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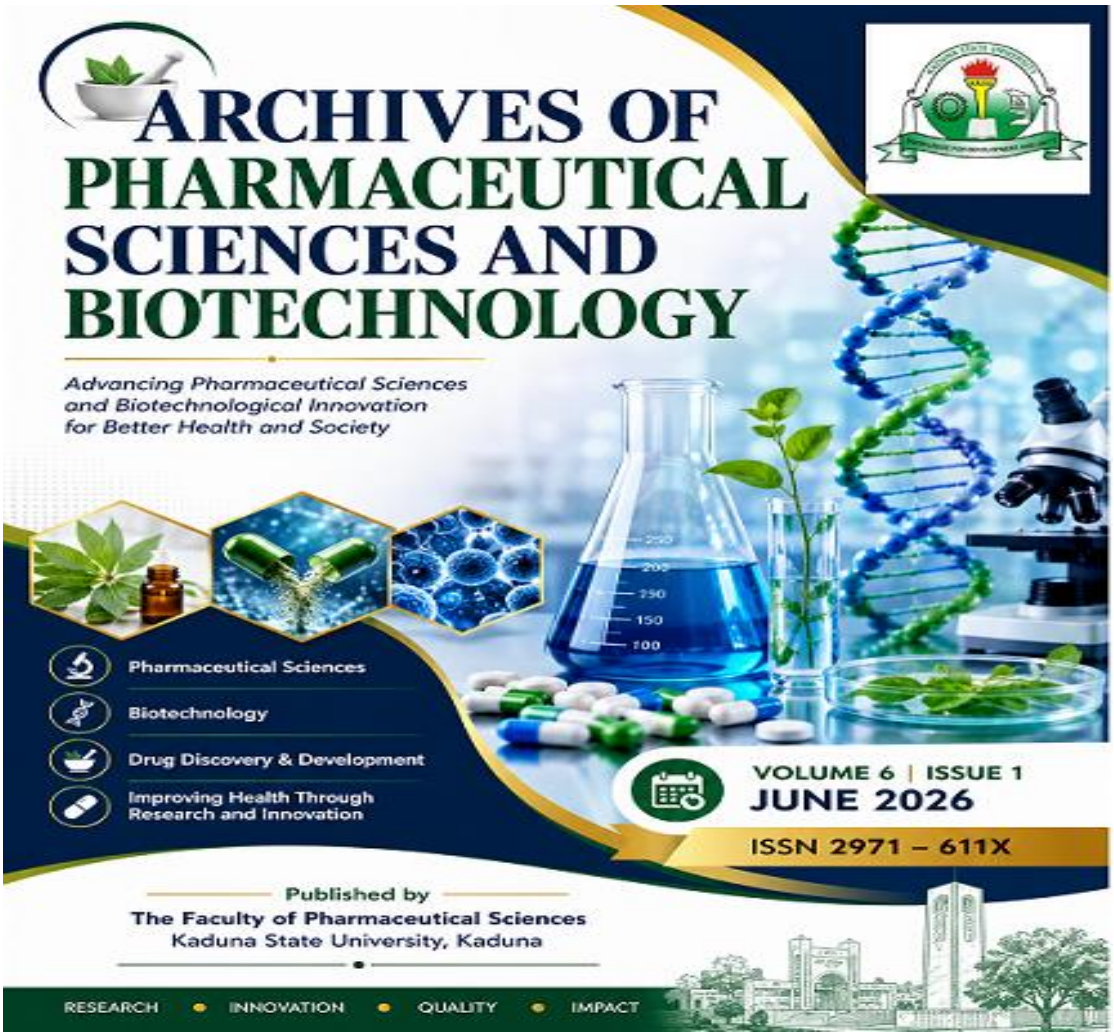


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# ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *Escherichia coli, Salmonella* spp, *Pasturella SPP* ISOLATED FROM QUAIL EGG SHELLS IN SOME FARMS IN KADUNA STATE, NIGERIA

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## ABSTRACT

**Background**: Food borne disease associated with inappropriately treated or untreated eggs is a major public health problem affecting developing and developed countries.

**Aim**: This study was undertaken to highlight the bacterial contamination associated with quail eggs and their antimicrobial susceptibility profile.

**Methods**: Bacteria isolation was carried out from 150 samples of quail eggs in some farms in Kaduna state, Nigeria. The isolates were screened for their antimicrobial susceptibility to ten commonly prescribed antibiotics used within Kaduna, Nigeria using disc diffusion method as described by Clinical Laboratory Standard Institute. Conjugation studies and curing experiments were also carried out on resistant bacteria to determine involvement of plasmid in their resistance using standard microbiological methods.

**Results**: A total of 191 bacteria colonies were isolated from the 150 samples of quail egg shells. The most occurring bacteria isolated were *Salmonella* spp, accounting for 60.7% (91) of the isolates followed by *Pasturella* species [34.67% (52)] and *E. coli* [32% (48)] were the least isolated**.** High percentage of the isolates were susceptible to Imipenem while significantly resistant to Bacitracin, Penicillin G, Nalidixic acid and Tetracycline. Out of 19 donor isolates only three (3) were observed to transfer their resistance factor. The minimum inhibitory concentration values of the tested transconjugants decreased significantly when compared with those obtained with the untreated transconjugants. Further assessment showed that 96.8% of the isolates had MAR index of 0.3 and above.

**Conclusion**: This study isolated bacteria with multidrug resistant profile, ability to transfer resistant drug genetic material and are of public health concern from quail egg sold in Kaduna. Therefore, stringent use of public health regulations for cleaning eggs before retailing is advocated.

**Keynote:** *Enterobacteriaceae, Pasturella spp,* quail egg, antibiotics resistance, plasmid conjugation.

## INTRODUCTION

Foodborne illnesses are major public health problem, which have led to an increase in morbidity and mortality worldwide (Igba *et al.,* 2021). Globally, an estimate of 2 million people died from diarrheal diseases in 2005 and approximately, 70% of these were foodborne-poisonous related (WHO, 2017). The reported extraordinary nutritional and

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medicinal properties of quail eggs have led to wide spread of it in Europe and America as well as in the Far East (Ismaila *et al*., 2026; Weerasinghe, 2024). The quail eggs contain bio-stimulator, a biologically active component indispensable to human (Olabode *et al.*, 2020). The quail eggs have been reported to contain more vitamins and mineral contents than hen's eggs (Ebenezer *et al.*, 2020). They are especially rich in the essential amino acids such as methionine, lysine, phenylalanine, and others (WHO, 2012).

Bacteria such as *E. coli*, *Salmonella* spp and *Pasturella* spp have been reported to be the common causes of poultry food borne illnesses and death in humans (Ezekiel *et al*., 2011; Abraham *et al.*, 2020). Cross-contamination of foods by these organisms can occur coupled with the spread of genes resistant to commonly used antibiotics along various points in the processing line of sale of poultry products (Abraham *et al.*, 2020). This is of both public health and economic concerns. The symptoms of food borne illnesses, resulting from the consumption of pathogen contaminated foods, can range from mild to more severe indications such as diarrhea, fever, nausea, vomiting, abdominal cramps, dehydration, meningitis, endocarditis, kidney failure and septicemia (Darwin and Miller, 1999; Abraham *et al.*, 2020)

*Escherichia coli*, *Salmonella* species, and *Pasteurella* spp. are the major bacterial pathogens isolated from poultry (Huang *et al*., 2009). Difference in susceptibility to antibiotics by these microorganisms has become a major factor in drug choice and success of treatment. Great concerns have been raised regarding emerging antimicrobial resistance among bacteria that may result in unpredictable antimicrobial susceptibility and failure of therapy (Apata, 2009). There is therefore, a need to evaluate and identification the presence of these pathogenic organism (*E.coli, Salmonella* spp., *and Pasturella* spp.) from quail egg shells.

Eggs constitute an important part of the human diet due to its high-quality of proteins, which makes them nutritious (Adabara *et al*., 2020). The interior of the egg is protected by the shell, making it unsusceptible to microbial contamination. However, the shell is always soiled with blood, manure, feathers and nest materials (Rehana *et al*., 2017). Such substances are most likely contaminated with bacteria, especially through the fecal materials.

It was observed that about 5-12% of eggshells are internally contaminated within few hours after they are laid (Rehana *et al*., 2017). The organisms of which some are pathogenic, are deposited on the shell from the intestinal tract of the quail and also from the environment, making the egg shells a reservoir of pathogenic bacteria (Jannatul *et al*., 2016). In the absence or poor implementation of public health regulations for cleaning eggs before retailing as is the case in most developing countries like Nigeria, handlers and consumers are at risk of contracting potentially pathogenic bacteria contaminant from contaminated eggshell surfaces.

The worldwide increase in the use of antibiotics in poultry and livestock production industry to treat and prevent infectious bacterial diseases and as growth promoters at sub-therapeutic levels in feeds has led to emergence of bacterial resistance to antibiotics during the past years (Oviasogie *et al.*, 2016). The increased use of

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antibiotics has also been reported to play a significant role in the emergence of antibiotic resistant bacteria (Jain *et al*., 2017), leading to increasing episodes of multi-drug resistant pathogens that can result in failure of antibiotic therapy in both animals and human. This can probably facilitate the transmission of antibiotic resistance between and among bacterial strains and species. There is an increase in public and government interest in phasing out inappropriate antibiotic use in animal husbandry (Adabar *et al*., 2020) because of the growing global concerns that antibiotic resistant bacteria can be transmitted from animals to humans. Improvement in the hygienic practice of handling raw animal products and adequate heat treatment to eliminate antibiotic resistant bacteria may play a role in preventing the spread.

## MATERIALS AND METHODS

### Study Population

Egg shells samples of quails were collected from three farms along Zaria-Kaduna road that gave expressed permission for this study. These farms include; Mai Doki Inter Agric Farm Limited (Gonar Salwa), Dinki Village Kaduna State, Amir Heritage Farms, Kwanan Farakwai, Kaduna State, Skypet Farms and Agric Limited, Birnin Yero, Kaduna State.

### Sample Collection

Egg shell samples were collected for a period of two months. They were collected into sterile wide containers and then transported to the Pharmaceutical Microbiology Laboratory at an ambient temperature ($15^0$C to $30^0$C).

### Isolation and Identification Test

Quail egg shells samples were rinsed in sterile distilled water and the rinsed samples were inoculated into sterile nutrient broth and incubated at $37^0$C overnight for 24 hours. The nutrient broth sample were sub-cultured on Triple Sugar (TSIA) iron agar, Eosin Methylene Blue (EMB) Agar, Horse Blood Agar (HBA) plates and further incubated at $37^0$C for 24hrs for isolation. Then the isolates were subjected to microscopic examination to determine the cell morphology and Gram stain reactions as described by Cheesbrough, (2000).

### Biochemical Tests

The isolated *E. coli*, *Salmonella* spp, and *Pasturella* spp were biochemically identified using various tests: citrate and non-citrate utilization, catalase production, indole formation, methyl red and Voges Proskauer tests, sugar fermentation, urease production, oxidase and hydrogen sulphite production (Cheesbrough, 2000). Triple sugar iron agar was used to further differentiate *Salmonella* species using a straight wire-loop, the butt was first stabbed and the slope was streaked in a zig-zag pattern. Pink-red (alkaline) slope and yellow (acid) butt, indicates fermentation of glucose, lactose and cracks in the medium depicts gas production from the serotype fermenting glucose. Blacking in the medium has been reported to be due to hydrogen sulphide ($H_2S$) production while grey and viscous colour with mucinous odour has been reported to be due to the presence of *Pasturella* spp (Cheesbrough, 2000).

### Antimicrobial Susceptibility Testing

The susceptibility of *E coli*, *Salmonella* spp, and *Pasturella* spp isolates to the ten (10) commonly prescribed panel of antibiotics

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were determined as modified by CLSI (2018); An overnight culture of each isolate was prepared on nutrient broth and incubated at $37^0$C for 18 hours. Standardized innoculum was in 1:5000 serial dilusions to reduce the microbial load. Dry sterile plates of prepared Mueller Hinton's agar (MHA) were flooded with the standardized innoculum ($10^5$CFU/ml) of 18hrs culture of the isolates. After inoculation, the plates were allowed to dry for 10minutes in properly disinfected incubator, before placing the susceptibility disc of the various antibiotics aseptically on the dried Mueller-Hinton agar plates. After incubation for 18 hours at $37^0$C, the plates were examined for zones of inhibition and measured appropriately. The results were interpreted according to the interpretation criteria published by CLSI (2018). Isolates were reported as susceptible (S), moderately susceptible or intermediate (I) and Resistant (R) to the various antibiotics, depending on the sizes of the zones of inhibition.

**Determination of Minimum Inhibitory Concentrations (MIC)**

The MIC determination were carried out using agar dilution method as described by Lennette *et al.,* (1990) with modifications viz: A freshly prepared stock solution of each antibiotic was filtered, and sterilized through milipore membrane filter (0.22µ). Graded concentrations of the various antibiotics in 5ml melted volume was prepared in triplicates using sterile distilled water, and then aseptically mixed with 5ml melted volume of double strength sterile Mueller-Hinton agar poured into a plate and allowed to set. Furthermore, 5ml of sterile distilled water mixed with 5ml double strength Mueller- Hinton agar was set up as positive control. Eighteen hours broth culture of the test isolates were standardized to an inoculum density of $10^5$cfu/ml (Wood and Washington, 1995). The dried Mueller Hinton agar surfaces were aseptically inoculated with ten micro litre of the test organisms per plate in triplicates at equidistant spacing. Similarly, positive control Mueller- Hinton agar inoculated with 10µl of the standardized overnight culture of standardized test isolates ($10^5$cfu/ml). The plates were allowed to stay for one hour (1 hour) and then incubated at $37^O$C for 18 hours. After the incubation the plates were examined for the presence or absence of growth. The lowest antibiotic concentration at which there were no visible growths was taken as the minimum inhibitory concentration (MIC).

**Conjugation Studies**

The transfer of resistant traits by ciprofloxacin resistant isolates of *E. coli*, *Salmonella* species, *Pasturella* species was investigated using the methods described by Onaolapo (1986) with some modifications. The minimum inhibitory concentrations (MIC) of the test antibiotics against the sensitive *Proteus* recipient was determined as described under section 3.2.4 above. The ciprofloxacin resistant isolates of the three (3) test organisms were each grown in sterile nutrient broth (5ml) each at 370C for 18hours. The ciprofloxacin sensitive *Proteus mirabilis* was sub-cultured into a sterile nutrient broth and incubated at 370C for 18 hours. The overnight cultures of the potential donor (R+) of the *E. coli, Salmonella* spp, *Pasturella* spp isolates and the recipient (R-) i.e sensitive *Proteus mirabilis* was grown in a ratio 10:1 respectively in 5ml of sterile nutrient broth and incubated in a static incubator at 370C for 18 hours. One loopful

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of transconjugants from the admixtures (*E. coli, Salmonella* spp, *Pasturella* spp and *Proteus mirabilis*) bottles were subcultured in triplicates on MacConkey agar plates incorporated with the antibiotics of MIC strength (4µg/ml) of ciprofloxacin in double strength MacConkey agar against sensitive *Proteus mirabilis* and incubated at 37$^{O}$C for 24 hours. The plates were examined for the presence or absence of cultural characteristics of *Proteus mirabilis* and lactose fermenting properties. Origianl culture of *Proteus* mirabilis and *Escherichia coli, Salmonella* species, *Pasturella* spp seperately was diluted in 1:100 ratio and a drop were spread on MacConkey agar containing ciprofloxacin (4µg/ml) for positive control. The colonies of *Proteus mirabilis* observed were aseptically picked and transferred to nutrient agar slant and sub-cultured after which the MIC of transconjugants were determined as previously described by (Lannette, *et al.*, 1990).

**Curing of Transconjugants**

The curing of transconjugants *Proteus mirabilis* (R-plasmid) was carried out by treating the *Proteus mirabilis* transconjugants with acridine orange dye as described by Onaolapo (1986). Each of the transconjugants (ER) was grown overnight in sterile nutrient broth and incubated at 370C for 18 hours in a static incubator. The overnight culture of the *Proteus mirabilis* transconjugants was standardized ($10^5$CFU/ml). A stock solution of acridine orange in sterile distilled water (10,000µg/ml), was prepared and 1.0ml of the solution dispensed into test tubes containing sterile nutrient broth (2ml). The content of each tube was vortexed and mixed properly, mixture was properly labeled and allowed to settle. Twenty microlitre (20µl) of standardized *Proteus mirabilis* transconjugants ($10^5$CFU/ml) was inoculated into the mixed solution of acridine orange and sterile nutrient broth and incubated at 37$^0$C for 18 hours. The growths from the overnight culture of the *Proteus mirabilis* transconjugants was sub-cultured on sterile MacConkey Agar plates. The colonies obtained from curing of transconjugants transfer experiment was further assessed for their antibiotic susceptibility using the MIC method. This was an attempt to determine whether the resistant pattern had changed or not.

## RESULTS

A total of 191 bacteria colonies were isolated from the 150 samples of quail egg shells. Of the bacteria isolated, 91 (60.67%) were *Salmonella* spp, 52 (34.67%) were *Pasturella* species while 48 (32.00%) were *E. coli*, (Table 1). Biochemical reaction performed showed that the colonies of *E. coli* appeared generally circular low convex, smooth and colourless on nutrient agar, rose-pink on MacConkey agar, green metallic sheen on EMB agar. It produced both acid and gas from fermentable carbohydrate such as lactose, glucose and sucrose. It gave positive result for methyl red (MR) and negative Voges-Proskauer (VP) reactions. Furthermore, samples showing red slope (lactose fermentation), yellow butt (glucose fermentation) small black spot (hydrogen sulphide production) and no gas production on triple sugar iron agar was considered as *Salmonella* spp. Samples showing colony growth accompany by characteristics mousy colour and odour, oxidase positive, catalase

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positive, fermenting large number of carbohydrates was considered as *Pasturella* spp (Table 2).

**Table 1: Percentage Distribution of Bacterial Isolates from the three Farms in Kaduna State**

| Farms | No. of samples collected | No. (%) of Bacteria isolated | | |
|---|---|---|---|---|
| | | *E. coli* | *Salmonella spp.* | *Pasturella spp* |
| Mai Doki Inter Agric Limited (Gonar Salwa) Dinki Village Kaduna State | 50 | 20(40.00) | 40(80.00) | 30(60.00) |
| Amir Heritage Farms, Kwanar Farakwai, Kaduna State. | 50 | 21(42.00) | 26(52.00) | 15(30.00) |
| Skypet Farms and Agric. Limited, Birnin Yero, Kaduna State | 50 | 7(2.00) | 25(50.00) | 7(14.00) |
| **Total** | 150 | 48(32.00) | 91(60.67) | 52(34.67) |

**Table 2: Biochemical reaction schemes of *E coli*, *Salmonella* and *Pasturella* species**

| Species | urea | lact | man | glu | suc | oc | cit | ind | Slop | Butt | $H_2S$ | Gas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | - | + | - | + | - | - | - | + | - | - | - | - |
| *Salmonella spp.* | - | - | + | + | - | - | + | - | R | Y | + | - |
| *Pasturella spp* | + | + | - | + | - | - | + | + | - | - | - | - |

Key: Urea = Urease, glu = glucose, cit = citrate, lact = lactose, suc = sucrose, ind = indole test, man = mannitol, ox = oxidase, H2S=hydrogen sulphide, R = Red pink (alkaline reaction), yellow (acid reaction) + = Production - = No production

*E. coli* isolated from quail birds in Kaduna, Nigeria were 100% resistant to Bacitracin, Penicillin G and Tetracycline. They were also 98% resistant to Nitrofuratoin and Erythromycin; 96% resistant to Trimethoprim and Nalidixic acid. *Salmonella* spp were resistant to Erythromycin (100%), Penicillin G (94.5%), and Bacitracin (84.6%) while mildly resistant to Nitrofuratoin (41.8%), Ciprofloxacin (51.6%) and Gentamicin (54.9%). *Paturella* spp were observed to show significant resistant to Penicillin G (100%), Bacitracin (98.1%), Nalidixic acid (96.1%) and Tetracycline (96.1%), Trimethoprim (94.1%). All the three pathogens evaluated were significantly susceptible to Imipenem: *E. coli* (93%), *Salmonella* spp (93.4%), *Paturella* spp (96%) (Table 3).

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**Table 3.0: Antibiotic Resistance Profile of *Escherichia coli, Salmonella* spp, *and Pasturella* spp to antibiotics**

| | | Resistance (%) | | |
|---|---|---|---|---|
| **S/N** | **Antibiotics** | ***E. coli n=48*** | ***Salmonella* spp *n=91*** | ***Paturella* spp *n=52*** |
| **1** | Ciprofloxacin | 86.0 | 51.6 | 70.6 |
| **2** | Bacitracin | 100.0 | 84.6 | 98.1 |
| **3** | Nitrofuratoin | 98.0 | 41.8 | 47.1 |
| **4** | Gentamicin | 61.0 | 54.9 | 55.0 |
| **5** | Penicillin G | 100.0 | 94.5 | 100.0 |
| **6** | Trimethoprim | 96.0 | 68.2 | 94.1 |
| **7** | Imipenem | 7.0 | 6.6 | 4.0 |
| **8** | Tetracycline | 100.0 | 69.2 | 96.1 |
| **9** | Nalidixic acid | 96.0 | 70.3 | 96.1 |
| **10** | Erythromycin | 98.0 | 100.0 | 100.0 |

The conjugation study among the multidrug resistant isolates showed that significant percentage (22.2% of the *Salmonella* spp., 12.5% of *Pasturella* species and 0% of *E. coli*) could transfer their resistant genes (R-plasmid) to sensitive *Proteus mirabilis* (Table 4). More so, the test transconjugants exhibited resistant pattern of donor cells before conjugation (Table 5).

**Table 4: Conjugation Studies Using Sensitive *Proteus Mirabilis* ($10^6$ Cfu/Ml) as Recipient**

| **S/N** | **Isolates** | **MIC of Ciprofloxacin against Donor test bacteria (µg/ml)** | **MIC of Ciprofloxacin against Recipient *Proteus mirabilis* before conjugation (µg/ml)** | **MIC of Ciprofloxacin against transconjugant (µg/ml)** |
|---|---|---|---|---|
| **1** | $S_{10}$ | 24 | 3 | 48.00 |
| **2** | $P_{22}$ | 24 | 3 | 96.00 |
| **3** | $S_{11}$ | 24 | 3 | 6.0 |
| **4** | $E_{23}$ | 24 | 3 | 3 |
| **5** | $S_{30}$ | 24 | 3 | 3 |
| **6** | $P_{12}$ | 24 | 3 | 3 |
| **7** | $P_{35}$ | 24 | 3 | 3 |
| **8** | $S_{79}$ | 24 | 3 | 3 |
| **9** | $P_4$ | 24 | 3 | 3 |
| **10** | $P_{33}$ | 24 | 3 | 3 |
| **11** | $P_{34}$ | 24 | 3 | 3 |
| **12** | $S_{20}$ | 24 | 3 | 3 |
| **13** | $P_{10}$ | 24 | 3 | 3 |
| **14** | $P_{40}$ | 24 | 3 | 3 |
| **15** | $S_3$ | 24 | 3 | 3 |
| **16** | $S_{25}$ | 24 | 3 | 3 |
| **17** | $S_{22}$ | 24 | 3 | 3 |

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| | | | | |
|---|---|---|---|---|
| **18** | $S_{18}$ | 24 | 3 | 3 |
| **19** | $E_{10}$ | 24 | 3 | 3 |

**Key:** E = *E. coli*, S = *Salmonella* spp, P = *Pasturella* spp

**Table 5: MIC of Ciprofloxacin against transconjugants**

| | | MIC of Ciprofloxacin (µg/ml) | |
|---|---|---|---|
| **S/N** | **Isolates No.** | **Before curing transconjugant(µg/ml)** | **After curing transconjugant (µg/ml)** |
| **1** | $S_{10}$ | 48 | 3 |
| **2** | $S_{11}$ | 6 | 3 |
| **3** | $P_{22}$ | 96 | 3 |

**Key:** S = *Salmonella* spp, P = *Pasturella* spp

The multiple antibiotics resistant index showed that *E. coli* were most resistant and pre-exposed to the sets of antibiotics tested with 100% expressing MARI ≥0.6, followed by *Pasturella* spp with 100% showing MARI ≥ 0.4 while *Salmonella* spp showed the list resistant profile with MARI 100% having MARI of ≥ 0.2 (Table 6).

**Table 6: Multiple antibiotics resistance indices of the test bacterial isolates**

| | Percentage of Isolates with MAR-Index | | |
|---|---|---|---|
| **MAR Index** | ***E. coli* (n=46)** | ***Salmonella* spp *(n=86)*** | ***Pasturella* spp *(n=51)*** |
| **0.20** | 0(0) | 6(6.98) | 0(0) |
| **0.30** | 0(0) | 13(15.12) | 0(0) |
| **0.40** | 0(0) | 7(8.14) | 0(0) |
| **0.50** | 0(0) | 2(2.33) | 3(5.88) |
| **0.60** | 2(4.35) | 6(6.98) | 3(5.88) |
| **0.70** | 5(10.87) | 11(12.79) | 13(25.49) |
| **0.80** | 15(32.61) | 17(19.77) | 20(39.22) |
| **0.90** | 24(52.17) | 27(31.40) | 10(19.61) |
| **1.00** | 2(4.35) | 0(0) | 3(5.88) |

MAR index = Ratio of no. of antibiotics to which isolates were resistant to over total of 10 antibiotics tested.

## DISCUSSION

Quail eggs have been widely reported to contain extraordinary, nutritional and medicinal properties needed for diet therapy (Apata, 2009). Quail eggs have been reported to have high therapeutic activity in nervous disorders, anaemia, high serum, diabetes, heart attack etc (Xu *et al*., 2024). Also, despite all the nutritional and economic attraction of the egg sector, there are also associated challenges, especially the risk of transmission of foodborne microbial disease

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and spoilage (Abraham *et al*., 2022). The results from this study showed that *E. coli, Salmonella* spp, *Pasturella* spp were highly prevalent in the investigated quail egg shells, which correlates with works documented by previous workers Bryce *et al.*, (2005), Afste, (2007), and Ifeanyi *et al.*, (2010). But dissimilar to the findings of Amal *et al*., (2015); Ebenezer *et al.*, (2020), Olabode *et al*., (2020); who observed fungi, gram positive bacteria, less Gram-negative bacteria, and less prevalence of *Samonella* spp (0.0%) of quail egg compared to this study. This could be possibly due to the poultry where these Quail eggs were obtained.

Another bacterium infecting food through contact with manure is *Escherichia coli*. This bacterium is found in the normal gut flora in humans and animals and have been isolated from quail eggs (Khan and Gupta 2020). However, there are some strains such as EHEC (0157:H7), which are pathogenic for humans. *Escherichia coli* population can be used as measures of quality and sanitary processing condition (Amal *et al*., 2015).

The presence of these pathogens in quail egg is a public health concern, as it has been implicated in several diseases such as diarrheoa, fever, nausea, vomiting, abdominal cramps, dehydration, meningitis, endocarditis, kidney failure and septicemia

The result of antibiotics susceptibility in this study also correlates with the reports of previous studies that document high resistant to Erythromycin, Tetracycline, Trimethoprim – sulfamethoxazole (Nwanze *et al*., 2010, Ngwai *et al.*, 2005). The reason for high level resistance to multiple antibiotics was common in this study. This situation is made worse as a result of ignorance or poor hygiene by quail eggs consumers and perhaps the poultry handlers. This study is in contrast to the work carried out by Nkang and co-workers (2009), who also observed that these test bacterial isolates were resistants to Ciprofloxacin, Nitrofuratoin. All of the isolates had 90% susceptibility percentage to Imipenem.

Furthermore, observations from the results obtained showed that there were substantial decrease in antibiotic activity of ciprofloxacin, Gentamicin, Nitrofuratoin and Cotrimoxazole against the bacterial isolates. This observation was similar to earlier reported cases of multidrug resistant *E. coli, Salmonella* spp (Ibrahim *et al.*, 1998, Threefall *et al.*, 2003). It showed increased bacteria resistance to test antibiotics by isolates from the quail farms. The high level of isolation of beta-lactam resistant *E. coli, Salmonella* spp and *Pasturella* spp 70% 80% 90% suggest frequent 88 usage of beta lactam antibiotics, bacitracin and others such as tetracycline, nitofuratoin nalidixic acid, and erythromycin in its environment (Ehinmidu and Ibrahim, 2004). Bacteria resistance to nalidixic acid predicts reduced susceptibility to flouroquinolones treatment (WHO, 2003) was observed in this study. This observation could suggest that the resistant isolates may probably originate from the environment where antibiotics are often used (Ngwai *et al.*, 2005) or resistance in the study could possibly be innate. The conjugation studies in this report showed that acquisition of R-plasmid by sensitive *Proteus mirabilis* from multiple antibiotic resistant *E. coli, Salmonella* spp *and Pasturella* species test bacterial isolates was obtained from the three quail farms. The antibiotic susceptibility testing result of the test transconjugants exhibited resistant pattern of donor cells before conjugation. This is because most of

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these isolates showed a common resistant pattern representing its environment and coupled with the fact that their resistant factor were transferable. Furthermore, the resistance isolates and their corresponding transconjugants co-migrated in the electro phoresis tank with some having the same band patterns.

Thirty (30) resistant bacterial test isolates were used for conjugation studies. The result of conjugation studies showed that some of the antibiotics resistance genes were likely plasmid encoded and transmissible. The MIC of ciprofloxacin was done before and after conjugation in order to ascertain if there was plasmid transfer between the donor and recipient organisms. The increase in the MIC value of ciprofloxacin after conjugation shows that the recipient had taken the characteristics of the donor resistant *E. coli, Salmonella* spp *and Pasturella* spp donors.

The curing of transcojugants showed a reduced value for ciprofloxacin against the same transconjugants. The MIC of ciprofloxacin against the cured transconjugants decreased as compared to the values obtained before the curing. This shows that the curing of the plasmid from the transconjugants with the acridine orange dye affected the change in MIC values observed.

## CONCLUSION

The result of this study showed that quail eggs evaluated in Kaduna Farms were contaminated with *E. coli Salmonella and Pasturella spp,* and those with MDR profile has possibility of transferring these gene by R-Plasmid to consumers through contaminated eggs. This could pose great risk to public health and economic loses by spoilage of the eggs. Therefore, to minimize the level of contamination of quail eggs by pathogenic microorganism, prevention and control measures should be put in place starting from the production farm and maintained through the market to the consumer level.

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