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Occurrence and Antibiotic Resistance of *Salmonella*, *Escherichia* and Other Bacteria Genera Isolated from Fresh Eggshell Surfaces Sold in Okada, Edo State, Nigeria

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ABSTRACT

Eggshell contamination by pathogenic and antimicrobial-resistant bacteria represents an important food-safety concern because eggs may serve as vehicles for bacterial transmission and exposure to resistant organisms. This study investigated the occurrence, phenotypic characteristics and antibiotic resistance profile of bacterial isolates recovered from eggshell surfaces of fresh eggs sold from ten retail outlets in Okada, Edo State, Nigeria. A total of 81 eggshell samples were examined using standard cultural, morphological and biochemical techniques, followed by antimicrobial susceptibility testing against ten antibiotics. No suspected *Salmonella* isolate was recovered, whereas seven suspected *Escherichia* isolates were detected at two outlets, giving an overall occurrence of 8.64%. Other isolates comprised *Enterobacter* (32.10%), *Pseudomonas* (32.10%), *Proteus* (11.11%), *Klebsiella* (9.88%) and *Enterococcus* (6.17%). Retail outlet was significantly associated with the occurrence of suspected *Escherichia* ($\chi^2 = 27.52$, $p = 0.001$), *Enterobacter* ($\chi^2 = 23.39$, $p = 0.01$) and *Proteus* ($\chi^2 = 72.79$, $p < 0.001$), but not *Klebsiella*, *Pseudomonas* or *Enterococcus*. Resistance to cefuroxime, cefoxitin, ceftriaxone, ceftazidime, pefloxacin, gentamicin and streptomycin was 100%, while amoxicillin-clavulanic acid and ciprofloxacin resistance were 98.77% and 90.12%, respectively. Ofloxacin showed the lowest resistance (2.47%). Cochran's Q test demonstrated significant differences in resistance across antibiotics ($Q = 655.56$, $df = 9$, $p < 0.001$). All isolates were multidrug-resistant, with an overall MAR index of 0.89. These findings indicate substantial bacterial contamination and a high antimicrobial resistance burden on retail eggshells, highlighting the need for improved poultry biosecurity, antimicrobial stewardship and hygienic egg handling as to safeguard public health.

Keywords: Retail eggs, Eggshell contamination, Antibiotic resistance, Multidrug resistance, Food safety, Public health.

Introduction

Eggs are an important component of the human diet because they provide relatively affordable, high-quality protein and several essential micronutrients. In Nigeria, poultry eggs are widely marketed through supermarkets, provision outlets, open markets and other retail outlets. However, before reaching consumers, eggs may be exposed to microorganisms originating from poultry houses, litter, fecal material, handlers, transport containers and contaminated surfaces. The eggshell, therefore, represents an important interface between poultry production and human food consumption. A recent systematic review found that bacterial contamination is generally more frequent on eggshells than within egg contents, with *Salmonella* spp. and *Escherichia* spp. among the major bacterial groups associated with poultry eggs (Zhang et al., 2025).

The presence of *Salmonella* and *Escherichia* on eggshell surfaces is particularly important because both organisms include strains capable of causing foodborne disease in humans. *Salmonella* is associated with gastroenteritis and systemic infections, while pathogenic *Escherichia* can cause diarrhoeal and other extraintestinal infections. Their importance is heightened when they acquire resistance to commonly used antibiotics. The World Health Organization (WHO, 2024) identified antimicrobial resistance (AMR) as a major global public-health threat and emphasized the need for coordinated surveillance across human, animal, food and environmental sectors. Antibiotics use in poultry production can create selective pressure that favours the persistence and dissemination of resistant bacteria. Resistant organisms may subsequently move between poultry, farm environments, food products and humans through the food chain.

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The WHO's 2024 guidance on antimicrobial resistance in the food chain emphasizes that controlling resistant bacteria requires interventions extending from primary animal production through food handling and consumption (WHO, 2024). This concern is especially relevant to *Salmonella* and *Escherichia*, which are recognized foodborne organisms capable of carrying and transmitting clinically important resistance determinants.

Ekpunobi et al., 2024, reported that antimicrobial resistance among *Salmonella* is already a significant concern along with substantial resistance to important antimicrobial groups, including fluoroquinolones and cephalosporins, and highlighted weaknesses in laboratory surveillance of *Salmonella* infections.

Despite these concerns, information on the resistance characteristics of *Salmonella* and *Escherichia* specifically associated with eggshells sold through retail outlets in Okada, Edo State, remains limited. Local surveillance is important because resistance patterns can differ according to antimicrobial-use practices, poultry management, environmental conditions and geographical location. Determining the antibiotic resistance patterns of these organisms in commercially available eggs can therefore provide useful evidence for food-safety monitoring, antimicrobial stewardship and One Health interventions.

This study was consequently designed to investigate the occurrence and antibiotic resistance patterns of *Salmonella* and *Escherichia* or other organisms isolated from eggshell surfaces sold at retail outlets in Okada, Edo State, Nigeria.

Materials and Methods

Study Area

The study was carried out in Okada (6º 43'N 5º23'E), Ovia North-East Local Government Area of Edo State, Nigeria. Okada is a growing semi-urban community characterized by residential, commercial and educational institutional activities. Poultry eggs are commonly sold through supermarkets, provision outlets, open-market stalls and other retail outlets within the community. The movement of eggs from poultry farms through transportation and retail handling provides several opportunities for contamination of eggshell surfaces by environmental and enteric bacteria.

The study area was selected because eggs constitute an important food commodity in the community and because retail handling conditions may differ considerably between outlets. These differences provide an appropriate setting for assessing the occurrence and antibiotic resistance characteristics of *Salmonella* and *Escherichia* or other bacteria associated with commercially available eggs.

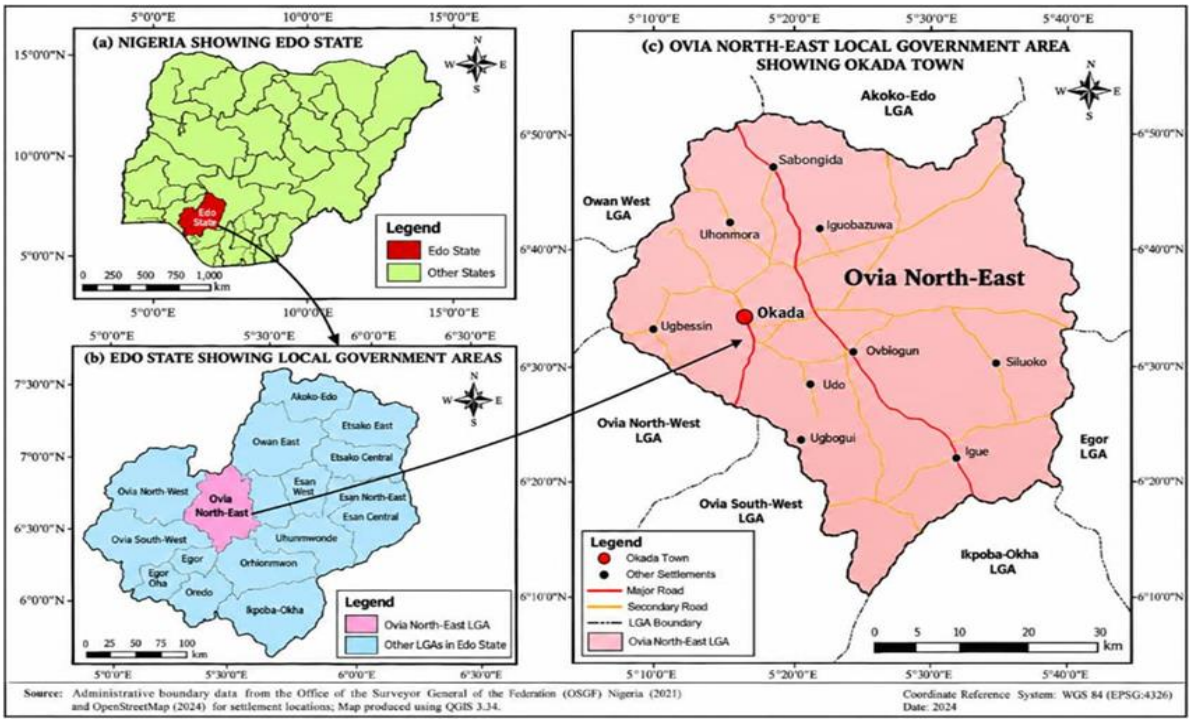


Figure 1: Map of the Study Area

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Experimental Design

A cross-sectional laboratory-based study was adopted to investigate the occurrence and antibiotic resistance characteristics of *Salmonella* spp. and *Escherichia* spp. recovered from eggshell surfaces. The investigation involved the collection of eggs from selected retail outlets, microbiological examination of their external surfaces, isolation and identification of the target organisms, and determination of antimicrobial susceptibility patterns of the isolated organisms. Each egg was considered an individual sampling unit. Samples collected from different retail outlets were assigned unique identification codes to allow the microbiological findings to be traced to their respective sampling locations.

Collection of Egg Samples

Thirty (30) fresh eggs were collected from ten selected retail outlets distributed within Okada (three eggs per retail outlet). Retail outlets were selected to provide representation of the different locations and types of establishments (small scale) where consumers commonly purchase poultry eggs. Eggs were selected without deliberately choosing visibly dirty or visibly clean samples so that the samples would reasonably represent the fresh eggs available to consumers at the point of purchase.

However, each egg was inspected visually and observations concerning visible dirt, cracks or other physical characteristics were recorded after purchase from retail outlets. Individual eggs were handled with sterile gloves and placed into sterile, appropriately labelled sample Polythene bags immediately after purchase. Each egg was placed separately in a sterile container and labelled appropriately. The sample identification code, sampling location, date of collection and relevant observations concerning the condition of the eggs and their storage environment were documented at the point of collection.

Samples were transported to the microbiology laboratory and bacteriological analysis done within four hours of sample collection. During transportation, the samples were protected from unnecessary exposure to environmental contamination and excessive temperature changes. The eggs were kept in labelled polythene bags and then transferred into a sterile plastic container with lid and soft packaging to prevent cracking during transportation.

Microbiological sample preparation was conducted in accordance with the general principles for preparation of food-chain samples described in International Standard Organization, (ISO, 2017) which specifies procedures for preparing initial suspensions and subsequent dilutions for microbiological examinations. The eggs were not washed or disinfected before microbiological examination because such treatment could remove naturally present bacteria on the external surface.

The external surface of each egg was sampled using a sterile cotton swab moistened with sterile Buffered Peptone Water (BPW). The swab was rolled and rubbed systematically over the eggshell surface, covering the major external areas of the egg while avoiding unnecessary contact with the surrounding environment. Following sampling, the swab was immediately transferred into a sterile tube containing 2 mL of sterile BPW. The tube was vigorously agitated to release bacteria from the swab into the suspension. The resulting suspension or eggshell wash constituted the primary sample preparation for bacterial isolation (ISO, 2017).

Cultivation, Enumeration and Isolation of Bacteria

Pre-Enrichment of Eggshell

Pre-enrichment was done by incubating the eggshell sample suspension in Buffered Peptone Water overnight at 35 ºC before selective isolation (ISO, 2017). The pre-enriched eggshell suspension was inoculated onto Nutrient agar plates and incubated at 35°C for 24 hours. Following incubation, the resulting bacterial colonies were purified by repeated subculturing to obtain pure cultures. The purified isolates were subsequently maintained on Nutrient agar slants and stored at 4°C until required for further analysis.

Isolation of *Salmonella-Shigella*

Following the pre-enrichment step, a measured portion of the resulting sample suspension was aseptically inoculated onto selective *Salmonella-Shigella* (SS) agar. The inoculated plates were incubated aerobically at 35 °C for 24 h. Following incubation, the plates were examined for presumptive *Salmonella* colonies, characterized by smooth, circular, translucent to colourless appearances, often exhibiting distinctive black centres (ISO, 2017).

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Suspected colonies were carefully picked and subcultured onto Nutrient Agar to obtain pure cultures. The purified isolates were subsequently preserved on appropriately labeled agar slants and outlet at 4 °C until they were required for further analyses.

Isolation of Potentially Pathogenic *Escherichia*

For the isolation of potentially pathogenic *Escherichia*, the pre-enriched eggshell suspension was inoculated onto sorbitol MacConkey agar and incubated at 37 °C for 18–24 h. Following incubation, colourless colonies were selected and further subcultured onto eosin methylene blue (EMB) agar. Colonies exhibiting the characteristic greenish metallic sheen associated with presumptive pathogenic *Escherichia* (Antony et al., 2016) were subsequently transferred to fresh nutrient agar to obtain pure cultures. The purified isolates were maintained on appropriately labelled agar slants and outlet at 4 °C until required for further laboratory analyses.

Phenotypic Characterization of the Bacterial Isolates

The phenotypic characterization of the bacterial isolates was performed using standard conventional microbiological techniques following the procedures described by Krieg and Holt (1984) and Cheesbrough (2006). Initially, the isolates were subjected to Gram staining to establish their Gram reaction and cellular morphology. This was followed by a series of biochemical assays to facilitate further identification. The identification process involved enzymatic, metabolic and carbohydrate utilization tests, including catalase, oxidase, urease, citrate utilization, indole, methyl red (MR) and Voges–Proskauer (VP) reactions. Fermentation of lactose was also evaluated by recording the production of acid and gas. The overall biochemical profiles obtained were subsequently compared with recognized bacteriological identification criteria to determine the presumptive identities of the bacterial isolates.

Antimicrobial Susceptibility Testing of Isolates

Antimicrobial susceptibility testing (AST) was conducted using the Kirby–Bauer disc diffusion method following the interpretive standards of the Clinical and Laboratory Standards Institute (CLSI, 2024). Fresh bacterial cultures were used to prepare suspensions adjusted to approximately 0.5 McFarland turbidity, equivalent to about 10^8 CFU/mL.

The standardized inoculum was evenly spread over Mueller–Hinton agar, after which antibiotic discs representing major antimicrobial classes were applied. The tested antibiotics included cefuroxime (30 µg); ceftazidime (30 µg); amoxicillin + clavulanic acid (20/10 µg); pefloxacin (5 µg); ofloxacin (5 µg); gentamicin (10 µg); streptomycin (10 µg); ciprofloxacin (5 µg); ceftriaxone (30 µg); and cefoxitin (30 µg). These agents represented the classes of cephalosporins, β-lactam/β-lactamase inhibitor combinations, aminoglycosides, fluoroquinolones. Inoculated plates were incubated at 35°C for 16–18 hours. Following incubation, inhibition-zone diameters around each antibiotic disc were measured and interpreted according to CLSI criteria. Each bacterial isolate was subsequently categorized as susceptible, intermediate, or resistant based on the corresponding zone-diameter breakpoints.

The antimicrobial resistance profiles of the bacterial isolates were evaluated to establish the occurrence of resistance to multiple antimicrobial classes. Each isolate was assessed based on its susceptibility pattern across the different antimicrobial agents included in the testing panel. An isolate was classified as multidrug resistant (MDR) when it exhibited acquired non-susceptibility to at least one antimicrobial agent belonging to three or more distinct antimicrobial categories (WHO, 2024). Accordingly, the antimicrobial categories represented in the susceptibility testing panel were recorded for each isolate, and the resistance pattern was used to determine its MDR status.

Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance (MAR) index was employed as an epidemiological measure to assess the extent of antimicrobial exposure associated with the bacterial isolates. MAR was determined using the conventional method proposed by Krumperman (1983). For each bacterial group, the MAR index was calculated from the cumulative number of antibiotics to which the isolates exhibited resistance relative to the total number of antibiotics tested and the number of isolates evaluated.

$$\text{MAR} = \frac{\sum(AS)}{A \times n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ldots\ldots (1)$$

In the calculation, $\sum AS$ denotes the total resistance score recorded for the isolates within a bacterial group, A represents the number of antibiotics tested, and n refers to the number of isolates examined. In this study, 10 antibiotics were considered for MAR analysis; therefore, A was assigned a value of 10.

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The mean MAR index for each bacterial group was obtained by dividing the cumulative resistance score by the product of the number of isolates and the total number of antibiotics tested. MAR values above 0.20 were regarded as indicative of isolates originating from environments with substantial antibiotic exposure or contamination, based on the interpretation described by Krumperman (1983).

Results

The result of the frequency of occurrence of *Salmonella* and Enterohaemorrhagic *Escherichia* on surfaces of eggshells sold in Okada is as presented in Table 1.

Table 1: Frequency of occurrence of *Salmonella* and Enterohaemorrhagic *Escherichia* on surfaces of eggshells sold in Okada

Retail outlet of Eggs	Number of bacteria Examined	*Salmonella* or Entero-haemorrhagic *Escherichia*	Frequency of occurrence F (%)
1	9	None	None
2	9	None	None
3	9	*Escherichia*	3 (33.33)
4	9	None	None
5	9	None	None
6	6	None	None
7	9	*Escherichia*	4 (44.44)
8	9	None	None
9	6	None	None
10	6	None	None

A total of 81 eggshell samples were examined across the ten retail outlets. No isolate presumptively identified as *Salmonella* was recovered from any of the sampled eggshells. *Escherichia* isolates were recovered from only Retail Outlet 3 and Retail Outlet 7. Biochemical characteristics supported the presumptive identification of the isolates as *Escherichia*.

At Retail Outlet 3, three suspected *Escherichia* isolates were recovered from nine isolates examined, representing a frequency of 33.33%.

Retail Outlet 7 yielded 4 isolates from nine examined isolates, corresponding to 44.44%.

No *Escherichia* isolate was recovered from Retail Outlets 1, 2, 4, 5, 6, 8, 9 or 10. Thus, the occurrence of *Escherichia* was restricted to two of the ten retail outlets examined. Overall, 7 *Escherichia* isolates were recovered from 81 eggshell samples, giving an overall occurrence of 8.64%, whereas *Salmonella* had an overall occurrence of 0%. The distribution of the bacterial isolates demonstrated considerable variation among the retail outlets.

The highest frequency of *Escherichia* was recorded at Retail Outlet 7 (44.44%), followed by Retail Outlet 3 (33.33%). The remaining eight outlets recorded no detectable occurrence. Consequently, the overall frequency of the two target organisms was 8.64% for *Escherichia* and 0% for *Salmonella*. A chi-square test of independence was initially used to examine whether the occurrence of *Escherichia* differed among the ten retail outlets.

The analysis produced a statistically significant association between retail outlet and *Escherichia* occurrence, χ^2 (9) = 27.52, p = 0.001. An exact comparison was also considered by grouping the two positive outlets against the eight outlets where no *Escherichia* was detected. The two positive outlets collectively had 7 positives among 18 samples (38.89%), compared with 0 of 63 samples (0%) from the other outlets. Fisher's exact test showed a statistically significant difference ($p < 0.001$). This indicates that the observed distribution was unlikely to have resulted from random variation alone. Because *Salmonella* was not recovered from any outlet, there was no meaningful between-outlet statistical comparison for *Salmonella* occurrence.

Similarly, inferential comparison between *Salmonella* and *Escherichia* prevalence is limited by the zero frequency recorded for *Salmonella*. The absence of *Salmonella* was therefore interpreted as a finding of non-detection within the sampled population rather than proof that the organism is completely absent from eggs sold in Okada.

Table 2 presents the frequency of occurrence (%) of other probable bacterial isolates from surface of egg shell surfaces purchased and collected from ten retail outlets in Okada, Edo State.

The identified genera included *Enterobacter, Klebsiella, Pseudomonas, Proteus,* and *Enterococcus*. Their distinctive colonial appearances, Gram reactions, and biochemical profiles provided presumptive evidence for identification.

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Table 2: Frequency of occurrence (%) of other probable bacterial isolates from surface of egg shells

Source of eggs (Retail outlet)	Frequency of occurrence (n %) of other probable bacterial isolates from surface of egg shells				
	Enterobacter	***Enterococcus***	***Klebsiella***	***Pseudomonas***	***Proteus***
1	5 (55.56)		3 (33.33)	1 (11.11)	-
2	1 (11.11)	2 (22.22)	1 (11.11)	5 (55.56)	1 (11.11)
3	-	1 (11.11)	2 (22.22)	3 (33.33)	-
4	4 (44.44)	-	-	5 (55.56)	-
5	-	-	-		(100)
6	3 (50.00)	2 (33.33)	-	1 (16.67)	-
7	-	1 (11.11)	-	4 (44.44)	-
8	5 (55.56)	-	1 (11.11)	3 (33.33)	-
9	3 (50.00)	-		3 (50.00)	-
10	4 (66.67)	-	1 (16.67)	1 (16.67)	-

Across 81 isolates, *Enterobacter* and *Pseudomonas* were most frequent, with 26 occurrences each (32.10%). *Proteus, Klebsiella,* and *Enterococcus* occurred 10 (12.35%), 8 (9.88%), and 4 (4.94%) times, respectively. Chi-square analysis showed significant outlet-related variation for *Enterobacter* ($\chi^2 = 23.39$, df = 9, p = 0.01) and *Proteus* ($\chi^2 = 72.79$, df = 9, $p < 0.001$). Conversely, differences were non-significant for *Klebsiella* ($\chi^2 = 11.72$, df = 9, p = 0.230), *Pseudomonas* ($\chi^2 = 13.45$, df = 9, p = 0.143), and *Enterococcus* ($\chi^2 = 14.73$, df = 9, p = 0.099). Thus, retail outlet was associated with the occurrence of *Enterobacter* and *Proteus*, but not the other three genera.

The antibiotic resistance profile of bacterial isolates recovered from the surfaces of eggshells sold at the ten retail outlets in Okada, Edo State, is presented in Table 3. A total of 81 bacterial isolates were subjected to antimicrobial susceptibility testing against ten antibiotics representing several antimicrobial classes. The isolates comprised *Enterobacter* (26; 32.10%), *Pseudomonas* (26; 32.10%), *Proteus* (9; 11.11%), *Klebsiella* (8; 9.88%), *Escherichia* (7; 8.64%) and *Enterococcus* (5; 6.17%).

The resistance pattern was characterized by very high resistance to most of the antibiotics tested. Resistance to cefuroxime (CEF), cefoxitin (FOX), ceftriaxone (TXN), ceftazidime (CTZ), pefloxacin (PEF), gentamicin (GN) and streptomycin (S) was observed in 81 of the 81 isolates (100.00%) for each antibiotic. Amoxicillin-clavulanic acid (AMC) showed resistance in 80 isolates (98.77%), whereas resistance to ciprofloxacin (CIP) occurred in 73 isolates (90.12%). Ofloxacin (OFX) demonstrated the lowest resistance, with only 2 isolates (2.47%) classified as resistant

Thus, the isolates showed particularly extensive resistance to the β-lactam antibiotics represented by cefuroxime, cefoxitin, ceftriaxone and ceftazidime, as well as to pefloxacin, gentamicin and streptomycin. In contrast, ofloxacin remained active against the overwhelming majority of isolates, with 79 isolates (97.53%) susceptible to the antibiotic. Resistance to the individual antibiotics varied somewhat among bacterial genera, although resistance to seven of the ten antibiotics was universal. All *Enterobacter*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Escherichia* and *Enterococcus* isolates were resistant to cefuroxime, cefoxitin, ceftriaxone, ceftazidime, pefloxacin, gentamicin and streptomycin.

The principal variation occurred with AMC, OFX and CIP. AMC resistance occurred in all isolates except one *Klebsiella* isolate recovered from Retail outlet 10, resulting in an overall resistance of 98.77%. Ofloxacin resistance was recorded in one *Pseudomonas* isolate from Retail outlet 2 and one *Proteus* isolate from Retail outlet 5, giving an overall resistance of 2.47%. Ciprofloxacin resistance was comparatively widespread. It was observed in 23 of 26 (88.46%) *Enterobacter* isolates, 22 of 26 (84.62%) *Pseudomonas* isolates, 9 of 9 (100.00%) *Proteus* isolates, 8 of 8 (100.00%) *Klebsiella* isolates, 6 of 7 (85.71%) *Escherichia* isolates and 5 of 5 (100.00%) *Enterococcus* isolates. A chi-square test was used to examine whether ciprofloxacin resistance was significantly associated with bacterial genus. The analysis gave $\chi^2 = 3.53$, df = 5, p = 0.619. Therefore, the observed differences in ciprofloxacin resistance among the bacterial genera were not statistically significant ($p > 0.05$). The result suggests that the high ciprofloxacin resistance was broadly distributed across the bacterial population rather than being restricted to a particular genus.

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Table 3: Antibiotic resistance profile of bacterial isolates on surfaces of eggshells from retain outlets in Okada

Retail Outlet of Eggs	Isolated bacteria		Prevalence of bacterial resistance to the tested antibiotics / N (%)									
		Number of isolates	CEF (30µg)	FOX (30µg)	TXN (30µg)	CTZ (30µg)	AMC (20/10µg)	OFX (5µg)	CIP (5µg)	PEF (5µg)	GN (10µg)	S (10µg)
1	*Enterobacter*	5	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	0 (0.00)	4 (80.00)	5 (100.00)	5 (100.00)	5 (100.00)
	Klebsiella	3	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	0 (0.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)
	Pseudomonas	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)
2	*Enterobacter*	2	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)	0 (0.00)	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)
	Pseudomonas	5	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	1 (20.00)	3 (60.00)	5 (100.00)	5 (100.00)	5 (100.00)
	Enterococcus	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)
	Klebsiella	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)
3	*Escherichia*	3	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	0 (0.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)
	Enterococcus	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)
	Pseudomonas	3	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	0 (0.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)
	Klebsiella	2	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)	0 (0.00)	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)
4	*Pseudomonas*	5	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	0 (0.00)	4 (80.00)	5 (100.00)	5 (100.00)	5 (100.00)
	Enterobacter	4	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)	0 (0.00)	3 (75.00)	4 (100.00)	4 (100.00)	4 (100.00)
5	*Proteus*	9	9 (100.00)	9 (100.00)	9 (100.00)	9 (100.00)	9 (100.00)	1 (11.11)	9 (100.00)	9 (100.00)	9 (100.00)	9 (100.00)
6	*Enterococcus*	2	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)	0 (0.00)	2 (100.00)	2 (100.00)	2 (100.00)	2 (100.00)
	Enterobacter	3	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	0 (0.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)
	Pseudomonas	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)
7	*Escherichia*	4	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)	0 (0.00)	3 (75.00)	4 (100.00)	4 (100.00)	4 (100.00)
	Pseudomonas	4	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)	0 (0.00)	3 (75.00)	4 (100.00)	4 (100.00)	4 (100.00)
	Enterococcus	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)
8	*Enterobacter*	5	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)	0 (0.00)	5 (100.00)	5 (100.00)	5 (100.00)	5 (100.00)
	Klebsiella	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)
	Pseudomonas	3	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	0 (0.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)
9	*Pseudomonas*	3	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	0 (0.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)
	Enterobacter	3	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)	0 (0.00)	3 (100.00)	3 (100.00)	3 (100.00)	3 (100.00)
10	*Enterobacter*	4	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)	4 (100.00)	0 (0.00)	3 (75.00)	4 (100.00)	4 (100.00)	4 (100.00)
	Pseudomonas	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)
	Klebsiella	1	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)	0 (0.00)	0 (0.00)	1 (100.00)	1 (100.00)	1 (100.00)	1 (100.00)

CEF: cefuroxime; CTZ: Ceftazidime; AMC: Amoxicillin + Clavulanic acid; PEF: Pefloxacin; OFX: Ofloxacin; GEN: Gentamicin; S: Streptomycin; CIP: Ciprofloxacin; TXN: Ceftriaxone; FOX: Cefoxitin

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The total number of isolates recovered from the individual retail outlets ranged from six to nine. Retail outlets 1, 2, 3, 4, 5, 7 and 8 each contributed nine isolates, whereas outlets 6, 9 and 10 contributed six isolates each, giving an overall total of 81 isolates. Ciprofloxacin resistance ranged from 5/6 isolates (83.33%) at Retail outlet 10 to 9/9 isolates (100.00%) at Retail outlets 3, 5 and 8. The corresponding resistance proportions were 8/9 (88.89%) at outlet 1, 7/9 (77.78%) at outlet 2, 7/9 (77.78%) at outlet 4, 6/6 (100.00%) at outlet 6, 7/9 (77.78%) at outlet 7 and 6/6 (100.00%) at outlet 9.

A chi-square test examining the association between retail outlet and ciprofloxacin resistance produced $\chi^2 = 9.22$, df = 9, p = 0.42. Consequently, the variation in ciprofloxacin resistance among the ten retail outlets was not statistically significant ($p > 0.05$). Although some outlets recorded complete ciprofloxacin resistance, the pattern did not provide sufficient statistical evidence that resistance differed systematically according to retail outlet. Based on the individual resistance of all 81 isolates tested against all ten antibiotics, the susceptibility observations represent paired/repeated measurements rather than independent observations. Consequently, Cochran's Q test was used to determine whether the proportion of resistant isolates differed significantly among the ten antibiotics. The analysis showed a highly significant difference in resistance proportions across the antibiotic panel (Cochran's Q = 655.56, df = 9, $p < 0.001$). This indicates that resistance was not distributed equally among the ten antibiotics. The result was primarily driven by the markedly different resistance profiles of OFX and CIP compared with the other antibiotics. Eight antibiotics showed complete resistance (100.00%), whereas OFX showed no resistance (0.00%) and CIP showed 22.22% resistance. Pairwise McNemar comparisons further demonstrated highly significant differences between OFX and each of the antibiotics to which all isolates were resistant ($p < 0.001$). The difference between OFX and CIP was also statistically significant (p = 0.000008). Similarly, CIP differed significantly from each of the antibiotics showing 100% resistance ($p < 0.001$). Because multiple pairwise comparisons were performed, these results were preferably interpreted with a multiplicity adjustment. Even after a conservative Bonferroni adjustment, the major differences remained statistically significant.

The isolate-level analysis therefore established two major statistical findings. First, antimicrobial resistance differed significantly across the ten antibiotics tested (Cochran's Q = 650.49, $p < 0.001$). Second, ofloxacin demonstrated significantly lower resistance than the other antibiotics.

The Multiple antibiotics resistance (MAR) index of bacteria isolated from surfaces of fresh eggshell is as presented in Table 4. Across the ten antibiotics, there were 722 resistance events out of 810 antibiotic-isolate combinations, corresponding to an overall resistance burden of 89.14%. On average, each bacterial isolate was resistant to approximately 8.91 of the ten antibiotics tested. Because every isolate was resistant to at least seven antibiotics, cefuroxime, cefoxitin, ceftriaxone, ceftazidime, pefloxacin, gentamicin and streptomycin, all 81 isolates (100.00%) met the conventional criterion for multidrug resistance (MDR). *Enterobacter* was recovered from several retail outlets and generally exhibited high resistance burdens. The mean MAR indices recorded for *Enterobacter* ranged from 0.88 to 0.90. At retail outlet 1, five *Enterobacter* isolates produced a cumulative resistance score of 44 and a mean MAR index of 0.88. At outlet 2, two isolates produced a resistance score of 18 and a mean MAR index of 0.90. Four *Enterobacter* isolates from outlet 4 recorded a cumulative resistance score of 35 and a mean MAR index of 0.88. Similarly, the three isolates recovered from outlet 6 recorded a mean MAR index of 0.90, while five isolates from outlet 8 also recorded a mean MAR index of 0.90. Three isolates from outlet 9 recorded a mean MAR index of 0.90, whereas four isolates from outlet 10 had a mean MAR index of 0.88. *Pseudomonas* was also widely distributed among the retail outlets and exhibited a similarly high resistance burden. The mean MAR index ranged from 0.88 to 0.90. Five *Pseudomonas* isolates from outlet 2 had a cumulative resistance score of 44 and a mean MAR index of 0.88. Five isolates from outlet 4 had the same mean MAR index. Other *Pseudomonas* populations, particularly those recovered from outlets 1, 3, 6, 8, 9 and 10, recorded mean MAR indices of 0.90. *Klebsiella* isolates consistently demonstrated high resistance. The mean MAR index was 0.90 at all outlets where the organism was recovered. Three isolates from outlet 1 recorded a cumulative resistance score of 27, while one isolate each from outlets 2, 8 and 10 recorded resistance scores of 9.

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Table 4: Multiple antibiotics resistance index of bacteria isolated from surfaces of fresh eggshell

Retail Outlet of Eggs	Isolated bacteria	n	∑AS	A	Mean MAR Index
1	*Enterobacter*	5	44	10	0.88
	Klebsiella	3	27	10	0.90
	Pseudomonas	1	9	10	0.90
2	*Enterobacter*	2	18	10	0.90
	Pseudomonas	5	44	10	0.88
	Enterococcus	1	9	10	0.90
	Klebsiella	1	9	10	0.90
3	*Escherichia*	3	27	10	0.90
	Enterococcus	1	9	10	0.90
	Pseudomonas	3	27	10	0.90
	Klebsiella	2	18	10	0.90
4	*Pseudomonas*	5	44	10	0.88
	Enterobacter	4	35	10	0.88
5	*Proteus*	9	82	10	0.91
6	*Enterococcus*	2	18	10	0.90
	Enterobacter	3	27	10	0.90
	Pseudomonas	1	9	10	0.90
7	*Escherichia*	4	35	10	0.88
	Pseudomonas	4	35	10	0.88
	Enterococcus	1	9	10	0.90
8	*Enterobacter*	5	45	10	0.90
	Klebsiella	1	9	10	0.90
	Pseudomonas	3	27	10	0.90
9	*Pseudomonas*	3	27	10	0.90
	Enterobacter	3	27	10	0.90
10	*Enterobacter*	4	35	10	0.88
	Pseudomonas	1	9	10	0.90
	Klebsiella	1	9	10	0.90
All retail outlets		81	723	10	0.89

Key: ∑*AS* denotes the total resistance score recorded for the isolates within a bacterial group, *A* represents the number of antibiotics tested, and *n* refers to the number of isolates examined.

Two isolates from outlet 3 recorded a resistance score of 18. The consistency of the MAR values indicates that the resistance burden in *Klebsiella* was relatively stable across the sampled retail locations The *Enterococcus* isolates also exhibited substantial resistance. One isolate from outlet 2, one from outlet 3, two from outlet 6 and one from outlet 7 all had mean MAR indices of 0.90. Similarly, the *Escherichia* isolates from outlets 3 and 7 had mean MAR indices of 0.90 and 0.88, respectively.

The highest individual organism-outlet MAR index was observed for *Proteus* at retail outlet 5. Nine *Proteus* isolates accumulated a resistance score of 82, giving a mean MAR index of approximately 0.91. This was the highest mean resistance burden recorded among the bacterial groups examined. The overall MAR index of 0.89 indicates that, on average, the isolates were resistant to approximately nine of the ten antibiotics tested. The resistance burden was therefore not restricted to a single bacterial genus or retail location. Rather, high MAR values occurred across *Enterobacter, Pseudomonas, Klebsiella, Enterococcus, Escherichia* and *Proteus*. The consistently high MAR values also indicate that the bacterial isolates had resistance profiles compatible with exposure to environments where antimicrobial agents may have been repeatedly used. However, the MAR index itself does not establish the source or cause of resistance; it only describes the observed resistance burden. The 28 organism-by-retail-outlet MAR observations were compared among the six bacterial genera using one-way analysis of variance. The analysis showed no statistically significant difference in mean MAR index among the bacterial genera: $F(5,22) = 1.59$, $p = 0.20$. Thus, although *Proteus* showed the highest observed mean MAR index (0.91) and *Enterobacter* and *Pseudomonas* included values as low as 0.88, the observed differences among genera were not statistically significant at the 5% level. Because MAR indices were highly clustered near 0.90 and the number of observations contributed by the different bacterial genera was unequal, a Kruskal-Wallis test was also considered. The non-parametric analysis similarly showed no statistically significant difference among bacterial genera: $H(5) = 8.71$, $p = 0.12$. Comparison of the organism-group MAR observations across the ten retail outlets also did not demonstrate a statistically significant difference: Kruskal-Wallis $H(9) = 15.07$, $p = 0.09$.

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The corresponding exploratory one-way ANOVA gave $F(9,18) = 2.18$, $p = 0.08$, which likewise did not reach statistical significance. Taken together, the inferential analyses suggest that the high MAR burden was broadly distributed rather than being concentrated in a particular bacterial genus or retail outlet. The absence of statistical significance should not be interpreted as evidence that the resistance profiles are identical; rather, the available aggregated data provide insufficient evidence of a statistically significant difference.

Discussion

The present study assessed the occurrence, phenotypic characteristics and antimicrobial resistance of bacteria recovered from eggshell surfaces sold at selected retail outlets in Okada, Edo State, Nigeria. A total of 81 eggshell samples were examined using cultural, morphological and biochemical procedures. *Escherichia* was recovered from seven samples (8.64%), whereas *Salmonella* was not detected. The recovery of enteric bacteria from eggshells is important because contamination may occur during laying, collection, transportation, storage and marketing. Ekpunobi et al. (2024) reported *Escherichia* and *Salmonella* among bacterial contaminants of table eggs marketed in Lagos, confirming the relevance of eggshell hygiene within the Nigerian food chain. The occurrence of *Escherichia* suggests exposure of some eggs to faecal or environmental contamination. Okorie-Kanu et al. (2016) reported considerably higher pathogenic *Escherichia* occurrence in eggs marketed in Enugu State, including substantial eggshell contamination. Differences in prevalence may reflect geographical location, production systems, handling practices, environmental conditions, sample size and laboratory procedures. *Escherichia* was concentrated in Retail Outlets 3 and 7, where occurrence was 33.33% and 44.44%, respectively, indicating uneven contamination. The significant association between retail outlet and *Escherichia* occurrence ($\chi^2 = 27.52$, $p = 0.001$) supports this observation. Kapena et al. (2021) similarly identified eggshells as an important site of *Escherichia* contamination in retail eggs in Zambia, while Hemeda et al. (2025) emphasized bacterial contamination of table eggs as a food-safety concern where hygiene and storage controls are inadequate.

The biochemical profile of the *Escherichia* isolates, characterized by Gram-negative rods, catalase, indole, methyl red and lactose positivity, together with negative urease, oxidase, citrate and Voges–Proskauer reactions, provided presumptive support for their identification. *Salmonella* was not recovered from any sample. This finding represents non-detection during the study period rather than proof of complete absence. Adesiyan et al. (2020) recovered *Salmonella* predominantly from eggshells in Ogun State, whereas Okorie-Kanu et al. (2016) and Addo et al. (2020) reported *Salmonella* contamination of eggshells in Nigeria and Ghana, respectively. Ekpunobi et al. (2024) also detected *Salmonella Typhimurium* in Nigerian eggshells. Zige and Alabere (2025) and Akintomide et al. (2025) further demonstrated continuing bacterial contamination of retail eggs and eggshell-associated materials in Nigeria. The USFDA (2025) have documented recent shell-egg-associated *Salmonella* outbreaks, demonstrating that continued surveillance and safe egg handling remain necessary.

Other bacterial genera recovered were *Enterobacter, Pseudomonas, Proteus, Klebsiella* and *Enterococcus*. *Enterobacter* and *Pseudomonas* were predominant, each occurring in 32.10% of the examined eggs. Jones et al. (2004) previously recovered *Enterobacter* and other Enterobacteriaceae from commercial eggshells, while Wang et al. (2024) demonstrated geographical and farm-related variation in eggshell microbial communities. *Pseudomonas* was particularly frequent at some outlets, consistent with its environmental persistence and association with moist environments. The occurrence of *Klebsiella* was 9.88%, and its detection across several outlets agrees with observations by Makinde et al. (2023) and Oladosu et al. (2023), who identified *Klebsiella* and related Enterobacterales in Nigerian retail food environments. *Proteus* showed the most pronounced outlet-specific distribution, occurring in all nine eggs examined at Retail Outlet 5. The association between retail outlet and *Proteus* occurrence was highly significant ($\chi^2 = 72.79$, $p < 0.001$), suggesting a localized contamination pattern. Ogunleye et al. (2025) likewise reported *Proteus mirabilis* among important multidrug-resistant poultry-associated bacteria. *Enterococcus* occurred less frequently, but its detection remains relevant because Araújo et al. (2017) demonstrated substantial *Enterococcus* contamination of eggshells from treated laying hens.

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The biochemical characteristics of the recovered organisms provided useful presumptive evidence for their identification, although molecular confirmation would provide greater taxonomic certainty.

The antimicrobial susceptibility findings revealed an exceptionally high resistance burden. Resistance to cefuroxime, cefoxitin, ceftriaxone, ceftazidime, pefloxacin, gentamicin and streptomycin was 100%, while resistance to amoxicillin-clavulanic acid and ciprofloxacin was 98.77% and 90.12%, respectively. Ofloxacin showed comparatively high activity, with 97.53% susceptibility. Hinson et al. (2025) documented substantial antimicrobial resistance among bacterial pathogens associated with table eggs globally and reported that eggshell contamination frequently exceeded contamination of egg contents. Chukwunwejim et al. (2025) and Ekpunobi et al. (2024) similarly reported multidrug-resistant bacteria on Nigerian eggshells. The complete resistance to several cephalosporins and the high ciprofloxacin resistance are particularly concerning because these drugs are clinically important. Ndahi et al. (2023) documented widespread antimicrobial use in Nigerian commercial poultry farms, while Hambolu et al. (2024) reported extensive therapeutic and prophylactic antibiotic use, including antibiotic combinations, in Nigerian poultry production. Such practices may create selection pressure favouring resistant organisms, although the present study cannot establish the precise source or mechanism of resistance. Ciprofloxacin resistance was high across all bacterial genera but did not differ significantly according to genus ($\chi^2 = 3.53$, $p = 0.619$) or retail outlet ($\chi^2 = 9.22$, $p = 0.42$). This suggests that resistance was broadly distributed rather than restricted to a particular organism or location. The marked susceptibility to ofloxacin despite its membership of the same fluoroquinolone class emphasizes the importance of antibiotic-specific susceptibility testing.

The study recorded 722 resistance events among 810 antibiotic-isolate combinations, corresponding to an overall resistance burden of 89.14%. Every isolate was resistant to at least seven antibiotics and consequently met the conventional criterion for multidrug resistance. The overall MAR index was 0.89, with organism-outlet values generally ranging from 0.88 to 0.91. *Proteus* at Retail Outlet 5 had the highest mean MAR index (0.91).

Statistical comparisons did not demonstrate significant differences in MAR indices among bacterial genera or retail outlets, indicating that the resistance burden was broadly distributed. Tadesse et al. (2024), similarly identified bacterial contamination and antimicrobial resistance in table eggs as important global food-safety concerns. The findings have important One Health implications because resistant bacteria on eggshells may be transferred to hands, utensils, food-contact surfaces and other foods during handling, even when the edible contents remain uncontaminated. Adesiyan et al. (2020) and Ekpunobi et al. (2024) highlighted the importance of biosecurity, market hygiene and handling practices in reducing egg contamination. Hambolu et al. (2024) further demonstrated that improved biosecurity measures were associated with reduced detection of non-typhoidal *Salmonella* in Nigerian poultry systems. Okorie-Kanu et al. (2016) documented antimicrobial resistance and plasmid-associated resistance among Enterobacteriaceae from Nigerian retail meat, reinforcing concerns about resistant bacteria within food environments. Overall, the study demonstrates that although *Salmonella* was not detected, eggshells sold in Okada carried diverse bacterial populations, including *Escherichia, Enterobacter, Pseudomonas, Proteus, Klebsiella* and *Enterococcus*. The high prevalence of multidrug resistance and overall MAR index of 0.89 indicate substantial antimicrobial-resistance pressure. The findings support strengthened poultry biosecurity, responsible antimicrobial use, veterinary oversight, adherence to withdrawal periods, hygienic egg collection and transportation, clean retail storage, consumer education and routine microbiological surveillance. Further molecular studies are recommended to confirm bacterial identities and characterize resistance genes and mobile genetic elements associated with the observed resistance patterns.

Conclusion

This study established that eggshells sold across the sampled retail outlets in Okada harboured a diverse range of bacterial organisms of potential public health importance. Although *Salmonella* was not detected, suspected *Escherichia* occurred in 8.64% of the samples, with its distribution significantly associated with retail outlet.

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Enterobacter and *Pseudomonas* were the predominant bacterial genera, while significant outlet-associated variation was observed for *Enterobacter* and *Proteus*. The antibiotic susceptibility findings revealed an exceptionally high resistance burden, with resistance to several β-lactam and non-β-lactam antibiotics reaching 100%. Ciprofloxacin resistance was also widespread, whereas ofloxacin demonstrated comparatively greater activity. All recovered isolates fulfilled the criterion for multidrug resistance, with an overall resistance burden of 89.14% and a mean MAR index of 0.89. The consistently high MAR values across bacterial genera and retail outlets indicate a substantial antimicrobial resistance problem associated with eggshell contamination. These findings underscore the importance of improved hygiene during egg production, transportation, storage and retail handling, together with strengthened antimicrobial stewardship and routine microbiological surveillance to minimize potential food-safety risks to consumers.

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