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Effect of Acidic pH on the Expression of Beta-Lactam Resistance Genes in *Escherichia coli*

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Abstract

Antibiotic resistance in *Escherichia coli* is a significant clinical challenge, particularly under environmental stress conditions such as pH change. This study aimed to evaluate the effect of acidic and alkaline environments on antibiotic susceptibility and the expression of the beta -lactam resistance gene (blaTEM) in clinical *E. coli* isolates.

Two hundred clinical samples were collected from patients with blood, urinary tract, and wound infections at hospitals in Anbar Governorate (Ramadi Teaching Hospital, Maternity and Children's Hospital, and Fallujah Teaching Hospital). After microbiological diagnosis using conventional biochemical assays, 50 *E. coli* isolates were obtained.

Susceptibility testing was performed on ten antibiotics under standard conditions (PH=7), which served as a control group. The same isolates were then retested under acidic (pH=5) and alkaline (PH=8.5) conditions to assess acid tolerance and the change in susceptibility pattern (MIC). Nine isolates exhibiting the greatest phenotypic changes across the three media were selected for molecular analysis. RNA was extracted and converted to cDNA, and the gene expression level of the blaTEM gene was measured using real -time quantitative PCR.

The results showed significant changes in antibiotic susceptibility patterns with varying pH levels. A clear effect of acidic stress was observed in increasing resistance levels to some antibiotic. Furthermore, gene expression analysis revealed a significant increase in blaTEM

gene expression in acidic media compared to neutral and alkaline media ($p<0.05$), suggesting that acidity may enhance beta-lactam resistance mechanisms in *E. coli*.

These findings underscore the importance of the infection site in modulating bacterial response to antibiotics and the need to consider this factor when selecting the appropriate clinical treatment.

Keywords: *Escherichia coli*, Acidic Stress, Gene Expression, Antibiotic resistance, Beta-Lactam

1. Introduction

The global emergence of antibiotic-resistant bacteria (ARBs) poses a significant danger to contemporary medicine, complicating the treatment of illnesses with standard medicines. The improper use of antimicrobials is the principal cause of this issue, whereas environmental stressors significantly influence the genetic pathways that allow bacteria to endure therapy. For instance, fluctuations in pH, especially acid stress, represent some of the most severe circumstances encountered by bacteria throughout various ecosystems, including the human stomach and urinary tracts (1) . *Escherichia coli*, a Gram-negative bacterium implicated in various human diseases, serves as an exemplary model organism for studying responses to acid stress (2) . It is a facultative anaerobe commonly located in the large intestine of warm-blooded mammals, with concentrations exceeding 10□ cells per gram of feces (2) . *Escherichia coli* is recognized for its rapid growth under optimal conditions, capable of doubling in approximately 20 minutes, rendering it a significant host bacterium in genetic manipulation systems. Over 4,800 E. coli genomes have been sequenced since the initial genome sequence was published in 1997. It remains the focus of extensive experimental microbial evolution studies encompassing over 50,000 generations (3) . *Escherichia coli* has evolved intricate methods to withstand acidic conditions, as they can inhabit environments with low ph. A recent study indicates that the physiological adaptations enabling survival in acidic settings may also confer protection against antibiotics. Acid stress not only influences the lifespan of E. coli but also significantly impacts gene transcription, particularly the expression of genes conferring antibiotic resistance. This occurs via several interconnected processes, wherein environmental stress accelerates the regulation of efflux pumps, horizontal gene transfer, and the rates of

conjugation and translocation of plasmid loops (4) . There is increasing interest in examining the impact of acid stress on antibiotic effectiveness and bacterial response. In certain therapeutic scenarios, such as urinary tract infections, alterations in environmental pH might affect antibiotic efficacy and bacterial adaptive mechanisms. Research suggests that several environmental factors, such as acidity, may influence the regulation of antibiotic resistance gene expression. *Escherichia coli* is a prevalent cause of bacterial infections and exhibits multiple mechanisms of antibiotic resistance, with the generation of β-lactamases being the most significant. The blaTEM gene is crucial for this resistance, as it encodes an enzyme that degrades β-lactams, diminishing their therapeutic effectiveness. This work seeks to assess the impact of acid stress on the expression levels of the blaTEM gene in *Escherichia coli* isolates, thereby enhancing the comprehension of how environmental circumstances regulate antibiotic resistance genes.

2.Methods

2.1 Sample Collection

Researchers collected 200 clinical specimens from Ramadi Teaching Hospital, Maternity and Children's Hospital, and Fallujah Teaching Hospital between September 17, 2025, and December 2, 2025. The majority originated from pee, at 68 percent, while blood constituted one-fifth of the total, and an additional 12 percent was obtained from wound swabs. Bacteria were identified in 140 of the initial cultures, paving the way for subsequent research. From that group, precisely fifty *E. coli* strains emerged, warranting detailed examination. These constituted the primary material analyzed herein.

2.2 Isolation and Identification

Fresh specimens were initially placed on specialized plates - MacConkey and EMB - to facilitate the differentiation of possible microorganisms from the mixture. Minuscule forms beneath glass, coupled with their growth patterns, provided initial insights into the potential identity of each germ. Subsequently, machine-assisted chemical reactions ensured that each suspect conformed to the profile of *Escherichia coli*. Each interconnected step bolstered confidence in the ultimate designation assigned to these insects(5).

2.3 Antibiotic Susceptibility Testing

The test assessed bacterial responses to ten antibiotics and quantified alterations in resistance, particularly concerning beta-lactams, following exposure to designated

environmental stressors. Response patterns were elucidated by comparing data prior to and following exposure to stressors during the experiment, employing the Kirby-Bauer disc diffusion method to ascertain the sensitivity of isolates to antibiotics. This method depends on quantifying the widths of growth-inhibition zones surrounding antibiotic discs and detecting alterations in resistance, including the bacteria's capacity to synthesize β-lactamases that breakdown the beta-lactam ring and enhance resistance(6),(7)**.**

2.4 Acidic Stress Induction

The onset was characterized by low acidity, during which bacterial proliferation occurred in broth amongst fluctuating pH levels. Under conditions approximating typical human internal states, one test-maintained stability precisely at seven. As the temperature approached five, the environment became markedly acidic and caustic. This experiment compelled microorganisms to inhabit more challenging environments instead of maintaining equilibrium. Simultaneously conducted yet distinct, each study adhered to modifications affecting development. As acidity escalated much beyond neutral levels, living circumstances deteriorated.

2.5 RNA Extraction

Following incubation at different pH values, bacterial samples were centrifuged. The total RNA was extracted using the Geneaid Genomic DNA Mini Kit (Korea), adhering strictly to the manufacturer's instructions.

2.6 cDNA synthesis

A pre-assembled kit from Pioneer in South Korea facilitated the conversion of RNA into cDNA. Ten microliters of isolated RNA were placed into a tiny tube. Incorporate four microliters of cDNA Master Mix next. Six microliters of water devoid of enzymes were added to achieve a total volume of twenty microliters. A machine heated the mixture to thirty-seven degrees Celsius; fifteen minutes elapsed. Subsequently, the temperature surged to eighty-five for a brief duration of five seconds. What remained was cDNA; one fraction was immediately sent to testing, while another was preserved at subzero temperatures for future use.

2.7 Measuring Gene expression with qRT PCR

Gene expression levels were evaluated from total RNA samples utilizing reverse transcription followed by quantitative reverse transcription polymerase chain reaction (qRT-PCR) with SYBR Green fluorescence detection. This study's β-lactamase gene primer pairs were created

using a reference gene sequence from the National Center for Biotechnology Information (NCBI) database, employing Primer3 software to guarantee amplification specificity and reaction efficiency. The primers were designed to amplify the β-lactamase gene, with the forward primer sequence (5'-GCTAAGCTCAGCCAGTGACA -3') and the reverse primer sequence (5'-CAAGTAAAGTGATGGCCGCG -3'). This produced an amplification fragment of 158 bp. The reaction was conducted at an annealing temperature of 59°C. The recA gene served as a reference gene (housekeeping gene) for normalizing gene expression levels, utilizing the following primers: Forward primer: 5'-CGCCTGATCGTCGTGTGTGA-3'; Reverse primer: 5'-TTCATCTCGGTCGCGGGTCAG-3'; Amplification product size: 210 base pairs. The relative alteration in gene expression was determined utilizing the Livak method (2^-ΔΔCT), with results presented as fold changes in relation to the control sample.

Ct Control – Ct housekeeping control = ΔCt control

ΔCt sample = Ct sample - Ct housekeeping sample

Δ Ct sample – Δ Ct control = ΔΔ Ct Fold of gene expression = $(2^{-}\Delta\Delta Ct)$ (8) .

2.8 Statistical Analysis

This research utilized IBM SPSS software to analyze the dataset. One-way ANOVA was employed to examine changes in gene expression among groups. Substantial alterations occurred exclusively when the P value was 0.05 or below. Results exceeding that threshold were statistically significant.

3 . Result

Table 1. shows the frequency and distribution of antibiotic use against *Escherichia coli* infections among the study samples.

Anti-microbial	Antibiotic susceptibility interpretation	Group of the study			X^2	p-value
		pH=5	pH=7	pH=8.5		
Aztreonam (AT)	R	25	45	29	20.927	0.0001
	S	14	1	12		
	I	11	4	9		
Amoxicillin (AX)	R	46	45	46	1.196	0.879
	S	3	4	4		
	I	1	1	0		
Ceftazidime (CAZ)	R	36	50	49	28.795	0.0001
	S	14	0	1		
	I	0	0	0		
Levofloxacin (LE)	R	15	9	9	16.882	0.002

	S	34	31	40		
	I	1	10	1		
Cefepime (CPM)	R	45	45	42	11.435	0.022
	S	0	4	7		
	I	5	1	1		
Ceftriaxone (CTR)	R	38	39	37	1.803	0.772
	S	7	9	8		
	I	5	2	5		
Ampicillin (AMP)	R	50	48	49	3.041	0.551
	S	0	1	0		
	I	0	1	1		
Meropenem (MR)	R	23	9	6	31.938	0.0001
	S	17	36	43		
	I	10	5	1		
Imipenem (IPM)	R	0	4	0	9.673	0.046
	S	48	42	45		
	I	2	4	5		
Amikacin (AK)	R	4	6	5	11.519	0.021
	S	32	32	43		
	I	14	12	2		

Experimental findings indicated that bacterial reactions to specific antibiotics may be influenced by alterations in the surrounding pH levels. A distinct variation in the efficacy of antibiotics, including Aztreonam, Ceftazidime, and Levofloxacin, was noted across different pH levels, indicating that environmental factors may influence bacterial sensitivity to these medications. Other antibiotics, including Meropenem, Imipenem, and Amikacin, had differing effects contingent upon the pH level being lower or higher. Conversely, the efficacy of several antibiotics remained largely constant; for instance, Amoxicillin, Ceftriaxone, and Ampicillin exhibited no significant variations in activity across varied pH levels. Statistical analysis indicated that the differences identified for certain antibiotics were statistically significant, whilst no comparable effects were noted for others. The results suggest that acidic or alkaline environmental factors may affect the efficacy of specific antibiotics, which is crucial for choosing suitable treatments in environments with variable pH, such as particular illnesses.

Table 2. Analysis of the gene expression level of the blaTEM gene in *Escherichia* coli isolates under acidic (pH=5) and basic (pH=8.5) stress conditions using qRT-PCR and the 2^-ΔΔCt method

Sample	CT blaTEM	CT recA	ΔCTE	ΔCTC	ΔΔCt	Expression Fold Change2^-ΔΔCt
control	32.51	34.2	0	-1.69	0	1
PH=5-1	32.77	35.51	-2.74	-2.37	-0.37	1.292353
PH=5-2	35.48	37.14	-1.66	-1.83	0.17	0.888843
PH=5-3	30.25	34.67	-4.42	6.82	-11.24	2418.673
PH=5-4	37.17	38.89	-1.72	-8.67	6.95	0.008088
PH=5-5	31.13	37.51	-6.38	-2.78	-3.6	12.12573
PH=5-6	34	36.66	-2.66	-7.46	4.8	0.035897
PH=5-7	33.89	33.69	0.2	-6.85	7.05	0.007546
PH=5-8	30.49	35.32	-4.83	3.09	-7.92	242.1908
PH=5-9	33.07	37.34	-4.27	4.79	-9.06	533.7425
PH=8.5-1	31.33	36.25	-4.92	-2.37	-2.55	5.856343
PH=8.5-2	32.65	33.42	-0.77	-1.83	1.06	0.479632
PH=8.5-3	38.2	34.11	4.09	6.82	-2.73	6.634556
PH=8.5-4	27.06	35.93	-8.87	-8.67	-0.2	1.148698
PH=8.5-5	24.27	33.72	-9.45	-2.78	-6.67	101.8287
PH=8.5-6	22.66	33.84	-11.18	-7.46	-3.72	13.1775
PH=8.5-7	20.34	26.42	-6.08	-6.85	0.77	0.586417
PH=8.5-8	23.22	32.7	-9.48	3.09	-12.57	6080.609
PH=8.5-9	33.76	37.2	-3.44	4.79	-8.23	300.2457

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) research demonstrated considerable variations in the expression levels of the blaTEM gene in *Escherichia coli* isolates subjected to acidic and alkaline stress conditions. Significant increases in expression were noted at pH 5, with several isolates demonstrating considerable fold changes above 200-fold in specific instances. This indicates that acidic stress may trigger the expression of this gene, which is linked to resistance against β-lactam antibiotics. Likewise, several isolates at pH 8.5 exhibited substantial increases in gene expression, with fold changes surpassing 500-fold in some cases. This suggests that alkaline stress may affect the control of blaTEM gene expression, however this effect

differed across the various isolates. The observed disparities in gene expression levels across the isolates indicate physiological and genetic variability, potentially linked to differences in genetic background or methods of gene regulation and environmental stress response.

Table 3. Comparison of gene expression (blaTEM) between study groups (pH=5, pH=7 and pH=8.5)

Gene	Categories	Groups			F	Sig.
		pH=7.0	pH=5.0	pH=8.5		
blaTEM	Mean±SD	1.0±0.0001	104.6±18.3	108.4±3.61	9.246	0.0001
	Min.	1	0.01	0.48		
	Max.	1	533.7	300.25		

The investigation of pH (5, 7, and 8.5) on the expression levels of the blaTEM gene revealed distinct variations among the treatments. The data were subjected to one-way ANOVA, which indicated statistically significant differences, evidenced by an F-value of 9.246 and a significance level of p = 0.0001, below the threshold of 0.05, demonstrating a significant impact of pH on the gene's expression level. The findings indicated that the minimal gene expression level occurred at pH = 7, with a mean of 1.0 ± 0.0001, signifying reduced gene activity under neutral conditions. Conversely, gene expression markedly elevated at pH = 5, attaining a mean of 104.6 ± 18.3, and likewise rose at pH = 8.5, reaching 108.4 ± 3.61. The data demonstrate that deviation from a neutral condition, either towards acidity or alkalinity, resulted in an elevated expression level of the blaTEM gene. The elevation in gene expression may indicate a bacterial adaptive response to environmental stress induced by pH fluctuations. This stress can trigger defensive mechanisms that enable bacterial cells to adapt and endure suboptimal environments. Consequently, these findings indicate that pH fluctuations may significantly influence the regulation of the blaTEM gene, associated with bacterial resistance mechanisms.

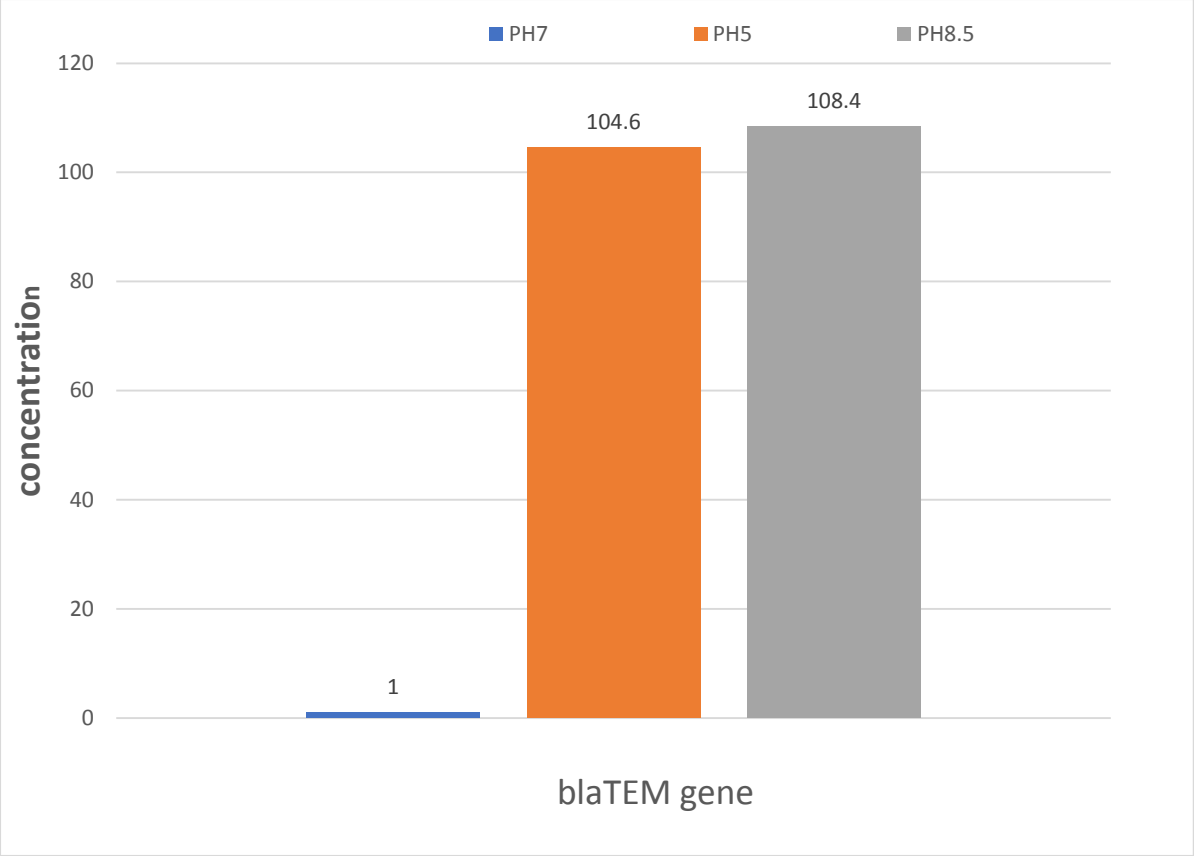

figure (1) Comparison of gene expression (blaTEM) between study groups (pH=5, pH=7 and pH=8.5)

The bar graph illustrates the fluctuations in blaTEM gene expression levels across varying pH values (pH 5, pH 7, and pH 8.5). Gene expression was minimal at pH 7 relative to other values, whereas it markedly increased at both pH 5 and pH 8.5, as evidenced by the elevated bar graphs at these two pH levels compared to the neutral state. The data indicate that deviation from a neutral environment, either towards acidity or alkalinity, resulting in an elevation of blaTEM environmental stress due to pH fluctuations. Disruption of pH equilibrium triggers certain regulatory pathways that activate genes linked to resistance mechanisms. The data reveal that non-neutral circumstances may boost blaTEM gene expression, indicating that environmental stress from pH fluctuations may significantly activate gene responses related to bacterial resistance.

Table 4. The relation of gene expression blaTEM with IMP and MR anti-microbial among study groups.

Anti-microbial	Gene	susceptibility interpretation	Groups Mean±SD	
			pH = 5.0	pH = 8.5
Imipenem	blaTEM	R	-	-
		S	104.6±18.3	86.60±11.84
		I	-	6.88±0.890
F			-	0.821
Sig.			-	0.395
Meropenem	blaTEM	R	4.47±0.665	38.53±5.51
		S	228.5±27.4	84.06±12.95
		I	80.74±13.98	-
F			1.229	0.322
Sig.			0.357	0.588

Table 4 illustrates the correlation between the expression levels of the blaTEM gene and the susceptibility of bacterial isolates to imipenem and meropenem at pH levels 5.0 and 8.5, indicating no statistically significant differences among the susceptibility groups (R, S, and I), as all p-values exceeded 0.05. While variations in mean gene expression levels were noted among resistant, sensitive, and intermediately sensitive isolates at both pH levels, these variations did not attain statistical significance. The results demonstrate no significant association between the expression level of the blaTEM gene and the susceptibility of isolates to imipenem and meropenem under the experimental conditions employed in this investigation.

4 Discussion

The data presented in the tables and graphs demonstrate a distinct influence of pH variation on the expression level of the blaTEM gene in *Escherichia coli*. The data indicate a substantial reduction in gene expression at pH 7 relative to other values, whereas gene expression significantly rises at pH 5 and pH 8.5. This pattern demonstrates the bacteria's adaptive response to environmental fluctuations, as deviations from neutral circumstances can trigger many regulatory pathways linked to bacterial resistance mechanisms. This observation corroborates findings from numerous recent research indicating that environmental stressors, such as pH fluctuations, might influence the control of gene expression of resistance-related genes, including β-lactamase genes like

blaTEM. This gene is significant as it encodes enzymes that may degrade β-lactam antibiotics, including penicillins and cephalosporins, hence allowing bacteria to endure in settings with these antibiotics (9)and again by (10) . Moreover, discrepancies in gene expression levels among bacterial isolates may result from genetic variances between strains. Certain *E. coli* isolates harbor numerous plasmids containing resistance genes, but others may depend on alternative regulatory systems to adapt to environmental challenges. Multiple studies have demonstrated that blaTEM genes are frequently located on plasmids, enabling their transfer across bacteria and resulting in considerable variations in gene expression levels among different isolates.. (11). Conversely, variations in pH can result in modifications to cell membrane architecture and the functionality of enzymatic systems within bacterial cells, thereby influencing antibiotic effectiveness. Research indicates that the efficacy of numerous antibiotics, especially those aimed at the cell wall, might be affected by the pH of the bacterial milieu, since acidic or alkaline stress may enhance the expression of specific resistance genes as part of bacterial adaptive mechanisms (12). Moreover, imbalanced environmental conditions can trigger several gene regulatory systems, including stress response mechanisms, which regulate the expression of numerous genes linked to survival and resistance. Research indicates that regulatory systems significantly enhance the production of β-lactamase genes under many environmental stressors, including alterations in pH (13),(14) . The present findings indicate that alterations in pH may significantly influence the regulation of blaTEM gene expression, hence augmenting the bacteria's capacity to adapt to varying environmental conditions. These results emphasize the significance of accounting for environmental factors in the research of antibiotic resistance, as the conditions at the infection site within the human body might affect treatment efficacy and the bacteria's response to medicines.

5 Fund

All expenses associated with sample collection, diagnostic testing, molecular analysis, and statistical analysis were entirely self-financed.

6 Conclusion

This study's results indicate that environmental stress from pH variations greatly influences the expression level of the blaTEM gene linked to β-lactam resistance in *Escherichia coli*. A considerable elevation in gene expression

was noted in acidic (pH=5.0) and alkaline (pH=8.5) conditions relative to neutral (pH=7.0), with a statistically significant difference ($P \leq 0.0001$). The findings indicate that acidic and alkaline stress may trigger bacterial adaptation mechanisms, including heightened expression of genes encoding β-lactamase enzymes, thus augmenting bacterial resistance to β-lactam antibiotics. This study establishes that pH variations significantly influence the expression of resistance genes, potentially affecting the effectiveness of antibiotic treatment across diverse physiological settings.

7 Authors contributions

The individual tasked with sample collecting and laboratory experimentation was graduate student Othman Muslih, while the oversight of this study was provided by Prof. Dr. Taisir Abdulelah Kadhim.

8 Data available

The datasets generated and/or analyzed during the current study are available in the Figshare repository https://doi.org/10.6084/m9.figshare.31427915 (15)

9 Acknowledgment

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