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Bacterial Filaments Induced by Antibiotic Minimal Inhibitory Concentrations in Persister Cells

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ABSTRACT: ***Background:*** *The ability of minor subpopulations among clonal populations to survive antibiotics is referred to as bacterial persistence. It is believed that persisters come from latent cells, where antibiotic target areas are less active and incapable of being affected.* ***Objective:*** *112 clinical Escherichia coli isolates were acquired out of diverse medical samples and genetically identified using the uspA gene, which is part of the housekeeping genes.* ***Methods:*** *The examination of persister cells was carried out by subjecting isolates of E. coli in the exponential phase with high dose of ciprofloxacin (20 fold MIC) and calculating the surviving persister cells using CFU (colony forming units) counts. The detection and measurement of bacterial filament production was done using scanning electron and light microscopy.* ***Results:*** *Results showed that the bacterial filament cells kept on lengthen but cease to divide (no septa formation) at sub-minimal inhibitory doses of ciprofloxacin. Persistent isolates were shown to exhibit a wide range of form and size variations, with cells up to 4.5 times longer than usual.* ***Conclusions:*** *The results showed the importance of antibiotic stress on persisted cells that result in the production of filaments as a means of survival and the need to examine these rare phenotypic variations. These occurrences may be the beginning of the spread of bacterial resistance.*

KEYWORDS: *E. coli; persister; filaments; ciprofloxacin; uspA gene*

INTRODUCTION

The inability to eliminate bacterial infections is often ascribed to the choice of antibiotic-Resistant mutations or the acquiring of resistance determinants via horizontal gene transfer [1]. Nonetheless, bacterial 'persistence' refers to the capacity of a subset of a bacterial populace to persist despite antibiotic treatment at high concentrations [2] it is a less understood process and seems to have a key role in infection recurrence and the development of antibiotic resistance [3]. Persistence is often noticed as the bulk of the bacterial clonal is immediately destroyed but a subpopulation survives for a significantly prolonged time in spite of the fact that the population is clonal.The characteristic feature of the persistence is the time-kill curve that results are going to be biphasic [4] quite the opposite with resistance and tolerance, that are characteristics of entire bacterial populace. When the antibiotic stress is eliminated, persisters resume growth and produce a new population that is the same to the initial strain cells [5].

The lower killing ratio of the persistent sub-set is not inherited: when persistent cells are detected, and then re-grown, and re-subjected to the exact same treatment, similar heterogeneous reaction towards treatment as in the initial population is observed with the population divided into persistent and non-persistent sub-populations [6]. As a result, persister cells are often assumed to be in a latent, metabolically in dormant state condition to shields them from antibiotic assaults [7]. This

inactive physiological state would allow resistance to many antibiotics, which is a characteristic of persistence. However, it is obvious that the non-dividing condition is not adequate for life, and only a little percentage of latent cells may regenerate after antibiotic treatment [8]. The SOS (save ourselves) response is a condition of intense DNA repair and mutagenesis that allows bacteria to quickly develop resistance to drugs [7].

Ciprofloxacin, a genotoxic antibiotic, triggers an SOS reaction that causes *E. coli* to transform from rod-shaped cells to multi-chromosome-containing filaments. It was shown that at Ciprofloxacin doses at sub-minimal inhibitory, the bacterial filament continually divides irregularly at its tip. [9] Some bacteria, like *E. coli*, have an abnormal growth pattern called filamentation, in which cells extend indefinitely but never split (there are no septa) [10].

Many investigations have confirmed the existence of filamentous *E. coli*. The function filamentation plays with the emergence of antibiotic resistance is discussed [11]. In response to lack of nutrients, extensive DNA damage via the SOS responding process, host natural immune responses, dehydration,and antibiotic treatment, many microorganisms follow a filamentous shape due to cell-division arrest but continue cell-size expansion [12].

The longer cells are harder to consume and they are protecting bacteria against protozoan predators and neutrophil phagocytosis. As well as being linked to virulence factors including biofilm development, it is believed that filamentation shields bacteria against antibiotics [13]. filamentation has been characterized as a more broader survival strategy only in recent times , despite the fact that it was originally thought to be an ill phenotype or exclusively for the finishing point of DNA replication prior to division [13], for instance, If the pressure is eliminated sufficiently quickly,division of the filamentous cell continues simultaneously and quickly at certain times along with the whole extent of the filament, leading to numerous viable progeny cells of typical dimensions [9].

Antibiotics Stimulate Filamentation

Through the SOS response, DNA synthesis- repressing and DNA-damaging antibiotics (such as metronidazole, mitomycin C, fluoroquinolones , and novobiocin) stimulate filamentation [13]. This interruption prevents the transmission of damaged DNA to offspring caused by the SOS response. Bacteria suppress septation by synthesizing protein SulA, a FtsZ inhibitor that prevents Z-ring formationand, a result, halts recruitment and activation of PBP3 [13].

The intend of this investigation was to explore the role of antimicrobial stress in persister cell filament formation as a survival mechanism.

MATERIALS AND METHODS

Bacterial Isolates, Detection, and Maturation Environments

One hundred and twelve clinical *E. coli* isolates were acquired out of various sources (urine, stool and blood). The isolates were identified employing their development characteristics in culture medium and validated via a Vitek-2 system (BioMe'rieux, France). Using particular primers for the *uspA* gene, conventional PCR was used to identify these isolates. The primers sequence (*uspA*- F-CCGATACGCTGCCAATCAGT) R-ACGCAGACCGTAGGCCAGAT.) with a product size of (884 bp) ,PCR conditions were performed as the following: Chromosomal DNA templates were heated at 94 °C for 5min and next augmented for 30 cycles, each including of 94 °C for 2 min, 70 °C for 1 min and 72 °C for 1 min [14]. The isolates were regularly cultured in Luria Bertani (LB) broth for 24 hours at 37 °C.

Determination of the MIC

E-test was used to evaluate the MIC values of the antibiotic ciprofloxacin. The MIC was established by determining the minimum dosage of ciprofloxacin antibiotic that was non-permissive for observable growth. The negative control strain was *Escherichia coli ATCC 25922*.

Persister Cell Phenotypic Detection

Escherichia coli E30 and E107 were grown overnight and diluted to a ratio of 1:100 LB broth to count the number of persister cells. At 37 °C, the bacterial culture was incubated until the absorbance at 600 nm approached 0.5 (exponential phase). Then these cultures were independently treated with 2 µg/ml ciprofloxacin (20 Fold MIC), concentration was then added to the culture. After 1, 3, 5, 7,

and 18 hours, 100 µL of culture was rinsed twice with 0.85% sterile saline solution to get rid of the antibiotic. A bacterial culture was serially diluted and every dilution was placed over LB agar. The plates were then incubated for 24 hours at 37 °C. Finally, colonies were counted to determine if the bacteria was present on the agar plate. To check that the MIC values had not altered, 2 colonies out of each test cell were re-inoculated into new LB broth and in vitro antimicrobial susceptibility assays were conducted [15].

Filament Formation Detection

With a few adjustments, filament production was detected using a microtiter wells plate, as described in [16]. In brief, eight sub-MIC ciprofloxacin antibiotic concentrations (5, 10, 20, 30, 40, 50, 100, as well as 120 mg/L) were prepared. Concentration of a negative control with no antibiotic was also utilized as a reference for the length of *E. coli* isolates.

The ciprofloxacin fluid was diluted in purified water, and 20 µL of each dilution was placed into the wells of microtiter plate, followed by 180 µL of *E. coli* suspension, which was gently mixed and incubated for 4 hours at 37 °C. Subsequently, a slide smear and Gram stain were performed on every well for examination under a light microscope.

Subsequent for the Gram's stain, the filament size, which is the most common feature utilized to assist indication of filament production bacteria, was determined, and a persistent *E. coli* isolate was discovered. Microscopy and a SEM were used to determine the filament diameters.

RESULTS AND DISCUSSION

Detection of Bacteria and Growth Conditions

E. coli Specimens were acquired from a variety of medical samples, consisting of (50, 38, 24) isolates from urine, stool, and blood respectively. The identity of the *E. coli* was validated by phenotypic characterization using the Vitek 2 method, which was subsequently verified again by Gel electrophoresis the *uspA* gene (-subunit of RNA polymerase), as depicted in Figure 1.

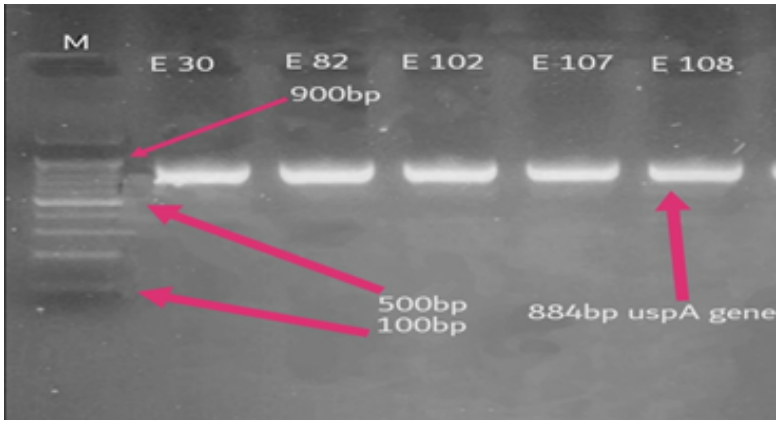


Figure 1. Gel electrophoresis (1% agarose, 70 volt for 50 min) of *uspA* gene (884 bp). Lane M 1000bp DNA Ladder, the other lanes bands are that the other lines are the positive results of *uspA*

The Determination of the MIC

Given that persister cells are able to bearing much higher concentrations of antibiotic MICs, first it was determined the MICs of ciprofloxacin for *E. coli* using E-test. The MIC values for the Ciprofloxacin were 0.094 µLg/ml for both (E30 and E107).

Persister cells, according to research, are the cause of antibiotic tolerance, bacterial survival in biofilms, and the progression of repeated infection resulting from various bacteria [17]. However, this work established that persister cells for *E. coli* were generated following antibiotic challenge, and the findings revealed that the proportion of viable cells varied according on colony count. The MIC of a persistent bacteria is identical the susceptible bacteria; however, the minimum duration for killing (MDK99.99) for 99.99% of bacterial cells in the population is much longer for a persistent strain than for a susceptible strain [18].

When the percentages of deaths of bacterial cells that are subjected to antibiotics become lower due to various mechanisms, the MDK rises. This is consistent with the fact that persister cells are sluggish or non-growing, rendering them "refractory" to antibiotics. Antibiotics target physiological mechanisms that are less active in slowly developing cells [19], [20].

Persister Cell Assay

To assess persister cells, *E. coli* culture was administered with Ciproloxacin during the exponential phase, (MIC = 0.094 µg/mL; sensitive) of *E. coli* experiment isolates with 2 µg/mL ciprofloxacin (20-fold MIC), the greater part of the population was capably killed. Colonies were enumerated according to the colony forming unit. Calculations revealed that the number of viable cells decreased considerably within 1 hour, except it was remained steady after 5 hours. Persister cell endurance was established using plateau-phase survival curves as shown in Figure 2.

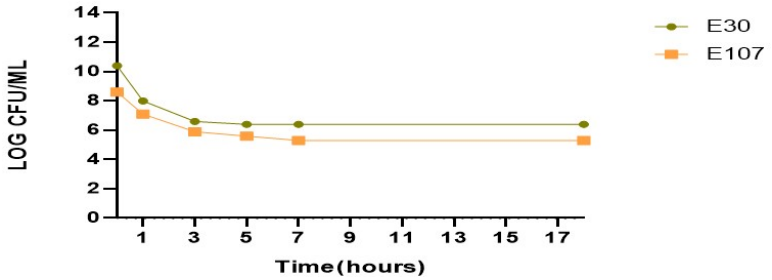
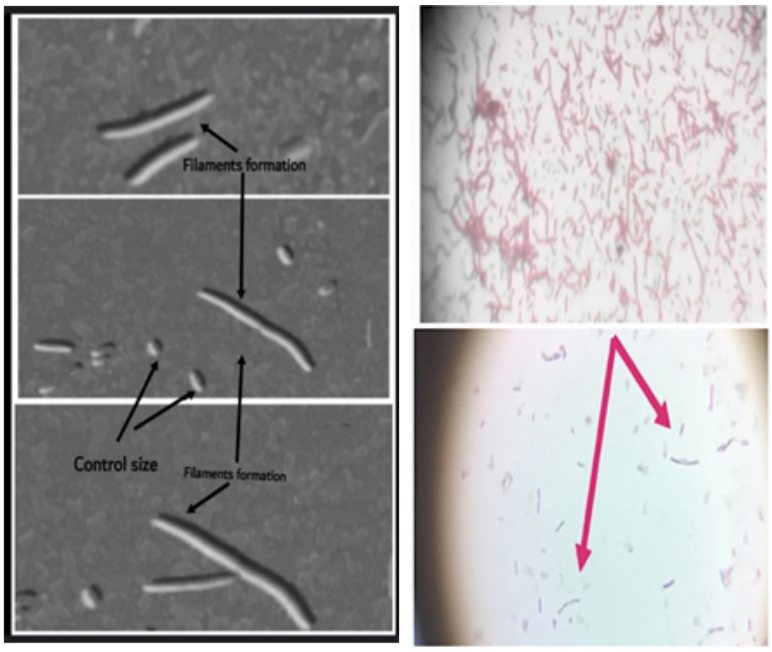


Figure 2. Stabilization test for bacteria through colony count (CFUs).Where the Experiment for persister cells heritability using Ciprofloxacin antibiotics against *E. coli* (E30) and (E107)

Filament Formation Detection Results

The results of subjecting persistent *E. coli* isolates to different Ciprofloxacin doses revealed that cell filaments developed at 10, 20, and 30 mg/L concentrations, indicating that the bacterial isolate was shape-shifted into a filamentous, as shown in Figure 3.



(a) (b)

Figure 3. Filaments of E.coli isolate, indicating that the bacterial isolate was shape-shifted into a filamentous (a) Scanning electron microscope, (b) light microscope, ×100 magnification

The way bacteria build their morphologies is influenced by their habitat and genetic composition. Morphological alterations were seen in *E. coli* when exposed to sub-MICs of ciprofloxacin., primarily filamentation. Under the microscope, *E. coli* was detected before and after treatment with fatal dosages of Ciprofloxacin (20 fold MIC).While most *E. coli* cells perished, a few persisters survived the treatment, and the surviving cells displayed filamentous appearance, indicating an induced SOS mechanism [21]. These findings revealed that antibiotic treatment and persistence development significantly altered the shape and size of persistent *E. coli* (filamentation).When studied under a scanning electron microscope, persistent *E. coli* isolates demonstrated cell elongation, with a 4.5-fold increase in cell length. Other researchers [22] observed morphological changes in bacterial cells caused by antibiotic exposure at sub-MIC levels, where they discovered persisters filamentous morphology after ampicillin treatment of *E. coli*, and the filaments are becoming longer with time. Changes in cell morphology at low antibiotic doses may give insight on diagnostic misunderstanding in clinical material morphological examination. Ciprofloxacin (inducing filamentation) inhibits DNA synthesis in prokaryotic cells, preventing cell division ; the bacteriostatic cause the repression of DNA and RNA synthesis due to the arrangement of a gyrase-DNA Ciprofloxacin intricate, which may hinder the expression of genes involved in septum development. Unusual elongation is thought to occur when the antibiotic concentration is high enough to prevent the production of enzymes that induce septation but not high enough to stop the synthesis of enzymes involved in elongation [23]. In this study when the bacterial cells exposed to sub-minimum inhibitory concentration (stress prolonged condition), bacteria demonstrated a changing in shape as a response. Bacteria have developed intricate methods to keep cell shapes constant. However, under some conditions, bacteria may change this carefully controlled process and evolve into filamentous shaped cell. Accumulating data suggests that filamentation plays crucial biological functions in stressed situations, as well as, areas of contact among pathogenic microorganisms and their hosts [24]. Chemical stress from poisonous and damaging substances may enhance the shift of bacteria from independent organisms to host-invading pathogens [25]. Filamentation might be intentional reactions to certain environmental signals that improve survival in the face of hazards like as ingesting and death .For example, the filamentous UPEC (Uro-pathogenic *E. coli*) withstand innate immune effectors cell death by escaping neutrophil phagocytosis. Furthermore, in immune-competent hosts, the bacterial cell-division suppressor SulA is required for UPEC pathogenicity and facilitates bacterial filamentation [26]. According to one research, the formation of filaments by persistent bacteria following the subjected to antibiotics or other stressful situations may be the first step of bacterial resistance [27].

CONCLUSION

Sub-minimal inhibitory quantities of the genotoxic antibiotic ciprofloxacin were employed to induce persister bacteria to leave in a filamentous condition. The results demonstrated that in response to ciprofloxacin at 20 fold MIC, the phenotypic morphological alterations of *E. coli* persister cells from rod form to multi-chromosome-containing filaments. The quantifiable phenotypic alterations were used to indicate methods through which persister cells resist antibiotics. The results suggest that persister cells resist antibiotics by growing longer and changing form into filaments. Persister cells were also shown to be heterogeneous. This variability may potentially be seen as a survival strategy for the cells which have been subjected to antibiotics.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Methodology, review and editing, Mohammed F.AL Marjani and Sawsan H.Authman; writing—original draft preparation, Haneen.N.mohammed; visualization and validation, Shivanthi Samarasinghe.

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DATA AVAILABILITY STATEMENT

None.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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