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Molecular identification of multidrug-resistant bacteria from eggshell surfaces in Nigeria: A growing threat to public health

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Abstract

Background: Eggs are an affordable dietary staple in Nigeria, yet they pose a potential public health risk when contaminated. This study assessed the prevalence of pathogenic bacteria and their antimicrobial resistance profiles on eggshells from markets in Lagos, Nigeria.

Methods: A total of 51 table eggs were randomly sampled from poultry farms and supermarkets. Bacterial isolation and identification were conducted using standard microbiological methods, biochemical testing, and 16S rRNA gene sequencing. Antimicrobial susceptibility was evaluated using the Kirby-Bauer disc diffusion method.

Results: All eggshell samples (100%) were contaminated with bacteria, with *Staphylococcus aureus* being the most prevalent organism. Other isolates included *Escherichia coli*, *Bacillus cereus*, and *Salmonella typhimurium*. Alarmingly, most isolates displayed complete resistance to first-line antibiotics like erythromycin and penicillin, while remaining susceptible to only gentamicin and ciprofloxacin.

Conclusion: The widespread bacterial contamination and high levels of antimicrobial resistance observed on eggshells represent a significant public health threat. These findings underscore the urgent need for improved biosecurity in poultry production, enhanced hygiene in market environments, public education on food safety, and systematic surveillance under the One Health framework to mitigate risks associated with contaminated eggs.

Keywords: Eggshell contamination; Antimicrobial resistance; Food safety; One Health; Public health; Pathogenic bacteria

1. Introduction

Eggs are a staple in the human diet, providing essential nutrients such as high-quality protein, vitamins, and minerals. They are widely consumed worldwide due to their affordability, ease of preparation, and versatility in cooking. In Nigeria, eggs are a common dietary component, often consumed in various forms such as boiled, fried, or in baked goods. However, despite their nutritional benefits, eggs are also known to be potential vehicles for food-borne pathogens,

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particularly when the eggshell is contaminated. The eggshell's outer surface can harbour various bacteria, some of which are pathogenic and pose significant public health risks (1).

The contamination of eggshells can occur at various production stages, including laying, collection, handling, and storage. Poor sanitary conditions in poultry farms and markets, as well as improper handling practices, can exacerbate the risk of contamination (2,3).

In Lagos, Nigeria's most congested city and a major commercial hub, the consumption of eggs is widespread. The city's bustling markets are a primary source of table eggs for the population. However, the conditions under which these eggs are sold and stored raise concerns about the potential for bacterial contamination. Lagos markets are characterized by unhealthy levels of hygiene and sanitation, which can influence the microbial load on eggshells. The potential for eggs to act as vectors for pathogenic bacteria underscores the need for regular monitoring and assessment of bacterial contamination on eggs sold in these markets.

The consumption of contaminated eggs poses a significant risk to public health in Lagos, where the open markets are crowded, and hygiene standards are upheld at zero level. Despite the widespread consumption of eggs, there is limited data on the prevalence of pathogenic bacteria on eggshells sold in Lagos markets. This lack of data hinders the ability of public health authorities to implement targeted interventions to reduce the risk of foodborne illnesses caused by pathogenic bacteria borne on eggshells. This research aims to fill an existing knowledge gap by isolating and identifying harmful bacteria present on eggshells collected from different markets in Lagos. The study's results will provide valuable insights into the microbial hazards linked to eggs, helping to shape effective strategies for improving food safety across Lagos markets.

2. Material and methods

2.1. Sampling

Fifty-one (51) fresh table eggs were collected randomly from three (3) individually owned poultry farms and three (3) supermarkets in Lagos state, Nigeria. A total of nine (9) eggs were collected from each poultry farm, while six (6) eggs were collected per supermarket.

Verbal informed consent was also gotten from the farm owners to use the collected eggs for the study. The sampled eggs were further subjected to bacterial analysis of the eggshells. The sample size was chosen by convenience. To preserve representative microbes on eggshells from farms and supermarkets for lab analysis, eggs were handled gently without washing, stored each in a sterile container, maintained cold temperatures (around 4°C) throughout collection and transport using coolers, labelled each container with source and collection details, and minimized transit time. Upon lab arrival, the eggs were sampled immediately to ensure the integrity of the microbial samples.

2.2. Isolation and identification of organisms

Each eggshell's surface was thoroughly swabbed with a sterile cotton swab dipped in 0.1% peptone water to cultivate bacteria from it. After that, the swab was aseptically streaked onto Nutrient and MacConkey agar plates. The plates were incubated for 24 hours at 37°C after inoculation. Following standard microbiological procedures, Gram staining was carried out on pure bacterial cultures, and several biochemical tests were carried out to identify the isolates. Before testing, the isolates were sub-cultured on nutrient agar to further purify them. The following biochemical tests were carried out for identification; coagulase, catalase, citrate utilization, motility, indole, urease, starch utilization, casein utilization and sugar fermentation tests.

2.3. Molecular identification/characterization of bacterial isolates

Molecular identification was conducted based on the nucleotide sequence of DNA. Genomic DNA was extracted using Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research), according to the recommended protocol. The ribosomal RNA gene was amplified using universal bacterial primers: 27F(5'AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' TACCTTGTTACGACTT 3'). The PCR reaction mixture was prepared as follows: 12.5µl of One Taq Quick-Load 2X Master Mix with Standard Buffer (NewEngland Biolabs Inc.); 0.5µl each of forward and reverse primers; 8.5µl of Nuclease-free water and 3µl of DNA template was used to prepare 25µl reaction volume of the PCR cocktail. The reaction was gently mixed and transferred to a thermal cycler (Eppendorf nexus gradient Mastercycler (Germany). PCR amplification was performed with the following cycling parameters: Initial denaturation for 30secs at 94^{o}C, followed by 35 cycles of denaturation at 94^{o}C for 20secs, primer annealing at 54^{o}C for 45secs and strand extension at 72^{o}C for 1 min. Final extension at 72^{o}C for 5 min. PCR products were separated on a 1.5% agarose gel and DNA bands were visualized with

Ethidium bromide under a UV transilluminator. Amplification products were sequenced and the resulting sequence was compared with other 16s rRNA gene sequences obtained from the NCBI GenBank database. Sequencing results were individually inputted online into the nucleotide BLAST program (BLASTN2.2.29) through the NCBI database to identify the isolates.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the isolates was performed by the Kirby-Bauer diffusion method and interpreted according to the Clinical and Laboratory Standards Institution guidelines (4). The following antibiotic discs were used for the susceptibility studies: Gentamicin, 10 µg (GN), Erythromycin, 15 µg (E), Penicillin G , 10 units (P), Oxacillin 1µg (OX), Amoxicillin-clavulanate 20/10 µg (AUG), Cefuroxime 30 µg (CFX), Ceftazidime 30 µg (CAZ), Ciprofloxacin 5 µg(CIP). The organisms were categorized as sensitive, resistant, or intermediate based on the measurement of the zone of inhibitions' diameter.

3. Results

Based on this investigation, bacteria from diverse genera were present in all 51 eggshell samples (100%) that were collected. Fifteen distinct bacterial isolates were found on the samples gathered; ten of these isolates were from the table eggs directly purchased from the poultry farms, and five were from the eggs purchased at supermarkets. Using colony-forming units (CFU), the bacterial load varied from 1.51 x 10^5 CFU in supermarket eggs to 2.96 x 10^5 CFU in eggsdirectly purchased from the farms.

Biochemical tests presumptively identified the different bacteria species present and were confirmed through blasting of their sequences on NCBI database using the tool, BLAST. Table 1 shows the bacteria isolated from samples had 97–100% similarity compared with closely related bacterial species in the GenBank. Through BLAST alignment analysis, the molecular identification results of the bacterial strains on the egg shells were known. *Staphylococcus aureus* was the most prevalent organism isolated from sampled egg shells obtained from both the poultry farms and supermarkets.

The Antimicrobial susceptibility tests showed that the isolates were 100% resistant to all the antibiotics used except Gentamycin and Ciprofloxacin. This finding highlighted the aforementioned health and biosafety risk of encountering pathogenic bacteria that are resistant to first-line of antibiotics on eggshells which the populace in Lagos are frequently exposed to.

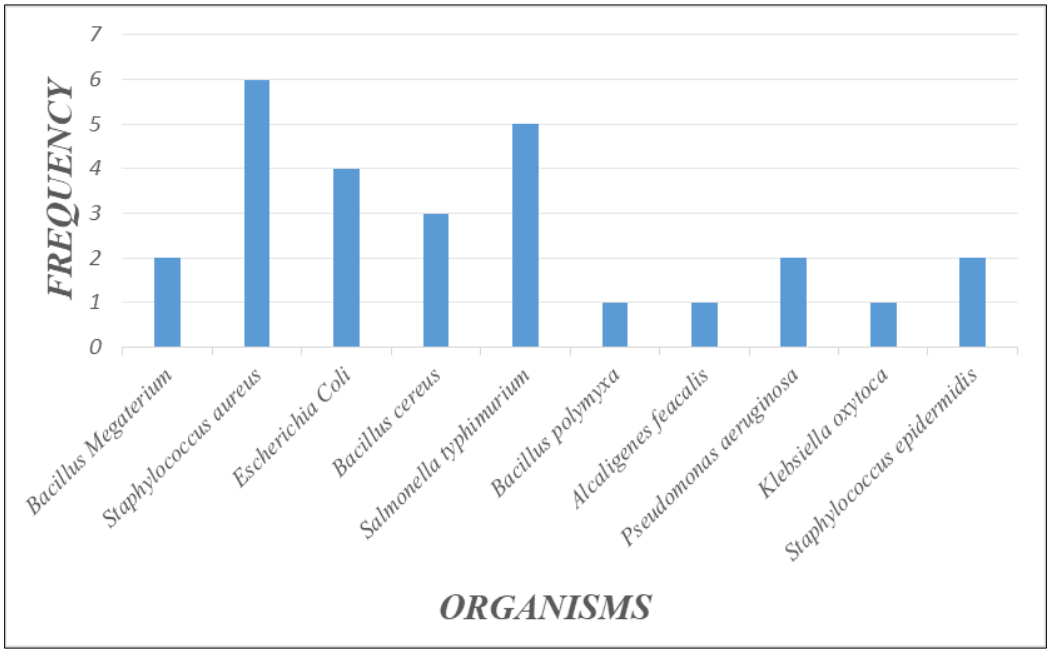

Figure 1 Distribution of bacteria from eggs collected directly from farms

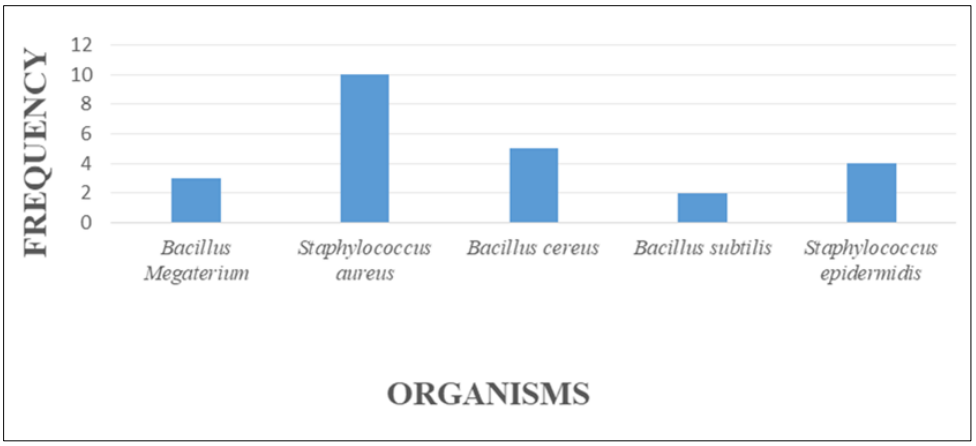

Figure 2 Distribution of bacteria from eggs collected from supermarkets

Table 1 Identification of Bacteria isolates recovered from table eggs gotten directly from the Farm by sequencing the 16s rRNA gene region and comparing with sequences on GenBank

Samples	**Accession number in Gen Bank**	**Organism**
Isolates from farm eggs		
SFA1	NR_043401.1	*Bacillus Megaterium*
SFA2	NR_118997.2	*Staphylococcus aureus*
SFA3	NR_024570.1	*Escherichia Coli*
SFA4	NR_074540.1	*Bacillus cereus*
SFA5	NR_074910.1	*Salmonella Typhimurium*
SFA6	NR_037006.1	*Bacillus polymyxa*
SFA7	NR_026078.1	*Pseudomonas aeruginosa*
SFA8	NR_113957.1	*Staphylococcus epidermidis*
SFA9	NR_113606.1	*Alcaligenes feacalis*
SFA10	NR_041749.1	*Klebsiella oxytoca*
ISOLATES FROM SUPERMARKET EGGS		
SSA1	KF_999668.1	*Staphylococcus aureus*
SSA2	NR_115714.1	*Bacillus cereus*
SSA3	NR_036904.1	*Staphylococcus epidermidis*
SSA4	NR_112116.2	*Bacillus subtilis*
SSA5	NR_117473.1	*Bacillus megaterium*

Table 2 Antimicrobial Susceptibility Pattern of Isolates from Table Eggs Collected Directly from Farms

Isolates	**No. of Isolates**		**CIP**	**CN**	**E**	**P**	**OX**	**AMC**	**CFX**	**CAZ**
Bacillus Megaterium	2	**S**	**2**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
		I	0	0	0	0	0	0	0	0
		R	0	0	2	2	2	2	2	2

Staphylococcus aureus	6	S	5	6	0	0	0	0	0	0
		I	1	0	0	0	0	0	0	0
		R	0	0	6	6	6	6	6	6
Escherichia Coli	4	S	3	4	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	1	0	4	4	4	4	4	4
Bacillus cereus	3	S	0	3	0	0	0	0	0	0
		I	3	0	0	0	0	0	0	0
		R	0	0	3	3	3	3	3	3
Salmonella Typhimurium	5	S	5	5	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	5	5	5	5	5	5
Bacillus polymyxa	1	S	1	1	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	1	1	1	1	1	1
Pseudomonas aeruginosa	2	S	2	2	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	2	2	2	2	2	2
Staphylococcus epidermidis	2	S	2	2	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	2	2	2	2	2	2
Alcaligenes feacalis	1	S	1	1	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	1	1	1	1	1	1
Klebsiella oxytoca	1	S	1	1	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	1	1	1	1	1	1

Keywords: Gentamicin (CN), Erythromycin (E), Penicillin G (P), Oxacillin (OX), Amoxycillin clavulanate (Amc), Cefuroxime (CFX), Ceftazidime (CAZ), Ciprofloxacin (CIP). S, Susceptible; I, Intermediate; R, Resistant.

Table 3 Antimicrobial Susceptibility Pattern of Isolates from Eggs Collected from Supermarkets

Isolates	**No. of Isolates**		**CIP**	**CN**	**E**	**P**	**OX**	**AMC**	**CFX**	**CAZ**
Staphylococcus aureus	10	S	10	10	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	10	10	10	10	10	10
Bacillus cereus	5	S	2	5	0	0	0	0	0	0
		I	3	0	0	0	0	0	0	0
		R	0	0	5	5	5	5	5	5
Staphylococcus epidermidis.	4	S	4	4	0	0	0	0	0	0

		I	0	0	0	0	0	0	0	0
		R	0	0	4	4	4	4	4	4
Bacillus subtilis	2	S	2	2	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	2	2	2	2	2	2
Bacillus megaterium	3	S	3	3	0	0	0	0	0	0
		I	0	0	0	0	0	0	0	0
		R	0	0	3	3	3	3	3	3

Keywords: Gentamicin (CN), Erythromycin (E), Penicillin G (P), Oxacillin (OX), Amoxycillin clavulanate (Amc), Cefuroxime (CFX), Ceftazidime (CAZ), Ciprofloxacin (CIP). S, Susceptible; I, Intermediate; R, Resistant.

4. Discussion

The findings of this study confirms significant contamination of table egg shells sold in Lagos markets with various pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Salmonella typhimurium*. Such contamination represents a substantial public health threat, especially in urban settings like Lagos, where population density and inadequate sanitation in food markets create an ideal environment for the transmission of foodborne pathogens. The presence of these bacteria on eggshells is particularly concerning because eggs are a dietary staple, often consumed in multiple forms that may not involve adequate cooking temperatures to neutralize bacteria.

This study's isolation of *Staphylococcus aureus* as the most prevalent contaminant raises concerns due to its association with food poisoning and its potential to cause a range of infections, from minor skin infections to life-threatening diseases such as septicemia and toxic shock syndrome. The identification of *Escherichia coli* and *Salmonella typhimurium* further underscores the risk, as these pathogens are known to cause severe gastrointestinal diseases. In immunocompromised individuals, the elderly, and young children, these infections can escalate quickly, potentially leading to systemic complications and increased mortality (5,6).

Of particular concern is the antibiotic resistance pattern observed among these isolates. All isolates exhibited resistance to common antibiotics such as erythromycin and penicillin, indicating a high level of antimicrobial resistance (AMR) that could complicate treatment outcomes. This resistance can be attributed to the prevalent use of antibiotics in poultry farming, where antibiotics are often administered not only for disease treatment but also as growth promoters, contributing to the selection and proliferation of resistant bacterial strains. Given that Lagos is a major commercial hub, the spread of resistant bacteria from contaminated eggs to other regions and across international borders becomes a tangible risk, very significant risk.

The implications of these findings extend to healthcare costs. In settings with limited healthcare resources, the increase in infections caused by multidrug-resistant bacteria can overwhelm health systems, placing a significant economic burden on both individual households and national health infrastructure. The lack of effective first-line antibiotics also necessitates the use of more expensive second or third line of antibiotics such that overexposure and heavy doses of these drugs could potentially result in organ toxicity and subsequent organ damage (kidney and liver) in patients thus, this strains healthcare resources and increased health complications (7).

The One Health framework, which emphasizes the connection between human, animal, and environmental health, is now being threatened as eggshells gets contaminated with pathogenic bacteria. Starting at the farm level, these pathogens can come from various locations along the supply chain (8). Inadequate hygiene and poor biosecurity practices in poultry farms provide a fertile environment for bacteria to thrive. Poultry farms with ineffective waste management methods certainly contributes to environmental contamination. This contamination does not affect only the animals but, the surrounding ecosystems. Contaminated runoffs from farms can enter water sources, which in turn may affect nearby human communities, leading to the circulation of pathogens and AMR genes between environmental reservoirs, animals, and humans (9,10).

Moreover, Lagos' markets represent a critical point in the One Health cycle. Here, eggs are exposed to conditions that may exacerbate contamination levels. Supermarkets and Farms often lack refrigeration and proper storage facilities, which can promote bacterial growth. In many cases, eggshells become compromised due to mishandling thus, allowing

pathogens to penetrate the inner egg. This highlights the need for a comprehensive, cross-sectoral approach from major agencies in the Agricultural, Environmental and Health sectors to address this issue holistically in a collaborative effort.

4.1. Strategies for Control and Prevention

The findings of this study call for targeted interventions to reduce the risks associated with the spread of antimicrobial resistance pathogens currently in circulation within Lagos State from contaminated eggs introduced to the markets daily. This is a call to identify entry route of AMR bacteria and to curb its spread. A multi-faceted approach is essential to mitigate the public health risks associated with these pathogens. This, suggestively, involves improved regulatory frameworks, farm-to-market hygiene improvements, public education, and enhanced surveillance as highlighted below:

4.1.1. Improved Biosecurity and Hygiene Practices on Farms

Strengthening biosecurity measures on poultry farms is essential to limit the initial contamination of eggs. This includes implementing stricter hygiene protocols for handling and packaging eggs and ensuring proper waste disposal to minimize environmental contamination. Furthermore, the use of antibiotics in poultry should be restricted to therapeutic applications, with a focus on preventive measures, such as improved vaccination and hygiene, to reduce reliance on antibiotics.

4.1.2. Enhanced Market Hygiene and Safe Handling Protocols

Since Lagos markets are a major point of contact for consumers, it is crucial to establish food safety standards that emphasize safe handling and storage practices of food items in these locations. Vendors should be educated on the importance of minimizing direct contact with eggshells, storing eggs under conditions that limit bacterial growth and hand hygiene practices post-handling of eggs. Local authorities should establish guidelines for regular sanitation of market facilities to reduce the accumulation of pathogens on surfaces that come into contact with food items.

4.1.3. Public Awareness and Safe Food Handling Practices

Educating the public on the risks that could arise from handling eggs and the importance of proper cooking can greatly reduce the incidence of egg-related infections. Public health campaigns could focus on promoting hygiene practices starting from each poultry farm such as washing poultry eggs before packaging and sealing for sales, washing hands after handling eggs, avoiding cross-contamination with other foods, and cooking eggs thoroughly. These measures would empower consumers to take active steps in reducing their exposure to bacterial pathogens especially pathogens with acquired resistant genes to readily available antimicrobials.

4.1.4. Surveillance and Monitoring

Establishing a systematic surveillance network that monitors bacterial contamination and AMR patterns in food products like eggs is essential. This study proposes that government should mandate that all medium and large scale Poultry farm must have a Microbiological Quality Control Laboratory. These laboratories should be under constant surveillance by the appropriate health body for compliance (11). Regular microbiological testing of eggs from farms and markets would provide valuable data to guide public health interventions. Additionally, monitoring antibiotic usage in the poultry industry could help track and limit the spread of AMR. This could be achieved through collaboration between agricultural, environmental, and health sectors, in alignment with the principles of One Health.

5. Conclusion

This study reveals a significant threat to public health posed by pathogenic bacteria and AMR on eggshells in Lagos markets. Addressing these risks requires a One Health approach that incorporates farm biosecurity, market hygiene improvements, public education, and systematic surveillance. By implementing these measures, Nigeria can reduce the health risks associated with contaminated eggs, enhance food safety standards, and contribute to the global effort to combat AMR. Through coordinated actions across sectors, Nigeria's food safety infrastructure can be strengthened, ensuring that the growing demand for eggs is met in a manner that safeguards both public health and environmental sustainability.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of informed consent

We obtained verbal informed consent from the owners of the farm to collect the egg samples and to use them for the study.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

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