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# Investigation of self-assembly and topological changes in the sessile droplets of *Klebsiella oxytoca* by Chronological study

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**Abstract**
The infectious droplet from the patient self-assembles into a novel pattern depending on bacterial interaction with substrate and liquid. The spatial location of bacteria inside the droplet fluctuates depending on the non-covalent forces. The deformation and dehydration induced stress on bacteria in evaporating contagious-fluid droplets alters the viability and infectivity. The self-assembly of *Klebsiella oxytoca* (KO) in contagious sessile droplets was studied by natural evaporation. KO forms novel patterns as the droplets exsiccate, thus revealing the unexplored topological changes that govern its survival and infection strategies. The droplets of both bacterial suspension in Milli-Q and SRF of volume 0.95 ± 0.1 µl were placed on the glass material for assessment of the self-assembly. The bacterial suspension was stained before allowing them to desicate. The bacterial chemotaxis and deposition near the end of evaporation are recorded using the bright and dark field optical method. The random time interval is also measured to track the bacterial movement. The investigation shows that the majority of the bacterial population moves toward the rim of the droplet because of edge closely packing, leading to enhanced viability and pathogenesis on the famously known “coffee ring” and few bacteria are present at the centre of the droplet which represents chemotaxis of bacteria. The mechanistic insight gained via our study can have far-reaching implications for bacterial infection through droplets e.g., through open wounds.
**Keywords—Self-assembly, Klebsiella oxytoca, Coffee ring, Pathogenesis, survivability, Bacterial infection, sessile droplet.**

## Introduction

Bacteria exhibit various responses based on their environment and these responses are different for various species in the same or different families or genera. They show hardy responses in various fluid types that vary based on their rheology, density, surface tension. The bacterial species mainly prefer only the effectively forceful or dynamic realms that are favourable for their motility and conditions that favour their nutrient and oxygen uptake[1]. Even Though the bacteria survive in flowy water, the cells that are prevailing at the midst of the fluid droplets experience a greater stress that includes scantiness of substrates, truncated levels of oxygen and so on the factors that influence the viability of the cells. This is because of the phenomena that causes the colloidal particles or the particles that reside in the fluid moves towards the sides of the droplets. In consideration of bacteria, they usually adhere to the rigid surfaces using flagella, pili or some other non-specific interactions but we consider the walls of the liquid droplet. As similar to the particles here the bacterial cells move parallel towards the walls[2]. The following property is not only due to the physical or environmental stress exerted over the droplets possessing those bacterial species but also the result of intercellular signalling which is considered as the Quorum Sensing, for the transmission of signals or information and this facilitates those cells to makes them assemble (self-assemble) near the walls of the droplets. This mode of signalling is impractical in the case of flowing fluid, making the self-assembling of cells improbable[3].

The sessile droplets, minuscule in size, that are immovable laid down on the surfaces come across various physical stresses exerted by the environment over the system(sessile droplets). The droplet genesis is utterly based on the surface tension (over the liquid) and the cohesive forces (that act inside

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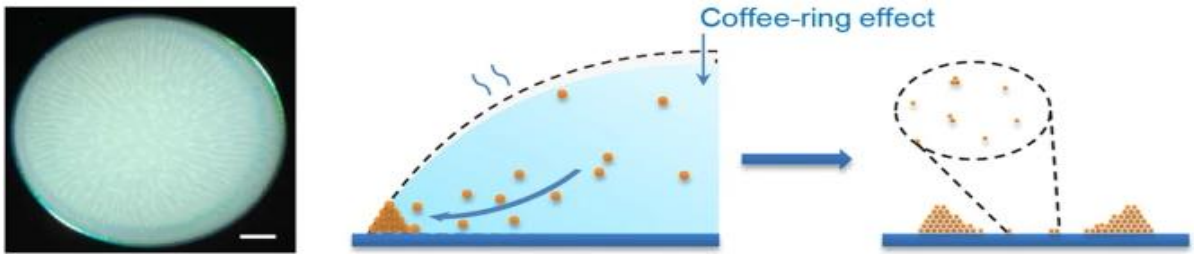
the droplets). The sessile droplets possess a volume ranging from micro to nano litres of liquid. The tremendous investigations had been done over these droplets that mainly emphasised over the evaporation of those sessile droplets that are initiated either naturally or artificially. The investigations and interpretations over the action of environmental conditions on these droplets explained by the curtailed level of atmospheric pressure sequels in escalating level of evaporation rate of solvents from these droplets[4].

The colloidal sessile droplets suspended with particles, when subjected to various rasping circs, results in the accumulation of these particles near the walls of the droplet and assists the formation of the ring-like structures that are similar to coffee ring stains. The stains leave depositions over the surface when these droplets are dried[5]. Furthermore, the droplets may not only contain suspended components but also some other active biotic components, probably. The coffee ring stains are commonly represented for these colloidal solutions due to the deposition of microscopic particles[6]. There are practicable changes in the deposition patterns and these patterns are influenced by various physical factors. The droplet ring formation property is implemented in various disquisitions of other sessile droplets and the above mentioned property is termed as Coffee Ring Effect[7,8]. The phenomenon is defined as the particulated droplets On desiccation of which is situated over a solid surface, the suspended particles inside the droplets tend to form a ring like structure. Usually, the ring boundaries will not form as soon as the droplet is placed. Once the droplet is spilled over a surface due to capillary flow the particles start to organise in the centre.The Initiation for drying starts from the boundaries of the droplets. In order to balance the moisture loss, the particles along with moisture move towards the boundaries as a reaction to the capillary flow that happened earlier [9]

Figure 1: Coffee Ring Effect[10]




*Klebsiella oxytoca*(KO), a bacterial species that belongs to the phylum Pseudomonadota under the family Enterobacteriaceae. It is an immotile, rod shaped gram negative bacterial species that are stratified in a classification under 279esearc-tolerant or faecal coliforms. In unconstrained outburst growth of *K. oxytoca* brings about an escalation in mortality rates amongst the 279esear-competent patients. In addition to these facultative anaerobes disseminate through the contact between the affected and non-affected individuals. Since the discovery these species have been positioned under pathogenic organisms. However, they show positive repercussions in the lumen of the intestines. These *Klebsiella* species primarily affect the nasal, urinary tracts and pave the way for certain infections in those regions. They exhibit highly outlasting responses over the antibiotics that are administered. This KO species has an action similar to *Klebsiella pneumonia*(KP)[11].

The conception of the 279esearchh is based on the hypothesis that an infectious droplet from the patient self-assembles into a novel pattern depending on bacterial interaction with substrate and liquid. The spatial location of bacteria inside the droplet fluctuates depending on the non-covalent forces. The deformation and dehydration induced stress on bacteria in evaporating contagious-fluid droplets alters the viability and infectivity. The self-assembly of KO in contagious sessile droplets was studied by natural evaporation.

As the precursory research, an identical genus *Klebsiella* but varied species of the similar genus-KP. By reviewing the literature, this study had investigated the flow and desiccation-driven self-assembly of KP in the naturally evaporating sessile droplets.The particular research had procured the results that these KP species were allowed to suspend, proceeding with the drying of droplets through the

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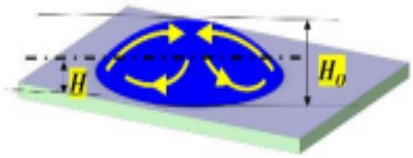
evaporation under environmental conditions, the minimal amount of cells gets scattered out in the middle of the desiccated droplets forming the unique pattern . The latter improvises the transmissivity of various droplet infections even after the droplets are being dried.[12] The another research represents a study of the evaporation of sessile drops containing silica rods to elucidate the structural arrangement of particles in the ring, an effect of the addition of surfactant and salt. The study reveals that several layers of silica rods close to the circumference of the drop are ordered such that the major axis of the rods are aligned parallel to the contact line by closer examination of the ring deposits. In addition, after the first few layers of ordered arrangement of particles, a random arrangement of particles in the drop interior is observed indicating an order-disorder transition in the ring.[13]

Figure 2: The motion of bacterial cells in both direction[11]




We aim to interpret the bacterial number density of the particular region in KO droplets and the objective of the study is to investigate self-assembly and topological changes in the sessile droplets of KO in Chronological order.

**Materials and Methods**

**Preparation of Bacterial culture:**

KO was cultured in Luria Bertani (LB) broth overnight at 170 rpm shakers and 37°C incubators. The overnight culture was cleaned by washing them 5 times with milli Q water by administering the centrifugation process to eliminate remnants from liquid culture media and then resuspended in milli Q water. At the overnight culture, the bacteria stay in the stationary phase. Aiming to shift to the Log phase,the bacterial culture was subcultured by adding 8.3 ml of bacteria in 250 ml of fresh media and incubated for 2 hours further at 37°C incubator shakers. The visualisation procedure was commenced by a staining with membrane dye called FM 4-64. The culture in stationary or log phase were incubated in ice bath with diluted dye in milliQ (concentration 1µg/mL of FM 4-64) for about 15mins, and then cells were further washed with Milli-Q to remove excess dye and resuspended in Milli-Q. This procedure was executed to isolate bacteria for further investigation and used as a control.

**Droplet Evaporation:**

The Surrogate respiratory fluid (SRF) is processed according to the procedure mentioned in Rasheed et.al[14]. The bacteria suspended in Milli-Q and SRF droplets are casted on the glass slide cleaned with 90% ethanol.. During the experiments, circumferential conditions are maintained at the relative humidity 40 ± 3% and temperature 25 ± 1°C. Droplets of volume 0.95 ± 0.1 µl are drop cast on glass substrates cleaned with 90% ethanol. The initial contact angle of the droplet was measured as 46 ± 2°; the droplet dries in pinned contact line mode with a wetting diameter of 2.0 ± 0.07 mm. The bacterial chemotaxis and deposition near the end of evaporation are recorded using the bright and dark field optical method. The random time interval is also measured to track the bacterial movement.

**Design Of Experiment for Self- Assembly Monitor:**

The droplets of both bacterial suspension in Milli-Q and SRF of volume 0.95 ± 0.1 µl were placed on the glass material for assessment of the self-assembly. The bacterial suspension was stained before allowing them to dessicate. The circumferential parameters were maintained as mentioned above. The total time of droplet evaporation($t_f$) was calculated to divide the regular interval for imaging the movement of prokaryotic cells. The cells' chemotaxis were imaged at various times (t). Finally, in order to quantify, the time parameters were represented into the ratio t/tf.

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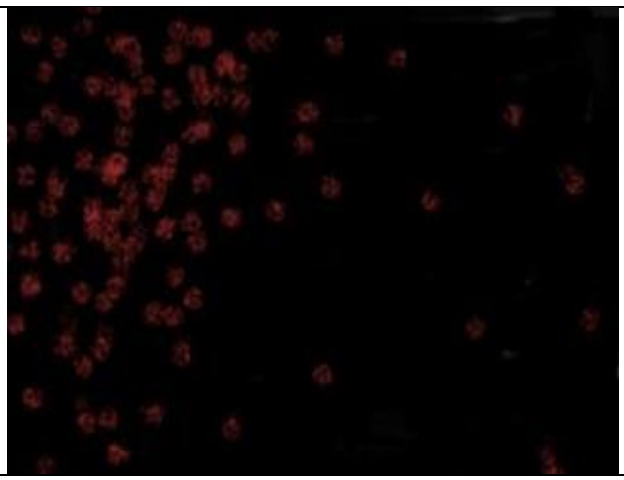
$$\frac{t}{t_f} = \frac{\text{image at any given time instant}}{\text{total time of evaporation of the droplet}}$$

**Cell imaging and live tracking:**
The bacterial chemotaxis was tracked by staining with membrane stain FM 4-63 which had excitation/emission maximum of 515/640 nm at 2.28 fps using a fluorescent microscope. Firstly, the bacterial movement was viewed in a compound microscope and then viewed through fluorescent microscope.

**Result And Discussion**
Our investigations show circulatory flow in the bacteria-laden Milli-Q water droplet. Near the glass 101 surface, the flow is directed radially outwards. The upwards movement along with the circulatory flow inside the droplet. Near the end of evaporation, the bacteria that has not settled in the coffee ring gets deposited in the rim of the local dewetting hole front. The ordered arrangement of KO is similar to the sequential arrangement of rod-shaped particles previously observed by Dugyala et al.[13] As the thickness of the droplet becomes reduced, the evaporative flux at the contact line increases. Eventually, the flow velocity inside the droplet towards the contact line also increases. The rapid increase in velocity near the end of evaporation, usually referred to as 'the rush hour effect.' The resulting deposition at the coffee ring generally exhibits an order to disorder transition. We observe a similar order to disorder transition self-assembly in the case of KO bacteria. At the median of the droplet, we observed an abrupt increase in the visible number of bacteria during the final stages of evaporation. The focus was adjusted to visualise a section plane of a droplet near the substrate. Near the air-water interface of the drop many bacteria reside, as the drop height reduces, the bacteria move along with the open surface of the droplet towards the substrate and thus become visible in the live-cell imaging in the end stage of evaporation.
The thin film of liquid and bacteria residue at the end stage of the evaporation of the droplet (t/tf~0.99) undergoes instability; subsequently, capillary forces dominate, leading to the formation of holes and compact packing of KO. The Fluorescence microscopy image in the particular realm shows the bacteria aggregates disregarding the region of holes. The figures in the table shows the compaction of KO happens in the last one percent of the total evaporation times, and the self-assembly is dominantly the result of capillary forces and thin-film instabilities at the end stage of evaporation.

| S.NO | TIME | $t/t_f$ | CELL IMAGE | OBSERVATION |
|---|---|---|---|---|
| 1 | 0.6 | 0.1 |  | The KO is present in colloidal suspension |

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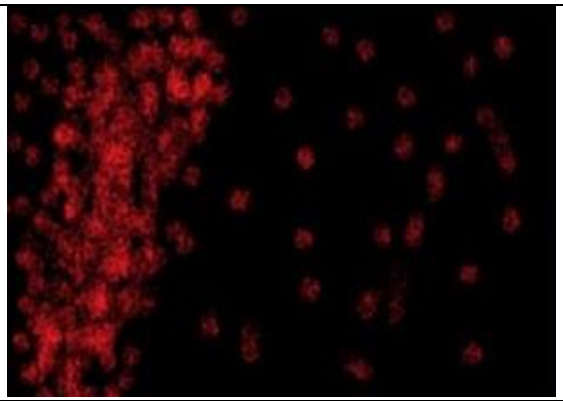
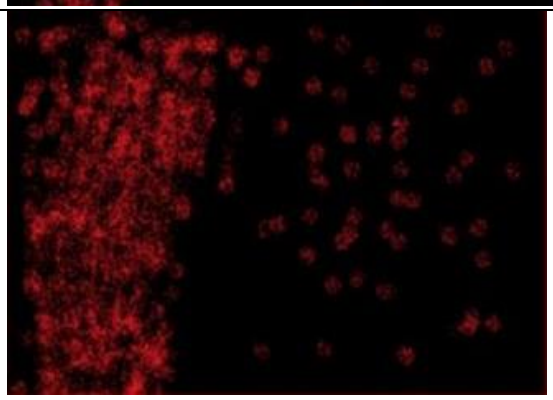
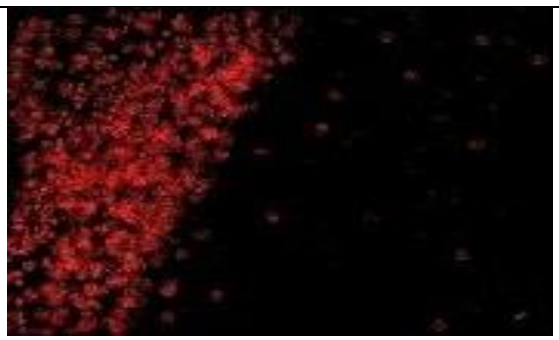
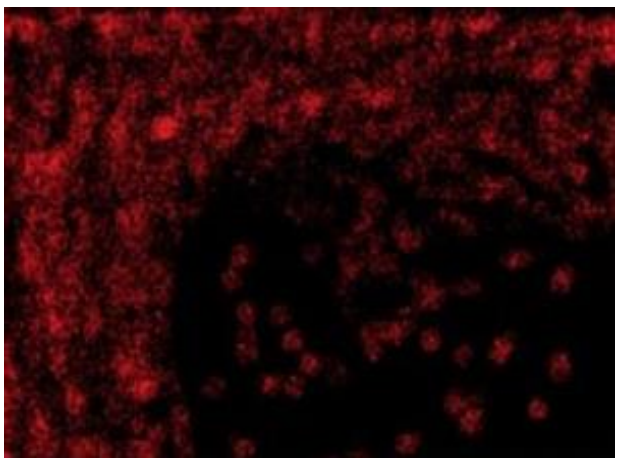
| | | | | |
|---|---|---|---|---|
| **2** | **1.2** | **0.2** |  | KO commence to deposit at the end circumference of the droplet |
| **3** | **1.8** | **0.3** |  | The deposition of bacteria progresses |
| **4** | **2.4** | **0.4** |  | Commencement of Coffee ring formation |
| **5** | **3.0** | **0.5** |  | The myriads of KO emerge into the coffee ring as thin runoff. |

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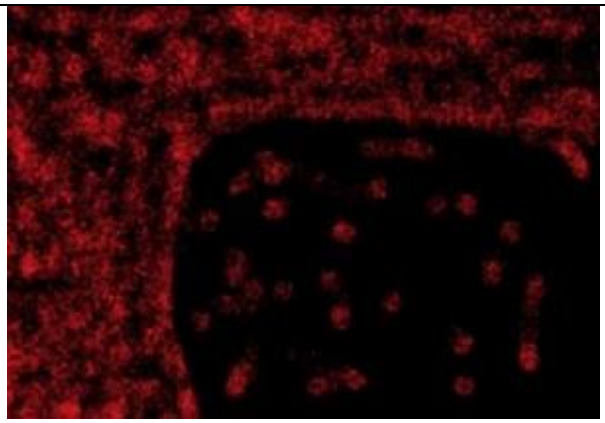
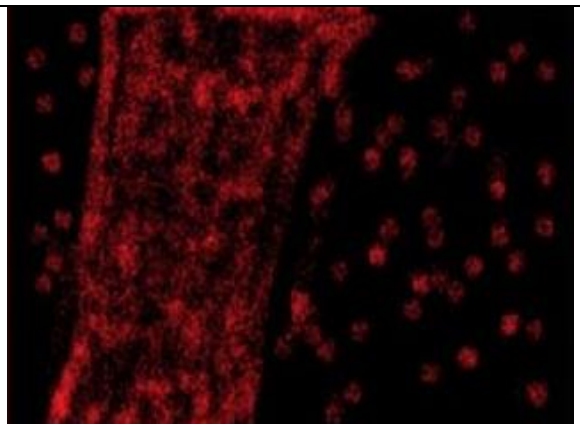
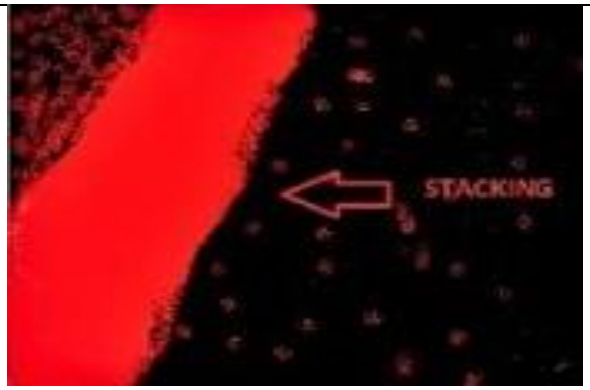
| | | | | |
|---|---|---|---|---|
| **6** | **3.6** | **0.6** |  | All KO does not faithfully flow towards the coffee ring. Few KO locomote away from the coffee ring. |
| **7** | **4.2** | **0.7** |  | KO concentration at the ring increases |
| **8** | **4.8** | **0.8** |   | Higher Bacterial Density and principle of stacking occurs. |
| **9** | **5.4** | **0.9** |  | Continuation of bacteria accumulation at the end of wall |

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| | | | | |
|---|---|---|---|---|
| **10** | **6.0** | **1.0** |  | The wider diameter of the coffee ring follows the principle of squeezing and results in the reduction of diameter size. The darkened realm represents the increase in bacterial density. |

## Conclusion

This paper investigates the self-assembly and topological changes in the sessile droplets of *Klebsiella oxytoca*.The bacterial chemotaxis was analysed by staining technique and deposition near the end of evaporation was recorded. The bacterial number density was calculated at a particular region of interest using the fluorescent microscopy. Higher KO bacterial density was observed by the mechanism of stacking and squeezing in the coffee ring. Hence, the results of self assembly and topological changes were interpreted.

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