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"The Effect of Some Physical Parameters on The Growth Rate of E. Coli and K. Pneumoniae"

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ABSTRACT:

This study aims to investigate the influence of some environmental factors including temperature, pH, and pressure on the growth rates of *Escherichia coli* and *Klebsiella pneumonia* due to its importance for developing effective prevention and treatment strategies. *E.* coli and *K. pneumonia* are common pathogens in humans and important members of the family *Enterobacteriaceae*, that are involved in many infections. In this study, growth rates were measured at different temperatures ranging between 25°C, 30°C, 37°C, and 42°C, over incubation periods of 24, 48, and 72 hours. Growth rates were also assessed at five pH levels: 4, 5, 7, 9, and 10. Moreover, we investigated the effect of five different pressure conditions on bacterial growth rates including 1 atm (standard atmospheric pressure), 5 atm, 10 atm, 20 atm, and 30 atm using liquid nutrient broth cultures and the usual nutrient agar plate count technique.

Our results demonstrated temperature-dependent variations in bacterial growth. As, it showed distinct growth patterns for each bacterium, with optimal growth observed at 37°C. Conversely, higher temperature (42°C) was accompanied by metabolic stress and reduced viability over prolonged incubation periods reaching 72 hours. However, *E. coli* showed better tolerance for higher temperatures compared to *K. pneumoniae*, which suggested that *E. coli* might have a higher adaptability to temperature fluctuations and explained its prevalence in a broader range of environments.

Moreover, both *E.* coli and *K. pneumonia* exhibit optimal growth at a neutral pH of 7, with significant declines in growth rates observed at more acidic and alkaline conditions. These findings are crucial for understanding the environmental preferences of these pathogens and can inform strategies for controlling infections. On the other hand, our results showed that *E.* coli and K. pneumonia exhibit optimal growth at 1 atm,

with marked decreases in growth observed at higher pressures. These findings provide quantitative data on the pressure sensitivity of these bacteria, which has various applications, including food preservation, and industrial microbiology.

KEYWORDS:

E. coli / K. pneumoniae/ Temperature/ pH/ Pressure/ Growth rate/ Colony forming unit

1. INTRODUCTION:

Escherichia coli and *Klebsiella pneumoniae* are gram-negative bacteria belonging to the *Enterobacteriaceae* family. They are common pathogens in humans, involved in many infections including UTIs, gastrointestinal infections, and pneumonia. *E. coli* is found in the lower intestine of warm-blooded organisms, including humans, while *K. pneumoniae* the rod-shaped bacteria, is an important antimicrobial-resistant bacteria and a common cause of pneumonia **(1).** Understanding the growth dynamics of these bacteria under different environmental conditions including temperature, pressure, and pH is crucial for optimizing experimental protocols and providing insights into their behavior which is essential for infection control and prevention strategies **(2).**

Temperature is a fundamental environmental factor known to influence microbial growth rates and plays a vital role in their growth and survival. Given the prevalence of *E. coli* and *K. pneumoniae* in mammalian hosts, it is reasonable to hypothesize that their growth would be optimal at temperatures resembling mammalian body temperature, typically around 37°C (98.6°F). However, they can survive and grow at a wide range of temperatures **(3).** So, exploring the growth behavior of the bacteria across a range of temperatures provides valuable insights into developing effective food safety measures and preventing foodborne illnesses **(4).**

For example, when *E. coli* and *K. pneumoniae* are exposed to suboptimal temperatures, they may exhibit a prolonged lag phase before entering the exponential growth phase. This lag phase allows the bacteria to adapt to the new temperature conditions. At refrigeration temperatures (4-7°C or 39-45°F), bacteria can still grow but at a much slower rate **(5).** This is a concern for food safety, as these bacteria can multiply in refrigerated foods if given enough time. On the other hand, high temperatures can inactivate or kill the bacteria. Pasteurization (typically at 63-65°C or 145-149°F for at least 30 minutes) is an effective way to ensure food safety **(6).**

In this study, we aimed to investigate the comparative growth rates of *E. coli* and *K. pneumoniae* at temperatures of 25°C, 30°C, 37°C, and 42°C over varying incubation periods (24 hours, 48 hours, and 72 hours). We hypothesized that optimal growth would occur at 37°C, mirroring the physiological temperature of mammalian hosts.

Also, pH is considered one of the most significant environmental factors that regulate bacterial growth. The hydrogen ion concentration affects the stability and function of cellular components, enzyme activity, and nutrient solubility. Bacteria are generally neutrophils, meaning that around 7.0 is the ideal pH for them to grow in. Acidophiles grow optimally at a pH near 3.0. Alkaliphiles are organisms that thrive best in the pH range of 8 to 10.5. Extreme acidophiles and alkaliphiles grow slowly or not at all near neutral pH.

Most familiar bacteria, like *Escherichia coli, K. pneumoniae,* and *Salmonella are* neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains *of E. coli, S. typhi***,** and other intestinal pathogens that are much more resistant to stomach acid, which is the reason behind their ability to cause GIT infections. In this study, growth rates were assessed at five pH levels: 4, 5, 7, 9, and 10.

Moreover, the effect of pressure on bacterial growth is a complex phenomenon influenced by various factors, including bacterial species, pressure range, and environmental conditions. Understanding these effects is crucial for understanding the distribution and activity of microorganisms in different environments, such as the ocean and space. In general, Gram-positive bacteria and bacteria in a stationary growth phase have higher resistance to high pressure than Gram-negative bacteria and bacteria that are in an exponential growth phase.

High-pressure processing (HPP) is used to inactivate both spoilage bacteria and pathogenic bacteria, such as *Listeria monocytogenes, Escherichia coli, Salmonella,* and *Vibrio*. However, the exact mechanisms behind the inactivation of bacteria under high pressure are not well known. High pressure may have an impact on some mechanisms including the inactivation of enzymes, cell membrane destruction, and changes in cell structures. In general, bacteria exposed to a pressure above 400 MPa will have both reversible and irreversible changes in their cell membrane.

Because microorganisms are subjected to various environmental pressures, both in natural settings and industrial applications, we aimed to study the effect of pressure on microbial growth by applying five different pressure conditions including 1 atm (standard atmospheric pressure), 5 atm, 10 atm, 20 atm, and 30 atm. To assess growth rates under these different circumstances of temperature, pressure, and pH, we employed both liquid nutrient broth cultures and nutrient agar plates, utilizing spectrophotometry to measure optical density in liquid media and colony counting on agar plates.

2. MATERIALS AND METHODS:

Source of Microorganisms

Standard *E. coli JM105* strain was obtained from Pharmacia (Rockville, USA), also *K. pneumoniae ATCC 13883* was purchased from the American Type Culture Collection (ATCC). These bacterial cultures were maintained on slant agar at 4°C. For long-term storage, they were preserved in tryptone soya broth (Oxoid, Hampshire, UK) with 20% glycerol and stored at -20 °C until use **(7).**

Effect of Different Temperatures on *in vitro* Growth of *E. Coli* and *K. pneumoniae*

On a nutrient agar plate, a single loopful of the bacterial culture was streaked, and the plate was incubated for 24 hours at 37°C. After that, one colony was inoculated with 10 mL of sterile Mueller Hinton Broth (MHB) and incubated overnight at 37°C. One milliliter of the overnight culture was then inoculated into a 100-milliliter conical flask that held 50 milliliters of sterile MHB. The flask was then incubated at different temperatures of 26°C, 30°C, 37°C, and 42°C in a shaking incubator (Binder, Germany) for incubation periods of 24 hours, 48 hours, and 72 hours. The optical density (OD) was measured at a wavelength of 600 nm using a UV/Vis spectrophotometer (Fisher Scientific, Germany). Each measurement was taken in triplicate to ensure accuracy and reproducibility.

Furthermore, the usual plate count technique was performed on nutrient agar plates after appropriate dilution of bacterial cultures to investigate the number of colony-forming units (CFU) **(8).**

Effect of pH levels on *in vitro* Growth of *E. Coli and K. pneumoniae*

Growth rates were measured at five different pH levels: 4, 5, 7, 9, and 10. The pH of the media was adjusted using HCl or NaOH. Triplicate samples were prepared for each pH condition. Bacterial growth was monitored by measuring the OD at 600 nm at hourly intervals for 24 hours using a spectrophotometer. The growth rate was determined using the exponential growth equation.

Effect of Different Pressure Conditions on *in vitro* Growth of *E. Coli* and *K. pneumoniae*

Nutrient broth was used as the growth medium. Bacterial cultures were inoculated into the growth media and incubated at 37°C. Growth rates were measured under five different pressure conditions: 1 atm (standard atmospheric pressure), 5 atm, 10 atm, 20 atm, and 30 atm. High-pressure conditions were maintained using a high-pressure chamber. Optical density (OD) measurements at 600 nm were taken at regular intervals to monitor bacterial growth. Growth rates were calculated using the exponential growth equation. Triplicate samples were prepared for each pressure condition.

3. RESULTS:

Effect of different temperatures and incubation periods on growth rates of *E. coli* and *K. pneumoniae*

The data represented in **Table (1)** showed the growth rates of *E. coli* and *K. pneumoniae* at different temperatures and incubation periods, measured by optical density OD at 600 nm. The results of this study demonstrated that their growth rates are temperature-dependent, where at lower temperatures (25 °C) they showed a slow growth rate ranging from 0.4 to 0.5 for *K. pneumoniae* and *E. coli*; respectively.

On the other hand, the optimal growth was observed at 37°C, where *E. coli* recorded an OD of 1.2 after 24 hours at 37°C, that was almost the same observation for *K. pneumoniae* which recorded an OD of 1.1 after 24 hours.

However, *E. coli* showed better tolerance for higher temperatures compared to *K. pneumoniae* as shown in **Figure (2),** where *K. pneumoniae* showed a significantly reduced growth rate, with an OD of 0.5 after 72 hours, while *E. coli* showed a slow to moderate growth rate, with an OD of 0.9 after 72 hours. Overall, our results suggested that the examined bacteria exhibited enhanced metabolic activity and reproduction rates at temperatures resembling their natural habitat.

Table 1: *E. coli* and *K. pneumoniae* growth rates at different temperatures and incubation periods (measured by optical density OD at 600 nm).

Temperature (°C)	Incubation Period (hours)					
	24		48		72	
	E. coli	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*

25°C	0.523	0.425	0.761	0.645	0.812	0.724
30°C	0.813	0.731	1.236	1.065	1.435	1.143
37°C	1.224	1.134	1.628	1.432	1.854	1.514
42°C	0.643	0.367	0.803	0.415	0.944	0.521

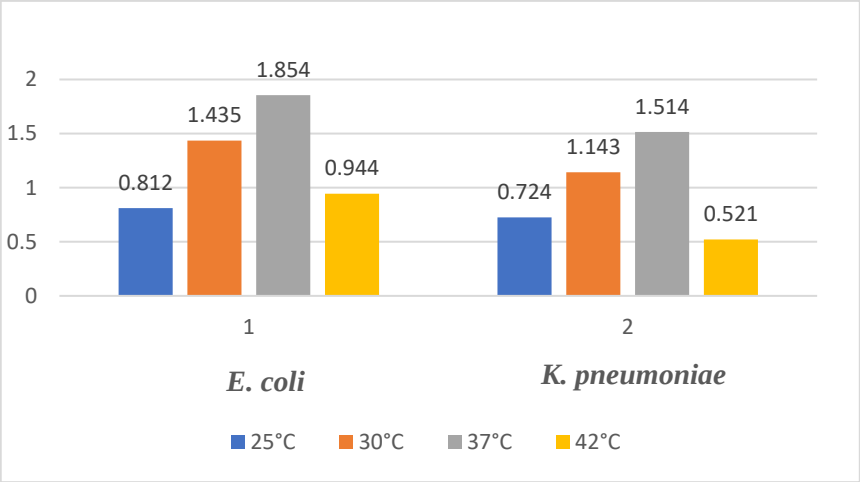

Figure 1: Comparison between *E. coli* and *K. pneumoniae* growth rates at different temperatures after an incubation period of 72 hours measured by OD at 600 nm.

The data represented in **Table (2)** demonstrated the nutrient agar plate count in colony-forming units (CFU) of *E. coli* and *Klebsiella pneumoniae* at temperatures of 25°C, 30°C, 37°C, and 42°C over incubation periods of 24 hours, 48 hours, and 72 hours. At 25°C, both bacteria showed lower CFU counts, indicating slower growth, while at 30°C, the CFU counts were slightly increased, indicating improved growth conditions.

On the other hand, at 37°C, *E. coli* and *Klebsiella pneumoniae* bacteria exhibited their highest CFU counts indicating optimal growth at this temperature. CFU counts increased from 3.0×10^6 to 5.5×10^6 in the case of *E. coli* and from 6.2×10^6 to 9.5×10^6 in the case of *K. pneumoniae*. However, at 42°C, there is a noticeable decrease in CFU counts for both bacteria, with *K. pneumoniae* showing a more significant reduction (1.5×10^6) compared to *E. coli* (3.8×10^6) suggesting a lower tolerance to higher temperatures.

Table 2: *E. coli* and *K. pneumoniae* CFU count at different temperatures.

Temperature (°C)	**Incubation Period (hours)**					
	24		**48**		**72**	
	E. coli	***K. pneumoniae***	***E. coli***	***K. pneumoniae***	***E. coli***	***K. pneumoniae***
25°C	1.2×10^6	1.0×10^6	2.4×10^6	1.8×10^6	3.0×10^6	2.4×10^6
30°C	3.0×10^6	2.5×10^6	5.0×10^6	4.0×10^6	6.2×10^6	5.2×10^6
37°C	5.5×10^6	4.5×10^6	8.0×10^6	6.8×10^6	9.5×10^6	8.0×10^6
42°C	2.5×10^6	0.8×10^6	3.2×10^6	1.2×10^6	3.8×10^6	1.5×10^6

Effect of pH on Bacterial Growth Rate:

As shown in **Table (3),** our results demonstrated that both *E. coli* and *K. pneumoniae* have a preference for neutral pH conditions, with optimal growth rates observed at pH 7. However, *E. coli* exhibited a broader pH tolerance range, maintaining relatively high growth rates even at slightly acidic (pH 4-5) and basic (pH 9-10) conditions.

At pH 7, *E. coli* showed the highest growth rate of 1.70±0.1. This rate decreased at acidic conditions (pH 5: 0.80±0.04) and basic conditions (pH 9: 0.90±0.06, pH 10: 0.30±0.03). This was likely due to denaturation of proteins and disruption of cellular processes.

On the other hand, *K. pneumoniae* was more sensitive to pH fluctuations, with a narrower optimal pH range of 6-8, where it showed optimal growth at pH 7, with the highest growth rate of 1.60±0.13. At pH 4 and pH 10, the growth rate was significantly reduced to 0.15±0.03 and 0.25±0.06; respectively, indicating inhibition of growth.

Table 3: *E. coli* and *K. pneumoniae* growth rates at different pH levels after incubation for 24 hours (measured by optical density OD at 600 nm).

pH level	Incubation Period (24 hours)	
	E. coli	***K. pneumoniae***
pH 4	0.20±0.02	0.15±0.03
pH 5	0.80±0.04	0.70±0.05
pH 7	1.70±0.1	1.60±0.13
pH 9	0.90±0.06.	0.80±0.07
pH 10	0.30±0.03	0.25±0.06

Effect of Pressure on Bacterial Growth Rate:

The data represented in **Table (4)** demonstrated that the growth rates of *E. coli* and *K. pneumoniae* exhibited clear pressure-dependent patterns. Both species showed optimal growth at standard atmospheric pressure (1 atm) ranging from (1.6 to 1.7).

However, the growth rates decreased significantly at higher pressures. The minimal growth was observed at 30 atm reaching 0.1 for *E. coli* and 0.08 for *K. pneumoniae.* These results indicated that the tested bacteria were sensitive to elevated pressure. The moderate growth at 5 atm and 10 atm ranging from (0.6 to 1.2) suggested some level of pressure tolerance, which may be relevant for their survival in certain high-pressure environments, such as deep-sea or industrial settings.

Table 4: *E. coli* and *K. pneumoniae* growth rates at different pressure conditions after incubation for 24 hours (measured by optical density OD at 600 nm).

Pressure (atm)	Incubation Period (24 hours)	
	E. coli	***K. pneumoniae***
1 atm	1.70±0.10	1.60±0.12
5 atm	1.20±0.08	1.10±0.09
10 atm	0.70±0.05	0.60±0.06
20 atm	0.30±0.04	0.25±0.05

30 atm	0.10±0.02	0.08±0.03

4. DISCUSSION:

Escherichia coli and *Klebsiella pneumoniae* are of the most frequent bacteria involved in a large number of human infections including UTIs, gastrointestinal infections, and pneumonia **(9).** The observed variations in growth rates underscore the importance of the different environmental conditions including temperature, pH, and pressure regulation in microbial cultivation and experimental design. By understanding the temperature-dependent responses of *E. coli* and *K. pneumoniae*, researchers can refine experimental protocols and optimize microbial cultivation strategies for various applications. The findings of this study are consistent with previous research indicating the influence of temperature on bacterial growth dynamics **(10).**

Our study demonstrated that both *E. coli* and *K. pneumoniae* have optimal growth temperatures at around 37°C, which is close to the human body temperature. This is consistent with a previous study by **Yang *et al.* (2018) (11).** While, at suboptimal temperatures such as 26°C, bacteria exhibited limited growth, suggesting that metabolic processes may be slowed or restricted under these conditions **(12)**.

While the reduced growth rates at 42°C indicated that higher temperatures can inhibit bacterial proliferation, and may be accompanied by metabolic stress and reduced viability over prolonged incubation periods that came in context with **Nguyen *et al.* (2019) (13).** These findings had significant implications for sterilization and disinfection practices. Understanding these temperature-dependent growth patterns can help in developing targeted strategies to control infections caused by these pathogens **(14).**

However, *E. coli* showed a higher tolerance for elevated temperatures compared to *K. pneumoniae*, these findings are consistent with previous studies by **Lambros *et al.* (2018)** who suggested that *E. coli* might have a higher adaptability to temperature fluctuations, which could explain its prevalence in a broader range of environments **(15)**.

pH can affect bacterial cell structure, enzyme activity, and overall metabolic processes **(16).** By studying the effect of different pH conditions on bacterial growth, we found that both *E. coli* and *K. pneumoniae* have a preference for neutral pH conditions, with optimal growth rates observed at pH 7, this is consistent with a previous study by **Salanitro *et al.* (1978) (17).** However, when the extracellular pH deviates from the optimal range, bacteria expend energy to regulate their internal pH, which can limit growth. *K. pneumoniae* was more sensitive to pH fluctuations, with a narrower optimal pH range of 6-8.

We found that *E. coli* exhibited a broader pH tolerance range, the higher tolerance of *E. coli* to pH changes may contribute to its prevalence in diverse environments. These findings are consistent with **Alya'ainun *et al.* (2021)** who stated that pH affects the growth rate and histamine formation of *K. pneumoniae CK02 and R. ornithinolytica TN01*, where the optimal growth rate of *K. pneumoniae* CK02 was in the range of 6-8 (0.304-0.380 log CFU/h). The differences in pH tolerance can be attributed to the bacteria's ability to maintain a stable intracellular pH **(18).**

Another important factor that influences bacterial growth is pressure which is a complex phenomenon influenced by various factors. The differences in pressure tolerance can be attributed to the bacteria's ability to maintain a stable cell membrane and cellular processes under varying pressure conditions **(19).** Understanding bacterial behavior under different pressure conditions has important implications for microbiology in high-pressure environments, including deep-sea exploration, food preservation, and industrial microbiology **(20).**

Our results demonstrated that both *E. coli* and *K. pneumoniae* are sensitive to pressure fluctuations. *E. coli* exhibited a higher growth rate at higher pressures, which came in agreement with **Hogan *et al.* (1975)** who studied how different levels of hydrostatic pressure affect the growth and survival of **E. coli** and confirmed that the tested bacteria were sensitive to elevated pressure, which is likely due to the mechanical stress exerted on cellular structures and the potential disruption of membrane integrity and enzyme function **(21).**

Furthermore, the methodological approach used in this study, combining spectrophotometry and colony counting, offered a comprehensive assessment of bacterial growth dynamics Spectrophotometry provided a rapid and non-destructive method for monitoring bacterial growth in liquid cultures, while colony counting allowed for accurate quantification of viable cells on solid media. This dual approach enhanced the reliability of growth rate measurements, ensuring accurate interpretation of experimental results **(22).**

In conclusion, this study provided insights into the temperature and pH-dependent growth rate of *E. coli* and *K. pneumoniae* in addition to the influence of different pressure conditions on bacterial growth rates. Optimal growth was observed at 37°C and neutral pH (7), with considerable declines in growth at more acidic and alkaline pH levels. *E.* coli and K. pneumonia exhibit optimal growth at 1 atm, with marked decreases in growth observed at higher pressures. These findings contribute to our understanding of the environmental factors influencing bacterial growth. Moreover, other factors including nutrient availability, and oxygen levels can also influence bacterial growth rates **(23).** So, future studies could explore the interactive effects of these variables on bacterial growth to further elucidate the underlying mechanisms governing microbial adaptation and metabolism **(24).**

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