytosis depends on various factors and fold proliferation is independent of phagocytosis. Irrespective of the number of bacteria engulfed, the virulence is determined by how the bacteria are able to survive in hostile environments of macrophages. More phagocytosis does not necessarily mean less virulence. *Klebsiella* inhibits the phagocytosis by macrophages due to the

presence of the capsule, however once phagocytosed by macrophages, they survive by deviating from the canonical endocytic pathway and fusion of lysosomes(2). So, it might be possible that stress conditions induce the virulence factor that is involved in surviving inside the macrophages. Hence, it would not be accurate to connect phagocytosis and proliferation directly with the data in hand.

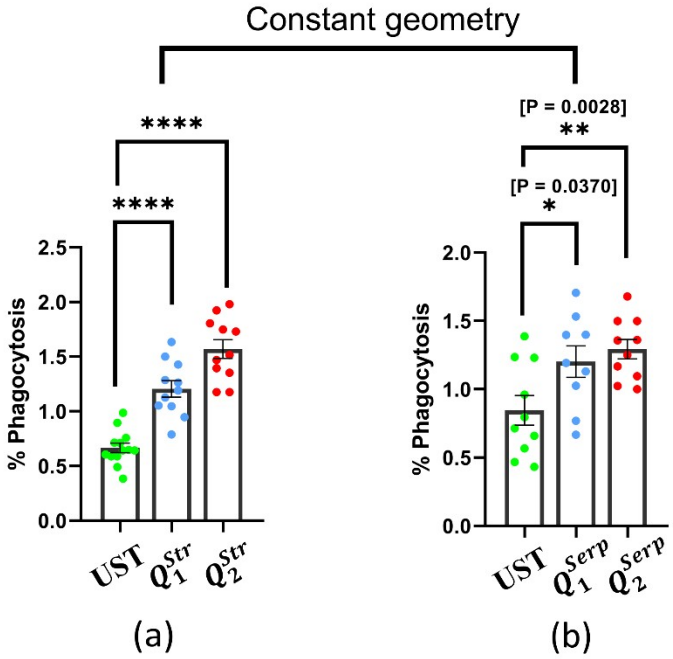

Figure S5: The comparison between the phagocytosis of the unstressed and stressed bacterial sample are shown by the RAW 264.7 macrophage cells at various stressing conditions. Here, UST represents the unstressed sample, and the other labels represent the different stressing condition as described in Table I (main manuscript). Figures (a) and (b) represent unique experimental set ($N \geq 3$, $n \geq 3$; N and n represents biological and technical replicates respectively, n values are plotted here) with different UST samples. (a) and (b) are carried out keeping the geometry constant as straight and serpentine respectively. The data represents the mean ± SEM. (P)* <0.05, (P)** < 0.005, (P)*** < 0.0005, (P)**** < 0.0001, ns = non-significant, Student's t test.

References

1. Hariharan V, Chowdhury AR, Rao S S, Chakravortty D, Basu S. phoP maintains the environmental persistence and virulence of pathogenic bacteria in mechanically stressed desiccated droplets. iScience. 2023;26(5):106580.

2. Cano V, March C, Insua JL, Aguiló N, Llobet E, Moranta D, et al. Klebsiella pneumoniae survives within macrophages by avoiding delivery to lysosomes. Cellular Microbiology. 2015;17(11):1537–60.