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Filamentous growth of laboratory *E. coli* DH5α cells with metallic green sheen in eosin-methylene blue broth containing ampicillin as a new strategy to escape the combined chemical and antibiotic-selection pressure.

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The laboratory *E. coli* DH5α strain cells are routinely used in the research. *E. coli* DH5α strain cells are typically rod shaped bacilli that are Gram negative. Certain conditions such as nutrients in the growth media or presence of antibiotics may induce stress on the bacterial cells that lead to changes in their shape as a response to escape the antibiotic pressure. In this study, the Gram positive *Lactobacillus* and Gram negative *E. coli* bacterial cells were grown in eosin-methylene blue (EMB) media containing 50 µg/ml ampicillin to compare their growth morphologies. As expected the EMB medium suppressed the growth of Gram positive *Lactobacillus* and promoted the growth of Gram negative *E. coli* cells with typical metallic green sheen. These *E. coli* cells were further cultured in the liquid EMB broth with ampicillin followed by microscopic observation. Long filamentous *E. coli* cells were seen that stained Gram negative. These filaments show demarcations for individual rod shaped bacilli but cytokinesis did not happen because of the absence of septa. We believe that the methylene blue in the presence of ampicillin caused the formation of such filaments as a morphological strategy for *E. coli* DH5α strain cells to escape the stress and antibiotic selection pressure.

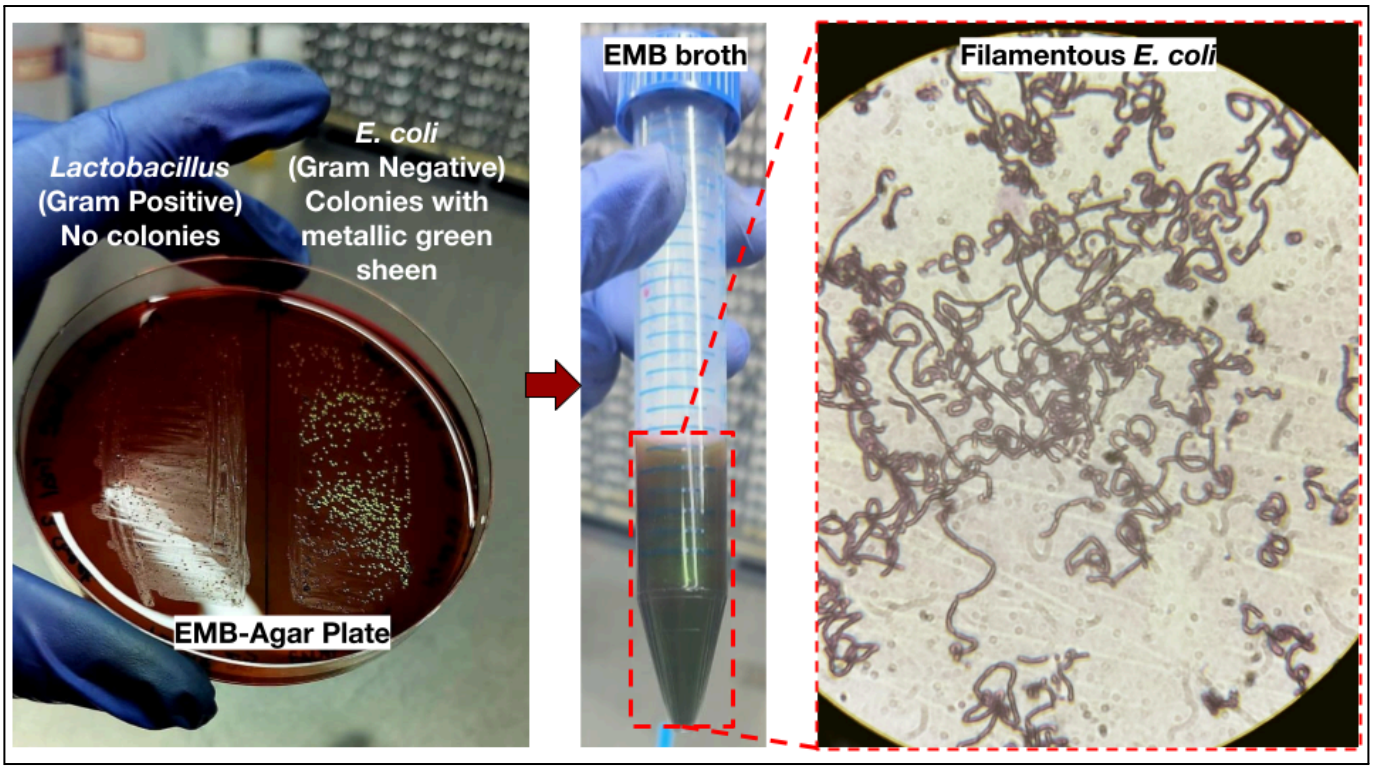

Figure 1. Gram negative *E. coli* cells show metallic green sheen on EMB agar plate and EMB broth with filamentous growth.

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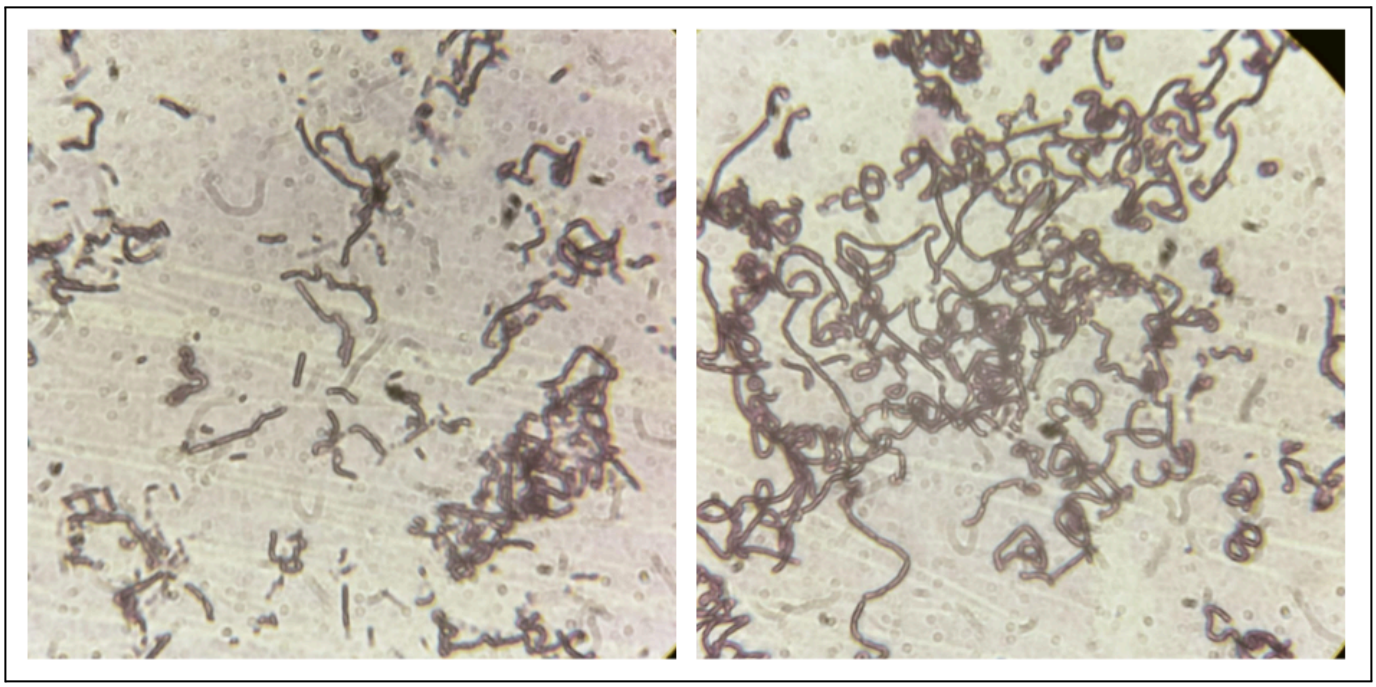

Figure 2. Microscopic images showing filamentation of *E. coli* cultures in the EMB broth. Both short filaments (left panel) and very long filaments (right panel) were observed under the same conditions.

Antibiotics belonging to different classes have different mechanisms of inhibiting the bacterial growth [1]. For example, all the beta-lactam ring-containing derivatives such as penicillin-G, ampicillin, amoxicillin, cephalexin, etc. inhibit cell wall formation by binding and inhibiting the activity of penicillin-binding protein (PBP) [2]. It has been previously shown that the usage of cephalexin [3] and other antineoplastic antibiotics such as azaserine [4] have induced bacterial filament formation in the case of *E. coli*. Typically, during the cell division in bacteria such as *E. coli*, the antiparallel-double stranded form of the FtsA protein aids in the activation of the septum formation so that the daughter cell is separated from the mother cell [5]. Along these lines, mutations in the kdsA protein may also inhibit the septum formation [6].

In addition to the internal factors responsible for bacterial filament formation explained above, certain external factors such as stress, nutrients, presence of drugs/antibiotics and other bacteria [7-11]. Filamentation is also used by bacteria as a mechanism of defense [12]. It is believed that filamentation has an evolutionary advantage for the bacteria to escape and survive from the macrophages and other predators because the long filamentous morphology would challenge the predator while getting engulfed which would have been easy otherwise. Bacteriophages are capable of inducing filamentation by inhibiting cell division [13]. It has been previously shown that the *Agrobacterium spp*. can exhibit filamentation in response to living tissues [14]. Small molecules such as methylene blue have been shown to cause minor DNA damage in response to which, *E. coli* displayed filamentation [15].

In this study, we used the EMB medium containing methylene blue dye in addition to the presence of 50 µg/ml ampicillin to select the *E. coli* cells that were previously transformed with a plasmid containing ampicillin-resistance causing gene. Initially we thought that this plasmid caused filamentation. However, the same transformants when plated on LB agar plates did not exhibit any filamentation suggesting that it is indeed the methylene blue dye present in the EMB agar plates that might have induced the filamentation in *E. coli* cells. Thus, our results suggest that the regular laboratory *E. coli* DH5α strain cells can exhibit filamentation in the presence of methylene blue under the selection pressure of antibiotics such as ampicillin.

Bacteria have been using the filamentation as a strategy to escape any stress causing evolutionary pressures in order to survive. Filamentation has been shown to accelerate the formation of biofilm [16] which would help further to protect the bacteria by itself from the external pressures. One can imagine the same scenario in the human gut and pathological situations where it can become a very big challenge to treat such bacteria. Additionally if multidrug-resistant bacteria form such biofilms then it could result in a global pandemic.

In conclusion, bacterial filamentation might pose a threat not only to the existing antibiotics but also may spread more lavishly into biofilms thus making the infection more serious and untreatable. Especially with artificial food containing chemical ingredients as preservatives may cause more evolutionary pressure on the gut bacteria either in the presence or absence of antibiotics. One should be careful while taking the antibiotics by following the clinicians prescriptions and advice rather than ingesting random antibiotics to avoid such pandemics.

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Conflict of interest: The authors declare no conflict of interest in this study.

Author contributions: M.V. and S.A. performed wet lab experiments. M.V. performed plating and imaging. S.A. performed liquid broth cultures. R.S.Y. is the principal investigator who designed the project, trained all authors, secured required material for the project, provided the laboratory space and facilities needed. R.S.Y. wrote, edited and finalized the manuscript.

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