South Asian Journal of Research in Microbiology

10(3): 1-6, 2021; Article no.SAJRM.71254
ISSN: 2582-1989

Bacterial Contaminants of New Unused Disposable Food Packs Used in Commercial Area of Gombe State University

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2021/v10i330228
Editor(s):
(1) Dr. Ana Claudia Coelho, University of Tras-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Taís Aragão Ishizawa, Federal University of Goiás, Brazil.
(2) Mabel k. Aworh, Ahmadu Bello University, Nigeria.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71254

Original Research Article

Received 11 May 2021
Accepted 21 July 2021
Published 11 August 2021

ABSTRACT

Aim: Consumable items frequently get contaminated with bacteria harbored by their packaging materials. These bacteria result in food-borne diseases when consumed along with the food by susceptible individuals, leading to illnesses and possibly death of these individuals.
Study Design: The study was designed to determine the presence of bacterial contaminants in new unused disposable food packs used in commercial area of Gombe State University.
Place and duration of study: This study was carried out in the department of microbiology, Gombe state university between March, 2018 and June, 2018.
Methodology: 30 disposable food packs were collected using simple random sampling method. Sterile swab sticks were used to swab the interior portion of the packs inside a disinfected glass

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cupboard, the swabs were serially diluted to tenth fold. Spread plate method was used to inoculate the samples on a nutrient agar plates and incubated at 36^{o}C for 24hours. Viable count method was used to enumerate the number of colonies formed, and the bacteria were identified based on their macroscopic characteristics, Gram's reaction, microscopy, and standard biochemical tests. Disc diffusion method was used to determine the sensitivity of these isolates to some antibiotics.

Results: Out of the 30 samples, 23 samples were positive for bacterial growths with discrete CFU/ml ranging from 3.0×10^5 to 5.9×10^5, these bacteria were identified to be *Staphylococcus aureus, Escherichia coli, Bacillus* spp. and *Streptococcus* spp. The sensitivity test results revealed that all the isolates were susceptible to chloramphenicol, augmentin, ciprofloxacin, and ampicillin, with the exception of *S. aureus* which was found to be resistant to ampicillin.

Conclusion: These disposable food packs have been shown to contain notable amounts of these bacteria, and so proper sanitation, such as rinsing in boiled water should be ensured before using the food packs.

Keywords: Bacteria; contaminants; disposable; food pack.

1. INTRODUCTION

Food packaging materials such as plastics, glass, metal, and paper are widely used in food applications [1]. These packaging materials have the characteristic of maintaining the safety of the food and prevent the food from contaminated air and moisture, as well as microbial spoilage [2]. Packaging has become an indispensable element in the food manufacturing process but these packagings have been found to represent a source of contamination itself through the migration of substances from the packaging material into food [3]. The routes of contamination from the packaging material to food usually include the surface, cutting board or direct contact with the raw edge of the packaging material [4]. The material coming into direct contact with food products must not represent a source of contamination for food, in accordance with the Framework Regulation (EC) No. 1935/2004 containing the general requirements on all food contact materials [5].

Also, the adhesion and persistence of microorganisms on surfaces can spread spoilage microorganisms to foods, influencing their shelf-life and safety. Several studies have shown the ability of microorganisms to attach to all the surfaces commonly found in the food processing environment, such as stainless steel, polystyrene, rubber, glass, wood [6]. However, if microorganisms remain on a given surface for a relatively long time, they can multiply and can eventually form biofilms if they are biofilm-producing microorganisms. Several studies showed that various foodborne pathogens, including *Escherichia coli* and *Listeria monocytogenes*, can survive on utensils and equipment surfaces for hours or days, and cross-contamination of foodborne pathogens into food is a major concern since it increases the health risk for humans due to the intake of such food [6].

Improvement of food service centers based on the principles of the Hazard Analysis and Critical Control Points (HACCP) system on food safety, and implementation of preventive measures focused on training of food handlers in hygiene practices and on improving the sanitary quality of meals will go a long way in improving the safety of food and food packaging materials [7].

Very limited and fragmentary information regarding the microbial cell loads present on the surfaces of packaging materials are available in the literature [8] and Gombe such information is unavailable, hence this study was set-up bridge this knowledge gap.

2. MATERIALS AND METHODS

2.1 Sample Collection

A simple random sampling method [9] was used to collect (purchase) a total of 30 new unused take-away food packs from the main commercial area of Gombe State University, Gombe State, Nigeria, in March 2018. These samples were collected while wearing hand gloves disinfected on-site, immediately placed inside disinfected sealable plastic bags, and transported to the Microbiology laboratory of Microbiology Department Gombe State University for further processing and analysis.

2.2 Isolation and Enumeration

A 10-fold serial dilution was carried out [10] and 100µl of sample from a tube with 10^{-5} dilution

was inoculated onto nutrient agar plates using spread plating method, these plates were then incubated at 37^{o}C for 24 hours, visible colonies counted using viable count method with colony counter, and CFU/mL were determined [11].

2.3 Identification

The isolates were identified using physical observation of colony color and shape, followed by Gram's staining, and then biochemical tests which included catalase test, coagulase test, citrate test, indole test, and urease test, which were chosen based on the results of the physical morphological observation and Gram's reaction of the isolates [12].

2.4 Antibiotic Susceptibility Assay

Firstly, standardization of inocula was done using the direct colony suspension method which involved making a suspension of 24-hour old sub-cultured identified bacteria by picking colonies with a sterile glass rod and adding to 2 mL aseptic normal saline in a test tube until the turbidity of the bacterial suspension matched that of the 0.5 McFarland turbidity standard [13]. The main assay was done using disc diffusion antibiotic susceptibility test whereby spread plating was used to inoculate Mueller Hinton Agar (MHA) plates with standardized inocula of the identified bacteria, then standard discs of ciprofloxacin, augmentin, ampicillin, and chloramphenicol were aseptically placed on the inoculated plates using sterile forceps except for the control dish which contained only inoculated MHA without antibiotic discs, the setup sensitivity plates were then incubated at 35^{o}C for 18 hours, zones of inhibition were observed, measured to the nearest millimeter using a meter rule, and interpreted as sensitive or resistant, zone of inhibition of ≤14 is considered as resistant, and ≥19 is considered as susceptible using documented guidelines for antimicrobial susceptibility testing [13].

3. RESULTS AND DISCUSSION

3.1 Isolation and Enumeration

The results of isolation and enumeration (Table 1) revealed that twenty-three (23) samples out of the total of thirty (30) presented visible bacterial growth, and the total bacterial counts isolated from these samples ranged from 3.0×10^{5} to 5.9×10^{5} CFU/mL. These findings agree with reports of Mohammadzadeh-Vazifeh et al., [14] who isolated bacteria including *Bacillaceae* from paper board food packaging. However, presence of microbial contaminants on the unused food packs indicates that they are not safe for use to serve food to the consumers because they can be a source of infection when they are consumes by the people.

3.2 Identification of Bacteria

The results of morphological identification (Table 2) revealed that representative isolates from samples 6, 21 and 18 were Gram-negative, rod-shaped, and white on nutrient agar, which led to the presumption that these organisms were *Escherichia coli*. The representative isolates from samples 8, 23 and 24 were Gram-positive cocci, yellowish grape-like color on nutrient agar, and thus the suspected organisms were *Staphylococcus aureus*. The representative isolates from samples 5 and 27 were Gram-positive rods, milk-colored on nutrient agar and hence were presumed as *Bacillus* spp. Representative isolates from samples 3 and 14 were Gram-negative cocci, milk-yellow colored, irregularly shaped on nutrient agar, and thus presumed to be *Streptococcus* spp. These findings agree with the reports of Adetutu et al. [15], who were able to carry out presumptive identification of *S. aureus* using its morphology. Microorganisms such as *E. coli* and *S. aureus* posses a health hazard to the individuals using the food packs and can result to serious complications when consumes.

The results of biochemical identification (Table 3) revealed that representative isolates from samples 8, 23, and 24, were catalase-positive, coagulase-positive, citrate-positive, urease-negative and indole-negative, and hence identified as *Staphylococcus aureus.* Representative isolates from samples 5 and 27 were catalase-positive, coagulase-negative, citrate-positive, indole-negative, urease-negative and hence identified as *Bacillus* spp*.* Representative isolates from samples 6, 18 and 21 were catalase-negative, coagulase-negative, urease-negative, indole-positive, citrate-negative and thus identified to be *Escherichia coli*, while representative isolates from samples 3 and 14 were catalase-negative, coagulase-negative, urease-positive, indole-positive, and citrate-negative, hence identified as *Streptococcus* spp**.** These findings agree with the work of Baron, [12], who described biochemical characteristics of bacteria including those isolated in this study.

Table 1. Results of isolation and enumeration of bacteria

Samples	Number of Colonies	CFU/ml
1	56	5.6×10^5
2	-	-
3	32	3.2×10^5
4	36	3.6×10^5
5	31	3.1×10^5
6	40	4.0×10^5
7	-	-
8	30	3.0×10^5
9	-	-
10	46	4.6×10^5
11	-	-
12	36	3.6×10^5
13	41	4.1×10^5
14	39	3.9×10^5
15	49	4.9×10^5
16	-	-
17	-	-
18	37	3.7×10^5
19	33	3.3×10^5
20	-	-
21	31	3.1×10^5
22	-	-
23	39	3.9×10^5
24	41	4.1×10^5
25	-	-
26	34	3.4×10^5
27	59	5.9×10^5
28	-	-
29	34	3.4×10^5
30	38	3.8×10^5

Key: - absent of colonies

Table 2. Results of morphological identification of bacteria

Representative Samples	Macroscopic examination on nutrient agar	Gram's reaction and shape	Presumed organism
6	Circular whitish	Gram-negative rod	*E. coli*
8	Yellowish Grapelike	Gram-positive cocci	*S. aureus*
5	Milk-colored	Gram-positive rod	*Bacillus subtilis*
3	Milk-Yellow, irregular shape	Gram-negative cocci	*Streptococcus* spp.
21	Circular whitish on NA	Gram-negative rod	*E. coli*
18	Circular whitish	Gram-negative rod	*E. coli*
23	Yellowish Grapelike	Gram-positive cocci	*S. aureus*
24	Yellowish Grapelike	Gram-positive cocci	*S. aureus*
14	Milk-Yellow, irregular shape	Gram-negative cocci	*Streptococcus* spp.
27	Milk-colored	Gram-positive rod	*Bacillus* spp.

Table 3. Results of biochemical identification of bacteria

Presumed organism	Catalase test	Coagulase test	Urease test	Indole test	Citrate test	Organisms identified
Bacillus spp.	+	-	-	-	+	*Bacillus* spp.
Streptococcus spp.	-	-	+	+	-	*Streptococcus* spp.
E. coli	-	-	-	+	-	*E. coli*
S. aureus	+	+	-	-	+	*S. aureus*

Key: + = Positive
- = Negative

Table 4. Results of antibiotic ssusceptibility test

	Zones of inhibition (mm)			
Bacteria	**Chloramphenicol**	**Ciprofloxacin**	**Ampicillin**	**Augmentin**
S. aureus	23 (S)	22 (S)	14 (R)	33 (S)
E. coli	26 (S)	24 (S)	33 (S)	27 (S)
Bacillus spp.	20 (S)	30 (S)	25 (S)	40 (S)
Streptococcus spp.	27 (S)	40 (S)	25 (S)	35 (S)

KEY: S = sensitive, R = resistant

3.3 Antibiotic Susceptibility Assay

The results of the sensitivity test (Table 4) revealed that *Streptococcus* spp. had the following mean zones of inhibition; Chloramphenicol = 27mm, Ciprofloxacin 40mm, Ampicillin = 25mm, and Augmentin = 35mm. For *Bacillus* spp., the mean zones of inhibition included Chloramphenicol = 20mm, Ciprofloxacin = 30mm, Ampicillin = 25mm, and Augmentin = 40mm. For *E. coli* the inhibition zones included Chloramphenicol = 26mm, Ciprofloxacin = 24mm, Ampicillin = 33mm, and Augmentin = 27mm, while *S. aureus* had zones of inhibition which included Chloramphenicol = 23mm, Ciprofloxacin = 22mm, Ampicillin = 14mm, and Augmentin = 33mm. The interpretation of these zones of inhibitions revealed that *Streptococcus* spp*., Bacillus* spp*.,* and *E. coli* were sensitive to Chloramphenicol, Ciprofloxacin, Ampicillin, and Augmentin, while *S. aureus* was found to be sensitive to Ciprofloxacin, Augmentin, and Chloramphenicol, but resistant to Ampicillin [13]. This study indicated that chloramphenicol, ciprofloxacin, ampicillin and augmentin can be used in the treatment of infections caused by *S. aureus, Streptococcus* spp*., Bacillus* spp*.,* and *E. coli* in the study area.

4. CONCLUSION

It has been established that food packaging materials used in Gombe State University main commercial area are contaminated with some bacteria including *E. coli* which can pose a health risk to individuals that patronize the foods been sold and packaged in the area.

5. RECOMMENDATION

The authors recommend that the unused disposable food packs should be sterilized before used. They can be sterilized by using disinfectants that are not harmful to humans. The unused disposable food packs can also be sterilized by boiling before use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://doi.org/10.3923/pjbs.2017.350.354

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71254