Survival of *Escherichia coli* in Food at Hot-Holding Temperatures

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**ABSTRACT**

Foods usually served hot were held at various hot-holding temperatures [40°C (104°F) - 60°C (140°F)] and were contaminated with fecal *Escherichia coli*. The contaminated hot foods were held for 1 h at each of the hot-holding temperatures during which the survival of the pathogen in each food type was evaluated. Results showed that *E. coli* survived hot-holding temperatures in each food type for the whole period of evaluation. A population increase occurred with time at temperatures below 50°C (122°F), while at and above this temperature there was a decrease in population with increasing time in each food type. A two-way analysis of variance using relative rates of increase or decrease (±b) showed food type to be unimportant for survival of the bacteria. A three-way analysis of variance of the same results using mean log CFU/g food showed holding temperature, food type, holding time, and the interactions of temperature and food type; and temperature and time to be significant important for survival of the bacteria. The public health significance of these findings are discussed.

Before 1968 *Escherichia coli* was not recognized as a foodborne pathogen; it was only regarded as a general inhabitant of the human intestinal system. A variety of foods which included coffee substitutes, ohagi (red bean-balls), stewed meat, gravy, ham, pies, cheese, pork, chicken, salmon, roast mutton, mashed potatoes, creamed fish and cream pies have since been implicated in *E. coli* diarrhea (1,7,8,22). Foodservice establishments have also reported 8% of 240 hospital personnel in San Antonio, Texas, suffered foodborne *E. coli* gastroenteritis. Eating in the hospital cafeteria was associated with the illness. Merson et al. (12) isolated enteropathogenic *E. coli* in 63% of conference participants in which 49% developed traveller’s diarrhea. Food was implicated as the likely vehicle. Sack and others (16) reported 8% of 240 *E. coli* isolates from food and that the microorganisms were responsible for 307 outbreaks of foodborne diseases in the U.S. in 1973. *E. coli* 06:H16 was responsible for 452 cases of a foodborne diarrhea outbreak in 1982 that resulted from dining in a restaurant that served both Mexican and American foods. A food handler was deduced as the source of the infection, which later spread to food ingredients by cross-contamination. Day et al. (5) isolated *E. coli* 0157:H7 from patients in two states who had consumed hamburgers before onset of illness.

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rather long periods before and during service. *E. coli* was chosen as test organism because it is ubiquitous in nature, and it is assumed that foods can easily become contaminated with it from a variety of sources.

**MATERIALS AND METHODS**

An isolate of enteropathogenic *E. coli* (0111:B4), was obtained from the Medical Microbiology Laboratory of Texas Children’s Hospital. The bacteria were transferred to 35 ml of alkaline peptone broth and were held as stock culture. Cultures on Endo Agar (BBL) produced shiny metallic or opaque red colonies after 24 h of incubation at 37°C (98.6°F). Cultures were made weekly. Fresh stock cultures were incubated for 4 h at 37°C (98.6°F) before use and were held at room temperature thereafter.

Cooked rice, peas, macaroni and cheese and meatballs were obtained from the Food Production unit of the University of Texas M.D. Anderson Hospital and Tumor Institute. Foods were formulated, cooked, and handled in the usual routines used in the cafeteria service of M.D. Anderson Hospital, and were given no special treatment. The foods were obtained hot and transferred from the cooking vessels to sanitized steel containers and taken to the laboratory for experimentation.

Five water baths were set to constant temperatures of 46°C (114.8°F), 50°C (122°F), 55°C (131°F), 60°C (140°F) and 64°C (147.2°F) to simulate food-holding temperatures of 40°C (104°F), 50°C (122°F), 55°C (131°F) and 60°C (140°F). A thermometer was put in each water bath to monitor the temperature fluctuations which were maintained within ±2°C (±3.6°F). In preliminary tests, higher temperatures killed test organisms in the shortest time periods, and therefore these higher temperatures were not used in these experiments.

Between 200 and 250 g of food were weighed on a Triple Beam Balance and put into an aluminum pan (Reynolds Redi-Pans) of dimensions 7.875 in. × 4.875 in. × 2.125 in. The pan was put in a constant temperature water bath. This provided an indirect way of bringing the foods to a constant/holding temperature. A thermometer was also put in each pan containing food to monitor the food temperature fluctuations. To allow equilibration of temperatures between the constant temperature water bath and the food in the pan, 30 min were allowed for heating depending on whether the food was used soon after collection or was refrigerated at 5°C before experimentation.

After heating the food to constant temperature, 0.05 ml of *E. coli* stock broth culture was added with a pipette drop by drop. This achieved a food contamination level of 10²-10⁶ *E. coli* organisms/g. One food type was experimented with at a time. After the bacteria were thoroughly mixed with the food, a 25-g food sample was immediately withdrawn. This sample was considered collected at time zero. Further 25-g food samples were withdrawn after holding the contaminated food for 10, 20, 40 and 60 min.

Each food sample was blended in a Waring Commercial Blender at low speed for 1 min with 100 ml of physiological saline solution. One ml was immediately pipetted into 99 ml of physiological saline solution in a dilution bottle. Serial dilutions were made and plated on Endo Agar. The plates were incubated for 24 h at 37°C (108.6°F). Colony forming units (cfu) in each plate were counted with the aid of a Darkfield Quebec colony counter. Bacterial survival was estimated as cfu/g food.

**RESULTS**

Logarithmic transformation of cfu/g food obtained for each food type at each holding temperature was done (13,18-20). Mean log cfu/g of food obtained from several experimental determinations for the survival of *E. coli* in each food type at each holding temperature was plotted against holding time (Fig. 1-4).

Relative rates of survival, depicted by growth or decline (±b) of the bacterial population in each food type at each holding temperature were computed by using the bivariate linear regression model (18-20). A two-way analysis of variance was made to determine the effect of temperature and food type on bacterial survival (Table 1). A three-way analysis of variance was also constructed using the mean log cfu/g food to determine the effects of food type, holding temperature and holding time and their interactions on the survival of bacteria (Table 2).

![Figure 1. Survival of E. coli in rice at hot-holding temperatures.](https://example.com/figure1.png)

![Figure 2. Survival of E. coli in peas at hot-holding temperatures.](https://example.com/figure2.png)
Figures 1-4 show the general survival trend for the bacteria in the different food types. Bacterial growth occurred when food was held at 40°C (104°F) and at 45°C (113°F). Above these holding temperatures, E. coli numbers decreased with increasing holding temperature and time within each food type. There were also differences in E. coli survival by food type. Although the finding (Table 1) that food type was unimportant for bacterial survival agrees well with the one reported earlier (9), analysis of the same data using the actual log_{10} cfu/g data showed that food type was an important factor in bacterial survival (Table 2). It does appear from these contradictory findings that the use of relative rates of growth/decline masks the true effect of food type in E. coli recovery. Interactions of the main effects, holding temperature and food type; and that of temperature and time (holding) were also found important to E. coli survival in hot-held foods. The interaction of food type and holding time was unimportant for the survival of the microorganism.

It is apparent from this study that hot-holding temperatures generally employed in the foodservice industry do not eliminate E. coli should the food become contaminated before or during hot-holding. E. coli survived even the usually recommended temperature of 60°C (140°F) for 1 h. Although cooking temperatures would practically kill this microorganism, its ubiquity in nature plus the unhygienic practices of foodhandlers, both compound the difficulty of controlling direct and indirect contamination, even of hot-held foods.

Although it is generally recommended that hot foods should be held at or above 60°C (140°F) (1,6,7,15), such...
foods may often be held below this recommended temperature to avoid overcooking and drying of food, and to preserve the flavor (14), or to maintain the aesthetic appeal of some foods. For example, green peas or beans if held at higher temperatures for a long time, will discolor and become aesthetically unacceptable. Although holding hot foods at lower temperatures has its own merits, it increases the chance of infection to the consumer since these temperatures may allow for proliferation of bacteria, should the food become contaminated with even low doses of microbes.

Preparation of sterile food is neither necessary, required, nor practical (17). However, a case is made in this study for serving food that is free from potentially pathogenic organisms in the interest of public health. Holding hot foods at high temperatures to control the growth of potentially contaminating pathogenic bacteria had been relied on for its implied safety. This study brings into question the reliability of these hot-holding temperatures should contamination of food occur after cooking and during hot-holding. It is also contended in light of the present findings that use of a single hot-holding temperature is not suitable for all food types, although temperatures of 50°C (122°F) and above tend to lower the survivability of the bacteria with increasing hot-holding time. The effectiveness of a hot-holding temperature on reducing or eliminating the contaminating pathogens therefore, depends on both the food type and the length of hot-holding.

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