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Prevalence of Pathogenic Bacterial and Antimicrobial Resistance on Eggshells from Markets in Lagos, Nigeria: Implications for Public Health and Food Safety

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Abstract: This study investigates the prevalence of pathogenic bacteria on eggshells from table eggs sold in Lagos markets, a major concern given eggs' role as a staple in the Nigerian diet. Fifty-one eggs were sampled from three poultry farms and three supermarkets, with bacterial isolation and identification conducted via microbiological assays and biochemical testing. The findings revealed that all eggshell samples (100%) were contaminated with various bacterial genera, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Salmonella typhimurium*. *Staphylococcus aureus* was the most frequently isolated organism, indicating a high potential risk for foodborne illness. Antimicrobial susceptibility tests indicated widespread resistance to first-line antibiotics, such as erythromycin and penicillin, with isolates only susceptible to gentamicin and ciprofloxacin. This study underscores the public health threat posed by contaminated eggs in Lagos and highlights the need for improved biosecurity measures, enhanced market hygiene, public awareness campaigns, and systematic surveillance under the One Health framework to combat bacterial contamination and antimicrobial resistance.

Keywords: Antimicrobial Resistance (AMR); Public Health; One Health, Food Safety; Foodborne-pathogen

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, 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 largest 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 varying 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 eggshells sold in these markets.

The consumption of contaminated eggs poses a significant risk to public health in Lagos, where markets are often crowded, and hygiene standards can be inconsistent. Despite the widespread consumption of eggs, there is limited data on the prevalence of pathogenic bacteria on eggshells sold

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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. 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. Materials and Methods

2.1. Sampling

Fifty-one (51) fresh table eggs were collected randomly from three [3] 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. The sampled eggs were further subjected to bacterial analysis of the eggshells. The sample size was chosen by convenience.

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 streaked onto nutrient and MacConkey agar plates. The plates were incubated for 24 hours at 37°C after inoculation. Following standard microbiological procedures, a Gram stain was carried out on pure bacterial cultures, and several biochemical tests were carried out to identify the isolates [4]. 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. 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 will be used for the susceptibility studies: Gentamicin (GN), Erythromycin (E), Penicillin G (P), Oxacillin (OX), Amoxicillin-clavulanate (AUG), Cefuroxime (CFX), Ceftazidime (CAZ), Ciprofloxacin (CIP).

2.4. Data Analysis

The data generated were collated and analyzed using descriptive statistics to determine the prevalence of different pathogenic bacteria and their antibiotic resistance profiles.

3. Result

Based on this investigation, bacteria from a variety of bacterial genera were present in all 51 eggshell samples (100%) that were collected. Fifteen distinct bacterial isolates were found in the samples that were gathered; ten of these isolates were from table eggs that were directly purchased from poultry farms, and five were from eggs that were bought 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 eggs that were directly purchased from farms.

Further biochemical tests confirmed the different bacteria genera present on the samples egg shells as Bacillus megaterium, Staphylococcus aureus, Escherichia Coli, Bacillus cereus, Salmonella typhimurium, Bacillus polymyxa, Pseudomonas aerogenes, Alcaligenes feacalis, Klebsiella oxytoca, Bacillus subtilis (Figures 1 and 2).

Staphylococcus aureus was the most prevalent organism isolated from sampled table egg shells obtained from both poultry farms and supermarkets.

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The Antimicrobial susceptibility tests showed that the isolates were 100% resistant to all the antibiotics used except Gentamycin and Ciprofloxacin. This finding highlights the potential risk of encountering bacteria on eggs that are not easily treated with first-line antibiotics.

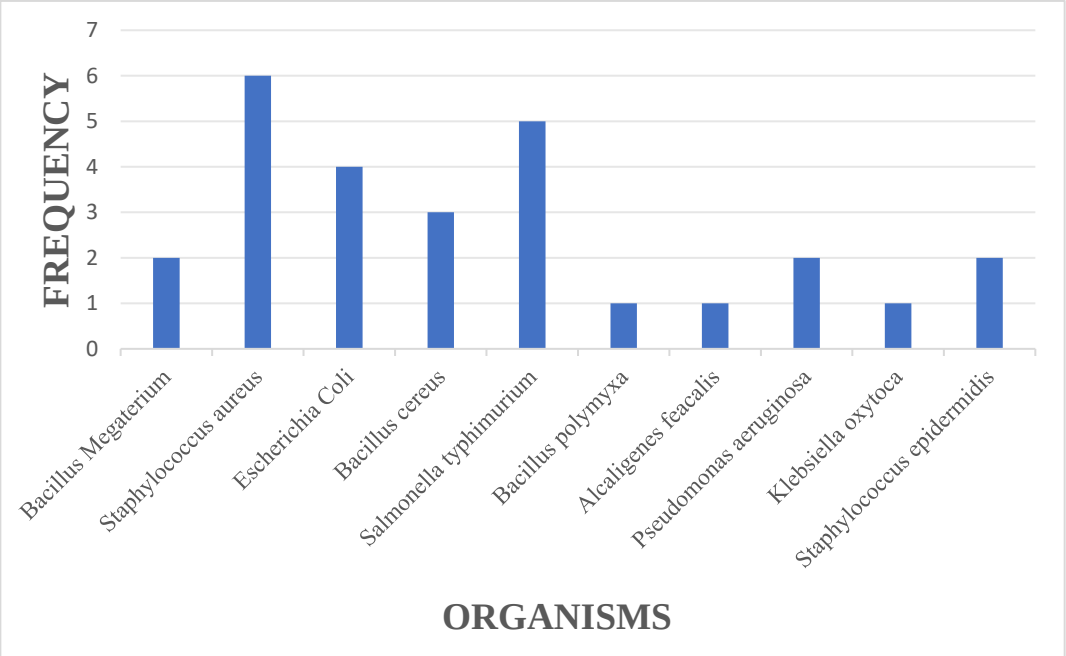

Figure 1. DISTRIBUTION OF ORGANISMS FROM EGGS COLLECTED DIRECTED FROM FARMS.

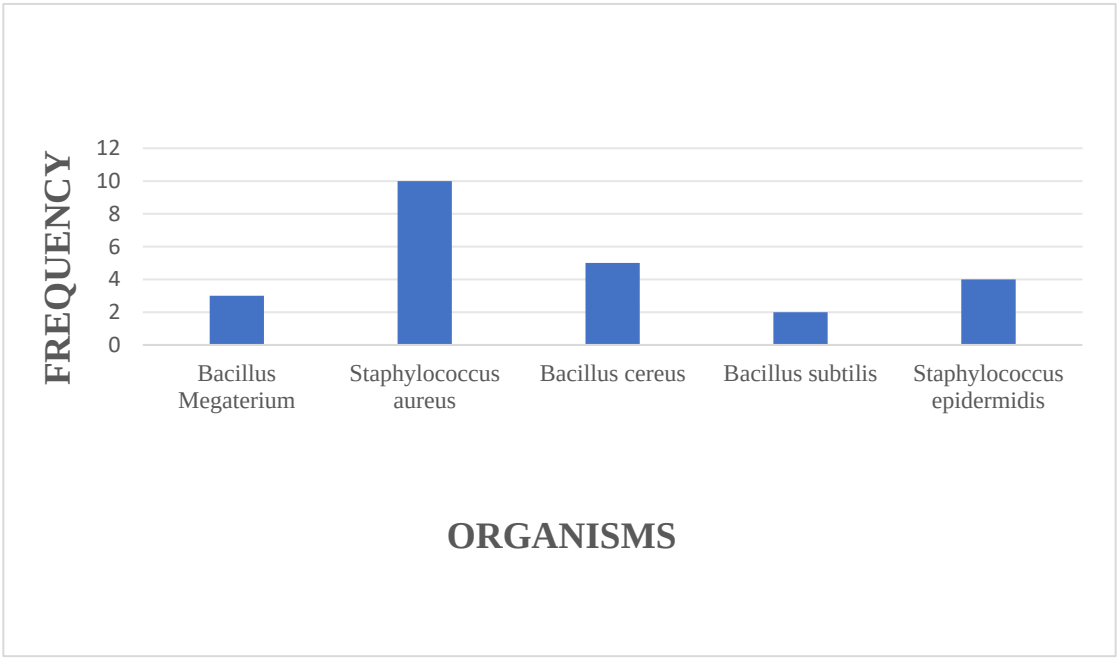

Figure 2. DISTRIBUTION OF ORGANISMS FROM EGGS COLLECTED FROM SUPERMARKETS.

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Table 1. 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
Staphylcoccus aureus	6	**S**	5	6						
		I	1	0	0	0	0	0	0	0
		R	0	0	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.

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Table 2. Antimicrobial susceptibility pattern of isolates from eggs collected from supermarkets.

Isolates	No. Of Isolates		CIP	CN	E	P	OX	AMC	CFX	CAZ
Staphylcoccus 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 confirm the 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 bacterial contaminants.

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

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high level of antimicrobial resistance (AMR) that could complicate treatment options. 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.

The implications of these findings extend to healthcare costs and capacity. In a setting 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 or potentially more toxic second or third-line treatments, further straining healthcare resources.

One Health Perspective

The One Health framework, which emphasizes the connection between human, animal, and environmental health, has serious repercussions when eggshells are contaminated with pathogenic bacteria. Starting at the farm level, these pathogens can come from various locations along the supply chain [7]. Inadequate hygiene and poor biosecurity practices in poultry farms provide a fertile environment for bacteria to thrive. Poultry farms with insufficient waste management can contribute to environmental contamination, which not only affects the animals themselves but also 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 [8,9].

Moreover, Lagos' markets represent a critical point in the One Health cycle. Here, eggs are exposed to conditions that may exacerbate contamination levels. Marketplaces often lack refrigeration and proper storage facilities, which can promote bacterial growth. In many cases, eggshells become compromised due to mishandling, allowing pathogens to penetrate the inner egg. This highlights the need for a comprehensive, cross-sectoral approach to address the issue, involving agricultural, environmental, and public health agencies working in concert.

Strategies for Control and Prevention

The findings of this study call for targeted interventions to reduce the risks associated with contaminated eggs and curb the spread of AMR. A multi-faceted approach is essential to mitigate the public health risks associated with these pathogens, involving improved regulatory frameworks, farm-to-market hygiene improvements, public education, and enhanced surveillance.

- **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.

- **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 in these locations. Vendors should be educated on the importance of minimizing direct contact with eggshells and storing eggs under conditions that limit bacterial growth. 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.

- **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, such as washing hands after handling eggs, avoiding cross-

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contamination with other foods, and cooking eggs thoroughly. These measures would empower consumers to take active steps in reducing their exposure to bacterial pathogens.

- **Surveillance and Monitoring**

Establishing a systematic surveillance network that monitors bacterial contamination and AMR patterns in food products like eggs is essential. 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 One Health principles.

5. Conclusions

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.

References

1. Whiley, H. and Ross, K. (2015). Salmonella and eggs: from production to plate. *Int J Environ Res Public. Health.* 26:12(3):2543-2556. doi: 10.3390/ijerph120302543. PMID: 25730295; PMCID: PMC4377917.
2. Gole, V. C., Roberts, J. R., Sexton, M., May, D., Kiermeier, A. and Chousalkar, K. K. (2014). Effect of egg washing and correlation between cuticle and egg penetration by various Salmonella strains. *Int J Food Microbiol*. 16:(182-183):18-25. doi: 10.1016/j.ijfoodmicro.2014.04.030. PMID: 24859187.
3. Jones, F. T., Rives, D. V. and Carey, J. B. (1995). Salmonella contamination in commercial eggs and an egg production facility. *Poult Sci.* 74(4):753-757. doi: 10.3382/ps.0740753. PMID: 7792248.
4. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Clinical Laboratory Standards Institute. 2021.
5. Kadariya, J., Smith, T. C. and Thapaliya, D. (2014). S*taphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. *Biomed Res Int*. 2014:827965
6. Ekpunobi, N. F. and Adeleye, I. A. (2020). Phenotypic characterization of biofilm formation and efflux pump activity in multidrug-resistant Staphylococcus species isolated from asymptomatic students. *J. Microbiol. Exp*. 8(6): 223-229. https://doi.org/10.15406/jmen.2020.08.00313
7. Horefti, E. (2023). The Importance of the One Health Concept in Combating Zoonoses. *Pathogens*. 12(8):977.
8. Ekpunobi, N. F., Adesanoye, S., Orababa, O., Adinnu, C., Okorie, C., & Akinsuyi, S. (2024). Public health perspective of public abattoirs in Nigeria, challenges and solutions. *GSC biol. pharm. Sci.* 26(2), 115-127.
9. Chukwurah, U., Ekwealor, C., & Ekpunobi, N. (2024). Assessment of the Fungal Contaminants of Dried Fishes Sold in Open Markets in Awka, Nigeria. Preprints, 2024, https://www.preprints.org/manuscript/202405.1880/v1