

## Physicochemical studies on fecal isolates of *Escherichia coli* O157:H7 from people in Edo state, Nigeria

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### Abstract

The aim of this study was to investigate some physicochemical properties of *Escherichia coli* O157:H7 isolated from people in Edo state. This organism belongs to the group classified as “diarrhoeagenic” due to its ability to cause diarrhea in humans. Its ability to produce toxins and survive stomach acidity, contributes to its success as a food-borne pathogen. In this study, fecal specimens obtained from one thousand persons with gastrointestinal complaints were inoculated onto Sorbitol MacConkey agar (Oxoid, CM 813) supplemented with Cefixime-Tellurite (Oxoid SR 172) and incubated at 37°C for 24hr. Twenty-seven(27) specimens yielded *Escherichia coli* O157:H7 strains identified serologically using the *E. coli* O157 latex agglutination kit (Oxoid DR620), and standard biochemical tests. The effects of exposure of local isolates of the organism to experimental temperatures of 37°C, 50°C and 70°C, at varying pH values of 3.0., 7.0, and 9.0, were assessed by their viability on Eosin Methylene Blue agar plates(Oxoid CM 0069). This was carried out by taking the average colony counts of growth at intervals of 5, 10, 15, and 30 minutes of incubation at 37°C for 24 hours. *E. coli* O157:H7 was found to survive at 4°C, irrespective of acid, neutral or alkaline conditions of growth. Colony counts were found to be higher for isolates kept at neutral pH of 7.0 and highest in broth kept at 37°C than those kept at other temperatures. Strains of *E. coli* O157:H7 appear to be hardy organisms and proper sanitation at both personal and community levels would be required to effectively control disease occurrence due this organism.

**Keywords:** *Escherichia coli* O157:H7, stool specimens, physicochemical studies, Edo State, Nigeria

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## Introduction

*Escherichia coli* first described in 1885 by the German pediatrician, Theodor Escherich, was for many years simply considered as a commensal organism of the colon. However, *E. coli* O157:H7, an emerging disease pathogen belonging to the group of “diarrhoeagenic *E. coli*”, because of their diarrhea-causing potentials (Nataro and Kaper, 1998), and other established serotypes have evolved pathogenic propensities due to the acquisition of specific virulence factors by means of mobile genetic elements such as plasmids, transposons, bacteriophages, etc. (Kaper *et al.*, 2004).

Food-borne transmission of *E. coli* remains the most important means of transmitting infection. Most cases of infection are caused by ingestion of contaminated foods, particularly of bovine origin (Nataro and Kaper, 1998); cattle having been identified as reservoirs of the organism (Effler *et al.*, 2001; Robinson *et al.*, 2004).

The incubation period of *E. coli* O157:H7 diarrhea is usually 3 to 4 days; it may be sometimes as long as 5 to 8 days or as short as 1 to 2 days and symptoms in infected persons range from non-bloody diarrhea (Ostroff *et al.*, 1990), to hemorrhagic colitis alone or may herald the development of hemolytic uremic syndrome (Su and Brandt, 1995).

*E. coli* O157:H7 thrives in diverse environments—from soil, sewage, and water ecosystems to the host gastrointestinal tract. It can survive for long periods of time in water, especially at cold temperatures (Wang and Doyle, 1998). Production of a potent toxin is essential for many of the pathological features as well as the life-threatening sequelae of STEC infection (Karmali *et al.*, 1983). Pathogenesis involves a complex interaction between a range of bacterial and host factors. Orally ingested bacterial organisms (often in very low initial doses) must initially survive the harsh environment of the stomach and then compete with other intestinal microorganisms to establish intestinal colonization.

This study is aimed at isolating *E. coli* O157:H7 from people with gastrointestinal complaints and determining the effects of some physicochemical properties on local isolates.

## Materials and methods

### Subjects

One thousand (1000) fecal specimens were collected from persons of both sexes and of all age groups. Patients reporting at the hospital clinics with gastrointestinal tract complaints and who gave their individual consent were requested to submit their fresh stool specimens. Those on antibiotic treatment for the preceding two weeks before the visit were excluded from the study.

### Specimen collection

A small quantity of fresh stool specimen was collected in a sterile plastic universal container (Sterilin, UK), and immediately taken to the Research and Diagnostic

Laboratory, College of Medicine, Ambrose Alli University Ekpoma, Nigeria, for analysis. Specimens from distant cities were transported in Cary- Blair transport medium (Oxoid CM 0519) and inoculated onto agar media within 1- 2 hr of receipt in the laboratory.

### **Ethical consideration**

The Edo State Ministry of Health gave ethical approval (Ref: HA 547/67 of 4<sup>th</sup> June, 2009) for this study. The purpose of the research was also explained to the subjects, while parental consent was also got for under aged persons before collection of specimens.

### **Bacteriological procedures**

The specimens were streak- inoculated onto MacConkey Agar (Oxoid CM7), Eosin Methylene Blue Agar (Oxoid CM 0069) and Sorbitol MacConkey Agar (Oxoid, CM 813) enriched with Cefixime-Tellurite supplement (Oxoid SR 172) (used to selectively differentiate the non-sorbitol-fermenting *E. coli* O157:H7 strains from other *E. coli* strains). Plates were incubated aerobically at 37°C for 24 hr to isolate *E. coli*. Each colonial morphology and reactions on agar media, like colony size, consistency, shape and pigmentation, lactose and sorbitol fermentation, were noted while the colonies were Gram stained, and their motility tested, using the simple hanging-drop technique.

### **Serotyping of *E.coli* isolates.**

All non-sorbitol-fermenting *E.coli* colonies (at least 5 colonies from each plate) were tested for agglutination with O157 latex reagents (Oxoid DR620) to determine if the isolates belonged to the O157 serogroup (Nataro and Kaper, 1998).

### **Biochemical identification of *E. coli* O157: H7.**

Presumptive *E. coli* colonies were identified using standard biochemical techniques (Cheesbrough, 2006). Strains of *E. coli* that agglutinated with O157 latex reagents were further tested for beta-glucuronidase activity using Sorbitol MacConkey agar with BCIG (Oxoid CM O981) as a differentiating chromogenic medium. Suspect colonies that were negative for beta-glucuronidase, and could not ferment cellubiose but fermented dulcitol and raffinose were confirmed as *E. coli* O157: H7 serotype (Thompson *et al.*, 1990).

### **Determination of the effect of temperature and pH changes on growth of *E. coli* O157:H7 isolates**

The effect of exposure to temperature and pH changes on the *E. coli* O157:H7 strains were determined by assessing their viability on culture media. Using a sterile wire loop, 2 to 3 colonies from a purity plate of the isolate were transferred into about 5 ml sterile peptone water and incubated aerobically overnight at 37 °C for 24 hr.

1 in 10 dilution of the overnight broth culture was made by transferring 1ml of it into 9ml sterile peptone water in three separate McCartney bottles with broth adjusted to the desired experimental pH values of 3.0, 7.0 and 9.0 respectively using a pH meter. These were left at 4°C. The above experimental protocol was repeated at 37 °C, 50°C, and 70°C. 1ml of each broth was in turn spread over the surface of each of two dried

EMB agar plates (Oshioma *et al.*,2009), at intervals of 5, 10, 15 and 30 minutes. Plates were incubated at 37°C for 24 hr after which the average colony counts were made.

## Results

A total of three hundred and sixteen (316) *E. coli* was isolated from 1000 stool specimens collected from people all over Edo state, giving a prevalence rate of 31.6 percent. Table 1 shows the distribution of organisms isolated from stool specimens of persons studied. *E.coli* alone occurred as the greatest proportion of microbial isolates (31.6%). While there were mixed bacterial growth in fifty specimens (5%), forty (4%) of the fecal samples yielded no microbial growth.

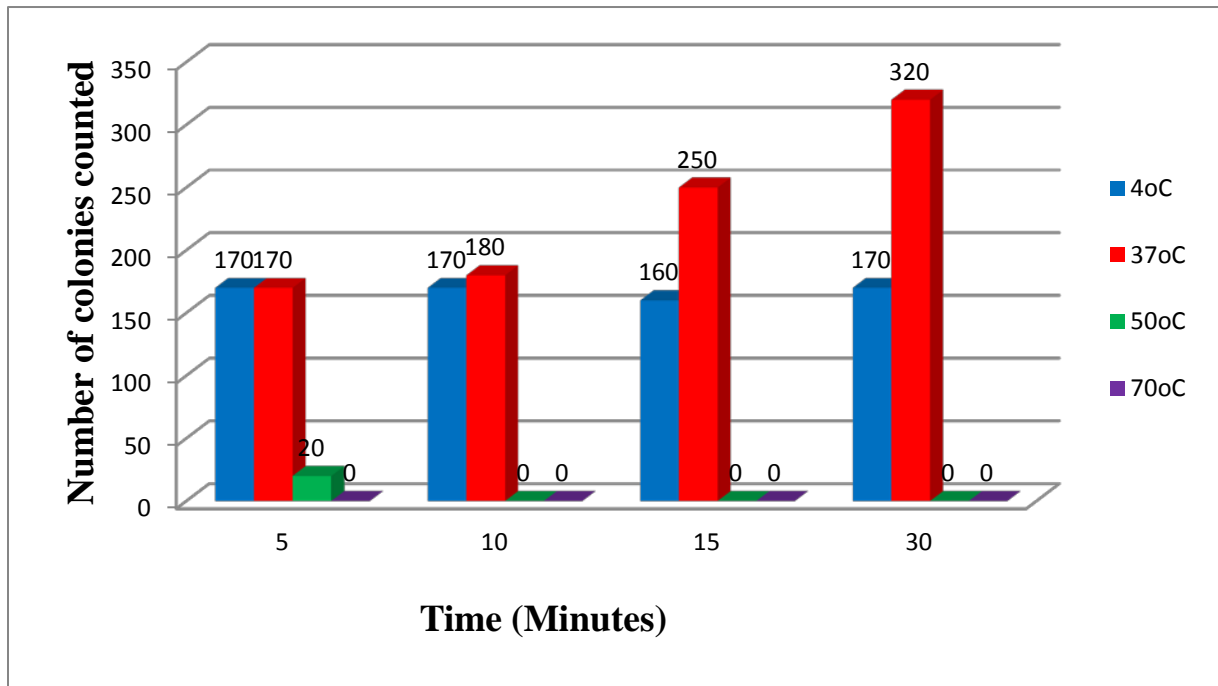
Of the 316 *E.coli* isolates, 27 (8.5%) were biochemically and serologically confirmed as *E. coli* O157:H7 (Table 2). The different colony counts of bacteria growing on media exposed to different experimental temperatures and pH are shown in Figures 1- 3.

**Table 1. Distribution of microbial isolates from fecal specimens**

Microbial growth	Number of isolates	% Isolation
Gram negative bacteria	402	40.2
<i>Escherichia coli</i>	316	31.6
Gram positive bacteria	135	13.5
Yeast cells	57	5.7
Mixed bacterial growth	50	5.0
No growth	40	4.0
<b>Total</b>	<b>1000</b>	<b>100</b>

**Table 2. Prevalence of *Escherichia coli* O157:H7 isolates**

<i>Escherichia coli</i> isolates	Number of Isolates	% Isolation
<i>E.coli</i> O157:H7 strains	27	8.5
Non- O157 <i>E.coli</i>	289	91.5
<b>Total</b>	<b>316</b>	<b>100</b>



**Figure 1. Colony counts at different temperatures and pH 3.0.**

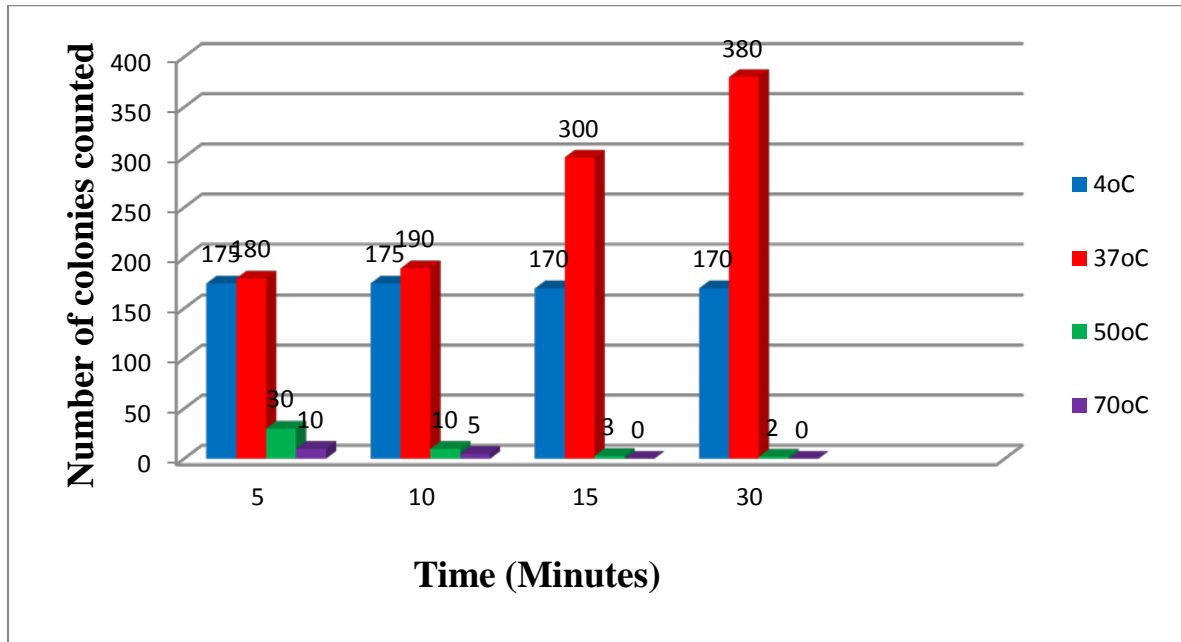


Figure 2. Colony counts at different temperatures and pH 7.0.

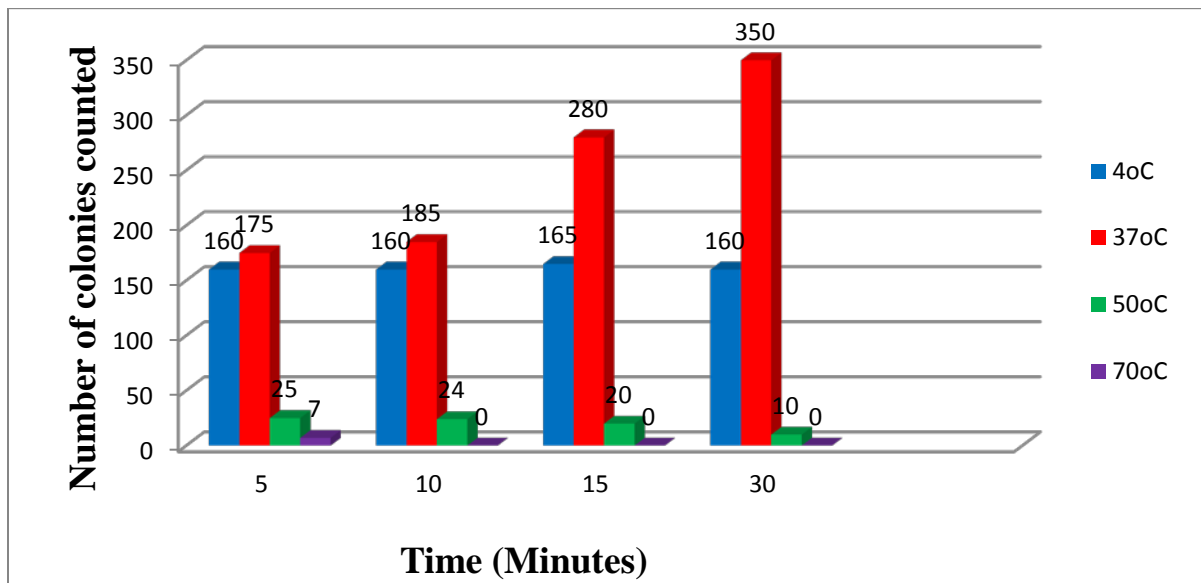


Figure 3. Colony counts at different temperatures and pH 9.0.

## Discussions

The results of colony counts made for *E. coli* isolates at different experimental temperatures and pH changes (Figures 1-3) show that temperature and pH changes affect growth of organism. Although it has been reported that the minimum growth temperature of *E. coli* O157:H7 under optimal conditions is approximately 8-10°C (Rajkowski and Marmer, 1995). *E. coli* O157:H7 can survive for a long time in water, especially at cold temperatures (Youn *et al.*, 2010). The present study has also shown that *E. coli* O157:H7 strains can survive at a temperature of 4° C, irrespective of acid, neutral or alkaline conditions of growth. As shown in Figures 1-3, there was no significant reduction in colony counts of organism at 4° C over the time periods;  $P > 0.05$ .

Ritchert *et al.* (2000) reported the survival of *E. coli* O157:H7 on produce held at 4° C. Arias *et al.* (2000) have equally demonstrated the survival and multiplication of this pathogen even at 0° C. The public health implication of this is that consumers of refrigerated foods such as fruits and vegetables should take extra care to decontaminate such foods before consuming them.

It was also observed that in all cases colony counts were highest in broth kept at 37° C than for those kept at other temperatures. 37° C is a suitable temperature for growth of most medically important bacteria (Cowan, 1993). There was enhanced growth of *E. coli* O157:H7 isolates at 37° C as shown by the increase in colony count with increasing time. More counts were recorded at 30 min than at 5 min (Fig. 2). However, this increase would have been halted by other factors involved in the growth dynamics of a bacterial population, were the sampling time further prolonged beyond 30 min.

Although *E. coli* are differentiated from other *Enterobacteriaceae* on the basis of their ability to grow and produce gas in *E. coli* broth at 44.5° C, many *E. coli* O157:H7 strains, however, do not grow well, if at all, above 44° C (Doyle and Schoeni, 1984). In this study, while growth was retarded at 50° C, as recorded by the scanty growth of discrete colonies on the agar medium, all broth cultures exposed to 70° C for 30 min recorded zero colonies on media. This observation conforms with the practical basis for the recommendation that foods could be made safe for consumption by cooking them to a minimum temperature of 161° F (71° C) (Buchanan and Doyle, 1997; CFSPH, 2009).

The greater colonial counts recorded for the isolate exposed to the neutral pH of 7.0 (Fig. 2), could be a reflection of the fact that the pH of mammalian blood and tissues is of this order. Most pathogenic bacteria have a fairly restricted pH range and grow best around pH 7.5, that is, at a slightly alkaline reaction (Cruickshank *et al.*, 1975). Growth rates of EHEC decline rapidly at low pH values and the minimum pH for *E. coli* O157:H7 to grow is 4.0 – 4.5. According to Buchanan and Doyle (1997), this is dependent on the interaction of pH with other growth parameters. EHEC strains have a high degree of acid tolerance and can survive almost unchanged after 2-7 hours of exposure to pH 2.5 and 37° C. This ability of the organism to grow in foods of low pH under conditions where other pathogens would not survive was a contributory factor in

previous outbreaks linked to consumption of unpasteurized apple juice (McCarthy, 1996)

Since growth of *E. coli* O157:H7 can withstand to some extent extreme physiological conditions as shown in this study, we cannot take for granted that foods and food practices that have been traditionally safe will always remain so in the future. Continued vigilance, proper sanitation at both personal and at the level of the community, as well as the ability to rapidly mobilize research capabilities is required mostly in developing countries of the world.

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