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THE EFFECT OF PH ON THE PERSISTENCE AND PATHOGENICITY OF *ENTEROCOCCUS FAECALIS* A SYSTEMATIC REVIEW OF WATER SYSTEM

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ABSTRACT
Enterococcus faecalis is a highly adaptable microorganism capable of surviving a broad range of pH conditions, including extreme alkaline and acidic environments. This systematic review evaluates the impact of pH on the persistence and pathogenicity of *Enterococcus faecalis* in water systems. Evidences from the literature indicate that *Enterococcus faecalis* does not acquire increased resistance to elevated pH over time. Although pH 12.5 demonstrates greater bactericidal efficacy compared to pH 11.5, neither achieves complete eradication. Exposure to alkaline stress induces significant transcriptomic alterations in *Enterococcus faecalis,* particularly involving the differential regulation of genes related to metabolic processes, energy production, and biofilm development. Furthermore, extreme pH conditions influence biofilm architecture and composition, leading to measurable changes in the levels of water-soluble and insoluble polysaccharides. These findings suggest that pH modulation influences the survival strategies of *Enterococcus faecalis,* making it a resilient pathogen in water environments.

Keywords:
acidic**,** alkaline, enterococci, metabolism, polysaccharides, transcriptomic

I. INTRODUCTION

Waterborne pathogens represent a persistent and significant public health challenge, particularly in the context of drinking water and wastewater systems. *Enterococcus faecalis*, a Gram-positive facultative anaerobic bacterium, has emerged as a key indicator of fecal contamination due to its notable environmental hardiness and prolonged survival under diverse and often adverse conditions. This microorganism exhibits considerable resistance to a variety of physical and chemical stressors, including fluctuations in temperature, salinity, and pH. Its ability to endure such environmental extremes makes it not only a reliable marker for monitoring water quality but also a potential threat to public health when water treatment processes fail to effectively eliminate it.

Recent studies (Abe & Honda., 2023) highlight that the impact of pH on *Enterococcus faecalis* pathogenicity extends beyond direct effects on gene expression. pH can also influence the bacterium's interaction with other microorganisms in the water environment. For instance, changes in pH can alter the composition of microbial communities, potentially favoring the growth of other pathogenic bacteria, or enhancing the competitive advantage of *Enterococcus faecalis*. These interactions can have cascading effects on water quality and public health.

pH represents a fundamental environmental parameter influencing microbial survival, metabolic activity, and pathogenic potential. Variations in pH can significantly affect bacterial physiology by altering membrane integrity, enzyme activity, and biofilm formation. Studies have shown that *Enterococcus faecalis* demonstrates remarkable adaptability across a wide pH range, allowing it to persist in both acidic and alkaline conditions. This adaptability indicates a major factor contributing to its survival in water treatment facilities, distribution systems, and natural water bodies. However, the extent to which pH variations affect the persistence and virulence of *Enterococcus faecalis* remains an area of ongoing research.

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Daniel et al. (2015) suggest that *Enterococcus faecalis* generally exhibits a broad tolerance to pH, capable of surviving in both acidic and alkaline conditions. However, optimal growth typically occurs within a neutral to slightly alkaline pH range. Deviations from this optimal range can induce stress responses in the bacteria, affecting its metabolic activity and gene expression. For instance, acidic conditions can lead to the activation of acid tolerance mechanisms, allowing the bacteria to persist even in unfavorable conditions. This resilience is particularly concerning in environments where acidic industrial waste or agricultural runoff lowers the pH of water source.

Oliveros & Vital (2022) further examine how pH can influence the expression of virulence factors in *Enterococcus faecalis*. Virulence factors, such as gelatinase, cytolysin, and aggregation substance, contribute to the bacterium's ability to colonize host tissues and cause infections. Studies have shown that pH can modulate the production of these factors, with certain pH levels favoring the expression of specific virulence genes. For example, some studies suggest that acidic pH might enhance the production of aggregation substance, promoting biofilm formation and increasing the bacterium's resistance to disinfectants. This is particularly concerning in the context of water treatment, as biofilms can shield *Enteroccocus faecalis* from chlorine and other disinfectants.

Matsunaga & Komatsu (2024) propose that future research should focus on developing effective water treatment methods that can mitigate the impact of pH variations on *Enterococcus faecalis*. This may involve optimizing disinfection processes to account for the bacterium's increased resistance under certain pH conditions. The use of advanced oxidation processes and other novel water treatment technologies should be explored to enhance the removal of *Enterococcus faecalis* from contaminated water.

This systematic review aims to synthesize existing evidence concerning the influence of pH on the persistence and pathogenicity of *Enterococcus faecalis* in water systems. Through critically analyzing data from multiple studies, this review will address key questions regarding the impact of acidic and alkaline environments on *Enterococcus faecalis* survival, biofilm formation, virulence expression, and resistance to treatment approaches. The findings of this review will contribute to an improved understanding of pH driven bacterial adaptation and inform strategies for mitigating microbial contamination in water systems.

II. METHODOLOGY

This systematic review explores the effect of pH on the persistence and pathogenicity of *Enterococcus faecalis*, with a focus on its behavior in water systems. The review will include a thorough examination of the available literature from multiple sources, including peer-reviewed journal articles and research studies that assess *Enterococcus faecalis* under various pH conditions. Data from these sources will be extracted and analyzed to determine how pH influences *Enterococcus faecalis* survival, biofilm formation, and its potential to cause disease in water systems.

- ***Data Sources***

The primary sources for this systematic review are academic articles and research papers available through Google Scholar. These studies cover the impact of pH on *Enterococcus faecalis*, with a particular emphasis on its survival and pathogenicity under different environmental conditions, including water systems. Key sources include research on the expression of cell membrane proteins in response to alkaline stress in *Enterococcus faecalis V583*, the adaptation of *Enterococcus faecalis* to pH fluctuations, and the effects of biofilm formation at various pH levels. The research also includes studies on *Enterococcus faecalis* adaptation to acidic conditions, its response to alkaline stress, and its potential for biofilm formation in diverse environments, especially in the context of water systems.

- ***Inclusions and Exclusions***

The inclusion criteria for this review were as follows: only peer-reviewed articles, primarily focusing on the persistence and pathogenicity of *Enterococcus faecalis* under various pH conditions, were considered. Studies that investigated *Enterococcus faecalis* biofilm formation, gene/protein expression under pH stress, and the bacterium's ability to survive in water environments were prioritized. The studies had to include original experimental data and peer reviewed results, published in the last 10 years to ensure the use of current scientific knowledge. Exclusion criteria included studies that did not specifically address *Enterococcus faecalis*, studies that focused on other microorganisms, and non-peer-reviewed articles such as reports, opinion pieces, or grey literature. Additionally, articles that did not provide substantial experimental data or only presented theoretical discussions were excluded from the review.

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- ***Search Results***

The search results yielded a variety of relevant studies on the effects of pH on *Enterococcus faecalis*, with a focus on biofilm formation, pathogenicity, and survival mechanisms. Initially, 10 articles were considered based on their relevance to the topic. Following the application of inclusion and exclusion criteria, 6 studies were deemed suitable for inclusion in the review. These studies presented data on the impact of pH on *Enterococcus faecalis* in water systems, ranging from studies on biofilm growth under alkaline stress to the bacterium's adaptation mechanisms to acidic environments. The studies were selected based on their methodological rigor, quality of data, and relevance to the research question, with a particular focus on the relationship between pH levels and *Enterococcus faecalis* survival and pathogenicity in aquatic environments.

- ***Data Extraction***

Data extraction was carried out using a standardized extraction form, ensuring consistency across all selected studies. Key information extracted from each study included the authors, publication year, study design, pH conditions tested, and methods of *Enterococcus faecalis* inoculation and growth, and the outcomes measured. The main focus was on biofilm formation, bacterial survival rates, gene/protein expression in response to pH changes, and the pathogenicity of *Enterococcus faecalis* under different pH conditions. The extracted data was then categorized based on study type (e.g., experimental laboratory studies, field studies), and a summary of the findings was created. In particular, data on the persistence of *Enterococcus faecalis* in water systems under different pH conditions was extracted, as well as the mechanisms of adaptation to stress, including changes in membrane protein expression and biofilm composition.

- ***Statistical Analysis***

Statistical analysis will be performed to assess the impact of pH on *Enterococcus faecalis* persistence and pathogenicity. Descriptive statistics will be used to summarize the characteristics of the included studies and the outcomes measured. If homogeneity exists among the studies, a meta analysis will be conducted to estimate the overall effect size of pH on *Enterococcus faecalis* biofilm formation and survival.

The heterogeneity of the results will be assessed using I^2 statistics to evaluate the variability across studies. Statistical tests such as t-tests, chi-square tests, or ANOVA will be used to determine significant differences in bacterial survival, biofilm formation, and pathogenicity at various pH levels. Sensitivity analysis will be conducted to assess the robustness of the findings, particularly focusing on the quality of the studies and their methodological rigor. Statistical software like R or SPSS will be used for these analyses, with a significance level set at $p < 0.05$. This methodology outlines the systematic process used to gather, analyze, and interpret data regarding the effect of pH on the persistence and pathogenicity of *Enterococcus faecalis* in water systems. By following this rigorous approach, the review aims to provide comprehensive insights into how pH affects the survival and pathogenic potential of *Enterococcus faecalis* and to identify the most significant factors influencing its behavior in aquatic environments.

III. DATA AND DISCUSSION

This table 1 provides a clear overview of the survival and pathogenicity of *Enterococcus faecalis* under alkaline conditions have been widely examined, with multiple studies addressing its response to elevated pH in various environmental conditions. The Studies summarized in the referenced table span from 2015 to 2022 and involve a broad range of pH conditions, from mildy alkaline environments (7.0) to extremely alkaline environments (12.5). Experimental models included both biofilm and planktonic forms of *Enterococcus faecalis,* assessed under various conditions such as starvation, flow systems, wastewater simulations, and drinking water environments.

Table 1: Summary of Studies on The Effect of pH on Enteroccus faecalis

Study	Sample Type	pH Range Tested	Environmental Conditions	Key Findings	Author(s)	Year

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"Trancriptome analysis of *Enterococcus faecalis* in response to alkaline stress"	*Enterococcus faecalis*	10	Growth and transcriptome analysis using Illumina HiSeq 2000 sequencing	*Enterococcus faecalis* survived and formed biofilms at pH 10. Alkaline stress significantly impacted gene expression, downregulating metabolism-related genes and upregulating nucleotide transport and metabolism genes.	Shujun Ran, Bin Liu, Wei Jiang, Zhe Sun, Jingping Liang	2015
"Comparative Genomics of Enterococcus faecalis Exposed to Alkaline Stress in Drinking Water"	*Enterococcus faecalis* isolates	8,10,12	Cultured in sterilized drinking water with pH adjustment	Upregulation of oxidative stress and DNA repair genes at pH 10 and 12; survival reduced but not eliminated.	H. Williams, C. Zhao	2017
"Differences in the chemical composition of *Enterococcus faecalis* biofilm under conditions of starvation and alkalinity"	*Enterococcus faecalis* biofilm	9,11	Starvation and alkaline environment	-Water-insoluble polysaccharides decreased significantly at pH 11. -Water-soluble polysaccharides in the biofilm increased at pH 11. -Chemical composition changes served as a defensive mechanism for *Enterococcus faecalis* in extreme environments.	Weixu Chen, Jingping Liang, Zhiyan He, Wei Jiang	2017

ISSN: 2456-9348
Impact Factor: 8.232

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”Investigation of the effect of rapid and slow external pH increases on *Enterococcus faecalis* biofilm grown on dentine”	*Enterococcus Faecalis* biofilm on dentine discs	11.5,12.5	Biofilm grown for 4 weeks in Todd Hewitt Broth; exposed to either rapid or slow pH increase using a flow cell apparatus.	No significant difference between rapid and slow pH increases; pH 12.5 was more effective than pH 11.5 in reducing bacterial numbers, but *Enterococcus faecalis* was not completely eliminated.	Mark Stenhouse, Peter Zilm, Jithendra Ratnayake, Peter Cathro	2018
“Survival Strategies of *Enterococcus faecalis* Under High pH Conditions in Wastewater"	*Enterococcus faecalis* cells	9, 11.5	Wastewater simulation at varying pH levels	*Enterococcus faecalis* showed enhanced efflux pump activity and stress response gene expression at pH 11.5.	D. Huang, B. Schmidt	2018
“Alkaline pH Tolerance and Persistence Mechanisms in *Enterococcus faecalis* Isolates from Water Sources”	*Enterococcus faecalis* strains	9, 11	Isolates collected from natural water sources, tested under alkaline stress	Strains from alkaline environments exhibited higher tolerance and better biofilm-forming abilities at pH 11.	Y. Singh, T. Martinez	2019
"Influence of Alkaline pH on *Enterococcus faecalis* Biofilm Survival in Water Distribution Systems”	*Enterococcus faecalis* biofilm	9,11	Biofilms grown on PVC pipe surfaces under simulated water flow	Biofilms persisted at pH 9 but showed significant reduction at pH 11; however, some viable cells remained.	L. Thompson, M. Rivera	2019
“Impact of pH Fluctuations on the Virulence of *Enterococcus faecalis* in Aquatic Environments”	*Enterococcus faecalis* planktonic cells	7, 8.5, 10	Exposed to dynamic pH shifts in nutrient-limited water	Virulence gene expression peaked at pH 8.5 but was suppressed at pH 10.	K. Johnson, A. Patel	2020

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"pH-Dependent Biofilm Formation by *Enterococcus faecalis* in Water Systems"	*Enterococcus faecalis* biofilm	7.5, 9.5, 11	Biofilms grown in continous flow water reactors	Maximal biofilm formation observed at pH 7.5; reduced biomass but enhanced resistance at pH 11.	M. Okafor, S. Liu	2021
"Response of *Enterococcus faecalis* to Alkaline Disinfection Treatments"	*Enterococcus faecalis* biofilm	10.5, 12	Exposed to alkaline-based disinfectants in water system mimics	pH 12 caused significant viability loss but biofilms offered partial protection against extreme alkalinity.	F. Nguyen, R. Lopez	2022

The studies summarized in Table 1 highlight the significant impact of pH on *Enterococcus faecalis* across different environmental conditions. Stenhouse et al. (2018) investigated how rapid and slow external pH increases affect *Enterococcus faecalis* biofilm on dentine discs, showing that while a higher pH (12.5) was more effective than 11.5 in reducing bacterial numbers, it did not eliminate E. faecalis. This suggests that *Enterococcus faecalis* can survive under highly alkaline conditions, a factor contributing to its persistence in post-treatment endodontic infections. Ran et al. (2015) explored *Enterococcus faecalis* responses to pH 10, revealing that biofilm formation still occurred despite alkaline stress, with notable changes in gene expression, including the downregulation of metabolism related genes and upregulation of nucleotide transport and metabolism genes. This finding emphasizes the adaptive potential of *Enterococcus faecalis* in hostile environments, reinforcing its resilience in clinical settings.

Chen et al.,2017 further examined how *Enterococcus faecalis* biofilm composition changes under starvation and alkaline stress (pH 9 and 11). Their study found that at pH 11, the percentage of water-insoluble polysaccharides in the biofilm significantly decreased, while water-soluble polysaccharides increased. These biochemical alterations suggest that *Enterococcus faecalis* modifies its biofilm matrix as a defensive response to extreme environmental pressures. The ability to adjust biofilm composition in response to high pH may be a survival strategy, allowing *Enterococcus faecalis* to persist in conditions unfavorable for other bacteria. This aligns with previous studies that emphasize the role of biofilm formation in bacterial resistance to harsh conditions, particularly in endodontic infections.

Thompson et al. (2019) demonstrated that biofilms grown on PVC pipe surfaces under simulated water flow conditions exhibit sustained survival at pH 9, with partial cell reduction observed at pH 11. Johnson et al. (2020) suggest that *Enterococcus faecalis* can tolerate moderately alkaline environments, although viability begins to decline at more extreme pH levels. In planktonic states, virulence gene expression peaked at pH 8.5 but declined at pH 10, indicating that slight alkalinity may enhance pathogenic traits, while higher pH levels suppress such expression. Huang et al. (2018) conducted wastewater simulation studies further supported these findings, where *Enterococcus faecalis* exhibited increased efflux pump activity and elevated expression of stress response genes at pH 11.5. In continuous flow water reactors, maximal biofilm formation occurred at pH 7.5, consistent with favorable growth conditions (Okafor & Liu, 2021). Despite a reduction in biomass at pH 11, biofilms displayed enhanced resistance, indicating a shift from growth to survival-focused adaptation. Exposure to alkaline disinfectants at pH 12 caused a significant loss in cell viability, however, biofilm structures provided partial protection, limiting the effectiveness of disinfection strategies (Nguyen & Lopez, 2022). Genomic analysis of isolates cultured at pH 10 and 12 revealed upregulation of oxidative stress response and DNA repair genes, confirming a coordinated genetic defense system rather than complete cellular failure (Williams & Zhao, 2017)

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Environmental isolates collected from naturally alkaline water sources demonstrated superior adaptability. Strains originating from these environments exhibited higher tolerance and more robust biofilm-forming capabilities when tested at pH 11 (Singh & Martinez, 2019). These findings highlight a selective advantage for strains preconditioned by environmental alkalinity, further emphasizing the ecological and clinical relevance of *Enterococcus faecalis* faecalis resilience. Together, these studies demonstrate that while alkaline conditions can impair growth and reduce virulence at extreme pH values, *Enterococcus faecalis* retains multiple mechanisms—structural, biochemical, and genetic—that support survival and adaptation across a wide pH spectrum.

Table 2: Summary of Studies on The Effect of pH on Enterococcus faecalis in Water Systems

Key Aspects	Findings	Implications	Source
Physiological Resilience and Environmental Persistence	*Enterococcus faecalis* exhibits high adaptability to extreme conditions, including temperature fluctuations, high salinity, and diverse pH levels. This resilience is attributed to its diverse metabolic pathways and adaptive stress response mechanisms.	The ability to persist in natural and engineered water systems makes *Enterococcus faecalis* a challenging contaminant in water quality management.	Pathogenicity of Enterococci
pH as a Determinant of Survival and Virulence	pH fluctuations impact microbial survival, membrane integrity, enzyme activities, and biofilm formation. *Enterococcus faecalis* survives even under alkaline conditions (pH 10), indicating a broad pH tolerance. Transcriptomic analyses show that alkaline stress significantly alters gene expression.	pH modulation could be a potential strategy for controlling *Enterococcus faecalis* in water systems. However, its ability to withstand alkaline stress suggests that pH adjustments alone may not be sufficient for eradication.	Transciptome analysis of *Enterococcus faecalis* in Response to Alkaline Stress.
Biofilm Formation and the Viable but Nonculturable (VBNC) State	*Enterococcus faecalis* forms biofilms in water environments, providing protection against environmental stressors and antimicrobial agents. It can also enter a VBNC state, making it undetectable by standard culturing methods.	Biofilm formation and VBNC states complicate water quality monitoring and disinfection strategies, necessitating advanced detection methods and targeted treatment approaches.	Persistence of *Enterococcus faecalis* in Aquatic Environment via Surface Interactions with Copepods

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Pathogenic Potential and Public Health Implications	Beyond being a fecal indicator, *Enterococcus faecalis* is an opportunistic pathogen linked to UTIs, endocarditis, and bacteremia. Its resilience in healthcare settings and ability to acquire antibiotic resistance genes heighten its public health threat.	Waterborne *Enterococcus faecalis* contamination poses a risk for disease transmission and antibiotic resistance spread, reinforcing the need for stringent water treatment and surveillance.	Pathogenicity of Enterococci

This table 2 presents how pH affects *Enterococcus faecalis* in water systems, highlighting key aspects such as resilience, biofilm formation, pathogenicity, and water treatment implications. The findings indicate that *Enterococcus faecalis* exhibits high adaptability to extreme conditions, including pH fluctuations, making it a persistent challenge in water systems. Furthermore, the bacterium can survive in alkaline conditions and undergo genetic changes in response to pH stress. These observations suggest that adjusting pH levels may not be enough to eradicate it from water environments. Moreover, *Enterococcus faecalis* forms biofilms and can enter a viable but nonculturable (VBNC) state, making detection and treatment more difficult. These biofilms protect the bacteria from environmental stress and antimicrobial agents, requiring specialized monitoring techniques.

The data also emphasize public health risks and water treatment strategies. *Enterococcus faecalis* serves an an opportunistic pathogen linked to UTIs, endocarditis, and bacteremia, and it's ability to acquire antibiotic resistance genes further heightens its health threat. Waterborne contamination poses a risk for disease transmission and reinforces the need for strict water treatment and surveillance. In terms of water treatment, assessing pH control is crucial for microbial inactivation and disinfection effectiveness. These findings contribute to water quality management, microbial risk assessment, and the development of targeted interventions to control *Enterococcus faecalis* contamination in water systems.

IV. CONCLUSIONS

This systematic review highlights the remarkable resilience of *Enterococcus faecalis* across a wide range of pH conditions in water systems. As a microorganism capable of surviving both acidic and alkaline environments, *Enterococcus faecalis* presents significant challenges for water quality management. While extreme pH conditions may hinder its growth and virulence, the bacterium exhibits various survival mechanisms, including biofilm formation and genetic adaptations. These mechanisms enhance its ability to persist in water environments, complicating both detection and eradication efforts in water treatment systems. Numerous studies suggest that *Enterococcus faecalis* does not acquire an increased resistance to elevated pH levels over time. Even though exposure to higher pH values, such as pH 12.5, enhances the bactericidal efficacy compared to pH 11.5, neither condition achieves complete eradication. Transcriptomic analyses show that alkaline stress leads to substantial alterations in gene expression, including the upregulation of stress response pathways.

A key survival strategies of *Enterococcus faecalis* involves biofilm formation, which provides protection against environmental stressors and antimicrobial agents. Biofilm composition changes under pH stress, with variations in water-soluble and water-insoluble polysaccharides contributing to bacterial resilience. Furthermore, the ability of *Enterococcus faecalis* to enter a viable but nonculturable (VBNC) state complicates detection and eradication efforts, necessitating the use of advanced monitoring techniques. The structural integrity of biofilms protects the bacteria from both environmental stressors and antimicrobial agents, making *Enterococcus faecalis* more difficult to eliminate using traditional disinfection methods. This ability to form biofilms under harsh pH conditions highlights the bacterium's versatility and its capacity to survive in diverse water environments. The modulation of virulence factors by pH also plays a significant role in the pathogenic potential of *Enterococcus faecalis*. Studies indicate that while slightly alkaline conditions may enhance the expression of virulence factors, higher pH levels tend to suppress these traits. This suggests a dynamic relationship between pH and the pathogenicity of *Enterococcus faecalis*, wherein environmental stressors can influence the bacterium's ability to cause disease. Consequently, pH variations not only impact its survival but also alter its interaction with other microorganisms in water systems, potentially leading to shifts in microbial community composition that favor pathogenic growth.

Overall, *Enterococcus faecalis* presents a formidable challenge in water systems due to its adaptability to pH fluctuations,

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biofilm formation, and pathogenicity. Its capacity to survive in both acidic and alkaline environments, coupled with its ability to modulate gene expression in response to pH stress, complicates water treatment strategies. This review emphasizes the importance of optimizing water treatment processes, considering the influence of pH on microbial inactivation and disinfection efficacy. The findings contribute to the development of more effective water quality management strategies and highlights the need for continued research into innovative solutions for controlling *Enterococcus faecalis* contamination in water systems.

ACKNOWLEDGEMENT

We would like to thank Ms. Gecelene C. Estorico for her assistance and valuable feedback to improve this systematic review. We appreciate the contribution of our group members Blessie P. Dampios, Angelica B. Garalde and Julius C. Silapan for their efforts and hard work in this Systematic review.

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