

## RESEARCH ARTICLE

## ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH EXPIRED SAUSAGE ROLLS

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## ARTICLE DETAILS

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## ABSTRACT

The study was undertaken to determine the microbial quality of expired sausage rolls. Samples were analyzed for the presence of microorganisms using appropriate selective media. Inoculation was done using spread plate techniques. The total viable count (TVC) of bacterial population in all the expired sausage rolls were in the range of  $2 \times 10^4$  to  $3 \times 10^4$  cfu/g. Beeti sausage roll recorded the highest number ( $3 \times 10^4$  cfu/g) of bacterial growth. The total coliform count (TCC) ranges from  $5 \times 10^3$  to  $1.3 \times 10^5$  cfu/g; Chi-Super Bite sausage rolls have the highest coliform count. The range of *Staphylococcus aureus* count was  $1 \times 10^4$  to  $4.1 \times 10^4$  cfu/g with Chi-Super Bite having the highest. The frequency of the six genera isolated showed *Pseudomonas spp* (10.7%) was the least predominant whereas *Escherichia coli* (17.9%), *Staphylococcus aureus* (17.9%), *Bacillus spp* (17.9%), *Enterococcus spp* (17.9%) and *Proteus spp* (17.9%) showed equal dominance. Considering the TVC and TCC of all the expired sausage rolls, they are unfit for human consumption. This study reveals the socio-economic benefits of monitoring the health standards of population especially the young generation.

## KEYWORDS

Sausage rolls, Selective media, Bacteria population, Total viable count, Total coliform count.

## 1. INTRODUCTION

Microorganisms are beneficial in some aspects of life and necessary for human well-being; food and microorganisms have had long and interesting history (Ogunrinade et al., 2022; Oranusi and Braide, 2012). Activities of microorganisms may be undesirable such as food spoilage and disease; some microbes cause food borne illness (Oyewole and Oguntoyinbo, 2022; Adestan et al., 2013). According to the study some of the food undergo spoilage in prolonged period of time (Willey et al., 2008).

According to Afolabi and Oyewole (2020) food and water account for 80% of all diseases worldwide. Food borne diseases results into death; in developing country food borne diseases results in a lot of morbidity and mortality and this is as a result of low level of hygiene maintain in this part of the world (Onuorah et al., 2015). Sausage rolls that have expired and produced under unhygienic condition could results in outbreak of food borne disease (Bello et al., 2013). Expired sausage rolls intoxication from bacterial cells and infection from viable bacteria can cause food borne infection (Ezeh et al., 2017).

Animal products such as sausage rolls and meat pie may harbor pathogens that originated from the animal itself (Clarence et al., 2009). This is the case with poultry that is contaminated with species of salmonella or campylobacter, using this in production of sausage will lead to contamination without proper production (Nester et al., 2009; Yusuf et al., 2012). During the preparation, harvesting of foods if proper care are not maintain some of these can retain microbes that are normal flora of the beef and meat use in sausage production (Odu and Akano, 2012). It has confirmed that the newspaper are filled with reports of beef being recalled due to *Campylobacter jejuni* and *E. coli* contamination transmitted by

uncooked or poorly cooked poultry products (Willey et al., 2008). It is obvious that from the raw materials such as beef, poultry in sausage production could allow contamination (Okonko et al., 2009).

## 2. MATERIALS AND METHODS

## 2.1 Samples Collection

Samples of different sausage rolls were purchased at different time, very near to their expiration date. The five samples of expired sausage rolls are: Gala sausage roll, Chi Super, Bite sausage roll, Bigi, Beeti and Rite. All these samples were taken into the laboratory in appropriate condition for analysis.

## 2.2 Isolation of Bacteria

The samples were collected from retailers and grounded with sterile pistil and mortar. 10 gram of the samples was put into 90ml of sterile water in a sterile test tube. From the solutions in the test tube, pour plate was done, transferring 0.1ml of the solution from  $10^{-2}$  and  $10^{-4}$  dilutions of the homogenate in replicate on MacConkey agar, Eosin Methylene Blue (EMB) Agar, Mueller Hinton Agar (MHA), Mannitol Salt Agar (MSA). The plates were incubated at 37°C for 24-48h.

2.3 Enumeration of Coliform and *Staphylococcus aureus*

MacConkey agar was use for coliform enumeration, while Mannitol salt agar was used for *Staphylococcus aureus*. At the end of the incubation periods, colonies were counted using the illuminated colony counter, the count was expressed as colony forming unit of the suspension (cfu/ml) Agar. Distinct isolates were subculture and subjected to biochemical test according to the method of (Hemraj et al., 2013).

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## 2.4 Enumeration of Total Viable Count

Total viable aerobic bacteria count was done on Mueller Hinton Agar. At the end of the incubation periods, colonies were counted using the using illuminated colony counter, the count was expressed as colony forming unit of the suspension (cfu/ml) Agar. Distinct isolates were subculture and subjected to biochemical test according to the method of (Hemraj et al., 2013).

## 2.5 Identification of Bacterial Isolates

The identification of bacterial isolates were based on the cultural, morphological and biochemical characteristics of the isolates. The identification was according to the method of (Hemraj et al., 2013; Willey et al., 2008).

## 3. RESULTS

Table1 shows the mean colony forming unit of the total bacteria count per gram of sausage roll. Sample of Chi Super Bite sausage roll was  $2.2 \times 10^4$  while that of Beeti sausage roll was  $3 \times 10^4$ , Gala sausage roll bacterial count per gram was  $2 \times 10^4$ . Bite sausage roll was  $2.1 \times 10^4$  and that of Bigi sausage roll was  $2.1 \times 10^4$ .

Table 1: Total Viable Bacterial Count of Expired Sausage Rolls	
Sausage roll samples	Mean total bacteria count
Chi super Bite	$2.2 \times 10^4$
Beeti	$3 \times 10^4$
Gala	$2 \times 10^4$
Rite	$2.1 \times 10^4$
Bigi	$2 \times 10^4$

Table 2, shows the coliform Count from the expired sausage, the mean colony count ranges from  $5.0 \times 10^4$  to  $1.3 \times 10^5$  cfu/g. The highest coliform count is from expired Chi Super Bite.

Table 2: Coliform Count of Expired Sausage Rolls	
Sausage roll samples	Count on MacConkey (cfu/g)
Chi super Bite	$1.3 \times 10^5$
Beeti	$5.0 \times 10^3$
Gala	$4.2 \times 10^4$
Rite	$2.0 \times 10^4$
Bigi	$5.0 \times 10^3$

Table 3 shows the mean total colony forming unit of incidence of *S.aureus* and *E.coli*. The *S.aureus* ranges from  $1 \times 10^4$  to  $4.1 \times 10^4$  cfu/g. *E.coli* ranges from  $2.0 \times 10^2$  -  $3.0 \times 10^4$  cfu/g.

Table 3: Total Colony Forming Unit for <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>		
Sausage roll samples	<i>S. aureus</i> count	<i>E.coli</i> count
Chi super Bite	$4.1 \times 10^4$	$1 \times 10^4$
Beeti	$1.0 \times 10^4$	$2 \times 10^2$
Gala	$2.0 \times 10^4$	$3 \times 10^4$
Rite	$4.0 \times 10^4$	$2 \times 10^4$
Bigi	$2.5 \times 10^4$	$2 \times 10^4$

Twenty eight isolates of six different bacteria species were isolated from five expired sausage roll samples and these are *Escherichia coli*, *Staphylococcus aureus*, *Bacillus spp*, *Enterococcus sp.*, *Klebsiella spp* and *Pseudomonas spp*.

Figure 1 shows the frequency of occurrence of bacteria isolated from expired sausage rolls samples. The figure showed that *Pseudomonas spp* (10.7%) was the least predominant; *Escherichia coli* (17.9%), *Bacillus spp* (17.9%), *Enterococcus spp* (17.9%), *Staphylococcus aureus* (17.9%) and *Klebsiella spp* (17.9%) were of equal dominance

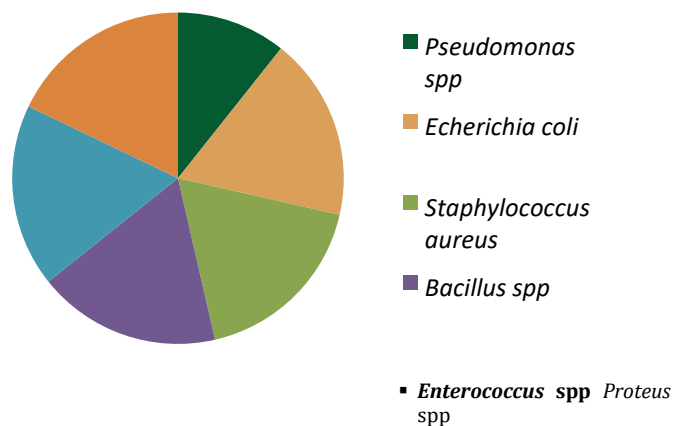


Figure 1: Frequency of Occurrence of Bacteria isolated from Expired Sausage Rolls

## 4. DISCUSSION

The total viable bacterial count ( $2 \times 10^4$ - $3 \times 10^4$ ) and coliform count ( $5 \times 10^3$ - $4.2 \times 10^4$ ) from expired sausage rolls is in agreement with the findings, they cultured high microbial load of bacteria from their ready to eat food samples (Yusuf et al., 2012; Ogunrinade et al., 2022). According to the study, high numbers of bacterial counts suggest heavy contamination of the food samples (Opeolu et al., 2010; Philips, 2003).

The predominant organisms isolated from the expired sausage rolls include *Staphylococcus aureus*, *Escherichia coli*, *Bacillus spp*, *Pseudomonas spp*, *Klebsiella spp* and *Enterobacter spp*; this agrees with the reports where similar organisms were isolated from meat pie and sausage rolls (Okonko et al., 2009; Adesulu and Oguntuyinbo, 2022). These biological contaminants of bacteria origin are present as a major cause of food borne diseases giving rise to acute illness such as *E.coli* gastroenteritis, *Brucellosis* and *campylobacteriosis* (Okonko et al., 2009 and Omemu et al., 2005).

The expired sausage rolls samples were contaminated with high level of *S. aureus* and *E. coli*; this result agrees to previous report; their food samples of animal origin cooked or uncooked were predominantly contaminated with *E. coli* and *S. aureus* (Onuorah et al., 2015). Itopined that the high level of the *E. coli* and *S. aureus* shows that the preservative method or substance used might have lost their potency given rise to the growth of small no of static organism (Afolabi and Oyewole, 2020). From scientific findings food mixture such as pastries, Salad sauces have been incriminated in food borne out breaks of *E. coli* and *S.aureus* origin (Harakeh et al., 2005).

The biochemical analysis of isolates showed that the samples were contaminated with high coagulase positive *Staphylococcus spp*; the *Staphylococcus aureus*, the microbial count of the *S. aureus* ranges from  $1 \times 10^4$  to  $4 \times 10^4$ , this result agreed with the results of (Bello et al., 2013). They isolated *Staphylococcus aureus*, a facultative anaerobe, Gram positive bacterium; from street vended food. Their presence indicates poor hygiene and poor manufacturing practices (Okonko et al., 2009). *Staphylococcus aureus* produce enterotoxins that can withstand high temperature which on ingestion can cause vomiting and diarrhea (Cencil et al., 2003 and Willey et al., 2008). They also withstand high sodium chloride concentration. Although death from *Staphylococcus* food poisoning is rare, it can cause death in children and the Immuno-compromised (Ibe, 2008). *Staphylococcus aureus*, a public health concern, might contaminate food from improper handling and hygiene (Hazariwala, et al., 2002).

*Bacillus spp* was also isolated from the expired sausage rolls; this agreed with the findings, they isolated *Bacillus spp* which have a well known pathogenic strain (*Bacillus cereus*) (Yusuf et al., 2012; Adestan et al., 2013). *Bacillus cereus* can cause two types of illness the emetic and the diarrheal syndrome this is due to the production of enterotoxin that can withstand high temperature and harsh condition (Willey et al., 2008).

The result obtained for bacteria growth was high ( $2 \times 10^4$  to  $3 \times 10^4$ ), by the standard of the public health laboratories (Olatunde et al., 2022) the total aerobic count/g for ready to eat foods is rated satisfactory for  $10^3$ , acceptable from  $10^3$  to  $10^4$  and unsatisfactory for  $>10^4$ . By this standard, all the five Samples of the expired sausage rolls can be rated as unsatisfactory.

Adequate temperature should be maintained during production, storage and usage of right quantity and quality of preservative to ensure safety of the sausage before expiration date (1sa and Akande, 2002; Abdalla et al, 2009).

## 5. CONCLUSION

This study shows that despite the satisfactory appearance of the expired sausage rolls, they have high level of total aerobic count, total coliform count and the expired sausage rolls contain bacterial isolates that can cause food borne infection. There should be awareness on the health implication of expired sausage rolls though it may appear satisfactory for consumption.

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