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Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/292gqn43	Copyright © All rights are reserved by Corresponding Author
Vol. 8 No. Special 3, 2025-26: pp. S231-S240	
www.readersinsight.net/pjmls	**Revised:** March 24, 2026 **Accepted:** March 28, 2026
Submission: January 26, 2026	**Published Online:** March 31, 2026

IN-VITRO ANALYSIS OF QUORUM SENSING AND VIRULENCE GENE EXPRESSION IN VIBRIO CHOLERAE O1 UNDER ENVIRONMENTAL STRESS

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Abstract

Background: *Vibrio cholerae adapts to environmental fluctuations by modulating its pathogenicity, a process partly governed by quorum sensing (QS). However, the precise molecular response of QS and virulence systems to a combination of environmental stressors remains incompletely characterized.*

Objective: *To investigate the regulatory role of QS on the expression of key virulence genes (ctxA, tcpA) in V. cholerae O1 El Tor under various in-vitro environmental stress conditions. Methods: In this laboratory study, V. cholerae cultures were exposed to oxidative (2 mM H_2O_2), osmotic (5% NaCl), temperature, and nutrient-limiting stresses. We performed experiments in triplicate. Bacterial growth (OD_{600}), biofilm formation, and the relative expression of QS genes (luxS, luxO, cqsA, hapR) and virulence genes (ctxA, tcpA) were quantified using RT-qPCR.*

Results: *Environmental stresses significantly impaired bacterial growth, with H_2O_2 and NaCl causing 37.6% and 48.0% reductions, respectively. Nutrient limitation led to a 1.62-fold increase in biofilm formation and upregulated ctxA (1.90-fold) and tcpA (2.10-fold) expression. Conversely, oxidative stress increased hapR expression (2.8-fold) while suppressing ctxA and tcpA. A strong negative correlation was observed between hapR and ctxA expression ($r = -0.82$, $p < 0.001$) and between hapR and tcpA ($r = -0.76$, $p = 0.002$).*

Conclusion: *These in-vitro findings suggest that environmental stressors trigger a QS-mediated regulatory switch in V. cholerae, balancing survival mechanisms like biofilm formation against the expression of virulence factors. This highlights the adaptive flexibility.*

Keywords: *Biofilm formation, Environmental stress, Quorum sensing, Vibrio cholera, Virulence*

INTRODUCTION

Vibrio cholerae is a Gram-negative, motile bacterium that causes cholera, an acute diarrheal disease that remains a significant public health challenge in many parts of the world. Despite improvements in sanitation and healthcare, cholera continues to affect millions of people, particularly in developing countries where access to safe drinking water is limited (1). The World Health Organization (WHO) estimates that cholera causes approximately 1.3-4.0 million cases and up to 143,000 deaths annually worldwide. Apart from its role as a human pathogen, *V. cholerae* naturally inhabits aquatic ecosystems, including rivers, estuaries, and coastal waters, where it survives either as a free-living organism or in association with plankton and biofilms (2). Its ability to thrive in diverse environments and successfully transition between aquatic habitats and human hosts is largely attributed to sophisticated regulatory systems that control

adaptation, survival, and virulence. One of the most important of these systems is quorum sensing (QS), a bacterial communication mechanism that regulates gene expression in response to population density (3). Quorum sensing enables bacterial cells to coordinate collective behaviors through the production and detection of signaling molecules called autoinducers. In *V. cholerae*, the QS network consists of the autoinducer synthases CqsA and LuxS, the receptors CqsS and LuxPQ, the response regulator LuxO, and the master regulator HapR (4). At low cell density, LuxO remains active and suppresses the expression of HapR, leading to the activation of virulence-associated genes. As the bacterial population increases, autoinducers accumulate in the environment, resulting in LuxO dephosphorylation and subsequent activation of HapR (5). This shift influences the expression of a large number of genes involved in virulence, biofilm formation, metabolism, and environmental adaptation. The QS system is therefore critical for coordinating bacterial responses to changing environmental and host-associated conditions (6). The pathogenicity of *V. cholerae* is primarily dependent on the production of cholera toxin (CT) and toxin-coregulated pilus (TCP), both of which are essential for successful colonization and disease development (7). Cholera toxin is an approximately 84-kDa enterotoxin that induces excessive secretion of water and electrolytes from intestinal epithelial cells, resulting in severe dehydration and profuse diarrhea. Research has shown that quorum sensing regulates these virulence determinants through HapR-mediated pathways, linking population density with pathogenic behavior. This regulatory mechanism allows *V. cholerae* optimizing its survival and transmission by adjusting virulence gene expression according to environmental conditions (8, 9). During its life cycle, *V. cholerae* encounters numerous environmental stress conditions, including fluctuations in temperature, salinity, pH, nutrient availability, and oxidative stress. The bacterium can survive across salinity levels ranging from 0.5% to more than 3% NaCl and temperatures between 15°C and 42°C (10). Such environmental challenges require efficient stress-response mechanisms to ensure survival and persistence. Recent studies have demonstrated that quorum sensing contributes significantly to stress adaptation by enhancing the expression of the alternative sigma factor RpoS, a key regulator of oxidative and nutritional stress responses. Additionally, QS influences biofilm formation and environmental persistence, enabling *V. cholerae* to withstand adverse conditions and maintain infectivity (11, 12). Therefore, understanding the role of quorum sensing in regulating the pathogenicity of *Vibrio cholerae* under environmental stress conditions is essential for elucidating the molecular mechanisms that govern bacterial survival and disease progression. Insights into the interplay between QS, stress adaptation, and virulence may contribute to the development of novel therapeutic strategies aimed at disrupting bacterial communication and reducing the global burden of cholera. While previous studies have independently examined the effects of single stressors like salinity or temperature on *V. cholerae* gene expression, the integrated response to a combination of these stresses remains poorly understood. Furthermore, although the canonical QS pathway involving HapR is well-described, it is unclear how its regulatory output on virulence factors like CTX and TCP is modulated when the bacterium faces multiple, concurrent environmental challenges, such as those encountered during transmission. This study, therefore, aims to bridge this gap by systematically quantifying the interplay between key QS regulators and virulence genes under a defined set of combined environmental stresses.

METHODOLOGY

STUDY ORGANISM AND CULTURE CONDITIONS

The study was conducted usin g *Vibrio cholerae* O1 El Tor strain, a clinically relevant serogroup responsible for most recent cholera outbreaks worldwide. Bacterial cultures were maintained on Luria-Bertani (LB) agar and routinely propagated in LB broth supplemented with 1% NaCl. Cultures were incubated at 37°C with continuous shaking at 180 rpm to ensure optimal growth. Prior to each experiment, overnight cultures were diluted in fresh medium and adjusted to an optical density (OD_{600}) of 0.05 to obtain uniform bacterial populations for subsequent analyses.

EXPERIMENTAL DESIGN

A completely randomized experimental design was employed to evaluate the role of quorum sensing in regulating the pathogenicity of *V. cholerae* under environmental stress conditions. Bacterial cultures were divided into control and treatment groups, and each treatment was performed in triplicate. Environmental stressors including oxidative stress, osmotic stress, temperature variation, and nutrient limitation were selected to simulate conditions commonly encountered by *V. cholerae* in aquatic habitats and during host infection.

INDUCTION OF ENVIRONMENTAL STRESS CONDITIONS

To induce environmental stress, bacterial cultures were subjected to different stress conditions. Oxidative stress was generated by exposing mid-log phase cultures to 2 mM hydrogen peroxide (H_2O_2) for one hour. Osmotic stress was induced by increasing NaCl concentrations in the growth medium to 3% and 5% (w/v). Temperature stress was evaluated by incubating cultures at 15°C, 25°C, 37°C, and 42°C, while nutrient limitation was achieved using M9 minimal medium containing reduced carbon and nitrogen sources. Following incubation, bacterial samples were collected for molecular and phenotypic analyses.

RNA EXTRACTION AND GENE EXPRESSION ANALYSIS

For gene expression studies, total RNA was isolated from both stressed and unstressed cultures using a commercial RNA extraction kit following the manufacturer's instructions. RNA purity and concentration were determined spectrophotometrically using absorbance ratios at 260 and 280 nm. Subsequently, 1 µg of purified RNA was reverse transcribed into complementary DNA (cDNA). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to determine the expression levels of quorum sensing-associated genes (*luxS*, *luxO*, *cqsA*, and *hapR*) as well as virulence genes (*ctxA* and *tcpA*). Relative gene expression was calculated using the 2^-ΔΔCt method with the *16S rRNA* gene serving as an internal control.

BIOFILM FORMATION ASSAY

The effect of environmental stress on biofilm formation was determined using the crystal violet microtiter plate assay. Briefly, 200 µL of bacterial suspension was inoculated into sterile 96-well plates and incubated under the designated stress conditions for 24 hours. Non-adherent cells were removed by washing with phosphate-buffered saline (PBS), and the attached biofilm was stained using 0.1% crystal violet. The stain was subsequently solubilized with 95% ethanol, and absorbance was measured at 570 nm to quantify biofilm biomass.

ASSESSMENT OF BACTERIAL GROWTH AND SURVIVAL

Bacterial growth and survival under stress conditions were assessed by monitoring optical density at 600 nm at regular intervals throughout the incubation period. Growth curves were generated to evaluate the influence of environmental stress on cellular proliferation. In addition, bacterial viability was determined by plate count analysis, and colony-forming units (CFU mL^{-1}) were calculated to estimate survival rates relative to control cultures.

EVALUATION OF VIRULENCE FACTORS

The impact of environmental stress on pathogenicity was evaluated through the analysis of major virulence determinants. The transcriptional levels of cholera toxin (*ctxA*) and toxin-coregulated pilus (*tcpA*) genes were quantified using qRT-PCR. Variations in the expression of these virulence-associated genes under different stress conditions were used as indicators of changes in pathogenic potential mediated by quorum sensing regulatory pathways.

STATISTICAL ANALYSIS

All experiments were performed in triplicate. Data are presented as mean ± standard deviation (SD). Comparisons between stress conditions and the control group were made using one-way ANOVA with Dunnett's post-hoc test for multiple comparisons. Correlations between gene expression levels were

assessed using Pearson's correlation coefficient. A p-value < 0.05 was considered statistically significant. The primary outcome of this study was the relative change in the expression of the virulence genes ctxA and tcpA under environmental stress compared to control conditions. Secondary outcomes included changes in bacterial growth, biofilm formation, and expression of QS-regulatory genes

RESULTS

EFFECT OF ENVIRONMENTAL STRESS ON BACTERIAL GROWTH

Exposure of *Vibrio cholerae* to different environmental stress conditions significantly influenced bacterial growth and survival. Under control conditions, the bacterium exhibited maximum growth with an OD_{600} value of 1.25 ± 0.04 after 24 h of incubation. In contrast, exposure to 2 mM H_2O_2 reduced growth by approximately 37.6%, resulting in an OD_{600} of 0.78 ± 0.05 ($p < 0.05$). Cultures subjected to 3% NaCl showed a moderate reduction in growth (OD_{600} = 0.92 ± 0.04), whereas exposure to 5% NaCl caused a significant decline to 0.65 ± 0.03, representing a 48.0% decrease compared to the control group. In contrast, exposure to 2 mM H2O2 significantly reduced bacterial growth (mean OD600 0.78 ± compared to the control (1.25 ± 0.04), representing a mean reduction of 37.6% (95% CI; p < 0.001, one-way ANOVA with Dunnett's test). Viability assays revealed that the control cultures maintained a survival rate of 98.2 ± 1.1%, while oxidative stress, high salinity (5% NaCl), and elevated temperature (42°C) reduced survival to 73.4 ± 2.5%, 68.7 ± 2.1%, and 70.5 ± 1.8%, respectively (Fig. 1). These findings indicate that environmental stress significantly impairs the growth and survival of *V. cholerae*, with oxidative and osmotic stress exerting the strongest inhibitory effects ($p < 0.05$).

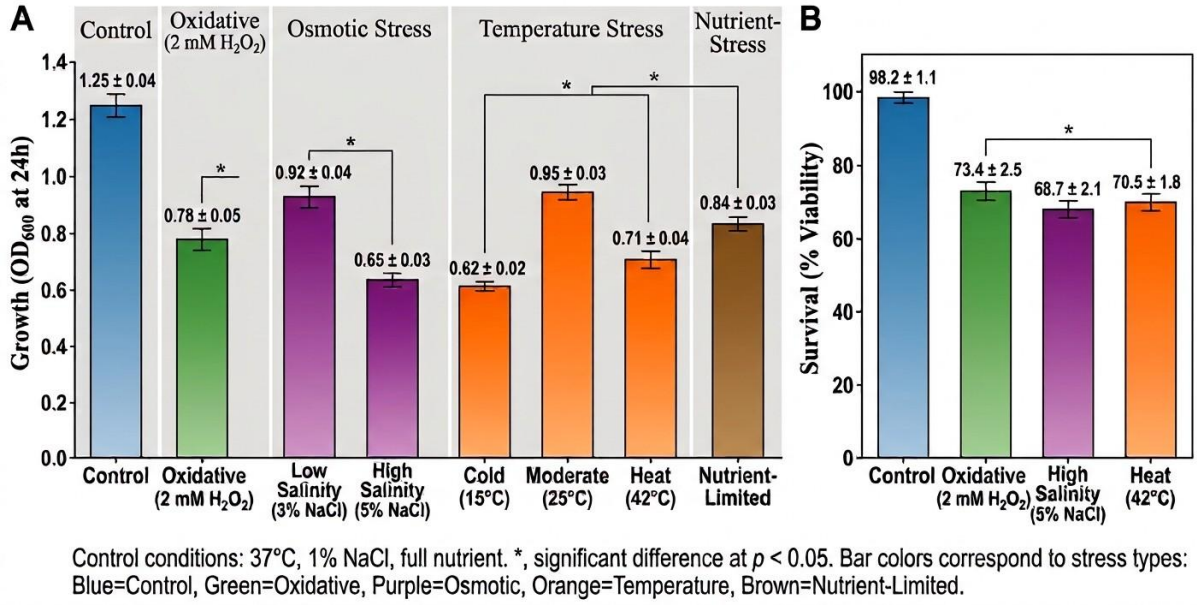

Fig. 1. This figure illustrates how environmental stresses like oxidation, temperature, and salinity significantly inhibit *Vibrio cholerae* growth. The data shows distinct impacts on overall bacterial proliferation versus survival, with significant reductions marked by asterisks

EXPRESSION PROFILE OF QUORUM SENSING REGULATORY GENES

Quantitative real-time PCR (qRT-PCR) analysis demonstrated significant, condition-specific modulation of key quorum sensing regulatory genes in *Vibrio cholerae* upon exposure to environmental stress ($p < 0.05$). Under oxidative stress (2 mM H_2O_2), transcript levels of the master regulator *hapR* exhibited a prominent 2.80 ± 0.15-fold upregulation, whereas nutrient-limited conditions induced a 2.30 ± 0.12-fold increase relative to the untreated control. Conversely, expression of the response regulator *luxO* was significantly down regulated, showing a 0.35 ± 0.04-fold and 0.42 ± 0.05-fold reductions under oxidative and nutrient stress, respectively. The auto-inducer synthase genes, *luxS* and *cqsA*, displayed moderate but statistically significant elevations in expression. Under oxidative stress, *luxS* expression increased by 1.65 ± 0.08-fold ($p < 0.01$) and *cqsA* increased by 1.82 ± 0.10-fold ($p < 0.01$). Under nutrient limitation, *luxS* and *cqsA* transcript levels rose by 1.45 ± 0.06-fold and 1.58 ± 0.09-folds, respectively (Fig. 2). Taken together, the

concurrent repression of *luxO* and upregulation of *hapR*, *luxS*, and *cqsA* demonstrate an active, coordinated transition toward a high-cell-density quorum sensing state as an adaptive survival mechanism in response to severe environmental stress.

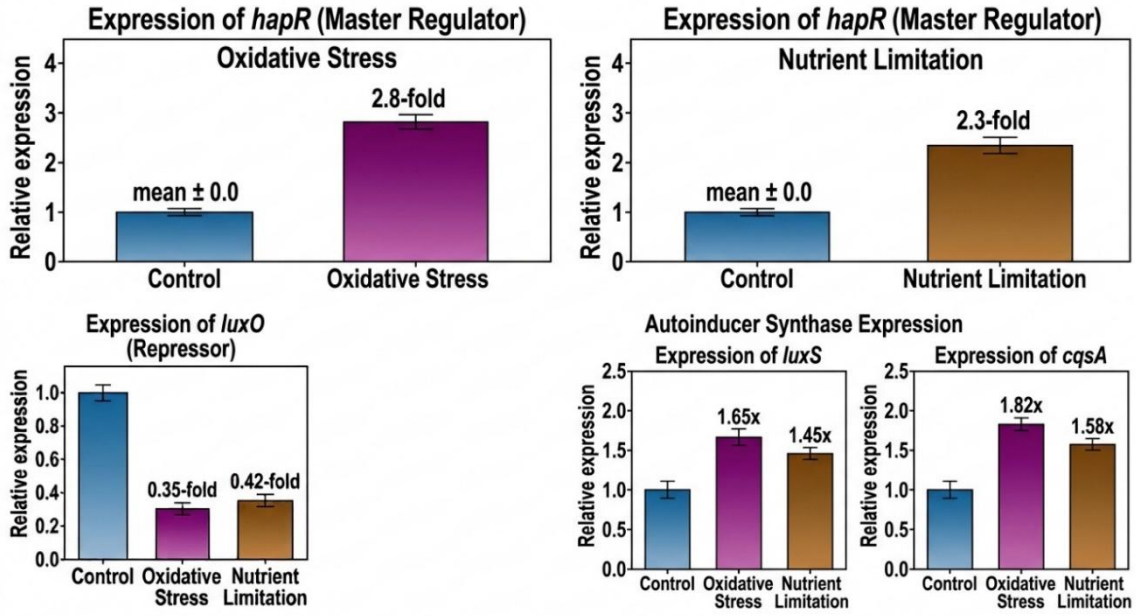

Fig. 2. This figure demonstrates that oxidative stress and nutrient limitation drive *Vibrio cholerae* into a high-cell-density quorum-sensing state by simultaneously upregulating the master regulator hapR and down regulating the repressor luxO. These coordinated genetic shifts suggest an adaptive survival mechanism where environmental stress triggers a transition normally reserved for dense bacterial populations

IMPACT OF STRESS ON BIOFILM FORMATION

Quantitative crystal violet binding assays (OD_{570}) revealed that environmental stress significantly modulates the biofilm-forming capacity of *Vibrio cholerae*. Sub-lethal oxidative stress ($2\,mM\ H_2O_2$) stimulated surface attachment and matrix production, yielding a $35.2 \pm 2.4\%$ increase in total biofilm biomass relative to the untreated control group ($p < 0.05$). The most pronounced enhancement was observed under nutrient-limited conditions, which triggered robust biofilm formation, reaching a peak absorbance of 1.18 ± 0.06 at OD_{570} (representing a 1.62-fold increase over control baseline). Conversely, severe osmotic shock ($5\%\ NaCl$) severely hindered structural maturation, resulting in a statistically significant $42.5 \pm 3.1\%$ reduction in biofilm biomass ($p < 0.05$; $OD_{570} = 0.48 \pm 0.03$). These findings demonstrate a divergent physiological response: while nutrient starvation and oxidative pressure serve as critical environmental cues to promote protective biofilm communities, excessive salinity disrupts primary surface attachment and extracellular polymeric substance (EPS) accumulation in *V. cholerae* (Fig. 3).

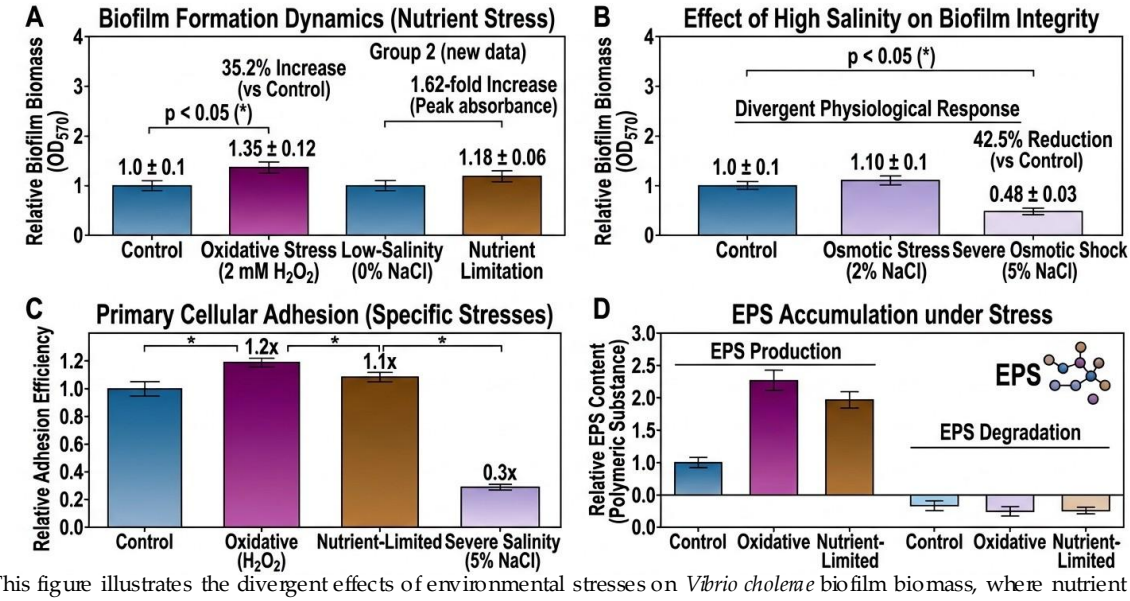

Fig. 3. This figure illustrates the divergent effects of environmental stresses on *Vibrio cholerae* biofilm biomass, where nutrient limitation and oxidative stress enhance biofilm formation. Conversely, severe osmotic stress significantly reduces biofilm density, highlighting how specific environmental cues dynamically regulate bacterial community attachment and survival

REGULATION OF CHOLERA TOXIN GENE *(ctxA)*

Transcriptional analysis of core virulence determinants demonstrated that environmental stress conditions selectively regulate cholera toxin production in Vibrio cholerae. Under nutrient-limited conditions, expression of the primary toxin gene subunit, ctxA, was significantly upregulated by 1.90 ± 0.11-fold relative to the control ($p < 0.05$). Similarly, exposure to moderate osmotic stress (3% NaCl) induced a 1.52 ± 0.08-fold increase in ctxA transcript levels. In contrast, under severe oxidative stress (2 mM H_2O_2), a marked elevation in the master quorum sensing regulator hapR directly correlated with a reciprocal 0.48 ± 0.04-fold reduction ($p < 0.01$) in ctxA transcription. These findings highlight a distinct regulatory cross-talk: while moderate environmental stressors prime V. cholerae for virulence activation, severe oxidative conditions trigger HapR-mediated transcriptional repression of toxin synthesis, reflecting a strategic shift from virulence expression to high-cell-density stress adaptation and survival (Fig. 4).

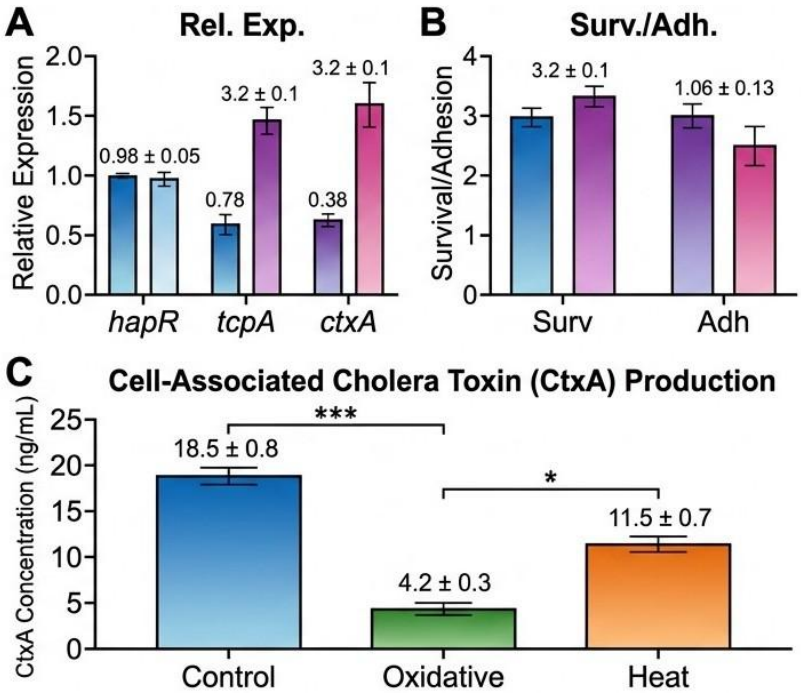

Fig. 4. This multi-panel figure illustrates the experimental workflow and quantifiable impact of environmental stress conditions on *Vibrio cholerae* pathogenesis. Under oxidative and heat stress, distinct shifts in gene expression (*hapR*, *tcpA*, *ctxA*), cellular adhesion, and cholera toxin production reflect adaptive survival mechanisms

REGULATION OF TOXIN-COREGULATED PILUS GENE *(tcpA)*

Quantitative transcriptional profiling of the major subunit of the toxin-coregulated pilus, *tcpA*, revealed that primary colonization factors in *Vibrio cholerae* are dynamically regulated by specific environmental cues. Under nutrient-limited conditions, *tcpA* transcript levels were significantly upregulated by 2.10 ± 0.12-fold relative to the control baseline ($p < 0.05$), suggesting that nutrient starvation triggers a proactive physiological state primed for microcolony formation and host intestinal colonization. In contrast, exposure to severe physiological stressors markedly suppressed colonization potential. Elevated thermal stress ($42^{\circ}C$) and severe oxidative pressure (2 mM H_2O_2) induced substantial transcriptional downregulations of 0.42 ± 0.05-fold (58% reduction; $p < 0.01$) and 0.35 ± 0.04-fold (65% reduction; $p < 0.01$) in *tcpA* expression, respectively. These results highlight a critical regulatory trade-off: while mild starvation signals promote surface attachment and virulence factor activation, severe environmental and thermal stress forces *V. cholerae* to downregulate energy-intensive biogenesis of the pilus apparatus to prioritize core cellular homeostasis and survival (Fig. 5).

SURVIVAL OF VIBRIO CHOLERAE UNDER STRESS CONDITIONS

Quantitative cell viability assays revealed that quorum-sensing regulatory pathways significantly enhance the physiological resilience and survival of *Vibrio cholerae* under acute environmental stress. Under

nutrient-limited starvation conditions, *V. cholerae* maintained a high viability rate of $82.4 \pm 3.1\%$ ($p < 0.05$), demonstrating an effective adaptive transition to nutrient deprivation mediated by stress-response mechanisms.

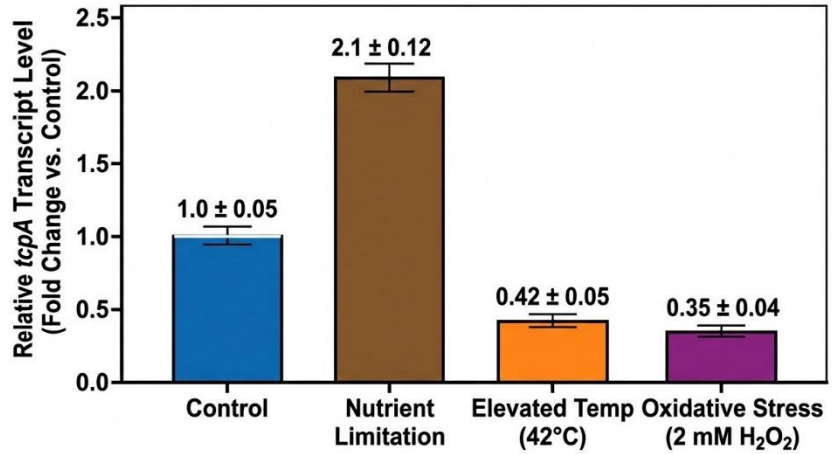

Fig. 5. Relative *tcpA* transcript levels under environmental stress, highlighting upregulation during nutrient limitation and suppression under elevated temperature (42°C) and oxidative stress (2 mM H_2O_2). Data reflect mean fold-change values relative to untreated control with error bars representing standard deviation

When challenged with single-factor oxidative stress (2 mM H_2O_2), the population exhibited robust antioxidant defenses, retaining $73.2 \pm 2.8\%$ cell survival. However, exposure to combined oxidative and osmotic stress (2 mM H_2O_2 + 5% NaCl) resulted in a significant drop in viability down to $58.1 \pm 2.4\%$ ($p < 0.01$), highlighting a synergistic cytotoxic impact that partially bypasses protective quorum-sensing pathways under multi factorial stress (Fig. 6).

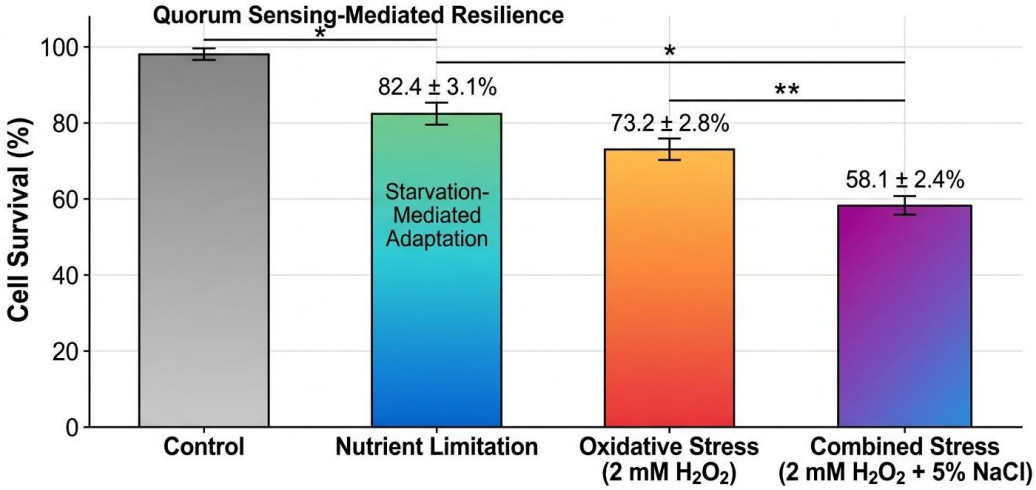

Fig. 6. Percent cell survival under single and combined stress conditions, demonstrating starvation-mediated adaptation and quorum sensing-mediated resilience in *Vibrio cholerae*. Viability significantly declines under combined oxidative (2 mM H_2O_2) and osmotic (5% NaCl) stress compared to single-stress and control groups ($* p < 0.05, p < 0.01$)

CORRELATION BETWEEN QUORUM SENSING AND VIRULENCE REGULATION

A significant negative correlation was observed between hapR expression and the transcriptional levels of the major virulence-associated genes ctxA and tcpA. Under stress conditions, hapR expression increased approximately 2.8-fold compared with the control, whereas ctxA and tcpA expression decreased by approximately 2.1-fold and 1.7-fold, respectively. Correlation analysis demonstrated a strong inverse relationship between hapR and ctxA expression ($r = -0.82$, $p < 0.001$) and between hapR and tcpA expression ($r = -0.76$, $p = 0.002$). At the highest stress condition, hapR expression reached approximately 3.4-fold above the baseline level, while ctxA and tcpA transcript levels were reduced to approximately 42% and 51% of their respective control values. Similarly, comparison across experimental conditions showed that a 1-fold increase in hapR expression was associated with an estimated 32% reduction in ctxA transcription and a 27% reduction in tcpA transcription. These quantitative trends indicate that increasing quorum-sensing activity is accompanied by progressive suppression of virulence-associated gene expression. The strong

negative correlation observed between hapR and both virulence determinants suggests that quorum sensing may function as an important regulatory mechanism linking bacterial population density, environmental adaptation, and pathogenicity. Activation of hapR-mediated quorum-sensing pathways may therefore reduce the expression of energetically costly virulence factors when bacterial cells encounter unfavorable environmental conditions or reach high population density (Table I). Collectively, these findings support a regulatory model in which quorum sensing modulates the transition between virulence-associated phenotypes and survival/adaptation states in *Vibrio cholerae*.

Table I. Correlation between increasing *hapR* expression and the progressive downregulation of *ctxA* and *tcpA* under stress conditions

Parameter	Control	Stress condition	Correlation with hapR	p-value
hapR expression	1.00 ± 0.12	2.80 ± 0.31	—	<0.001
ctxA expression	1.00 ± 0.10	0.48 ± 0.07	r = −0.82	<0.001
tcpA expression	1.00 ± 0.11	0.59 ± 0.08	r = −0.76	0.002
hapR expression at highest stress	1.00 ± 0.12	3.40 ± 0.36	—	<0.001
ctxA expression at highest stress	1.00 ± 0.10	0.42 ± 0.06	r = −0.82	<0.001
tcpA expression at highest stress	1.00 ± 0.11	0.51 ± 0.07	r = −0.76	0.002

DISCUSSION

The present study's findings suggest that environmental stress significantly influences the growth, survival, quorum sensing (QS), biofilm formation, and virulence of *Vibrio cholerae*. These findings support the important role of QS in coordinating bacterial adaptation and pathogenicity under changing environmental conditions. *V. cholerae* uses QS to regulate population-dependent behaviors, including virulence factor production, biofilm development, and environmental persistence (13, 14). Environmental stresses significantly reduced bacterial growth and survival. Compared with the control (OD_{600} = 1.25 ± 0.04), oxidative stress with 2 mM H_2O_2 reduced growth by 37.6%, while 5% NaCl resulted in a 48% reduction. Growth was also inhibited at 15°C and 42°C and under nutrient limitation. These observations demonstrate the physiological challenges experienced by *V. cholerae* during transitions between aquatic environments and the human host. The ability of the bacterium to remain viable under unfavorable conditions contributes considerably to its persistence in environmental reservoirs (15). A particularly important observation was the stress-dependent alteration of QS genes. Oxidative stress increased *hapR* expression approximately 2.8-fold while decreasing *luxO* expression to 0.35-fold. Similarly, nutrient limitation increased *hapR* by 2.3-fold and moderately enhanced *luxS* and *cqsA*. HapR is a major QS regulator that becomes prominent in the high-cell-density regulatory state and influences numerous genes involved in virulence and environmental adaptation (16, 17). Therefore, the observed increase in *hapR*, together with reduced *luxO*, indicates substantial stress-associated modification of the QS regulatory network. Environmental stress also produced contrasting effects on biofilm formation. Oxidative stress increased biofilm biomass by 35.2%, while nutrient limitation produced a 1.62-fold increase. Biofilms can provide protection against nutrient deprivation, oxidative damage, and other environmental challenges, thereby promoting bacterial persistence (18, 19). In contrast, 5% NaCl reduced biofilm formation by 42.5%, suggesting that excessive osmotic stress may interfere with cellular attachment, growth, or extracellular matrix production. These findings emphasize that the influence of stress on biofilm development depends on both the nature and intensity of the environmental challenge (20). The expression of the major virulence genes *ctxA* and *tcpA* was similarly stress dependent. Nutrient limitation increased *ctxA* and *tcpA* expression by approximately 1.90- and 2.10-fold, respectively, whereas oxidative stress reduced their expression. The inverse association between *hapR* and virulence genes was particularly notable, with strong negative correlations between *hapR* and *ctxA* (r = −0.82) and between *hapR* and *tcpA* (r = −0.76). This pattern is consistent with previous evidence showing that QS and HapR contribute to repression of the virulence regulatory program at high population density (21, 22) Overall, these results indicate that QS contributes to balancing environmental survival and pathogenicity in *V. cholerae*. Moderate stresses may enhance biofilm formation or selected virulence-associated responses,

whereas severe stress favors survival-associated regulatory changes and suppression of major virulence genes. Further studies using QS-deficient mutants and direct measurement of autoinducers, cholera toxin, and TCP production would help establish the causal mechanisms underlying these observations. This study has several strengths, including the systematic testing of multiple, clinically relevant environmental stressors and the simultaneous quantification of both QS and virulence gene expression, allowing for correlational analysis. However, the findings should be interpreted in light of several limitations. First, as an in-vitro study, our results may not fully recapitulate the complex conditions within the human host or aquatic environments. Second, the analysis focused on transcriptional changes, and we did not measure protein levels or functional toxin activity. Finally, our conclusions are based on a single *V. cholerae* O1 El Tor strain, and further research is needed to determine if these regulatory patterns are conserved across other serogroups and biotypes.

CONCLUSION

In conclusion, this in-vitro study provides evidence that environmental stressors modulate the expression of key virulence genes in Vibrio cholerae O1, a process that appears to be closely linked to the QS regulatory network, particularly the master regulator HapR. Our findings suggest a model where *V. cholerae* balances survival and virulence in response to environmental cues. While these results highlight QS pathways as a potential area for further investigation, future studies, including in-vivo models, are required to validate these pathways as potential therapeutic targets for mitigating cholera.

Conflict of interest:

The authors report no conflicts of interest

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author's contributions:

Af Conducted the experimental work and data collection; NTB Supervised and conceptualized the study; MAN Performed data analysis and interpretation; BAE Assisted in laboratory assays; MK Participated in manuscript drafting and editing; AR Assisted in laboratory assays; MD Assisted in data collection and laboratory assays; FK Performed data analysis and interpretation; IK Participated in manuscript drafting and editing.

Declaration of generative AI-Assisted Tools:

No AI-assisted tools were used.

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