**The Asia Journal of Applied Microbiology**
*2023 Vol. 10, No. 1, pp. 51-60.*
*ISSN(e): 2313-8157*
*ISSN(p): 2409-2177*
*DOI: 10.18488/33.v10i1.3411*


# Microbiological investigation of bacteria in raw eggs obtained from Obafemi Awolowo University, Nigeria

**Eunice Damilola Wilkie**[1+]
**Jude Oluwapelumi Alao**[2]
**Anthonia Olufunke Oniduro**[3]
**Deborah Oluwasola Olasehinde**[4]
**Daisy Adedoyin Kayode-Ogun**[5]

[1,2]*Department of Microbiology, Faculty of Science, Adeleke University, Ede, Osun State, Nigeria.*
[1]*Email: wilkie.eunice@adelekeuniversity.edu.ng*
[2]*Email: alaojude@adelekeuniversity.edu.ng*
[3,4,5]*Department of Microbiology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.*
[3]*Email: aoluduro2003@yahoo.co.uk*
[4]*Email: olugbogisola@yahoo.com*
[5]*Email: adedoyinkayode@gmail.com*


*(+ Corresponding author)*

**Article History**
Received: 29 April 2023
Revised: 9 June 2023
Accepted: 13 July 2023
Published: 2 August 2023

**Keywords**
Antibiotics
Bacteria
Eggs
Resistant
Sample
Susceptible.

## *ABSTRACT*

This study aimed to investigate the bacterial load and antibiotic susceptibility of bacteria isolated from uncooked eggs, with the goal of assessing potential health risks associated with their consumption. Six uncooked egg samples were collected, including samples from the eggshell, egg yolk, egg albumen, and their mixture. Bacterial enumeration was performed using the serial dilution and pour plate method on nutrient agar and MacConkey agar. Bacterial isolates were identified based on morphological and biochemical characteristics using Bergey's Manual of Determinative Bacteriology. Antibiotic susceptibility testing was conducted using the disc diffusion method on Mueller Hinton agar. The mean total bacterial load in the egg samples ranged from 3.6 x 10^4 to 5.0 x $10^4$ CFU/ml, with coliforms ranging from 2.5 x $10^3$ to 3.0 x $10^4$ colony-forming unit per ml. A total of 48 isolates were obtained and identified, consisting of 13 Gram-negative and 35 Gram-positive bacteria. The identified bacteria exhibited varied susceptibility and resistance patterns to antibiotics. Gram-negative bacteria showed 100% susceptibility to gentamycin, ofloxacin, pefloxacin, and ciprofloxacin, except for *Neisseria denitrificans*, while demonstrating 100% resistance to nitrofurantoin, augmentin, and ceftriaxone. Most Gram-positive bacteria were susceptible to Pefloxacin and Streptomycin. Some isolates displayed resistance to multiple antibiotic classes, with *Micrococcus varians* and *Bacillus laterosporus* showing resistance to 6 and 7 different classes of antibiotics, respectively. The study findings emphasize the potential health risks associated with consuming uncooked eggs due to the presence of multiple antibiotic-resistant bacteria. These findings highlight the importance of proper cooking and handling practices for eggs to minimize the risk of foodborne infections caused by antibiotic-resistant bacteria. It underscores the need for awareness and implementation of appropriate food safety measures to safeguard public health.

**Contribution/Originality:** This study contributes to the existing body of knowledge by providing original findings on the bacterial load, antibiotic susceptibility, and multiple antibiotic resistance patterns of bacteria isolated from uncooked eggs. It offers new insights into the potential health risks associated with consuming uncooked eggs and emphasizes the need for proper food safety measures.

## 1. INTRODUCTION

The consumption of eggs is popular worldwide, and they are commonly consumed in various forms, including uncooked or undercooked dishes. However, uncooked eggs have been associated with a higher risk of bacterial contamination and foodborne illness due to the presence of pathogenic bacteria [1]. One important aspect of food safety is the antibiotic susceptibility of bacteria present in food, as antibiotic resistance is a growing global health concern. Understanding the antibiotic susceptibility patterns of bacteria in uncooked eggs is crucial for assessing the potential risks associated with their consumption.

This research paper focuses on conducting a bacteriological assessment of uncooked eggs to investigate the antibiotic susceptibility and patterns of multiple antibiotic resistance (MAR) in the isolated bacteria. A total of 6 uncooked egg samples were obtained, and various parts of the eggs, including the eggshell, egg yolk, egg albumen, and their mixture, were sampled [2]. Bacterial enumeration was performed using the serial dilution and pour plate method, and the isolated bacteria were identified by examining their morphological and biochemical characteristics following the guidelines provided by Bergey's Manual of Determinative Bacteriology. To assess antibiotic susceptibility, the disc diffusion method on Mueller Hinton agar was employed.

This study aimed to examine the antibiotic susceptibility patterns of bacteria obtained from uncooked eggs, encompassing both Gram-negative and Gram-positive bacteria. Additionally, the research sought to determine the prevalence of multiple antibiotic resistance among the isolated strains. The findings of this research will contribute to our understanding of the potential health risks associated with consumption of uncooked eggs and provide valuable information for food safety regulations and interventions to minimize the spread of antibiotic-resistant bacteria through food.

## 2. METHODOLOGY

### *2.1. Sample Collection*

Samples were collected from a store located in Obafemi Awolowo University (Moremi Hostel) using standard aseptic laboratory methods for sample collection. A total of 6 uncooked egg samples were randomly selected using sterile sampling nylon bags. Hands were thoroughly disinfected before handling the samples, and the bags were tied immediately after collection to prevent contamination. The samples were immediately transferred to the Department of Microbiology, Obafemi Awolowo University, Ile-Ife for immediate processing and analysis to ensure the freshness of the samples.

### *2.2. Sample Preparation*

All samples obtained had similar physical appearance and were freshly obtained and clean. The samples included the eggshells, the yolk, the albumen, and the yolk and albumen mixture of the egg. Each sample was appropriately labelled with a unique sample identification code to ensure traceability and accuracy in subsequent analyses.

### *2.3. Bacterial Enumeration*

The identification of the isolated colonies was accomplished by assessing their morphological and biochemical characteristics, following the guidelines provided in Bergey's Manual of Determinative Bacteriology. To determine the Gram status of the isolates, Gram staining was conducted. Further identification of the pure isolates was carried out through the implementation of biochemical tests.

#### *2.3.1. Serial Dilution*

To determine the total bacterial count, serial dilution of the stock solution and pour plate techniques were carried out.

Microbial analysis of the uncooked egg samples was determined by serial dilution using pour plate method. Analysis was carried out on the eggshell, egg albumen, egg yolk, and the mixture of the egg yolk and egg albumen. For the eggshell, the eggshell was rinsed aseptically with 9 ml of sterile distilled water, and 1ml was transferred to a test tube containing 9 ml of sterile distilled water which was then serially diluted in about five (5) additional test tubes with 9 ml of sterile distilled water to ensure proper thinning out of the microbial load [2]. This procedure was repeated for the other samples (egg yolk, egg albumen and mixture of egg yolk and egg albumen). A quantity of 1 ml of each of the dilution was then transferred into sterile Petri dishes and 20 ml of sterile molten nutrient agar and 20 ml of sterile MacConkey agar was then poured into each plate separately, and the media were allowed to set, and incubated at 37°C for 24 hours.

### *2.4. Antibiotic Susceptibility Test*

Susceptibility test was carried out using disc diffusion method and the susceptibility test was interpreted following Clinical and Laboratory Standard Institute guidelines [3]. Discs immersed into concentrations of different antibiotics (Amoxicillin 25µg, Ofloxacin 5 µg, Cotrimozazole 25 µg, Augmentin 30 µg, Nitrofuranton 200 µg, Tetracycline at 30 µg, Streptomycin 10 µg, Chloramphenicol 30 µg, Erythromycin 5 µg and Gentamycin 10 µg) were carefully inserted on the inoculated Mueller –Hinton agar plate with the aid of sterile forceps and incubated for 18-24 h at 37°C. The dimensions of inhibition were taken with a transparent calibrated ruler. The results were recorded in line with the guideline of Clinical and Laboratory Standard Institute [3].

## 3. RESULTS

### *3.1. Enumeration and Characterization of Bacterial Load, including Coliforms, in Uncooked Egg Samples*

In this study, we used the standard plate count method to enumerate the bacterial load and coliforms present in various egg samples. Nutrient agar and MacConkey agar were used to culture the bacteria, and the results are presented as mean bacterial load and coliforms in Table 1. The mean total bacterial load ranged from 3.6 x $10^4$ to 5.0 x $10^4$ CFU/ml, and coliforms ranged from 2.5 x $10^3$ to 3.0 x $10^4$ CFU/ml in the egg samples. The egg shell sample had the highest microbial load, with a bacterial load of 4.2 x $10^6$ CFU/ml.

**Table 1**. Mean bacterial load of the uncooked egg samples (CFU/ml).

| Sample | Mean total bacterial load on nutrient agar (CFU/ml) | Mean coliform load on macconkey agar (CFU/ml) |
|---|---|---|
| Eggshell | 4.2 x $10^6$ | 3.0 x $10^4$ |
| Egg albumen | 0 | 0 |
| Egg Yolk | 5.0 x $10^4$ | 3.0 x$10^3$ |
| Mixture of egg yolk and albumen | 3.6 x $10^4$ | 2.5 x $10^3$ |

### *3.2. Prevalence and Relative Abundance of Probable Bacteria in a Sample Population*

The incidence and percentage occurrence of Gram-positive and Gram-negative bacteria in a microbial population are presented in Table 2. The identified species include *Bacillus alvei* (2.08%), *Bacillus badius* (2.08%), *Bacillus brevis* (2.08%), *Bacillus cereus* (2.08%), *Bacillus insolitus* (2.08%), *Bacillus laterosporous* (2.08%), *Bacillus pantothenicus* (2.08%), *Bacillus pasteurii* (2.08%), *Bacillus polymyxa* (4.17%), *Bacillus sphaericus* (2.08%), *Bacillus subtilis* (2.08%), *Staphylococcus epidermidis* (10.42%), *Staphylococcus saprophyticus* (10.42%), *Streptococcus pneumoniae* (2.08%), *Streptococcus pyogenes* (2.08%), Streptococcus mitis (2.08%), *Micrococcus varians* (8.33%), *Micrococcus luteus* (4.17%), *Klebsiella pneumoniae* (2.08%), *Escherichia coli* (2.08%), *Yersinia pseudotuberculosis* (2.08%), *Enterococcus hirae* (2.08%), *Lactobacillus delbreuckii* (2.08%), *Corynebacterium kutsceri* (2.08%), *Corynebacterium xerosis* (2.08%), *Neisseria lactamica* (8.33%), *Neisseria ovis* (2.08%), *Neisseria meningitidis* (2.08%), *Neisseria denitrificans* (2.08%), *Neisseria subflava* (2.08%), *Neisseria perflava* (2.08%), and *Neisseria sicca* (2.08%).

**Table 2**. Incidence and percentage occurrence of the probable bacteria.

| Probable bacteria | Frequency | Percentage occurrence (%) |
|---|---|---|
| *Bacillus alvei* | 1 | 2.08% |
| *Bacillus badius* | 1 | 2.08 |
| *Bacillus brevis* | 1 | 2.08% |
| *Bacillus cereus* | 1 | 2.08% |
| *Bacillus insolitus* | 1 | 2.08% |
| *Bacillus laterosporous* | 1 | 2.08% |
| *Bacillus pantothenicus* | 1 | 2.08% |
| *Bacillus pasteurii* | 1 | 2.08% |
| *Bacillus polymyxa* | 2 | 4.17 |
| *Bacillus sphaericus* | 1 | 2.08% |
| *Bacillus subtilis* | 1 | 2.08% |
| *Staphylococcus epidermidis* | 5 | 10.42% |
| *Staphylococcus saprophyticus* | 5 | 10.42% |
| *Streptococcus pneumoniae* | 1 | 2.08% |
| *Streptococcus pyogenes* | 1 | 2.08% |
| *Streptococcus mitis* | 1 | 2.08% |
| *Micrococcus varians* | 4 | 8.33% |
| *Micrococcus luteus* | 2 | 4.17% |
| *Klebsiella pneumoniae* | 1 | 2.08% |
| *Escherichia coli* | 1 | 2.08% |
| *Yersinia pseudotuberculosis* | 1 | 2.08% |
| *Enterococcus hirae* | 1 | 2.08% |
| *Lactobacillus delbreuckii* | 1 | 2.08% |
| *Corynebacterium kutsceri* | 1 | 2.08% |
| *Corynebacterium xerosis* | 1 | 2.08% |
| *Neisseria lactamica* | 4 | 8.33% |
| *Neisseria ovis* | 1 | 2.08% |
| *Neisseria meningitidis* | 1 | 2.08% |
| *Neisseria denitrificans* | 1 | 2.08% |
| *Neisseria subflava* | 1 | 2.08% |
| *Neisseria perflava* | 1 | 2.08% |
| *Neisseria sicca* | 1 | 2.08% |
| Total | 48 | 100% |

### *3.3. Antimicrobial Susceptibility of the Isolates*

Tables 3 and 4 present the results of antibiotic susceptibility tests carried out on both Gram-positive and Gram-negative bacteria. The zones of inhibition produced by each isolate against various antibiotics were measured and recorded. Out of the 48 bacterial isolates tested, 38 (79.17%) were found to be susceptible to perfloxacin, while only 2 (4.17%) isolates were resistant. Resistance to co-trimoxazole was observed in 24 (50%) isolates, whereas 20 (41.67%) isolates were found to be susceptible. Among the isolates, 12 (25%) were susceptible to ciprofloxacin, while 21 (43.75%) were resistant. All 13 (100%) of the Gram-negative bacteria tested against Augmentin at 30µg were found to be resistant. In addition, 24 (50%) isolates were found to be susceptible to ofloxacin, and all 13 (100%) of the Gram-negative bacteria were susceptible to it. Furthermore, 23 (47.92%) isolates were found to be resistant to ofloxacin, and 11 (22.92%) showed intermediate susceptibility.

### *3.4. Antibiotic Resistance Profiles of Gram-Positive and Gram-Negative Bacteria Isolated*

Using the standards published by the Clinical and Laboratory Standard Institute (CLSI), the antimicrobial susceptibility testing was interpreted. Tables 5 and 6, respectively, show the resistance patterns of the Gram-positive and Gram-negative bacteria. Twenty nine (82.86%) of the 35 Gram-positive bacteria tested demonstrated resistance to one or more antibiotics. Similar to this, all 13 (100%) Gram-negative bacteria showed resistance to at least one antibiotic.

**Table 3.** Antibiotic susceptibility patterns of Gram-positive isolates.

| Isolate | PEF | COT | CPX | ERY | AMX | OFL | STR | CHL | CEF | GEN |
|---|---|---|---|---|---|---|---|---|---|---|
| *Streptococcus pneumoniae* | 20(S) | 20(S) | 19(I) | 18(I) | 21(S) | 7(R) | 21(S) | 19(S) | 20(I) | 15(S) |
| *Staphylococcus epidermidis* | 13(I) | 23(S) | 0(R) | 20(I) | 21(S) | 0(R) | 24(S) | 18(S) | 20(I) | 21(S) |
| *Staphylococcus epidermidis* | 26(S) | 23(S) | 15(R) | 21(I) | 26(S) | 10(R) | 28(S) | 19(S) | 28(S) | 15(S) |
| *Staphylococcus epidermidis* | 15(I) | 13(I) | 0(R) | 14(I) | 12(R) | 0(R) | 12(I) | 0(R) | 15(I) | 0(R) |
| *Staphylococcus epidermidis* | 15(I) | 21(S) | 0(R) | 20(I) | 20(S) | 0(R) | 21(S) | 12(R) | 16(I) | 10(R) |
| *Staphylococcus epidermidis* | 18(S) | 17(S) | 0(R) | 20(I) | 18(S) | 0(R) | 18(S) | 0(R) | 0(R) | 9(R) |
| *Bacillus sphaericus* | 19(S) | 0(R) | 17(I) | 13(R) | 0(R) | 22(S) | 16(S) | 10(R) | 0(R) | 15(S) |
| *Bacillus brevis* | 20(S) | 22(S) | 0(R) | 22(I) | 23(S) | 0(R) | 22(S) | 16(I) | 20(I) | 0(R) |
| *Staphylococcus saprophyticus* | 18(S) | 21(S) | 0(R) | 23(S) | 12(R) | 0(R) | 20(S) | 0(R) | 0(R) | 10(R) |
| *Staphylococcus saprophyticus* | 23(S) | 24(S) | 18(I) | 24(S) | 25(S) | 10(R) | 25(S) | 18(S) | 23(S) | 20(S) |
| *Staphylococcus saprophyticus* | 0(R) | 18(S) | 12(R) | 21(I) | 15(I) | 21(S) | 16(S) | 11(R) | 0(R) | 7(R) |
| *Staphylococcus saprophyticus* | 26(S) | 18(S) | 17(I) | 20(I) | 14(I) | 0(R) | 21(S) | 22(S) | 25(S) | 20(S) |
| *Staphylococcus saprophyticus* | 20(S) | 21(S) | 0(R) | 22(I) | 19(S) | 0(R) | 19(S) | 0(R) | 0(R) | 0(R) |
| *Bacillus subtilis* | 22(S) | 10(R) | 22(S) | 21(I) | 0(R) | 25(S) | 20(S) | 19(S) | 9(R) | 17(S) |
| *Bacillus polymyxa* | 19(S) | 19(S) | 0(R) | 20(I) | 18(S) | 0(R) | 20(S) | 0(R) | 16(I) | 8(R) |
| *Bacillus polymyxa* | 15(I) | 16(S) | 0(R) | 21(I) | 19(S) | 0(R) | 18(S) | 18(S) | 0(R) | 0(R) |
| *Micrococcus varians* | 21(S) | 0(R) | 0(R) | 13(R) | 0(R) | 25(S) | 0(R) | 0(R) | 21(S) | 27(S) |
| *Micrococcus varians* | 17(S) | 0(R) | 15(R) | 20(I) | 27(S) | 8(R) | 18(S) | 20(S) | 25(S) | 23(S) |
| *Micrococcus varians* | 18(S) | 19(S) | 0(R) | 21(I) | 16(I) | 0(R) | 19(S) | 0(R) | 0(R) | 10(R) |
| *Micrococcus varians* | 20(S) | 0(R) | 21(S) | 10(R) | 0(R) | 20(S) | 18(S) | 0(R) | 0(R) | 11(I) |
| *Bacillus cereus* | 15(I) | 16(S) | 0(R) | 16(I) | 18(S) | 0(R) | 15(S) | 13(I) | 13(R) | 0(R) |
| *Streptococcus mitis* | 17(S) | 13(I) | 19(I) | 9(S) | 17(I) | 0(R) | 11(R) | 0(R) | 17(I) | 15(S) |
| *Bacillus pasteurii* | 22(S) | 16(S) | 23(S) | 20(I) | 20(S) | 0(R) | 20(S) | 20(S) | 26(S) | 16(S) |
| *Bacillus alvei* | 21(S) | 0(R) | 19(I) | 19(I) | 0(R) | 21(S) | 18(S) | 15(I) | 10(R) | 18(S) |
| *Bacillus pantothenicus* | 13(I) | 18(S) | 0(R) | 20(I) | 18(S) | 0(R) | 21(S) | 0(R) | 6(R) | 0(R) |
| *Enterococcus hirae* | 22(S) | 14(I) | 24(S) | 16(I) | 18(S) | 0(R) | 23(S) | 19(S) | 22(S) | 20(S) |
| *Bacillus laterosporus* | 10(R) | 0(R) | 14(R) | 0(R) | 0(R) | 15(I) | 10(R) | 0(R) | 0(R) | 0(R) |
| *Streptococcus pyogenes* | 24(S) | 19(S) | 18(I) | 20(I) | 10(R) | 29(S) | 15(S) | 24(S) | 22(S) | 18(S) |
| *Lactobacillus delbreuckii* | 20(S) | 21(S) | 23(S) | 24(S) | 19(S) | 20(S) | 23(S) | 17(I) | 25(S) | 14(I) |
| *Corynebacterium kutsceri* | 17(S) | 20(S) | 0(R) | 17(I) | 23(S) | 0(R) | 20(S) | 17(I) | 18(I) | 0(R) |
| *Bacillus insolitus* | 16(S) | 18(S) | 0(R) | 21(I) | 16(I) | 0(R) | 18(S) | 0(R) | 13(R) | 0(R) |
| *Micrococcus luteus* | 19(S) | 0(R) | 20(I) | 14(I) | 0(R) | 24(S) | 0(R) | 0(R) | 11(R) | 13(I) |
| *Micrococcus luteus* | 16(S) | 15(I) | 0(R) | 17(I) | 20(S) | 0(R) | 16(S) | 16(I) | 17(I) | 0(R) |
| *Bacillus badius* | 13(I) | 0(R) | 16(I) | 0(R) | 0(R) | 18(S) | 0(R) | 0(R) | 21(S) | 17(S) |
| *Corynebacterium xerosis* | 16(S) | 0(R) | 16(I) | 10(R) | 0(R) | 22(S) | 17(S) | 0(R) | 0(R) | 14(I) |

**Table 4**. Antibiotic susceptibility patterns of Gram-negative isolates.

| Isolate | NIT | GEN | COT | OFL | AMX | CPX | TET | PFX | AUG | CRO |
|---|---|---|---|---|---|---|---|---|---|---|
| *Klebsiella pnemoniae* | 11(R) | 21(S) | 24(S) | 29(S) | 0(R) | 27(S) | 13(I) | 26(S) | 0(R) | 0(R) |
| *Neisseria denitrificans* | 14(R) | 20(S) | 0(R) | 20(S) | 0(R) | 15(R) | 0(R) | 13(I) | 0(R) | 0(R) |
| *Neisseria ovis* | 12(R) | 22(S) | 24(S) | 23(S) | 0(R) | 21(S) | 0(R) | 18(S) | 0(R) | 15(I) |
| *Escherichia coli* | 14(R) | 14(I) | 0(R) | 21(S) | 0(R) | 21(S) | 12(I) | 19(S) | 0(R) | 8(R) |
| *Neisseria lactamica* | 16(I) | 14(I) | 0(R) | 20(S) | 20(S) | 17(I) | 9(R) | 17(S) | 0(R) | 0(R) |
| *Neisseria lactamica* | 16(I) | 19(S) | 0(R) | 20(S) | 0(R) | 20(I) | 6(R) | 19(S) | 0(R) | 0(R) |
| *Neisseria lactamica* | 12(R) | 22(S) | 24(S) | 23(S) | 0(R) | 25(S) | 20(S) | 22(S) | 0(R) | 0(R) |
| *Neisseria lactamica* | 16(I) | 20(S) | 11(R) | 20(S) | 14(I) | 22(S) | 0(R) | 20(S) | 0(R) | 0(R) |
| *Neisseria perflava* | 12(R) | 19(S) | 10R) | 20(S) | 10(R) | 20(I) | 0(R) | 17(S) | 0(R) | 0(R) |
| *Neisseria subflava* | 10(R) | 20(S) | 0(R) | 22(S) | 7(R) | 19(I) | 0(R) | 17(S) | 0(R) | 0(R) |
| *Neisseria meningitidis* | 13(R) | 18(S) | 0(R) | 22(S) | 15(I) | 21(S) | 8(R) | 19(S) | 0(R) | 0(R) |
| *Neisseria sicca* | 0(R) | 16(S) | 11(R) | 24(S) | 12(R) | 22(S) | 0(R) | 21(S) | 0(R) | 0(R) |
| *Yersinia pseudotuberculosis* | 16(I) | 18(S) | 0(R) | 20(S) | 0(R) | 18(I) | 0(R) | 20(S) | 0(R) | 0(R) |

**Note**: S: Susceptible, I: Intermediate, R: Resistant, PEF: Pefloxacin (5µg), COT: Cotrimoxazole (5µg), CPX: Ciprofloxacin (10µg), AMX : Amoxicillin (25µg), OFL : Ofloxacin (5µg), STR: Streptomycin (10µg), CHL: Chloramphenicol (30µg), CEF: Ceftriazone (30µg), GEN: Gentamycin (10µg), ERY: Erythromycin (5µg), AUG: Augmentin (30µg), CRO: Ceftriazone (30µg), PFX:Pefloxacin (5µg), NIT: Nitrofuranton (200µg), TET: Tetracycline (30µg).

**Table 5**. Antibiotic resistance patterns of Gram-positive bacterial isolates.

| Isolate | Antibiotics |
|---|---|
| *Streptococcus pneumoniae* | OFL |
| *Staphylococcus epidermidis* | CPX, OFL |
| *Staphylococcus epidermidis* | CPX, OFL |
| *Staphylococcus epidermidis* | AMX, CHL, CPX, GEN, OFL, |
| *Staphylococcus epidermidis* | CHL, CPX, GEN, OFL |
| *Staphylococcus epidermidis* | CEF, CHL, CPX, GEN, OFL, |
| *Bacillus sphaericus* | AMX, CEF, CHL, COT, ERY |
| *Bacillus brevis* | CPX, GEN, OFL |
| *Staphylococcus saprophyticus* | AMX, CEF, CHL, CPX, GEN, OFL |
| *Staphylococcus saprophyticus* | OFL |
| *Staphylococcus saprophyticus* | CEF, CHL, CPX, GEN, PEF |
| *Staphylococcus saprophyticus* | OFL |
| *Staphylococcus saprophyticus* | CEF, CHL, CPX, GEN, OFL |
| *Bacillus subtilis* | AMX, CEF, COT |
| *Bacillus polymyxa* | CHL, CPX, GEN, OFL |
| *Bacillus polymyxa* | CEF, CPX, GEN, OFL |
| *Micrococcus varians* | AMX, CHL, COT, CPX, ERY, STR |
| *Micrococcus varians* | COT, CPX, OFL |
| *Micrococcus varians* | CEF, CHL, CPX, GEN, OFL |
| *Micrococcus varians* | AMX, CEF, CHL, COT, ERY |
| *Bacillus cereus* | CEF, CPX, GEN, OFL |
| *Streptococcus mitis* | CHL, OFL, STR |
| *Bacillus pasteurii* | OFL |
| *Bacillus alvei* | AMX, CEF, COT |
| *Bacillus pantothenicus* | CEF, CHL, CPX, GEN, OFL |
| *Enterococcus hirae* | OFL |
| *Bacillus laterosporus* | AMX, CEF, CHL, COT, CPX, ERY, GEN, PEF, STR |
| *Streptococcus pyogenes* | AMX |
| *Corynebacterium kutsceri* | CPX, GEN, OFL |
| *Bacillus insolitus* | CEF, CHL, CPX, GEN, OFL |
| *Micrococcus luteus* | AMX, CEF, CHL, COT, STR |
| *Micrococcus luteus* | CPX, GEN, OFL |
| *Bacillus badius* | AMX, CHL, COT, ERY, STR |
| *Corynebacterium xerosis* | AMX, CEF, CHL, COT, ERY |

### *3.5. Characterization of Multiple Antibiotic Resistance Patterns among Bacterial Isolates*

The results of the antibiotic resistance patterns of the isolates are presented in Tables 7 and 8. The majority of the isolates exhibited resistance to several antibiotics, as demonstrated by their susceptibility profiles. Among the bacteria tested, 35 (72.92%) isolates, both Gram-positive and Gram-negative, displayed multiple antibiotic

resistance, showing resistance to three or more classes of antibiotics. *Micrococcus varians* and *Bacillus laterosporus* demonstrated the highest incidence of multiple antibiotic resistance patterns, with *M. varians* exhibiting resistance to six distinct classes of antibiotics, while *B. laterosporus* demonstrated resistance to seven different classes of antibiotics.

**Table 6**. Antibiotic resistance patterns of Gram-negative bacterial isolates.

| Isolate | Antibiotics |
|---|---|
| *Klebsiella pneumoniae* | AMX, AUG, CRO, NIT |
| *Neisseria denitrificans* | AMX, AUG, COT, CPX, CRO, NIT, TET |
| *Neisseria ovis* | AMX, AUG, NIT, TET |
| *Escherichia coli* | AMX, AUG, COT, CRO, NIT |
| *Neisseria lactamica* | AUG, COT, CRO, TET |
| *Neisseria lactamica* | AMX, AUG, COT, CRO, TET |
| *Neisseria lactamica* | AMX, AUG, CRO, NIT |
| *Neisseria lactamica* | AUG, COT, CRO, TET |
| *Neisseria perflava* | AMX, AUG, COT, CRO, NIT, TET |
| *Neisseria subflava* | AMX, AUG, COT, CRO, NIT, TET |
| *Neisseria meningitidis* | AUG, COT, CRO, NIT, TET |
| *Neisseria sicca* | AMX, AUG, COT, CRO NIT, TET |
| *Yersinia pseudotuberculosis* | AMX, AUG, COT, CRO, TET |

**Note:** PEF: Pefloxacin (5µg) COT: Cotrimoxazole(5µg) CPX: Ciprofloxacin (10µg).
AMX: Amoxicillin (25µg) OFL: Ofloxacin (5µg) STR: Streptomycin (10µg).
CHL: Chloramphenicol (30µg) CEF: Ceftriaxone (30µg) GEN: Gentamycin(10µg).
ERY: Erythromycin (5µg) AUG: Augmentin (30µg) CRO: Ceftriaxone (30µg).
PFX: Pefloxacin (5µg) NIT: Nitrofuranton (200µg) TET: Tetracycline (30µg).

**Table 7**. Multiple antibiotic resistance patterns of the Gram-positive bacterial isolates.

| Isolate (n) | Resistance Pattern | Frequency | Number of MAR | Percentage |
|---|---|---|---|---|
| *Bacillus sphaericus* (1) | AMX, CHL, CEF, COT, ERY | 1 | 1 | 2.08% |
| *Staphylococcus saprophytyicus* (3) | AMX, CEF, CHL, CPX, GEN, OFL | 1 | 1 | 2.08% |
| | CEF, CHL, CPX, GEN, PEF | 1 | 1 | 2.08% |
| | CEF, CHL, CPX, GEN, OFL | 1 | 1 | 2.08% |
| *Bacillus subtilis* (1) | AMX, CEF,COT | 1 | 1 | 2.08% |
| *Bacillus polymyxa* (2) | AMX, CHL, CPX, GEN, OFL | 1 | 1 | 2.08% |
| | CEF, CPX, GEN, OFL | 1 | 1 | 2.08% |
| *Micrococcus varians* (3) | AMX, CHL, COT, CPX, ERY, STR | 1 | 1 | 2.08% |
| | CEF, CHL, CPX, GEN, OFL | 1 | 1 | 2.08% |
| | AMX, CEF CHL, CPX, ERY | 1 | 1 | 2.08% |
| *Bacillus cereus* (1) | CEF, CPX, GEN, OFL | 1 | 1 | 2.08% |
| *Streptococcus mitis* (1) | CHL, OFL, STR | 1 | 1 | 2.08% |
| *Bacillus alvei* (1) | AMX, CEF, COT | 1 | 1 | 2.08% |
| *Bacillus pantothenicus* (1) | CEF, CHL, CPX, GEN, OFL | 1 | 1 | 2.08% |
| *Bacillus laterosporus* (1) | AMX, CEF, CHL, COT, CPX, ERY, GEN, PEF, STR, | 1 | 1 | 2.08% |
| *Staphylococcus epidermidis* (3) | AMX, CHL, CPX, GEN, OFL | 1 | 1 | 2.08% |
| | CHL, CPX, GEN, OFL, | 1 | 1 | 2.08% |
| | CEF, CHL, CPX, GEN, OFL, | 1 | 1 | 2.08% |
| *Bacillus insolitus* (1) | CEF, CHL, CPX, GEN, OFL | 1 | 1 | 2.08% |
| *Micrococcus luteus* (1) | AMX, CEF, CHL, CPX, STR | 1 | 1 | 2.08% |
| *Bacillus badius* (1) | AMX, CEF, CHL, CPX, ERY | 1 | 1 | 2.08% |
| *Corynebacterium xerosis* (1) | AMX, CHL, CPX, ERY, STR | 1 | 1 | 2.08% |

## 4. DISCUSSION

Bacterial contamination in eggs and egg products is a growing concern, posing significant risks to public health. Poor handling and storage practices can lead to spoilage and the transmission of pathogens, resulting in foodborne infections or intoxications for consumers. The presence of pathogenic bacteria on the surface and within eggs necessitates periodic assessments to ensure the safety and quality of eggs, considering the ongoing global demand. Uncooked eggs can be contaminated by a consortium of microorganisms, with the albumen generally considered sterile unless exposed to influencing factors like Gram-positive and Gram-negative bacteria. The egg yolk and shell

are commonly associated with various bacteria. Factors such as improper handling practices, egg source, and storage conditions contribute to the contamination of uncooked egg samples. The temperature at which eggs are stored plays a crucial role in bacterial growth and contamination. The risk of illness from contaminated eggs depends not only on the quantity of bacteria present on the shells and in the contents but also on the specific bacterial strains. Salmonella infections, commonly associated with egg consumption, originate from warm-blooded animals, manure, and soil. While carrier animals may not show symptoms, the introduction of Salmonella into the human food supply can cause illness. Although the risk of foodborne illnesses from eggs is generally low, their nutrient-rich composition provides an ideal environment for bacterial growth. Any food, especially protein-rich animal products like eggs, has the potential to harbor pathogenic microorganisms and contribute to food spoilage.

**Table 8**. Multiple antibiotic resistance patterns of the Gram-negative bacterial isolate.

| Isolate (n) | Resistance pattern | Frequency |
|---|---|---|
| Klebsiella pneumoniae (1) | AMX, AUG, CRO, NIT | 1 |
| Neisseria denitrificans (1) | AMX, AUG, COT, CPX, CRO, NIT, TET | 1 |
| Neisseria ovis (1) | AMX, AUG, CRO, NIT, TET | 1 |
| Escherichia coli (1) | AMX, AUG, COT, CRO, NIT | 1 |
| Neisseria lactamica (4) | AUG, COT, CRO, TET | 2 |
| | AMX, AUG, COT, CRO, TET | 1 |
| | AMX, AUG, CRO, NIT | 1 |
| Neisseria perflava (1) | AMX, AUG, COT, CRO, NIT, TET | 1 |
| Neisseria subflava (1) | AMX, AUG, COT, CRO, NIT, TET | 1 |
| Neisseria meningitides (1) | AUG, COT, CRO, NIT, TET | 1 |
| Neisseria sicca (1) | AMX, AUG, COT, CRO, NIT, TET | 1 |
| Yersinia pseudotuberculosis (1) | AMX, AUG, COT, CRO,TET | 1 |

**Note:** PEF: Pefloxacin (5µg) COT: Cotrimoxazole (5µg) CPX: Ciprofloxacin (10µg) AMX : Amoxicillin (25µg) OFL : Ofloxacin (5µg)STR: Streptomycin (10µg) CHL: Chloramphenicol (30µg) CEF: Ceftriaxone (30µg) GEN: Gentamycin (10µg) ERY: Erythromycin (5µg) AUG: Augmentin (30µg) CRO: Ceftriazone (30µg) PFX: Pefloxacin (5µg) NIT: Nitrofuranton (200µg) TET: Tetracycline (30µg).

Microbiological analysis was conducted on uncooked egg samples purchased from a store located in Moremi Hostel, Obafemi Awolowo University, and bacterial load ranged from 2.5 x $10^3$ to 4.2 x $10^6$ CFU/ml. No bacteria were isolated from the egg albumen, indicating high sterility. Samples cultured on Nutrient agar and MacConkey agar showed no growth. The research yielded 48 bacteria from 11 genera, including *Bacillus spp.*, *Micrococcus spp.*, *Neisseria spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, *Corynebacterium spp.*, *Lactobacillus spp.*, *Escherichia coli*, *Yersinia pseudotuberculosis*, *Klebsiella pneumoniae*, *and Enterococcus spp.* Isolation was mostly from other parts of the egg samples, such as the egg yolk, eggshell, and mixture of yolk and albumen.

The prevalence of Gram-positive bacteria exceeded that of Gram-negative bacteria in the egg samples. This may be attributed to the multiple handling procedures performed by the carriers of these organisms, which inevitably contaminate the egg. Furthermore, the egg yolk had a greater amount of Gram-negative bacteria than Gram-positive bacteria. Additionally, bacteria were isolated from the mixture of the egg yolk and egg albumen, demonstrating that the majority of the bacteria found were present in the egg yolk. These findings contrast with previous research, where Gram-negative bacteria were more frequently isolated than Gram-positive bacteria [4, 5]. The bacteria identified were diverse, ranging from potentially pathogenic to non-pathogenic bacteria, although the risk of pathogenicity is greater when poor hygiene practices are followed during the handling and cooking of uncooked egg samples, such as consuming uncooked eggs or handling cooked eggs with contaminated or unclean hands, leading to cross-contamination. The potential routes of contamination for egg contents by bacteria include both penetration and withdrawal through the pores of eggshells, as well as via the transovarian route [6-8]. In addition to these routes, environmental factors such as temperature and humidity may play a significant role in bacterial penetration, ultimately leading to increased rates of infection and spoilage [9, 10].

The antimicrobial susceptibility patterns of bacteria were evaluated in this study, with Gram-positive bacteria exhibiting resistance ranging from 20% to 100%, while Gram-negative bacteria showed resistance ranging from 25% to 100%. Multiple resistance patterns were observed in some of the bacteria, particularly for Ofloxacin (5µg), Ciprofloxacin (10µg), Chloramphenicol (30µg), Augmentin (30µg), Ceftriaxone (30µg), and Nitrofuranton (200µg), indicating a high level of resistance to these antibiotics. The high resistance of most of the Gram-negative bacteria to antibiotics suggests that these antibiotics would be ineffective in treating infections caused by these organisms. Ineffectiveness of antibiotics in treatment can contribute to antibiotic resistance and worsen the patient's condition. Bacteria resistant to only a few antibiotics pose a lesser threat. Bacteria with high susceptibility to all antibiotics tested are ideal for effective treatment. These findings support previous reports that have shown higher counts and prevalence of bacteria on unwashed eggshells than in the egg contents [11, 12]. Additionally, another study noted that the microbial load of egg contents is dependent on storage duration and temperature [13].

*Enterobacteriaceae* is a commonly used indicator for assessing the sanitary or hygienic quality of raw foods and during food processing [14]. The absence of *Salmonella* in the egg samples analysed is consistent with a previous study [15], which reported a low incidence of Salmonella in uncooked eggs, possibly due to the implementation of strict control measures against these bacteria. However, it is important to note that some bacteria, particularly Gram-positive bacteria, have a high potential to revert to virulence when subjected to conditions that promote their virulence. Therefore, consumption of raw or uncooked eggs without proper preparation or cooking is not recommended.

The results also showed that *Micrococcus varians* and *Bacillus laterosporus* exhibited the highest multiple antibiotic resistance patterns, being resistant to 6 and 7 classes of antibiotics, respectively. This may be due to these bacteria being implicated in human diseases where antibiotics have been overused without proper medical consultation, or through the acquisition of mechanisms to evade antibiotics, leading to the development of multiple antibiotic resistance patterns.

## 5. CONCLUSION

The presence of both Gram-positive and Gram-negative bacteria in uncooked egg samples indicates a high level of bacterial contamination in such samples. The degree of pathogenicity of these bacteria is largely determined by the processing methods employed on the egg samples. It is evident that the consortia of bacteria penetrated and colonized the inner parts of the eggs due to heavy contamination and unfavourable storage conditions. It is likely that these bacteria were introduced into the egg samples via various routes, such as faecal contamination and poor handling practices.

To minimize the pathogenicity of the isolated bacteria, it is recommended that processing methods utilize safe, clean and hygienic procedures to reduce or eliminate the bacteria present in the egg samples. It is also advisable that individuals infected with any of the isolated bacteria seek medical attention promptly to prevent adverse health consequences and potential mortality.

**Funding:** This study received no specific financial support.
**Institutional Review Board Statement:** Not applicable.
**Transparency:** The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
**Competing Interests:** The authors declare that they have no competing interests.
**Authors' Contributions:** Designed the study and wrote the protocol, A.O.O.; wrote the first draft of the manuscript and managed the analyses of the study, D.O.O. and D.A.K.O.; managed the literature searches and prepared the final draft of the manuscript for publication, E.D.W. and J.O.A. All authors have read and agreed to the published version of the manuscript.

## REFERENCES

[1] T. Bintsis, "Foodborne pathogens," *AIMS Microbiology*, vol. 3, no. 3, pp. 529-563, 2017.

[2] C. McAuley, L. Duffy, N. Subasinghe, G. Hogg, J. Coventry, and N. Fegan, "Salmonella typhimurium and salmonella sofia: Growth in and persistence on eggs under production and retail conditions," *BioMed Research International*, vol. 2015, pp. 1-8, 2015. https://doi.org/10.1155/2015/914987

[3] Clinical and Laboratory Standards Institute, *Performance standards for antimicrobial disk susceptibility tests CLSI standard M02* 13th ed. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.

[4] D. Stepień-Pyśniak, "Occurrence of gram-negative bacteria in hens' eggs depending on their source and storage conditions," *Polish Journal of Veterinary Sciences*, vol. 13, no. 3, pp. 507-513, 2010.

[5] F. El Ftouhy *et al.*, "Bacteriological quality of table eggs in moroccan formal and informal sector," *International Journal of Food Science*, vol. 22, pp. 1-9, 2022. https://doi.org/10.1155/2022/6223404

[6] K. De Reu *et al.*, "Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella enteritidis," *International Journal of Food Microbiology*, vol. 112, no. 3, pp. 253-260, 2006. https://doi.org/10.1016/j.ijfoodmicro.2006.04.011

[7] V. Gole *et al.*, "Effect of egg washing and correlation between eggshell characteristics and egg penetration by various salmonella typhimurium strains," *PLoS One*, vol. 9, no. 3, pp. 1-12, 2014. https://doi.org/10.1371/journal.pone.0090987

[8] I. Gantois *et al.*, "Mechanisms of egg contamination by salmonella enteritidis," *FEMS Microbiology Reviews*, vol. 33, no. 4, pp. 718–738, 2009. https://doi.org/10.1111/j.1574-6976.2008.00161.x

[9] L. D'Alba and M. D. Shawkey, "Mechanisms of antimicrobial defense in avian eggs," *Journal of Ornithology*, vol. 156, no. Suppl 1, pp. 399-408, 2015. https://doi.org/10.1007/s10336-015-1226-1

[10] L. D'Alba, R. Maia, M. E. Hauber, and M. D. Shawkey, "The evolution of eggshell cuticle in relation to nesting ecology," *Proceedings of the Royal Society B: Biological Sciences*, vol. 283, no. 1836, p. 20160687, 2016. https://doi.org/10.1098/rspb.2016.0687

[11] K. De Reu, W. Messens, M. Heyndrickx, T. Rodenburg, M. Uyttendaele, and L. Herman, "Bacterial contamination of table eggs and the influence of housing systems," *World's Poultry Science Journal*, vol. 64, no. 1, pp. 5-19, 2008. https://doi.org/10.1017/s0043933907001687

[12] S.-C. Hsu, H.-L. Chen, C.-F. Chou, W.-C. Liu, and C.-T. Wu, "Characterization of microbial contamination of retail washed and unwashed shell eggs in Taiwan," *Food Control*, vol. 149, p. 109718, 2023. https://doi.org/10.1016/j.foodcont.2023.109718

[13] G. Saleh, N. El Darra, S. Kharroubi, and M. Farran, "Influence of storage conditions on quality and safety of eggs collected from lebanese farms," *Food Control*, vol. 111, p. 107058, 2020. https://doi.org/10.1016/j.foodcont.2019.107058

[14] K. Mladenović *et al.*, "Enterobacteriaceae in food safety with an emphasis on raw milk and meat," *Appl Microbiol Biotechnol*, vol. 105, no. 23, pp. 8615-8627, 2021. https://doi.org/10.1007/s00253-021-11655-7

[15] J. M. Kingsbury, K. Thom, H. Erskine, L. Olsen, and T. Soboleva, "Prevalence and genetic analysis of Salmonella enterica from a cross-sectional survey of the New Zealand egg production environment," *Journal of Food Protection*, vol. 82, no. 12, pp. 2201-2214, 2019. https://doi.org/10.4315/0362-028x.jfp-19-159