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In Vitro Quantitative Assessment of Some Virulence Factors Produced by *Escherichia coli* in Different pH, Temperature and Oxygen Conditions

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Abstract

Background: Bacteria virulence is modulated by different factors in the gut and other host environments. Aim: This study was to quantitatively determine some virulence factors produced in *Escherichia coli* when exposed to different conditions. Method: The different conditions of temperature and pH were used. The growth response and biofilm production of *E. coli* in these conditions was quantified. The production of secretory molecules was analyzed using the thin layer chromatography (TLC) method. The isolates exposed to these conditions were analyzed for antibiotic susceptibility using the disk diffusion method. Results: For the acidic condition, the comparisons of the growth in the other temperatures were lower than the neutral pH and significant results were noted in 25˚C (P = 0.0036), 4˚C (P = 0.0006) and −5˚C (P = 0.0011), while non-significant result was seen in 37˚C (P = 0.2453). In the alkaline condition, at 37˚C the degree of significance was P = 0.0102, 25˚C (P = 0.0007), 4˚C (P = 0.0009), and at −5˚C (P = 0.0006). The formation of biofilm, when compared to the normal body temperature of 37˚C, was significantly higher at 4˚C (P = 0.0382). TLC showed production of bands at −5˚C, (Rf = 0.5), at 25˚C two bands in both the acidic and alkaline condition (Rf = 0.4) and at 37˚C, two bands were seen: one in the acidic condition with an Rf of 0.6 and 0.5 in the alkaline condition. Antibiotic susceptibility testing showed varying zones of susceptibility and resistance in the different conditions to the antibiotics used. In the temperature condition, the isolates became resistant to Cefotaxime (CTX) and ColistinSulphate (CT) at −5˚C, Ceftolozane/Tazobactam (CT) at 4˚C, Cefotaxime (CTX) at 25˚C, and at 37˚C, it was resistant to Ceftolozane/Tazobactam (CT). Resistance was also seen in

the pH condition of Cefotaxime (CTX) in the alkaline condition. Conclusion: Exposure to these different conditions shows an increase in growth, biofilm, antibiotic resistance, and susceptibility in some conditions which indicates that these conditions promote virulence. The results in the study showed that different conditions of temperature and pH induce some virulence factors such as growth and biofilm. Different secretory proteins were found in different treatment conditions. These observations could be responsible for the resistance to some antibiotics seen in this study.

Keywords

Virulence, Biofilm, Antimicrobial Resistance, Secretory Molecules

1. Introduction

Escherichia coli are one of the most implicated bacteria in urinary tract infections, traveler's diarrhea, neonatal meningitis, and pneumonia. This bacterium is responsible for 12% - 50% of nosocomial infections and 4% of diarrheal diseases [1]. Most strains of *E. coli* colonize the gut and consequently prevent the attachment of pathogenic bacteria from binding to the host [2]. However, there are a hand full of strains that are responsible for morbidity and mortality; often associated with medical devices, such as prosthetic grafts, prosthetic joints, and shunts as well as catheters [3].

Escherichia coli are found in the gut of humans. Gut microbiota is exposed to different stress factors such as diet, temperature, pH, and microbial products. According to Falling Borg *et al*. [4], the intraluminal pH in the human stomach is 1.5 which can rapidly increase to 6.0 in the duodenum. There is a steady upsurge in the small intestinal pH from 6.0 to about 7.4 in the terminal ileum. This rise drops to 5.7 in the caecum, but further increases reaching a pH of 6.7 in the rectum.

Biotic and abiotic factors can influence the growth and survival of *E. coli* in natural environments [5]. The gastrointestinal tract is regarded as the main habitat of *Escherichia coli* in humans and generally not harmful to the host. These biotic and abiotic factors affect regulatory systems which eventually affect morphological and physiological changes that enable the organisms to adapt and ensure survival efficiently. Temperature and pH changes are amongst the major abiotic factors influencing the survival and its susceptibility under antibiotic stress [6]. The temperature may be the most important factor influencing the growth and survival of *E. coli* in the environment. While temperature is stable and optimal growth of *E. coli* in the intestinal tract of warm-blooded animals is obtainable at 36˚C - 40˚C, the temperature in the natural environment is generally low (<30˚C). For example, *E. coli* survived in sun-dried algal mats stored in airtight plastic bags at 4˚C for 6 months [7], thus indicating that *E. coli* can survive long term under temperature conditions lower than those of the hosts. Environmental pH can also influence the survival and growth of *E. coli* and the lev-

el of pH resistance varies by strains [8]. Environmental stresses can induce antibiotic resistance by a range of means such as the production of genes that regulate the expression of efflux pumps which are strongly expressed under environmental stress.

Bacteria experience stress from their initial moment of contact with the host, for most pathogens, this entails a temperature change. For bacteria transmitted by arthropod vectors, this also involves a transition from the insect gut to mammalian subcutaneous tissue or the bloodstream [9]. Respiratory pathogens must cope with an array of host-derived antimicrobial mediators, including bactericidal peptides produced by epithelial cells [10], and may also be required to adapt to nitrosative stress, hyperosmolarity, and oxygen limitation [11]. In contrast, enteric pathogens are ingested and must survive the hostile environment of the stomach, which is notable for a strongly acidic pH and the presence of reactive nitrogen species generated from dietary nitrate. Within the intestinal lumen, enteric pathogens encounter membrane-active antimicrobial peptides [12], bile salts, free fatty acids, enhanced osmolarity, and changing oxygen tensions. Host inflammatory responses recruit phagocytic cells, subjecting pathogens to oxidative and nitrosative stress [13]. To survive these changing environments, bacteria have developed exquisite systems that not only sense these stresses but also trigger appropriate responses that allow survival and propagation under these conditions. These conditions include; pH stress response: Upon exposure to acidic conditions, these bacteria upregulate expression of defense enzymes such as amino acid carboxylases [14] [15], deiminases, ureases, and F1F0 ATPase pumps [16] to help maintain pH homeostasis within the cell, Osmotic Stress Response: The long-term bacterial responses to osmotic stress involve the differential expression of transport systems as well as modifications to the composition of the cell membrane to survive hyper- or hypo-osmotic conditions [17], Pathogens encode for numerous direct oxidant detoxification enzymes to survive the harmful effects of oxidative stress encountered during host colonization and pathogenesis [18], Protein secretion plays a vital role in modulating the bacterial interactions with their environments, bacterial ribosomes synthesize up to 8000 different proteins [19].

This study aimed to determine the production of secretory molecules when *E. coli* is exposed to different conditions of pH, temperature, and oxygen. This research hypothesized that on exposure to these conditions, *E. coli* will be able to grow and produce certain virulence factors.

2. Methods

2.1. Collection and Storage of Organism

The organism used for the experiment was *E. coli* ATCC 252922 which was identified using PCR amplification of the 16SrRNA at Lahor Research Laboratories, Benin, Edo State, Nigeria. The organism was stored in 10% glycerol and kept at −20˚C. The organism was confirmed as *E. coli* using MacConkey agar.

2.2. Media Preparation

Tryptic Soy Broth (TSB) was prepared by dissolving 30 g of TSB in 1 l of distilled water. It was mixed thoroughly and sterilized by autoclaving at 15 psi and 120˚C for 15 minutes. Tryptic Soy Agar (TSA) was prepared by dissolving 28 g of TSA in 1l of distilled water. It was mixed thoroughly and sterilized by aut*oc*laving at 15 psi and 120˚C for 15 minutes.

2.3. Exposure Studies of *E. coli* in Acid and Base Conditions

Ten milliliters (10 ml) of TSB were aseptically aliquot into a sterile container. A wire loop was used to in*oc*ulate *E. coli* into the prepared TSB and incubated at 37˚C overnight. The overnight culture was diluted as 1:500 TSB. Two thousand microliter (2000 µl) of the diluted culture was put into the 24-well plate. 2 µl of concentrated hydro*ch*loric acid of final pH 4.0 and 2 µl of sodium hydroxide solution (of final pH 11.7) was added to the24-well plate. This was then incubated at different conditions including temperature; −5˚C, 4˚C, 25˚C, 37˚C, and oxygen condition (anaerobic and aerobic) for 48 hours. The anaerobic condition was provided by plating in a jar of citrate and sodium bicarbonate solution.

2.4. Antibiotic Susceptibility Testing

One hundred microliters (100 µl) of the growth from each of the exposure conditions in section 3.4 were cultured on TSA and antibiotics were added. Antibiotic disks (Amoxicillin/Clavulanate-AMC (30 µg), Cefotaxime-CTX (30 µg), Colistinsulphate-CT (10 µg), Imipenem-IPM (10 µg), and Meropenem-MEM (10 µg). The TSA plate was incubated at 37˚C overnight and zones of inhibition were measured in millimeters.

2.5. Biofilm Assay

The experiment in Section 3.4 above was performed and incubated for 48 hours. The broth was carefully removed and plates were air-dried. The biofilm was stained using 20% crystal violet and allowed to dry. The biofilm was re-suspended in 1000 µl of ethanol and the turbidity was measured at 590 nm using a spectrometer. The crystal violet stains the biofilm produced which is detected by the spectrometer as a complex composed of crystal violet and biofilm.

2.6. Spectrophotometric Analysis of Growth

The experiment in Section 3.4 above was performed and incubated for 48 hours at 37˚C. The growth of the bacteria was determined by measuring the optical densities in the different treatment options.

2.7. Thin Layer Chromatography Assay

With the aid of a capillary tube, about 5 µl of the different conditions were spotted on the TLC plate and air-dried for 10 minutes. The TLC plate was then placed in a beaker containing 10 ml of the mobile phase. The mobile phase was

obtained by adding 2 ml of ethyl acetate to 1 ml of methanol. Two grams of potassium permanganate in 10 ml of distilled water was used as the developer. It was then sealed completely and allowed to get up to the marked point. It was then allowed to air dry before placing in the developer and then rinsed with running tap water

2.8. Data Analyses

All experiments were performed at least in duplicate and on at least two independent occasions. Results were presented as mean ± SD where necessary. Where appropriate, statistical analyses were performed using an unpaired t-test in which a two-tailed P value was calculated (Graph Pad Prism Software Version 5.03, San Diego, CA). Statistical significance was defined as a P-value of less than 0.05 at a 95% confidence interval.

3. Results

3.1. Effect of Temperature and pH on the Growth of *E. coli*

Figure 1 shows the growth of *E. coli* exposed to different temperature conditions (−5˚C, 4˚C, 25˚C, and 37˚C) and pH condition [acid (4.0) and alkaline (11.7)]. For the acidic condition, the neutral condition was significantly higher by 1.25-fold than the 37˚C (P = 0.2453), by 3.84-fold significantly higher than the 25˚C (P = 0.0036), by 25-fold higher than the 4˚C (P = 0.0006) and by 6.25-fold higher than the −5˚C (0.0011). For the alkaline condition, the neutral condition is significantly higher by 2.38-fold than the 37˚C (P = 0.0102), by 7.14-fold significantly higher than the 25˚C (P = 0.0007), and 62.5-fold significantly higher

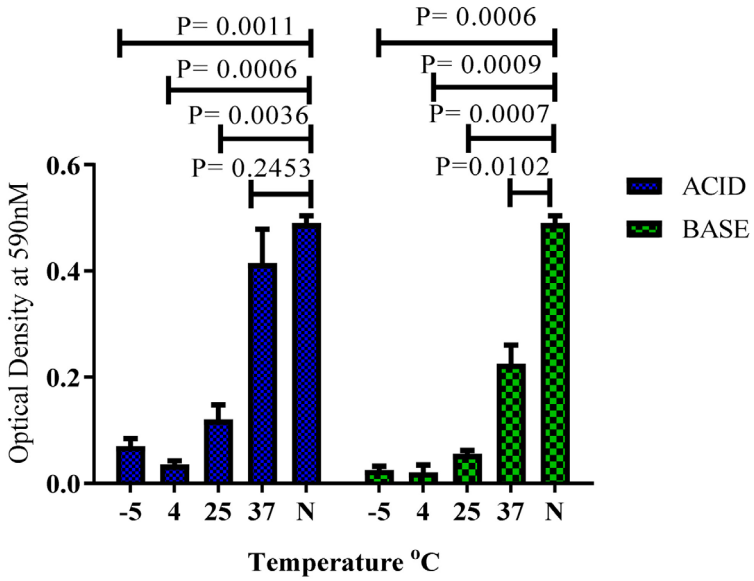

Figure 1. Growth Responses of *E. coli* under Different Temperatures and PH. *E. coli* treated in acid and alkaline were incubated at different temperatures and turbidity measured at 590 nm. Results were expressed as Mean ± SD. (P-value is significant <0.05). Key: N-Neutral.

than the 4˚C (P = 0.0009) and 50-fold significantly higher than −5˚C (0.0006). Generally, the organism grew better in the acidic conditions when compared to the alkaline condition at various temperatures.

3.2. Effect of pH and Oxygen Condition (Aerobic) on the Growth *E. coli*

Figure 2 shows the growth of *E. coli* exposed to pH condition [acid (4.0) and alkaline (11.7)] and grown in anaerobic conditions. In the acidic condition when compared with the control, there was no significant difference (P = 0.7515) and the control was 1.05-fold significantly higher in the acidic condition. The control was significantly higher (P = 0.0026) and was 4.44-fold significantly higher than the alkaline condition. Generally, growth was observed more in the acidic condition than in the alkaline condition.

3.3. Effect of pH and Oxygen Condition (Anaerobic) on the Growth *E. coli*

Figure 3 shows the growth of *E. coli* exposed to pH condition [acid (4.0) and alkaline (11.7)] and grown in an anaerobic condition. In the acidic condition, the control was significantly higher (P = 0.0009) by 2.5-fold. The control was significantly higher (P = 0.0396) by 2.5-fold than the alkaline condition. Generally, growth observed was the same in both conditions.

3.4. Effect of Temperature on Biofilm Formation of *E. coli*

Figure 4 shows the biofilm formation of *E. coli* when exposed to different

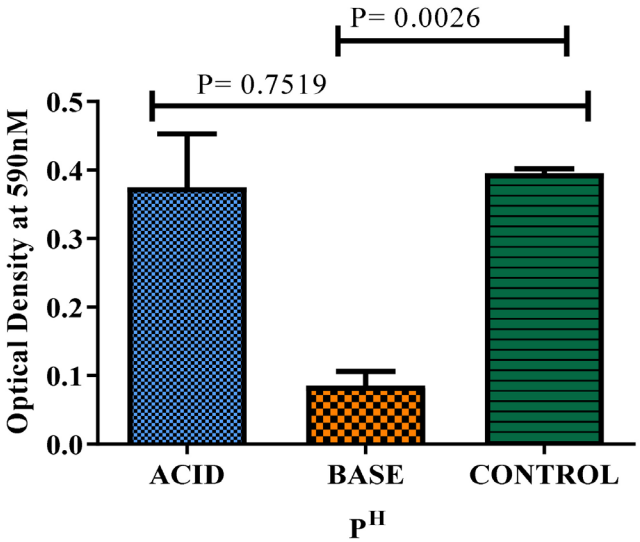

Figure 2. Growth Responses of *E. coli* under pH and Aerobic Condition. Isolate of *E. coli* treated with acid and alkaline under aerobic conditions. The growth was measured spectrophotometrically at 590 nm after overnight incubation. Results were expressed as Mean ± SD and the P-value was considered significant at a 95% confidence interval.

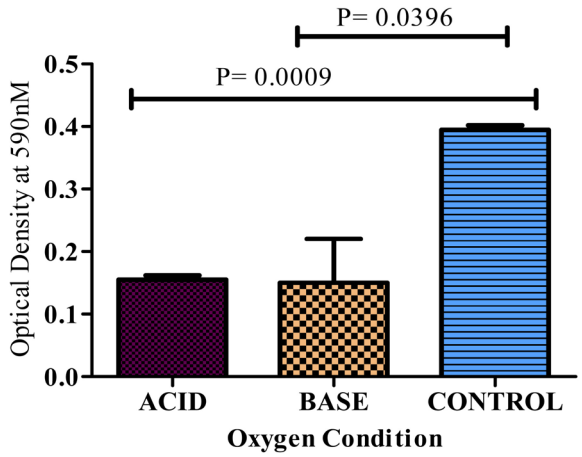

Figure 3. Growth Responses of *E. coli* under pH and Anaerobic Condition. Isolate of *E. coli* treated with acid and alkaline and incubated under anaerobic condition. The growth was measured spectrophotometrically at 590 nm. Results were expressed as Mean ± SD and the P-value was considered significant at 95% confidence interval.

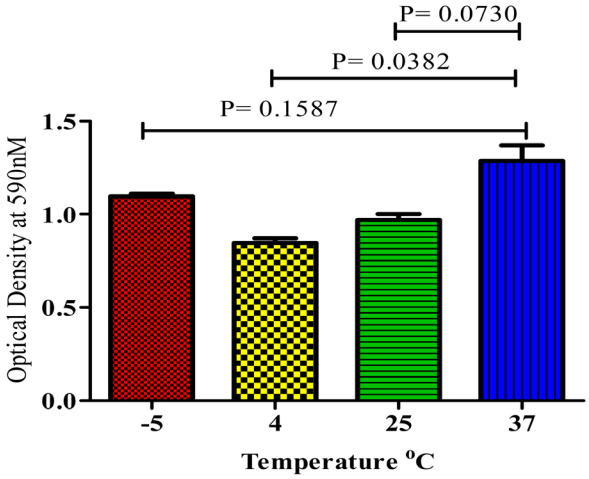

Figure 4. Effect of Temperature on Biofilm of *E. coli*. Isolates of *E. coli* at different temperature; (−5˚C, 4˚C, 25˚C, and 37˚C). The biofilm was measured spectrophotometrically at 590 nm. Results were expressed as Mean ± SD and the P-value was considered significant at 95% confidence interval.

temperatures (−5˚C, 4˚C, 25˚C, and 37˚C). Compared to 37˚C, there was no significance at 25˚C (P = 0.0730) but biofilm produced was 1.70-fold higher. There was significance at 4˚C (P = 0.0382) and was 1.70-fold higher. There was no significance at −5˚C (P = 0.1587) and was 1.14-fold higher.

3.5. Effect of pH on Biofilm Formation of *E. coli* under Aerobic Condition

Figure 5 shows the effect of pH in aerobic conditions. There was a decrease in

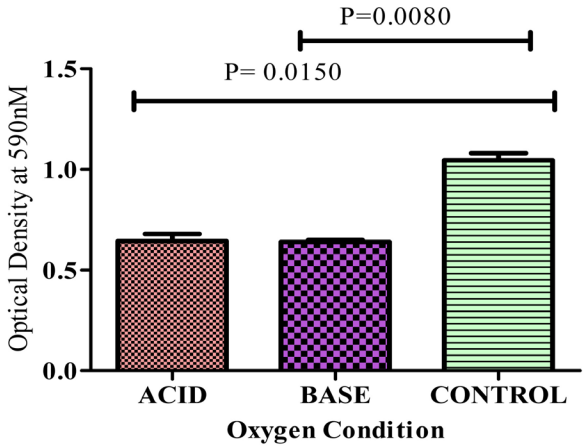

Figure 5. Effect of pH on Biofilm of *E. coli* in Aerobic Condition. Isolates of *E. coli* were incubated at 37˚C under aerobic condition. The biofilm was measured spectrophotometrically at 590 nm. Results were expressed as Mean ± SD. P-values were considered significant at 95% confidence interval.

the level of biofilm produced when compared to the control. The control was significantly higher than the acid (P = 0.0150) and alkaline (P = 0.0080) and by 1.42 fold in both conditions.

3.6. Effect of pH on Biofilm Formation of *E. coli* under Anaerobic Condition

Figure 6 shows the effect of pH in anaerobic conditions. There was a decrease in the level of biofilm formed when compared to the control. The control was significantly higher than the acid (P = 0.0483) and alkaline (P = 0.0290) and by 1.25-fold in both conditions.

3.7. Effect of Temperature, pH and Oxygen Conditions on the Level of Secretory Molecules by *E. coli*

Figure 7 shows the level of secretory molecules when *E. coli* isolates are treated with different temperatures (−5˚C, 4˚C, 25˚C, and 37˚C) and pH (acidic and alkaline conditions). At −5˚C, a band was spotted with a retardation factor (Rf) of 0.5. Also, at 25˚C degrees one band was seen in acid condition and another in the alkaline condition with Rf of 0.4 in both conditions. At 37˚C, two bands were also seen with Rf of 0.6 in the acidic condition and 0.5 in the alkaline condition. No band was seen at 4˚C.

3.8. Effect of pH and Oxygen Condition on the Level of Secretory Molecules by *E. coli*

Figure 8 shows the level of secretory molecules when *E. coli* isolates are treated with pH (acidic and alkaline conditions) and incubated under aerobic conditions. There was no band formation seen.

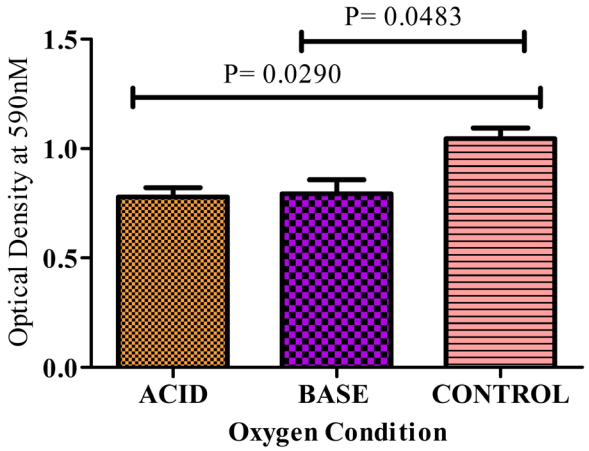

Figure 6. Effect of pH and on Biofilm of *E. coli* in Anaerobic Condition. Isolates of *E. coli* were incubated at 37˚C under anaerobic condition. The biofilm was measured spectrophotometrically at 590 nm. Results were expressed as Mean ± SD. P-values were considered significant at 95% confidence interval.

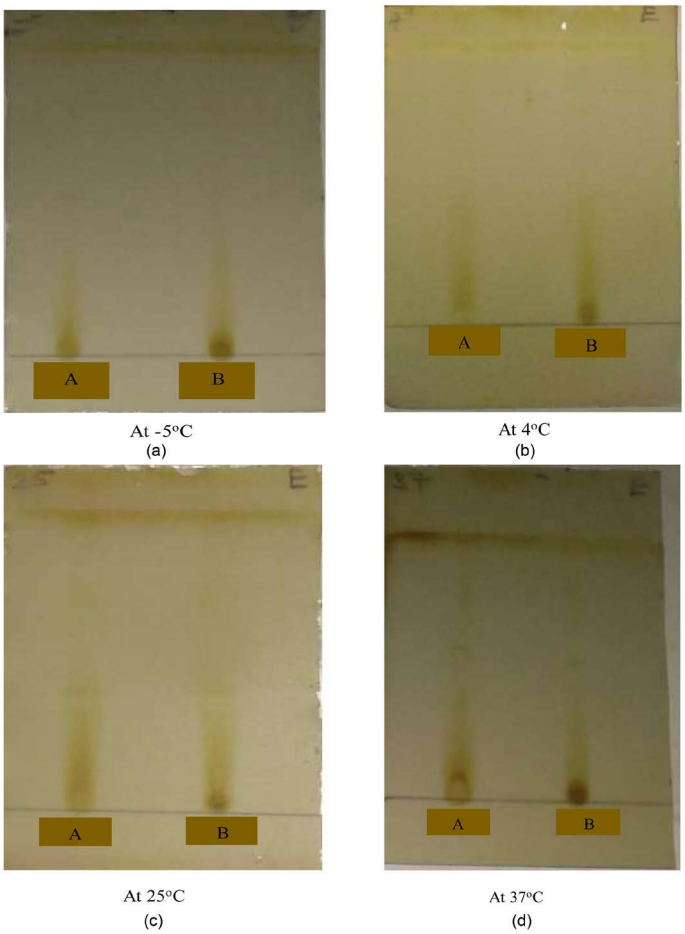

Figure 7. Effect of temperature and pH in the level of secretory molecules by *E. coli.* Isolates of *E. coli* were treated with acid and alkaline. Key: A-Acid, B-Alkaline.

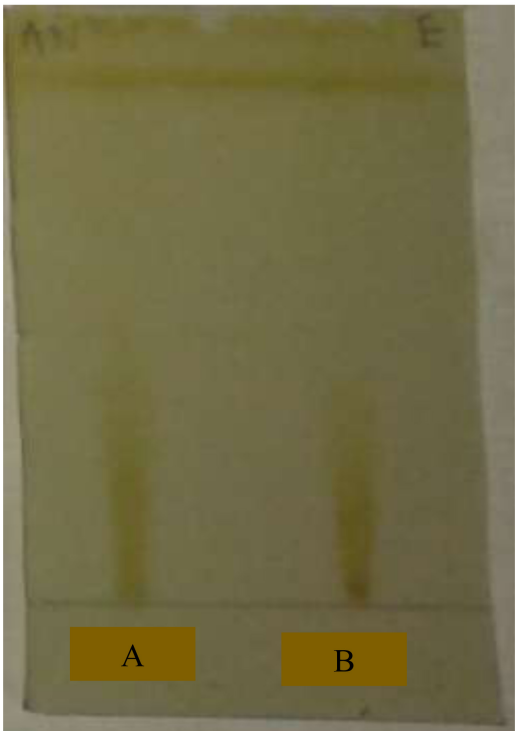

Figure 8. Effect of pH in aerobic condition on the level of secretory molecules by *E. coli*. Isolates of *E. coli* were treated with acid and alkaline. Key: A-Acid, B-Alkaline.

3.9. Effect of pH and Oxygen Conditions on Antibiotic Susceptibility of *E. coli*

Table 1 shows isolates of *E. coli* which were exposed to different pH and oxygen conditions (aerobic and anaerobic) and showed varying degrees of susceptibility when treated with known antibiotics (AMC, CTX, CT, IPM, and MEM). According to NCCLS, resistance is seen in bacteria when the zone of clearance is ≤20 millimeters (20 mm), moderately sensitive at 21 - 26 mm, and sensitive at >26 mm.

4. Discussion

For *E. coli* to colonize the human gastrointestinal tract, it must be able to grow between pH 4.5 and 9.0. Over this wide range of pH, *E. coli* preserves enzyme activity as well as protein and nucleic acid stability by maintaining the cytoplasm in the range of 7.2 to 7.8 [10]. At optimal temperature, pH, and oxygen conditions, organisms tend to grow and flourish. When these organisms find themselves in extreme conditions (e.g. high or low temperature) bacterial cells to prevent these hostile changes, selectively produce a specific set of proteins that enhance their survival [11]. This study was carried out to evaluate the survival and growth response of *E. coli* when subjected to varying temperature, pH, and oxygen conditions, which are similar to the conditions in humans and the environment.

Table 1. Effect of antibiotics on *E. coli* in different temperature, pH and oxygen conditions.

Temperature	AMC (mm)	CTX (mm)	CT (mm)	IPM (mm)	MEM (mm)
−5˚C	30 (S)	18 (R)	15 (R)	42 (S)	36 (S)
4˚C	24 (M)	36 (S)	20 (R)	28 (S)	30 (S)
25˚C	22 (M)	16 (R)	22 (M)	32 (S)	32 (S)
37˚C	28 (S)	24 (M)	10 (R)	34 (S)	36 (S)
		Aerobic pH			
EA	40 (S)	36 (S)	28 (S)	26 (M)	24 (M)
EB	30 (S)	20 (R)	28 (S)	34 (S)	36 (S)
EC	26 (M)	16 (R)	18 (R)	32 (S)	36 (S)
		Anaerobic pH			
EANA	32 (S)	24 (M)	14 (R)	26 (M)	30 (S)
EANB	30 (S)	18 (R)	12 (R)	24 (M)	28 (S)
EANC	26 (M)	16 (R)	18 (R)	32 (S)	36 (S)

Key: AMC; Amoxicillin/Clavulanate, CTX; Cefotaxime, CT; Colistin Sulphate, IPM; Imipenem, MEM; Meropenem, S; Sensitive, M; Moderately resistant, R; Resistant, EA; *E. coli* treated with Acid, EB; *E. coli* treated with Alkaline, EC; *E. coli* Control, EANA; *E. coli* in Anaerobic condition (acid), EANB; *E. coli* in Anaerobic condition (alkaline). R-Resistant with interval of ≤20 mm; M-Moderately Resistant with interval of 20 - 26 mm; S-Sensitive with interval >26 mm.

In this study, it was observed that *E. coli* growth was higher in the acidic condition than in the basic condition, although there were varying growth responses across the temperature conditions. At −5˚C, it was observed that growth was higher in the acidic condition than in the basic condition. The cold temperature slows down the growth of organisms but does not kill them. At 4˚C growth was also observed in both acid and basic conditions but there is a decrease in the growth level when compared to other conditions. Optimum growth was observed a 37˚C when compared to the neutral condition which showed a marked decrease at 37˚C. *Escherichia coli* is a mesophilic organism with a temperature range of 20˚C - 45˚C with an optimum temperature at 37˚C and a pH range of 3.4 - 3.6. This implies that there were growth responses in all conditions but optimum growth was observed at 37˚C when compared to the neutral followed by 25˚C and then −5˚C with the least growth response at 4˚C (**Figure 1**). In the anaerobic condition when *E. coli* was treated with acid and base, the growth response was the same, but there was a significant decrease when compared to the control. This implies that the organism grows optimally at neutral pH in anaerobic conditions than in acidic or basic conditions.

The survival of *E. coli* in these conditions might be due to the formation of biofilm and secretion of molecules and as such further analysis was carried out. Crystal violet biofilm assay was carried out to evaluate the level of biofilm formation under the various temperature conditions, pH, and oxygen conditions. In the temperature condition, the highest level of biofilm formation was highest

at 37˚C (serving as a control for the normal body temperature), this was followed by −5˚C, 25˚C and the least level of biofilm formation at 4˚C (**Figure 4**). In the pH condition, the level of biofilm formation was the same in both conditions but there was a significant decrease when compared with the control (neutral), and the same was seen in the anaerobic condition. The variation in the level of biofilm formation could be due to the varying temperature, pH, and oxygen conditions with optimal production at 37˚C and neutral pH.

One of the most important virulence factors of Enterotoxigenic *Escherichia coli* (ETEC) is its ability to produce and release toxins [12]. When organisms are stressed, they secrete certain molecules that help them survive in these stress conditions. In this study, these molecules were assayed for using thin-layer chromatography. Secretory molecules were assayed for under different temperature pH and oxygen conditions. In the temperature and pH treated condition, one band was spotted at −5˚C with a retardation factor of 0.5 in the acidic condition and none in the basic condition (**Figure 7(a)**). No bands were seen at 4˚C in both acid and basic conditions (**Figure 7(b)**) while at 25˚C two bands were seen; at the acidic condition with a retardation factor of 0.4 and at the basic condition with a retardation factor of 0.4 (**Figure 7(c)**). Also at 37˚C two bands were seen in both the acidic and basic condition with a retardation factor of 0.6 and 0.5 respectively (**Figure 7(d)**). In the pH treated condition grown in anaerobic condition, no band was seen (**Figure 8**). The bands seen indicate the secretion of certain molecules. This implies that the bands seen are secretory molecules that were produced by *E. coli*. The molecules could include proteins, amino acids, siderophores, bacteriocins, etc. that could have been produced and have helped the organism survive in these stress conditions. Also in the conditions where no bands were seen it could suggest that the secretions were inhibited.

Antimicrobial testing is carried out to determine the sensitivity and susceptibility of an organism to a wide range of antimicrobial agents [13]. According to NCCLS [20], resistance is seen in bacteria when the zone of clearance is ≤20 millimeters (20 mm), moderately sensitive at 21 - 26 mm, and sensitive at >26 mm. Antimicrobial susceptibility testing was carried out on the organisms treated in temperature and pH using the following drugs; Amoxicillin/Clavulanate (AMC), Cefotaxime (CTX), Ceftolozane/Tazobactam (CT), Imipenem (IPM), and Meropenem (MEM). In the temperature condition, at −5˚C AMC, IPM, and MEM were sensitive with a zone of clearance (Z˚C) of 30, 42, and 36 mm respectively while CTX, and CT were resistant with a zone of clearance of 18 and 15 mm respectively. At 4˚C, AMC was moderately sensitive (24 mm), CTX was sensitive (36 mm), and CT was resistant (20 mm), IPM, and MEM were sensitive (with a Z˚C of 28 and 30 mm respectively). At 25˚C, sensitivity was seen in AMC (22 mm), CT (22 mm), IPM (32 mm), and MEM (32 mm) while CTX was resistant (16 mm). At 37˚C CT was resistant (10 mm) while AMC, CTX, IPM and MEM were sensitive with Z˚C 28 mm, 24 mm, 34 mm and 36 mm respectively. The degree of susceptibility and resistance seen in the different temperature conditions suggests that the temperature might have affected the virulence of the or-

ganism. Pathogenic bacteria sense environmental cues including temperature, to regulate the production of key virulence factors [21]. It was noticed that AMC was sensitive most at −5˚C, 37˚C and 4˚C with the least level of sensitivity at 25˚C. CTX was most active against the isolates at 4˚C and 37˚C and the organism became resistant at −5˚C and 25˚C. CT was only sensitive at 25˚C and resistant at −5˚C, 4˚C and 37˚C. IPM and MEM were sensitive at all temperature conditions. This difference in bactericidal capacity of a drug usually depends on the cell wall composition of the bacterial cell [19] [22] [23]. This could be responsible for the phenomenon observed in this study where higher temperatures were showing better antimicrobial efficacies on the bacterium.

In the pH condition, the isolate was treated in acidic and basic conditions in aerobic and anaerobic conditions, antibiotic susceptibility testing was done using AMC, CTX, CT, IPM and MEM. The pH has previously been reported to have an important effect on the efficacy of antibiotics [22] [23] [24]. In the acidic (aerobic) condition, all the antibiotics were sensitive with the following zone of clearance; AMC (40 mm), CTX (36 mm), CT (28 mm), IPM (26 mm) MEM (24 mm). There were variations when compared with the control which gave the following zones of clearance, AMC (26 mm), CTX (16 mm), CT (18 mm), IPM (32 mm), and MEM (36 mm). In the control, CTX, and CT were resistant, while AMC, IPM and MEM were sensitive. This implies that the acidic pH might have caused the organism to secrete virulent factors [24] [25]. Previous studies have shown that *Klebsiella pneumoniae* [26] and *Staphylococcus aureus* [27] develop resistance when exposed to alcoholic herbal mixture called Goko alcoholic bitters. Alcohols are known to be weak acids, hence this study has replicate similar results. In the anaerobic acid condition, AMC (32 mm), CTX (24 mm), IPM (26 mm), MEM (30 mm) were sensitive while CT was resistant (14 mm). In the basic (anaerobic) condition, CTX (18 mm) and CT (12 mm) were resistant while the rest were sensitive having a zone of clearance as follows; AMC (30 mm), IPM (24 mm), and MEM (28 mm). Variations in the basic control were not seen in any of the antibiotics, as the sensitivity of the isolates was the same when compared to the control. Generally, the isolate was more sensitive in the aerobic condition than in the anaerobic condition.

5. Conclusion

Escherichia coli, one of the leading causes of UTI and diarrheal infections, are found in the gut of humans and animals as normal microbiota. They contaminate foods stored at different temperatures. This study showed that different temperatures of storage and pH condition potentiate virulence in *E. coli* via the production of biofilm, enhancement of their growth, and production of protein molecules. Some exposure conditions also showed the bacteria could acquire resistance to certain antibiotics.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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