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Co-Regulation Of Fimh, Luxs, And Hlya Genes In E. Coli Isolates Under Chemical Stress: A Study On Cross-Gene Expression Dynamics

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Abstract
Antimicrobial treatments, while designed to eradicate bacterial pathogens, can unexpectedly encourage bacterial virulence abilities. This study explored the influence of various antimicrobial agents (Dettol, surgical soap, ciprofloxacin, ceftriaxone, and gentamicin) on the expression of three key virulence genes: hyl (hyaluronidase), fim (fimbrial adhesin), and lux (quorum sensing) in Escherichia coli using quantitative PCR. Clinical samples were collected from Al Ramadi Teaching Hospital and Al Ramadi Teaching Hospital for Gynecology and Pediatrics, during the period between December 2024 to March 2025. Gene expression was standardized using the $2-\Delta\Delta$Ct method, with fold changes calculated relative to an untreated control. All antimicrobial treatments reliably stimulated the upregulation of these virulence genes, representing complex stress-responsive way activation in bacteria.
The hyl gene, encoding a spreading factor, exhibited consistent upregulation through all treatments, with gentamicin inducing the highest rise (6.45-fold). This proposes that protein synthesis inhibition and oxidative stress may powerfully promote bacterial tissue penetration. The fim gene, serious for adhesion and biofilm formation, exhibited significant upregulation, mainly with ciprofloxacin (8.98-fold) and ceftriaxone (8.79-fold), suggesting that DNA damage and cell wall stress activate enhanced surface association for perseverance. Most strangely, the lux gene, elaborate in quorum sensing, revealed a dramatic 14.44-fold upregulation with ciprofloxacin, emphasizing an unprecedented activation of bacterial communication networks. These results reveal that sub-lethal levels of antimicrobials can trigger integrated stress response systems in bacteria, leading to the corresponding expression of multiple virulence factors. This phenomenon poses a significant challenge to antimicrobial therapy, as treatments intended to eliminate bacteria may inadvertently enhance their pathogenic potential. In conclusions, antimicrobial treatments paradoxically enhance bacterial virulence by upregulating key genes for spreading, adhesion, and communication.
Key words *Escherichia coli, hyl, fim, lux genes, and antimicrobials.*

INTRODUCTION

Escherichia coli is arguably the most scrutinized bacterial species in microbiology, and both a potentially beneficial commensal organism in our gut microbiome, and a dangerous pathogen that can cause a wide variety of clinical disease manifestations, including urinary tract infections to more severe and life-threatening conditions such as septicemia (Cho et al., 2024). This gram-negative, facultative anaerobe has gained a reputation as the most studied microorganism in the world, providing scientists with foundational knowledge about bacterial physiology, genetics, and pathogenesis (Singh et al., 2025). However, the same properties that make E. coli a model organism for investigating processes within the organism have also led to its definition as a public health menace, especially in the context of antimicrobial resistance. The footprints of antimicrobial resistance in E. coli have created an unparalleled challenge for public health systems worldwide, impacting not only those who are resistant but also producing multidrug-resistant (MDR) E. coli, which threatens traditional treatment regimens and renders common infections untreatable (Nasrollahian et al., 2024).

The evolution of antibiotic resistance in bacterial populations is a classic example of natural selection under intense selective pressure, and antimicrobial use can exert strong selective force on bacteria to kill the susceptible variants and select resistant variants, as the antimicrobials are exerting pressure on the bacterial population as well (Ramos-Martín & D'amelio, 2023).

The fimH gene is encoded by the adhesion unit of type 1 fimbriae, described as one of the most studied virulence factors found in uropathogenic E. coli (UPEC) strains, which are present in 68% to 100% of clinical isolates (Hojati et al., 2015). The hly gene cluster encodes α-hemolysin, a cytolysin that acts as a pore and is a virulence factor in intestinal and extraintestinal pathogenic strains. The regulation of hly is often coordinated with other important resistance mechanisms in environmental pathogenic strains

(Arafi et al., 2023). Functionally, the luxS gene encodes S-ribosylhomocysteine lyase, which also acts as a metabolite and synthase of the autoinducer-2 (AI-2) signaling molecule(s) involved in biofilm development and quorum sensing, as well as in phenotypes associated with antibiotic resistance in bacteria (Meng et al., 2022). This study aimed to explore the effect of numerous antimicrobial treatments on bacterial virulence, precisely examining the gene expression of hyl, fim, and lux. And for determine if sub-lethal antimicrobial exposure could encourage or upregulate these virulence abilities.

MATERIALS AND METHODS

This study was conducted at General Al-Ramadi Hospital and the Women and Children's Hospital in Al-Ramadi, Iraq. A total of 180 urine samples were collected from patients clinically diagnosed with urinary tract infections (UTIs). Urine specimens were inoculated onto Blood Agar, MacConkey Agar, and Eosin Methylene Blue (EMB) culture media, followed by incubation at 37°C for a 24-hour period. Presumptive colonies were subjected to preliminary identification based on colony morphology, Gram staining, and biochemical characteristics. Gene expression of the following genes was analyzed using quantitative polymerase chain reaction (qPCR) before and after exposure to stress: **fimH** (associated with adhesion), **hly** (encoding hemolysin), and **luxS** (involved in quorum sensing).

Chemical Stress Application

Bacterial suspensions were treated with sub-inhibitory concentrations of selected antibiotics (ciprofloxacin, cefotaxime, gentamicin), Dettol antiseptic, and surgical hand soap before performing gene expression analyses.

Molecular Identification and Gene Expression Analysis

PCR Equipment: Real-time PCR was performed using a Stratagene MX3000 (USA). Other applicable equipment involved a Nanodrop Spectrophotometer (USA, Thermo Fisher Scientific) for nucleic acid quantification.

- Chemicals and Biological Materials for PCR:
- Absolute ethanol and Chloroform (USA, SIGMA).
- Nuclease-free water (China, TransGen Biotech).
- Oligonucleotide (Primers) (Canada, Alpha DNA).

Kits:

Table (1) provides comprehensive documentation of all commercial kits employed in this research work, including details of their respective manufacturers and procurement sources:

Table (1): Commercial kit specifications and supplier information for the current study.

Biological materials	Source	Company
EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix	China	TransGen biote
EasyPure® miRNA Kit		
TransZol Up Plus RNA Kit		
TransStart® Top Green qPCR Super Mix		

Primers

The primers which were used in the current work are demonstrated in Table (2).

Oligonucleotide primers were developed utilizing Primer3plus software version 4.0, with validation performed through University of California Santa Cruz (UCSC) bioinformatics tools, based on reference sequences obtained from the National Center for Biotechnology Information (NCBI) GenBank database. Commercial synthesis and freeze-drying of all primers were conducted by Alpha DNA Ltd. (Canada). Table (2) presents the complete set of primer sequences employed in the experimental assays of this investigation.

Table (2): Oligonucleotide primer sequences developed for this investigation.

Primer	Sequence (5′→3′ direction)	primer size bp	Product size bp	Ta °C
HlyA				
Forward	GTTGAAGAGCTCATTGGCGGA	21	300	62.4
Reverse	TAACTGGTCGTCTCCCTGTCC	21		
FimH				

Forward	CTGGTCGGTAAATGCCTGGTC	21	300	63.6
Reverse	GACGGGCGCAAGGTTTACATA	21		
LuxS				
Forward.	AGATCCGGAACTGAACGTCT	21	300	63.4
Reverse	TCCTGCAACTTCTCTTTCGGC	21		

RESULTS AND DISCUSSIONS

Gene Expression Analysis by Quantitative PCR

The expression levels of three target genes (hyl, fim, and lux) were analyzed using quantitative PCR following exposure to various antimicrobial treatments. Gene expression was normalized using the 2^-ΔΔCt method, with fold changes calculated relative to the untreated control group.

The obvious stimulation of upregulation of the hyl, fim, and lux genes due to different antimicrobial stresses observed in this paper shows that antimicrobial therapies can induce bacterial virulence capability, in a convoluted way, that should be alarming to any practitioner or investigator studying the influence of antimicrobials on disease. We have seen increases in virulence gene expression with sub-lethal levels of antimicrobials on bacteria and many studies have shown that sub-inhibitory levels of antimicrobials cause bacteria to invoke complex stress responsive pathways that can negate the effect of antimicrobials (Andersson & Hughes, 2014; Yin et al., 2023; Liu et al., 2024). This paradoxical phenomenon represents an existential threat to antibiotics since both antimicrobial therapies designed to eliminate bacteria could additionally enhance their abilities to become pathogens by activating compensatory or alternative virulence pathways.

The range of differential gene response observed in this study, vary from a moderate 1.4-fold increase with microbicide antimicrobial treatments to a higher 14.44 - upregulation with an antibiotic treatment, ciprofloxacin, indicates bacteria have sensitive and complex regulatory-domain networks that can detect antimicrobial-stress in an organized response to surface environmental antimicrobial stressors. These responses, in turn, also appeared to be coordinated and gradated in that different surface formatting led to different pathways and levels of virulence upregulation. This coordinated activation response, likely because many of the virulence pathways are interconnected, demonstrates that bacteria also have the capacity for integrated-stress-response systems that respond to antimicrobial challenge by activating several pathogenic capabilities (Zhang et al., 2025; Sharma et al., 2023).

Meaningful differential responses of activation from the three gene systems provides information on the different molecular processes which are contributing to antimicrobial-enhanced virulence. It is likely that the activation of virulence through the different gene systems also indicate that the three systems are responding to different types of antimicrobial sensing and that there are different pathways of sensory and regulatory control connecting antimicrobials to the virulence gene activation.

Bacteria are likely to have several layers of activation for virulence genes once they have sensed antimicrobials have been allotted. In support of this hypothesis, the hyl gene was consistently increased across all treatments although gentamicin had the greatest effect with the most significant 6.45-fold increase in gene expression. In this case, it seems like they were most likely effective mechanisms of bacteria survival which influence both processes that were penetrating circularly into tissue with the cost of inhibiting innate immune processes.

Hyaluronidase clearly had its instrumental functions as a "spreading factor", as it is a substrate for this bacterial product that works to hydrolyze hyaluronic acid found in the extracellular matrix which was essential for the bacteria to disseminate in the host and develop highways for further penetration into tissue (Saeed et al., 2020; Handa et al., 2020). Such spreading capability was initially important for the bacteria in terms of survival during the antimicrobials treatment, in that hyaluronidase gives the bacterial cell a rapid capability to escape an unfavourable microenvironment and gain entry into a protected pocket of tissue that could be used for as a niche infection, where they could remain persistently colonized at non-lethally high numbers.

For gentamicin, this large effect was likely due to the specific cellular stress involved because of process of disrupting ribosomal activity due to the aminoglycoside antibiotic and the ROS that were also produced (Kohanski et al., 2010; Ellermann & Sperandio, 2020). The aminoglycosides produced a broad stress perspective and the disruption of protein production, or membrane depolarization and oxidative stress came together to create a stressful cocktail. All this molecular stress may have demanded a progressive

effect on hyaluronidase expression as potentially the bacteria would be unfavourably motivated to want to make a quick escape out of the antimicrobial enriched environment, utilizing every resource they could to enhance how they would penetrate tissue.

Hyl Gene Expression:

The hyl gene demonstrated upregulation across all treatment groups compared to control (1.02 ± 0.214 fold). Expression levels progressively increased with different treatments: Dettol showed modest upregulation (1.40 ± 0.461 fold), followed by surgical treatment (2.57 ± 0.895 fold), ciprofloxacin (3.43 ± 0.725 fold), ceftriaxone (4.27 ± 0.801 fold), and gentamicin showing the highest expression (6.45 ± 1.725 fold) (Table 4.7, Figure 4.4).

Table 3: Hyl Gene Expression

Genes	Group Treatment	Mean ΔCt	Mean ΔΔCt	Mean±SD 2^-ΔΔCt (Fold)
hyl	Control	14.088	-0.002	1.02 ± 0.214
	Detol	13.678	-0.412	1.40 ± 0.461
	Surg	12.822	-1.268	2.57±0.8946
	Cip	12.336	-1.754	3.43±0.725
	Ctx	12.018	-2.072	4.27±0.801
	Genta	11.436	-2.654	6.45±1.725

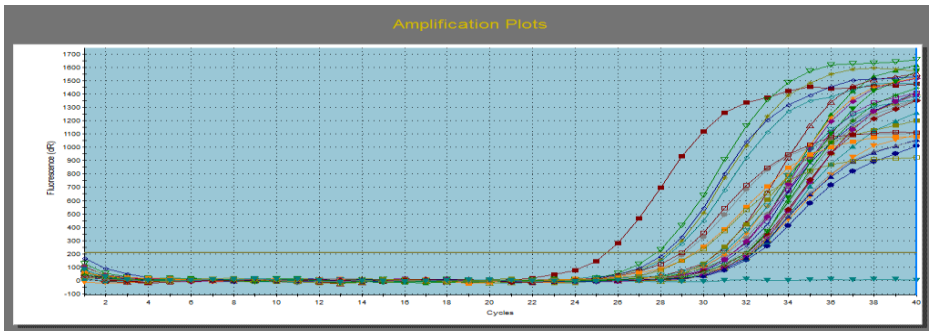

Figure 1. : Multing Curve of hlyA gene

The upregulation pattern demonstrated of hyaluronidase (Gentamicin > Ceftriaxone > Ciprofloxacin > Surgical > Dettol) suggests that inhibition of protein synthesis and disruption of the cell wall may be stronger stimuli for hyaluronidase expression than inhibition of DNA gyrase or membrane disruption. This order may represent cellular prioritization related to the evolutionary history of bacteria who faced extreme pressures to invade tissues (Liu et al., 2024; Ramdani et al., 2022). The ranking indicates that antimicrobials which target essential cellular functions, such as protein synthesis or the cell wall would be recognized by bacteria as severer threats calling for an immediate escape mode to dampen the activation of these functions.

The molecular pathways through which hyl gene upregulation occurs probably involve multiple pathways of stress sensing which operate on transcriptional regulation of hyaluronidase. Ribosomal stress from aminoglycosides could induce the stringent response linked to accumulation of (p)ppGpp, which would create conditions more favourable to activating alternative sigma factors which govern the stress response of the gene/s including regulation of relative virulence factor production or release (Liu et al., 2024; Ramdani et al., 2022). The Reactive Oxygen Species from aminoglycosides could also activate oxidative stress response pathways which directly cross link to regulation of virulence gene/s (Wicaksono et al., 2022). The cell wall stress provoked by the β-lactams commonly like cefrtriaxone could activate envelope stress response systems including Cpx and Rcs two-component regulatory systems. Activation of large envelope stress response system has influenced virulence factor gene expression studied within numerous species including human pathogenic variants.

Fim Gene Expression:

The fim gene exhibited significant upregulation in response to antimicrobial treatments. While Dettol and surgical treatments showed moderate increases (2.47 ± 0.732 and 2.23 ± 0.538 fold, respectively), the antibiotic treatments produced substantially higher expression levels. Ciprofloxacin and ceftriaxone treatments resulted in the most pronounced upregulation (8.98 ± 0.539 and 8.79 ± 1.801 fold, respectively), while gentamicin induced 7.44 ± 0.896 fold increase compared to control (Table 4.8, Figure 4.5).

Table 4: Fim Gene Expression

Genes	Group Treatment	Mean ΔCt	Mean ΔΔCt	Mean±SD 2^-ΔΔCt (Fold)
Fim	Control	14.034	0.004	1.020± 0.238
	Detol	12.772	-1.258	2.47±0.732
	Surg	12.918	-1.112	2.23±0.538
	Cip	10.866	-3.164	8.98±0.539
	Ctx	10.918	-3.112	8.79±1.801
	Genta	11.144	-2.886	7.44±0.896

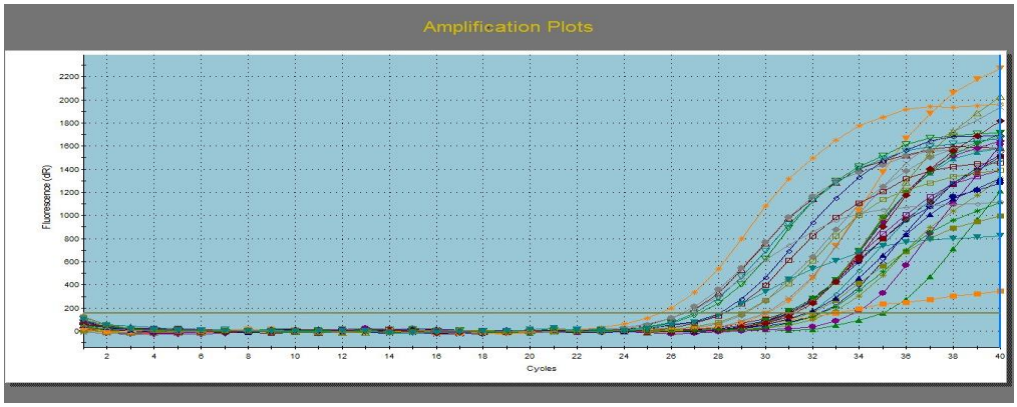

Figure 2: Multing Curve of Fim Gene

The significant upregulation of fim genes, particularly the 8-9 fold changes observed with ciprofloxacin and ceftriaxone shows a significant change towards increased adhesion and biofilm ability for creating an association with the surface. Fimbrial adhesins are critical right from the beginning of attaching to either eukaryotic cells or abiotic surfaces. Fimbrial adhesion is an important first step to start creating biofilms (Farzand et al, 2021; Guillaume et al, 2022). It is not surprising that it was mainly successful with fluoroquinolones and β-lactams, since an SOS response is triggered by DNA damage and, maybe a different SOS response triggered by cell wall stress. The dramatic increase of fimbrial gene expression associated with antimicrobial stress represents an adaptation model for the bacteria's survival priority to persist and not multiply. By increasing adhesion potential, bacteria can make more stable associations with surfaces or host tissue to create protected microenvironments where penetration of these antimicrobials is limited and proliferation is no longer a concern due to the underlying antimicrobial pressure if they happen to be susceptible to it.

It was reasonable to expect a significant fimbrial response to ciprofloxacin (8.98 -fold) because of the DNA damage caused by activation of the SOS response. The SOS response is a known global regulatory network that is activated by DNA, formed through a series of DNA strand breaks caused when fluoroquinolones stabilized the previously known DNA gyrase-DNA complexes. The increased amount of single-stranded DNA activated the self-cleavage of the RecA protein, leading to the self-cleavage of the LexA repressor and transient de-repression of the downstream ~60-expressed SOS genes. There is now some new evidence in the literature which suggests the SOS response may enhance fimbrial gene expression associated with complex regulatory cross-talk mechanisms that connect DNA damage sensing to enhancing surface adhesion (Hernando-Amado et al, 2019; Zhang et al, 2025).

Based on the combination of traditional approaches and molecular biological methods, the likely mechanism involved stress-induced transcriptional regulatory element, which includes stress-responsive transcription factors such as RpoS (sigma-38) and the stringent response, to coordinate the expression of genes associated with survival and persistence (Delago et al, 2021). RpoS is known as the general stress

response sigma factor because it is typically activated by environmental stress, along with other genes involved as stress-resistance genes, biofilm formation and adhesion at surfaces. The fimbrial increase (8.79-fold) associated with ceftriaxone treatment suggests that stress associated with the cell wall can also be able to activate adhesion enhancement mechanisms. The β-lactam class structure is a good starting point for interacting with peptidoglycan synthesis and thus the disruption they cause leads to weakening of a cell wall, including possible cell lysis. Therefore, the envelope stress of the bacteria caused either by variations in the size of the structure or by total disintegration, may activate specific two-component regulatory systems, i.e. Cpx and Rcs to detect the perturbation in the cell envelope to format adaptive response.

The enhanced fim expression when exposed to antibiotics may explain the clinical observation of biofilm formation increasing, considered in the last decade as an effect of the antimicrobial exposure during infection, and even leading to high burden of chronic and recurrent infections (Ciofu et al, 2022; Nahum et al, 2025). Biofilm lifestyle affords the bacterium many forms of survival advantages, including but not limited to penetration rate of antibiotics, growth rate alterations that result in less antibiotic effectiveness, subsequent promotion of persistence in a dormant state. The connection of antimicrobial treatment to increased biofilm-forming potential creates an entirely inconvenient clinical paradigm because therapeutic solutions promoted the establishment of bacterial communities that are now highly resistant to treatment.

Lux Gene Expression:

The lux gene showed the most dramatic response to antimicrobial treatments. Ciprofloxacin treatment produced the highest fold change (14.44 ± 3.912), followed by ceftriaxone (9.10 ± 0.869) and gentamicin (8.74 ± 1.511). Dettol and surgical treatments showed more moderate increases (3.40 ± 0.965 and 2.22 ± 0.607 fold, respectively) (Table 4.9; Figure 4.6).

Table 5: Lux Gene Expression

Genes	Group Teretment	Mean ΔCt	Mean ΔΔCt	Mean±SD 2^-ΔΔCt (Fold)
Lux	Control	13.406	-0.004	1.04±0.347
	Detol	11.688	-1.722	3.40±0.965
	Surg	12.304	-1.106	2.22±0.607
	Cip	9.594	-3.816	14.44±3.912
	Ctx	10.230	-3.180	9.10±0.869
	Genta	10.300	-3.110	8.74±1.511

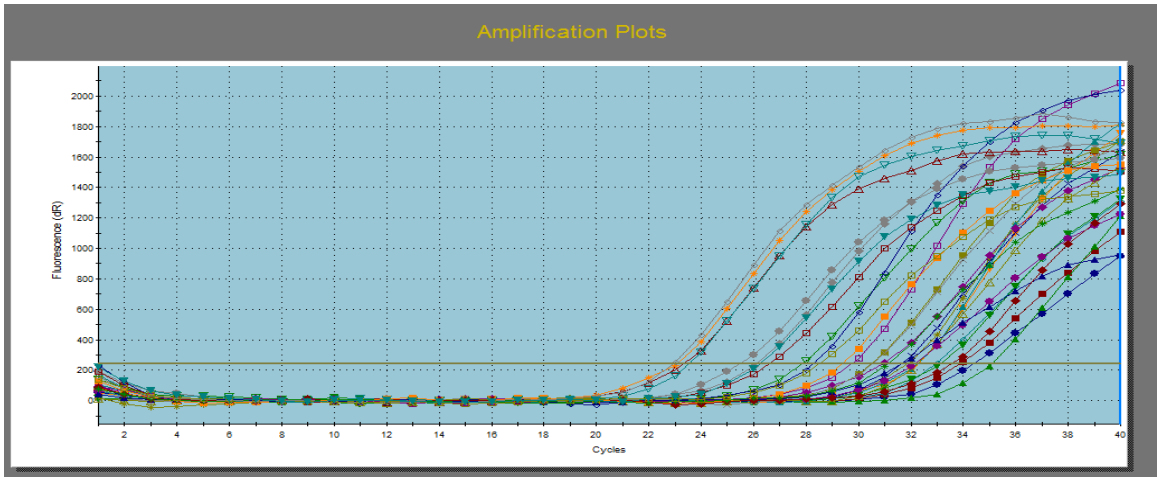

Figure 3: Multing Curve of Lux Gene

The significant upregulation of lux genes in this study, with ciprofloxacin inducing a whopping 14.44-fold upregulation, illustrates an unprecedented upregulation of quorum sensing networks that is particularly pertinent to this study, as quorum sensing is the biological mechanism by which bacteria "communicate" and coordinate group behaviors at the population level, including but not limited to producing virulence factors, biofilm maturation, and antimicrobial resistance mechanisms (Wang et al., 2023; Zhou et al., 2025; Zhao et al., 2020). The scale of this response, the largest of all three gene systems,

suggests that in the face of antimicrobial-induced stress, responding to a community collaboratively may be the preferred strategy to promote survival of individuals.

This 14.44-fold upregulation of lux genes induced by ciprofloxacin was the most remarkable finding in this study, and it deserves further investigation into the underlying mechanisms. This extreme responsivity suggests that fluoroquinolone-inducing stress may also exert significant capacity to induce bacterial communication networks due to the specific nature of the DNA damage attributed to these antibiotics. Furthermore, the magnitude of the response to ciprofloxacin could be confounded by dual stress caused by DNA damage and subsequent SOS response; there are significant amounts of research that show that SOS response has upregulated quorum sensing in many different bacteria (Hernando-Amado et al., 2019; Scoffone et al., 2019). Fluoroquinolones target two enzymes directly, gyrase and topoisomerase IV, and induce a novel DNA stress signature that results in protein-linked DNA breaks through binding to the DNA, which are an effective trigger for SOS response. Investigating the relationship between DNA damage and quorum sensing activation is complex, with regulatory cross talk mechanisms that were only recently sustainable to explain. SOS regulatory response (RecA/LexA dependent) can modulate luminescence production through a variety of strategies that include several path routes, with direct transcriptional regulation, indirect regulatory cascades, or metabolic coupling. SOS transcription factors can bind with the operon promoter and increase transcription of quorum sensing genes, with SOS-derepressed genes that include regulatory elements leading to quorum sensing operon (Delago et al., 2021; Ahmed et al., 2019).

This creates a concerning positive feedback loop, where treatment with an antimicrobial increases bacterial communications, thus, potentially leading to coordinated resistance strategies being employed including virulence strategies across a bacterial population (Hernando-Amado et al., 2019; Yin et al., 2023). The establishment of this positive feedback loop represents a fundamental hurdle to antimicrobial therapy, since it has converted stress at the level of the individual cell, to a change in behavior at the level of the community, which increases bacterial viability and pathogenicity. With the increased quorum sensing capability, there are numerous population level adaptive responses associated including coordinated gene expression, resource allocation and metabolic coordination, and spatial organization and biofilm formation.

The differential quorum sensing responses to the various antimicrobials (Ciprofloxacin 14.44-fold > Ceftriaxone 9.10-fold > Gentamicin 8.74-fold > Dettol 3.40-fold > Surgical 2.22-fold) is also important, in that, they provide insight into the specificity of how communication enhancement mechanisms are orchestrated. The relative rankings, seem to suggest that antimicrobials causing DNA damage, induce bacterial communication particularly well, possibly because DNA damage is a particularly serious threat that may require coordinated population responses. The large effect seen for ceftriaxone (9.10-fold), indicates that, although not as pronounced as DNA damage, sub-lethal cell wall stress also forms a significant trigger for quorum sensing activation. The more modest responses benevolent to gentamicin (8.74-fold), where it ranked so high for hyaluronidase activation, may mean that protein synthesis inhibition activates mechanisms of individual survival over the mechanisms of collective coordination (Zhou et al., 2025; Wang et al., 2023).

The profound activation of quorum sensing networks from treatment by an antimicrobial has numerous notable clinical implications. Bacterial communication enhancement facilitates the rapid dissemination of resistance elements by horizontal gene transfer, which are often governed by quorum sensing (Madsen et al., 2012; Ramdani et al., 2022). Also of importance is the coordinated expression of virulence factors at the level of entire bacterial populations where community behavior translates to pathogenic potential from the infected host, potentially leading to more severe clinical outcomes. The data show that antimicrobial treatment approaches should consider more potential communication enhancement when selecting agents and dosing regimens. Specifically, rapid bactericidal treatments that minimize exposure to even sub-lethal concentrations of antimicrobials may help impede quorum sensing networks from being activated. Alternatively, changing the standard of care to combination therapies including quorum sensing inhibitors could further assist with reducing the aforementioned unintended consequences of activating bacterial communication (Ahmed et al., 2019; Scoffone et al., 2019).

CONCLUSION

Results of this study reveal that antimicrobial treatments paradoxically enhance bacterial virulence by upregulating key genes for spreading, adhesion, and communication. This proposes bacteria activate complex survival strategies under antimicrobial stress, leading to amplified pathogenic potential. Such unintentional consequences underscore the urgent requirement for therapeutic approaches that reflect these intricate bacterial responses, potentially by reducing sub-lethal exposures or incorporating quorum sensing inhibitors.

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