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PREVALENCE OF *ESCHERICHIA COLI* 0157:H7 IN FRESH AND ROASTED BEEF IN KANO CITY, NIGERIA

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ABSTRACT
The prevalence of Enterohemorrhagic Escherichia coli 0157:H7 in 300 fresh beef and 150 roasted beef samples from Kano city Nigeria was determined, by direct plating on Sorbitol MacConkey agar (CT-SMAC) supplemented with Cefexime 50µg/L and Potassium tellurite 25mg/L and resuscitation on Trypticase Soy Broth (TSB) at 25^{0}C for two hours for roasted beef samples. Presumptive colonies were confirmed by using E. coli 0157:H7 latex agglutination test kit. Prevalence rate of 53% was obtained in fresh beef and 25.3% in roasted beef. Consumption of inadequately cooked beef poses a serious risk of infection. The study therefore stressed the need for health authorities to educate and put in place efficient surveillance strategies for detection and control of possible outbreaks of E. coli O157:H7 in the society.

Keywords: Pravalence, Escherichia coli 0157:H7, Beef

INTRODUCTION

Enterohemorrhagic *Escherichia coli* 0157:H7 is an important food-born pathogen (Benjamin and Datta, 1995) which was first identified in 1982 as a cause of hemorrhagic colitis during outbreaks of bloody diarrhea in Oregon and Michigan, USA. After these cases, several outbreaks of hemorrhagic colitis and hemolytic uremic syndrome caused by this organism have been epidemiologically linked to consumption of ground beef (Schlundt, 2001). The likely cause of *E. coli* O157:H7 infection is undercooked ground beef. Additionally, unchlorinated water, raw milk, cold sandwiches and vegetables have been implicated as sources of some outbreaks. Other implicated foods include unpasteurized apple cider and juice, salad dressing containing mayonnaise, home-made yoghurt, frozen meats, turkey roll and clams (FAO/WHO, 2003). Since then a number of works have reported the incidence of *E. coli* O157:H7 in food and during outbreaks (Hancock *et al*., 1994; Karch *et al* 1996; Uzeh *et al.,* 2006; Agbogu *et al.,* 2006). Mead *et al* (1999) has estimated the incidence of *E. coli* 0157:H7 to 50% among EHEC serotype in relation to public health problems. This study was planned to investigate the presence of *E. coli* O157:H7 in retail fresh beef and roasted beef sold in Kano metropolis.

MATERIAL AND METHODS

Three hundred (300) samples of fresh beef and one hundred and fifty (150) roasted beef samples each were randomly collected from retail markets in a sterile polyethene bags, from the six metropolitan Local Government Areas of Kano State. The samples were analyzed within two hours of collection.

One gram (1g) of fresh beef samples each was aseptically collected placed in a sterile blender and homogenized in 9 ml of (1%) peptone water for 1 minute to obtain a homogenate. The homogenate was further serially diluted up to 10^4 (FAO, 1979). It was then cultured on CT-SMAC for *E. coli* 0157:H7. However, for roasted beef, 1g of each sample was transferred into 9ml Trypticase Soy Broth (Lab M) shaken and incubated at 20^{o}C for 2h (Kudo *et al.,* 2000), then 1ml each of the incubated broth was serially diluted in peptone water to 10^2. All the samples were cultured on Sorbitol MacConkey agar CT-SMAC (U.S Biological, Swampsctt) supplemented with Cefexime (50µg/L) and Potassium tellurite (2.5 mg/L) at 45^{o}C (March and Ratnam, 1986). The plates were allowed to solidify and incubated at 37^{o}C for 24 hours (Chapman *et al*., 1994; Karch *et al.,* 1996). The population of the non-sorbitol fermenters in the samples was determined by counting the colony forming unit (cfu/ml) (Wells *et al*., 2005), and confirmed by using Latex agglutination test Kit (anti-o157:H7 antibody for *E. coli* 0157:H7) test kit DR0620M (Oxoid LTD Hamspshire, England) as described by Nataro and Kaper (1998). The data obtained was analyzed by the use of ANOVA statistical analysis on SPSS package.

RESULTS

The result of the analysis of fresh beef and roasted beef samples is as presented in Table 1. The fresh beef has a mean NSFC count of 1.5 x 10^6 cfu/g and roasted beef had a mean of 3.0 x 10^2 cfu/g. Out of 300 fresh beef sampled, 163 yielded NSFC out of which 158 were confirmed to be serologically positive *E. coli* 0157:H7. Of the 150 sampled roasted beef samples, 38 yielded NSFC and the 38 were confirmed to be *E. coli* 0157:H7.

Table 1: A comparative distribution of serologically confirmed *E. coli* 0157:H7 isolated between fresh and roasted beef

Meat sample	No. of sample Collected	Mean SFC counts (cfu/g)	Non-sorbitol fermented colonies	Serologically Confirmed	Percentages
Fresh Beef	300	1.5 x 10^6	163	158	53%
Roasted Beef	150	3.0 x 10^2	38	38	25%

Key:

SFC = Sorbitol fermenting colonies, NSFC = Non Sorbitol fermenting colonies, cfu/g = Colony forming unit per gram, EHEC = Enterohemorragic *E. coli* (0157:H7)

DISCUSSION

Fresh beef was found to be more contaminated than Roasted beef samples, with mean SFC counts of 1.5 x 10^6 cfu/g (Table 1). This might be as a result of so many factors at the slaughtering and skinning points. These include contamination from external sources like air, soil, use of non portable water and improperly washed utensils. It might also be from internal sources like intestines content, lymph nodes, as well as cross contamination by meat handlers. All these could contribute to the microbial contamination of fresh beef as described by Umoh (2001). The evidence of contamination as the most potential source of *E. coli* 0157:H7 in beef was vividly highlighted further by Elder *et al.* (2000) that meat only becomes contaminated with *E. coli* 0157:H7, when in contact with contaminated hide and or feaces during slaughter process. Evidently, *E. coli* 0157:H7 has been isolated from feaces or gastrointestinal tract of cattle, sheep, horses, pigs, turkeys, dogs and a variety of wild animals (Hancock *et al.,* 1998). However carcass contamination may vary with season, plant design and operation, geographic area, location within the plant, and to some extent, anatomical carcass site (Sofos *et al*., 1999). The 53% prevalence rate in fresh beef is a clear indication of heavy contamination. This agrees with the work of Petridis *et al.* (2006) who reported undercook ground beef implicated as a source of contamination that led to the infection of 243 in Montana USA. Hancock *et al.* (1994) also reported a prevalence rate of *E. coli* 0157:H7 in herds of dairy cattle to be 8.3% and 16% from pastured beef cattle herds.

Although Roasted beef is least contaminated (25%) having a mean counts of 3.0 x 10^2 cfu/g (Table 1) and showed no significance difference between counts of samples. Uzeh *et al.* (2006) reported a total bacterial count on *Trire-suya*, (a local delicacy) in Nigeria, to be in the range of 7x 10^1 to 42 x 10^1 cfu/g an indication of high contamination, thus the mean counts of 3.0 x 10^2 cfu/g of NSF shows more contamination (Uzeh *et al.*, 2006). Addition of spices is a tradition in local roasted beef preparation and have been shown to exhibit antimicrobial effect, majority of which is linked to their Phenolic compounds (Shelef, 1984), however spices have played an insignificant inhibitory role, if any, as to the growth and survival of *E. coli* 0157:H7. According to Frazier and Westhoff (2006) spices and condiments do not have a marked bacteriostatic activity in the concentrations normally used and unless they are treated to reduce their microbial loads, they may add high numbers and undesirable types of microorganisms in the food in which they are used. Similarly, Uzeh *et al.* (2006) have reported the resistance of the activity of *Afromomum melagueta*, *Piper quinense*, and *Capsicum fructescens* spices by *Psuedomonas aeruginosa* on *Tsire-suya.* The percentage prevalence *E. coli* 0157:H7 in roasted beef (25.3%) might be partly due to pre-enrichment on liquid and non selective medium, which enhances recovery of the injured cells (Kudo *et al*., 2000; Clavero and Beuchat, 1995).

In addition, during roasting of beef, heat is expected to have denatured microorganisms present, yet *E. coli* 0157:H7 was isolated, though it's optimal growth temperature is approximately 37^0C (98.6^0F), and is not reported to grow at temperatures below 8°C to 10°C (46^0F to 50^0F) or above 44^0C to 45^0C (Buchanan and Doyle, 1997). However, Ansay *et al.* (1999) reported *E. coli* O157:H7 survives freezing with some decline in concentration. Equally, Clavero and Beuchat, (1995), recovered 5 strains of *E. coli* 0157:H7 at 568^0C for 0, 15 and 30 minutes by using a non selective medium (Trypticase Soy Broth), with decrease in the microbial load in the samples, as the heating time increased. This signifies that, *E. coli* 0157:H7 could survive temperatures above 45^0C.

CONCLUSION

The results of this investigation in Kano metropolis show a high prevalence rate of *E. coli* 0157:H7 (53% in fresh beef and 25% in roasted beef) could be attributed to lack of good hygienic practice right from abattoir, during handling and transportation of carcass to the market. Thus, intensive sanitary measures should be taken to establish reliable hygienic standard in all operations. In view of this, health authorities and researchers have a duty to educate and put in place efficient surveillance strategies for detection and control of outbreaks of *E. coli* O157:H7 in the society.

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