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# Antibiotic Resistance of *Escherichia coli* and *Salmonella* Isolates from Table Eggs, Poultry Sausages, and Clinical Samples in Southwest Benin

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## Abstract

Antimicrobial resistance (AMR) in foodborne pathogens poses a major public health risk in Benin. Table eggs and poultry sausages have been identified as potential reservoirs of resistant bacteria. Nevertheless, the lack of integrated AMR data from food and clinical sources limits the ability to assess public health risks and design evidence-based interventions. Therefore, this study aimed to determine the prevalence of *Escherichia coli* and *Salmonella* in table eggs and poultry sausages and to evaluate the antibiotic resistance profile of isolates from these foods and clinical samples alongside clinical isolates. A total of 135 table egg pools, 90 poultry sausages, and 81 clinical isolates of *E. coli* (56) and *Salmonella* (25) were collected between August and December 2023. Table eggs and poultry sausages were analysed for *Escherichia coli* and *Salmonella* using conventional methods. Antibiotic resistance ($n$ = 99 isolates) was tested using Kirby–Bauer disc diffusion. Clinical isolates ($n$ = 77) were included for comparison. High *Escherichia coli* prevalence (eggs: 59.3%, 95% CI: 50.9–67.3%; sausages: 14.4%, 95% CI: 8.6–23.2%) and lower *Salmonella* prevalence (eggs: 2.2%, 95% CI: 0.8–6.3%; sausages: 5.6%, 95% CI: 2.4–12.4%) were observed. According to antibiotic resistance data, predominant multidrug resistance to tetracyclines, streptomycin, and fluoroquinolones, followed by preserved sensitivity to nitrofurans and chloramphenicol, was noticed. These findings highlight the urgent need to regulate antibiotic use in Benin's poultry and medical sectors.

**Keywords:** antibiotic resistance; *Escherichia coli*; *Salmonella*; poultry products; Benin



Academic Editor: Eugenia Bezirtzoglou

Received: 23 December 2025
Revised: 4 February 2026
Accepted: 5 February 2026
Published: 11 February 2026



## 1. Introduction

In sub-Saharan Africa, poultry farming is becoming more intensive, yet in many countries, such as Benin, it remains largely informal and continues to play a major role in family agriculture and poverty reduction [1–3]. Eggs are widely consumed worldwide because they are affordable, highly nutritious, and can be stored for several weeks in their natural state, although storage conditions may affect their internal and external quality over time [4,5]. However, data on antimicrobial resistance (AMR) in poultry products (eggs and sausages) and associated clinical isolates remain scarce, limiting targeted intervention [6–8]. In 2023, the laying hen population in Benin was estimated at 1,068,000 birds,

producing approximately 17,388 tonnes of eggs in 2023 [9]. This nearly corresponds to 289.8 million table eggs with an average unit weight of 60 g from both traditional and modern systems [10]. In parallel, the demand for processed poultry products, particularly sausages, has increased steadily in Benin, driven by urbanization and changing dietary habits. Local needs are partly met by imports, which can reach around 280 tonnes of poultry sausages per month, and partly by the recent development of domestic charcuterie industries producing finely textured sausages [11,12]. These consumption trends heighten AMR contamination risks, as imported and processed poultry products often harbor multidrug-resistant bacteria (e.g., *E. coli*, *Salmonella*) introduced via chicks/eggs and amplified by inadequate hygiene and cold chain controls. These products are highly perishable and require strict compliance with hygiene standards, food safety regulations, and cold chain management, yet in Benin, food processing beyond fresh meat slaughter remains limited, and controls over manufacturing, storage, and retail conditions are often insufficient [12]. Eggs and poultry meat products harbor pathogens such as *Salmonella enterica*, pathogenic *E. coli*, *Enterobacter cloacae*, *Klebsiella*, *Enterococcus*, and *Staphylococcus*, contaminating shells, contents, and minced meat [13]. Sausages are particularly vulnerable due to the grinding, mixing, and stuffing steps, favoring cross-contamination and biofilms persisting on surfaces under suboptimal cleaning [14]. *Escherichia coli* is a commensal inhabitant of the intestinal tract of humans and warm-blooded animals and is released into the environment through faeces and wastewater [15]. *Salmonella enterica* is one of the most important foodborne pathogens globally and has been associated with tens of millions of gastroenteritis cases and hundreds of thousands of deaths each year, with poultry meat and eggs identified as major vehicles in many outbreaks [16]. Furthermore, biofilms formed by *Salmonella* and pathogenic *E. coli* on eggshells, processing equipment, and food-contacting surfaces significantly enhance pathogen persistence and resistance to sanitizers, as their extracellular matrix shields bacteria from disinfectants [17–19]. These structured communities facilitate cross-contamination during egg handling, sausage mincing, and storage, particularly under Benin's limited cold chain and hygiene controls. The widespread use of antibiotics in poultry production has historically improved animal health and productivity, helping to ensure regular supplies of affordable meat and eggs. However, the inclusion of antimicrobial agents in feed or water to treat, control, or prevent diseases has exerted a strong selective pressure on bacterial populations, promoting the emergence and dissemination of resistance genes, metabolic adaptation, and biofilm formation [20]. In developing countries, poultry meat consumption is projected to grow markedly in the coming years, which is likely to be accompanied by intensified production and, in the absence of strict regulations, by sustained or increased antibiotic use [21]. In Benin, veterinary drugs can often be obtained directly from clinics or pharmacies on customer request, sometimes without proper prescription, and self-medication by farmers [22,23], misuse of antimicrobial molecules, incorrect dosing, inadequate treatment duration, and failure to respect withdrawal periods are frequently reported [24]. In the human sector, antibiotic overuse and self-medication further accelerate resistance. Studies in Benin have reported high rates of hospital-acquired infections and high levels of resistance among Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* and third-generation cephalosporin-resistant *E. coli* [25]. However, data on AMR in food products of animal origin, particularly table eggs and poultry sausages, are still scarce. Possible links between foodborne isolates and clinical strains circulating in hospitals are poorly documented, which limits risk assessment and control. The key gaps hindering effective AMR control in Benin include weak multisectoral coordination, limited surveillance systems, insufficient resources, and inadequate communication and education efforts. Additionally, there are gaps in laboratory capacity, antimicrobial use monitoring,

infection prevention programs, and integration of WASH practices, which collectively impede progress against antimicrobial resistance [22,26,27]. WASH refers to integrated water, sanitation, and hygiene interventions aimed at ensuring access to safe water, adequate sanitation facilities, and basic hygiene behaviours (e.g., handwashing with soap), which are essential for preventing infections and reducing antimicrobial resistance. In Benin, most available data focus either on clinical settings or on food, but rarely on both. It can be hypothesized that *Escherichia coli* and *Salmonella* isolated from table eggs and poultry sausages show high levels of resistance to antimicrobials commonly used in veterinary and human medicine. Phenotypic analyses of AMR may reveal similarities between foodborne (eggs/sausages) and clinical *E. coli* and *Salmonella* isolates, potentially indicating shared selective pressures or circulation within Benin's food–animal–human interface. This study, therefore, aims to adopt an integrated approach to determine the prevalence and antimicrobial resistance profiles of *E. coli* and *Salmonella* in table eggs and poultry sausages, as well as in clinical samples from the National University Hospital Centre in Cotonou. It also aims to compare resistance patterns across these compartments to identify overlapping phenotypes and explore potential food–human AMR linkages to inform surveillance priorities and stewardship measures. By combining data from the food and hospital sectors, the study describes the prevalence.

## 2. Materials and Methods

### *2.1. Study Area*

The present study was carried out from August to December 2023 (average temperature of 31 °C), corresponding to a dry period with minimal rainfall. Five departments in southwest Benin, Atlantique, Littoral, Mono, Ouémé, and Couffo (Figure 1), organized into two agricultural development poles (ADPs), were selected on account of their high levels of poultry meat production and consumption.

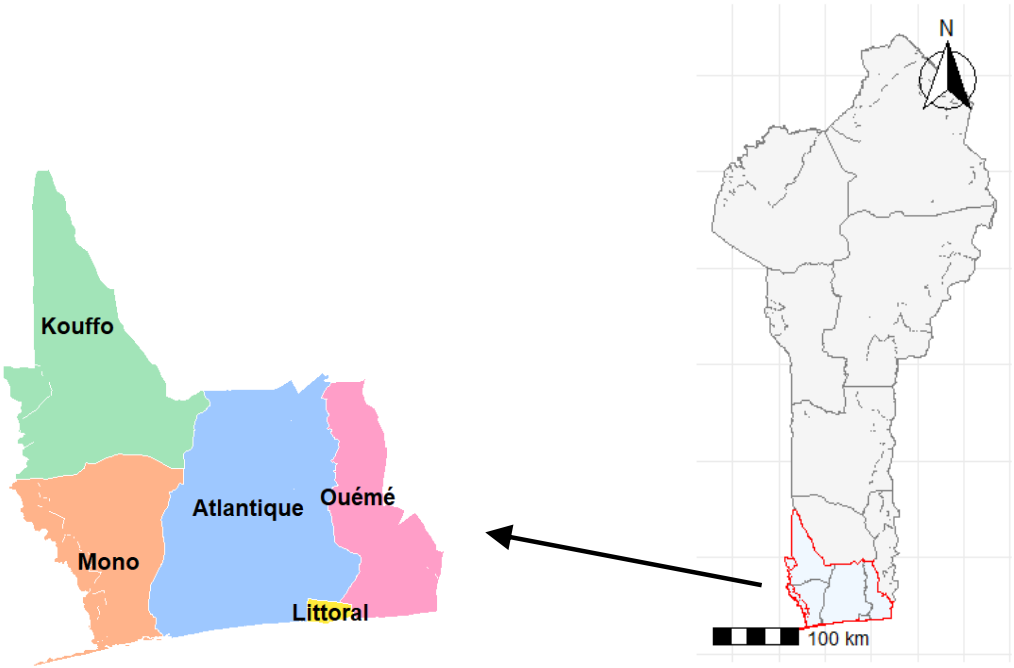


**Figure 1.** Geographical location of the study area in southwest Benin, including the different departments. Legend: eggs and poultry sausages were collected in Mono, Atlantique, Couffo, Ouémé, and Littoral; clinical samples were collected in the Littoral Department only.

### 2.2. Sampling

A convenience sampling approach incorporating snowball techniques was employed to collect table egg and poultry sausage samples across the five departments. This was necessary because no exhaustive up-to-date list of poultry producers and vendors was available. Ministry lists are outdated, and the majority of farms and vendors were identified through word of mouth from local stakeholders. We analysed 135 pooled egg samples (675 eggs in total, 60 from farms and 75 commercial samples) and 90 pre-cooked poultry sausages (15 from the three most recognized local producers who agreed to collaborate and 75 imported samples). For eggs, composites of five eggs per farm were taken, and for sausages, one package of ten pieces per producer or vendor was taken. Samples were transported at 4 °C to the laboratory. Sample sizes provided reasonable precision for detecting contamination at moderate prevalence (e.g., > 99% probability of detecting $\geq$ 5% contaminated egg pools with $n$ = 135, assuming independence). The results reflect sampling period conditions and may not capture full seasonality. Source distribution mirrored product availability at accessible sites rather than stratified balancing.

Additionally, 56 *Escherichia coli* isolates and 25 *Salmonella* isolates from urine, blood, and stool samples at the National University Hospital Centre of Cotonou in Benin were included. These clinical isolates were anonymised, precluding patient identification.

### 2.3. Microbial Analysis of Table Eggs

Microbial analysis of the table eggs involved separate enumeration, isolation, and identification of *Escherichia coli* and *Salmonella* spp. from eggshells and egg contents. Egg samples were pooled in groups of five to form composite samples and processed as per Adesiyun et al. [28] with adaptations. For eggshells, each composite sample's surface was swabbed using a sterile, Buffered Peptone Water-moistened (BPW) swab covering the entire area. Swabs were placed in 9 mL of BPW, diluted in 90 mL BPW, and incubated at 37 °C for *Salmonella* detection [29]. For *E. coli*, 0.1 mL of the main suspension was plated onto Tryptone Bile X-Glucuronide agar (TBX) and incubated at 44 °C for 24 h [30]. Representative blue–green colonies were presumptively re-streaked on TBX for purification. For egg contents, the same composite sample was disinfected (70% ethanol, 2 min). The blunt end was flame-sterilised, and eggs were aseptically opened. Contents were pooled, homogenised, and 25 mL of the mixture were combined with 225 mL BPW. For *E. coli* enumeration, 0.1 mL of the neat and serial dilutions was plated on TBX agar and incubated at 44 °C for 24 h. Presumptive *E. coli* were identified as typical blue–green colonies purified by re-streaking on TBX.

### 2.4. Microbial Analysis of Poultry Sausages

Twenty-five-gram portions of both locally produced and imported poultry sausages were homogenized with 225 mL BPW for simultaneous *Salmonella* spp. detection and *E. coli* enumeration. *Salmonella* spp. detection followed ISO 6579-1:2017 [29], incorporating selective enrichment and plating. *E. coli* were quantified on TBX agar, incubated at 44 °C for 24 h. Presumptive *E. coli*, typical blue–green colonies were re-isolated on a fresh TBX agar for purity.

### 2.5. Serotyping of Salmonella Isolates and Preservation

Presumptive *Salmonella* isolates obtained from a xylose lysine deoxycholate (XLD) agar were confirmed biochemically with a Triple Sugar Iron (TSI) agar and API 20E strips (bioMérieux, Brussels, Belgium). Serotyping was carried out by the Belgian National Reference Laboratory for *Salmonella* (Sciensano, Brussels, Belgium) using classical slide agglutination according to the Kauffmann–White scheme combined with molecular genoserotyping

(Luminex-based method). All isolates (*Escherichia coli* and *Salmonella* spp.) were preserved in a BHI broth containing 50% glycerol and stored at −80 °C.

### 2.6. Antibiotic Susceptibility Testing

Antibiograms were conducted using the Kirby–Bauer disk diffusion technique on ready-prepared Mueller–Hinton agar, in line with Clinical and Laboratory Standards Institute (CLSI) M100 30th ed. 2020 guidelines [31]. The isolates tested comprised 86 *Escherichia coli* and 3 *Salmonella* spp. from table eggs, 13 *E. coli* and 5 *Salmonella* spp. from poultry sausages, and 56 *E. coli* and 25 *Salmonella* spp. clinical isolates from urine, blood, and stool. Bacterial suspensions were standardised to 0.5 McFarland using sterile saline. Suspensions were swabbed onto Mueller–Hinton agar, and antibiotic discs (Becton Dickinson Benelux, Aalst, Belgium) were applied aseptically. Plates were incubated aerobically at 37 °C for 18–24 h. The diameter of inhibition zones was measured and interpreted according to the CLSI criteria. Ten antibiotics from six classes were included: cephalosporins (cefotaxime (CTX): 30 µg; cefuroxime (CXM): 30 µg), aminoglycoside (gentamicin (GM): 10 µg; streptomycin (S): 10 µg), cyclins (tetracycline (TE): 30 µg), quinolones and fluoroquinolones (nalidixic acid (NA): 30 µg; ciprofloxacin: 5 µg), penicillins/β-lactamase inhibitors (amoxicillin/clavulanic acid (AMC): 20/10 µg), phenicols (chloramphenicol (C): 30 µg), and furans (nitrofurantoin (F/M): 300 µg). Table 1 below presents the inhibition zone diameters (in mm) for the interpretation of antibiotic resistance test results. Multidrug resistance (MDR) was defined as resistance to ≥3 antibiotic classes [32]. Susceptibility categories (S, I, and R) were recorded and reported separately in all AST tables and figures. For multidrug resistance (MDR) classification, only resistant (R) results were considered; isolates with intermediate (I) results were not counted as resistant. Supplementary Materials (Tables S1–S3) provide the inhibition zone diameters measured for all isolates.

**Table 1.** Inhibition zone diameters (in mm) for interpreting antibiotic resistance test results.

| Bacteria | Antibiotic | Quantity (µg) | S | I | R |
|---|---|---|---|---|---|
| *Salmonella* spp. | Cefotaxime | 30 | ≥26 | 23–25 | ≤22 |
| | Tetracycline | 30 | ≥15 | 12–15 | ≤11 |
| | Ciprofloxacin | 5 | ≥31 | 21–30 | ≤20 |
| | Chloramphenicol | 30 | ≥18 | 13–17 | ≤12 |
| *Enterobacteriaceae* | Cefuroxime | 30 | ≥18 | 15–17 | ≤14 |
| | Cefotaxime | 30 | ≥26 | 23–25 | ≤22 |
| | Gentamicin | 10 | ≥18 | 15–17 | ≤14 |
| | Streptomycin | 10 | ≥15 | 12–14 | ≤11 |
| | Tetracycline | 30 | ≥15 | 12–14 | ≤11 |
| | Ciprofloxacin | 5 | ≥26 | 22–25 | ≤21 |
| | Chloramphenicol | 30 | ≥18 | 13–17 | ≤12 |
| | Nitrofurantoin | 300 | ≥17 | 15–16 | ≤14 |
| | Nalidixic acid | 30 | ≥19 | 14–18 | ≤13 |
| | Amoxicillin + clavulanic acid | 20/10 | ≥18 | 14–17 | ≤13 |

Legend: antimicrobial susceptibility testing was performed using the disk diffusion method according to the CLSI guidelines. Breakpoints were interpreted as per the Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. (M100): S: susceptible, I: intermediate, R: resistant.

### 2.7. Statistical Analysis

All analyses were performed in R version 4.5.0. Contamination prevalence was assessed by origin (farm/commercial eggs; local/imported sausages), matrix (shell/contents), and locality via chi-squared test with 95% CI. Antibiotic resistance prevalence (%R/I/S) of shells and contents was calculated in R using Fisher's exact test. All analyses were conducted at a 95% confidence level, with significance set at $p < 0.05$.

## 3. Results

### *3.1. Prevalence of Escherichia coli and Salmonella spp. in Table Eggs*

Of the 135 composite egg samples analyzed, *Escherichia coli* was isolated from 80 samples (59.3%) across eggshells and contents. Specifically, *E. coli* was detected on eggshells in 76 samples (56.3%) and in egg contents in 10 samples (7.4%); six samples showed co-contamination. The prevalence was significantly higher on eggshells than in contents (56.3% vs. 7.4%, $p < 0.001$). For *Salmonella* spp., 3 of the 135 eggs (2.2%) tested positive, with 2 isolates from shells (farm—1, retail—1) and 1 from contents (farm) (see Table 2). The identified serotypes included Muenster, Larochelle, and Subsp. I 3,10:eh:-. Prevalence of *E. coli* varied by sampling area, with the highest in Mono (83.3%, 25/30), followed by Atlantique (46.7%), Couffo (56.7%), Ouémé (60%), and Littoral (73.3%). However, these differences were not significant ($p > 0.05$). *Escherichia coli* was found in 61.7% of farm eggs (36/60) and 66.6% of commercial eggs (50/75), with no significant differences between the sources ($p \geq 0.05$).

**Table 2.** Prevalence of *E. coli* and *Salmonella* spp. in table eggs by Matrix (shells/contents) and sampling site (farm/retail).

| Sampling Site | *Escherichia coli* | | *Salmonella* spp. | |
|---|---|---|---|---|
| | **Content** | **Shell** | **Content** | **Shell** |
| Farm ($n$ = 60) | 6.7% (4) | 55.0% (33) | 1.7% (1) | 1.7% (1) |
| 95% CI | 2.6–15.9% | 42.5–66.9% | 0.3–8.9% | 0.3–8.9% |
| Retail ($n$ = 75) | 8.0% (6) | 57.3% (43) | 1.3% (1) | 0.0% (0) |
| 95% CI | 3.7–16.4% | 46.1–67.9% | 0.2–7.2% | 0.0–4.9% |
| Total ($n$ = 135) | 7.4% (10) | 56.3% (76) | 1.5% (2) | 0.7% (1) |
| 95% CI | 4.1–13.1 | 47.9–64.4 | 0.4–5.2% | 0.1–4.1% |
| $p$-value (Fisher) | NS | NS | NS | NS |

Legend: number in parentheses = positive samples; NS = not significant ($p \geq 0.05$).

### *3.2. Prevalence of Escherichia coli and Salmonella spp. in Poultry Sausages*

In poultry sausages, microbiological analysis revealed contamination rates of 14.4% for *Escherichia coli* and 5.6% for *Salmonella* spp., with higher prevalence detected in locally produced samples. Fisher's exact test revealed significant differences ($p < 0.01$) in *E. coli* and *Salmonella* spp. prevalence according to product origin (imported vs. local) (see Table 3). *Salmonella* serotypes identified were Hadar and Give (two isolates each from local sausages) and Infantis (one isolate from an imported sausage).

**Table 3.** Prevalence of *E. coli* and *Salmonella* spp. in imported vs. local poultry sausages.

| Pathogen | Imported ($n$ = 75) | 95% CI | Local ($n$ = 15) | 95% CI | $p$-Value (Fisher's Test) | Total ($n$ = 90) | 95% CI |
|---|---|---|---|---|---|---|---|
| *Escherichia coli* | 4.0% (3) | 1.4–11.1 | 66.7% (10) | 41.7–84.4 | *** | 14.4% | 8.6–23.2 |
| *Salmonella* spp. | 1.3% (1) | 0.2–7.2 | 26.7% (4) | 10.9–52.0 | *** | 5.6% | 2.4–12.4 |

Legend: values in parentheses indicate positive samples; *** $p < 0.05$ (significant difference).

### *3.3. Antimicrobial Resistance Profiles in Table Eggs*

Among the 86 *Escherichia coli* isolates from table eggs, 88.4% (95% CI: 80.1–94.0) were resistant to tetracycline. Resistance exceeded 50% for amoxicillin–clavulanic acid (54.7%; 95% CI: 43.6–65.4) and streptomycin (65.1%; 95% CI: 53.3–75.3). A significant difference ($p$ = 0.048) in resistance to cefuroxime was found between isolates from egg contents (50.0%; 95% CI: 23.7–76.3) and shells (19.7%; 95% CI: 12.3–30.0); other antibiotics showed no significant differences. Sensitivity was highest for nitrofurantoin (91.9%), cefuroxime (76.7%), and chloramphenicol (74.4%). Overall, 95.4% (82/86) of the isolates were resistant

to at least one antibiotic, and 59.3% (51/86) exhibited multidrug resistance (see Figure 2a,b). Of the three *Salmonella* isolates from table eggs, the Muenster serotype showed intermediate sensitivity to amoxicillin–clavulanic acid and ciprofloxacin, but was resistant to nalidixic acid, streptomycin, and tetracycline. The Larochelle serotype was resistant to tetracycline and showed intermediate sensitivity to amoxicillin–clavulanic acid, ciprofloxacin, and streptomycin. The Subsp. I 3,10:eh:- serotype was resistant to all the tested antibiotics except gentamicin. Except for Larochelle, the isolates were MDR. Table 4 details the various resistance patterns.

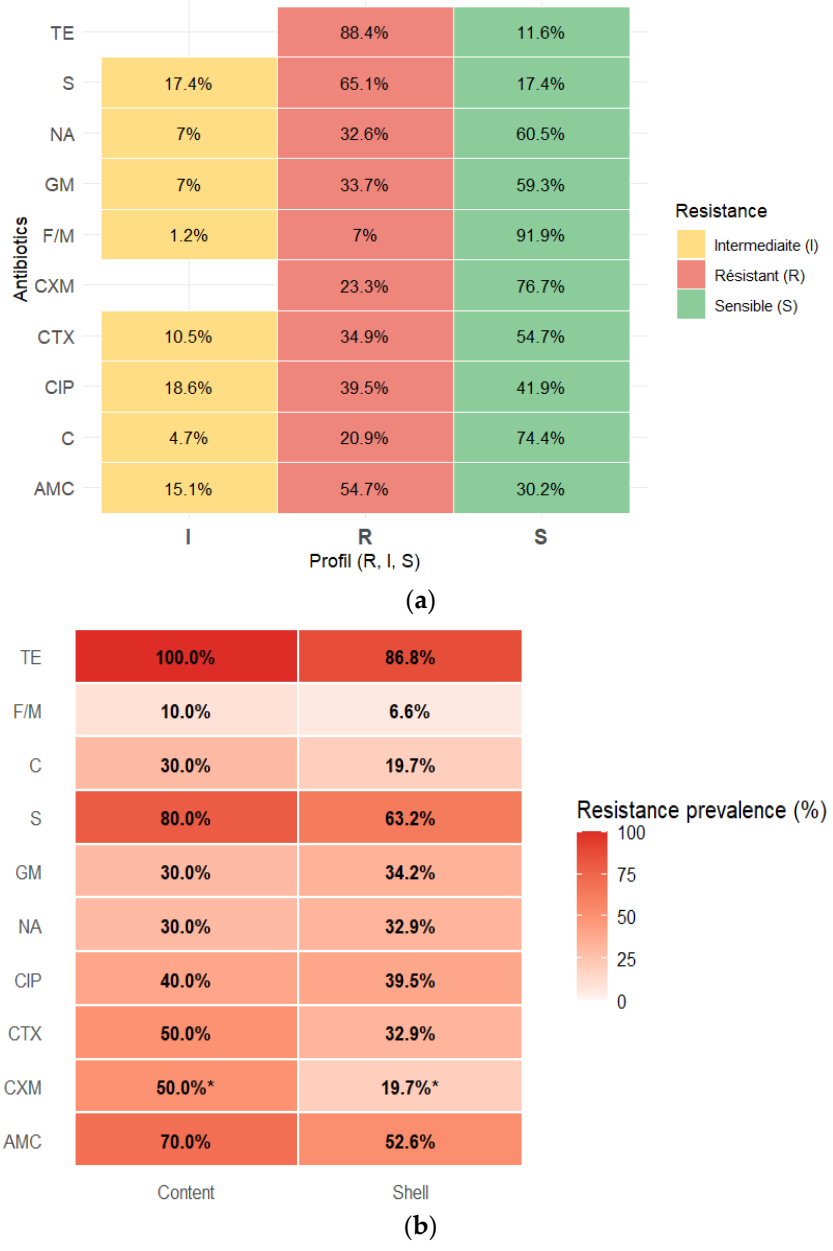


**Figure 2.** (**a**) Distribution of resistance (R), intermediate susceptibility (I), and susceptibility (S) to antibiotics among the *E. coli* isolates from table eggs. (**b**) Comparison of resistance prevalence (%) to antibiotics among *E. coli* isolates from egg contents and eggshells. Legend: AMC: amoxicillin + clavulanic acid, CTX: cefotaxime, CXM: cefuroxime, CIP: ciprofloxacin, C: chloramphenicol, S: streptomycin, NA: nalidixic acid, GM: gentamicin, F/M: nitrofurantoin, TE: tetracycline; *: CXM values marked with an asterisk indicate a significant difference in resistance between egg contents and shells ($p = 0.048$); no significant differences were observed for the other antibiotics.

**Table 4.** Antibiotic multidrug resistance profiles of the *Salmonella* spp. isolates from eggshells, egg contents, poultry sausages, and clinical samples.

| Origin | Serotypes | Resistance Patterns | Number |
|---|---|---|---|
| Eggshells | Subsp. I 3,10: eh:- | AMC/CIP/CTX/C/F/M/NA/S/CXM/TE | 1 |
| Egg contents | Muenster | NA/S/TE | 1 |
| Clinical samples | Shubra | AMC/CTX/CIP/S/C/TE | 1 |
| | Enteritidis | AMC/CIP/S/C/TE | 1 |
| | Typhimurium, Enteritidis, Typhi | AMC/CIP/S/C | 3 |
| | Enteritidis | CIP/GM/S/TE | 1 |
| | Typhi | CIP/S/C/TE | 1 |
| | Enteritidis, Typhi, Agbeni, Agona | CIP/S/TE | 5 |
| | Oakland | S/C/TE | 1 |
| | Typhimurium | AMC/CTX/C/F/M/NA/S/CXM/TE | 1 |
| Sausages | Give | AMC/CTX/GM/F/M/NA/S/CXM/TE | 1 |
| | Hadar | AMC/CIP/S/C/TE | 2 |

Legend AMC: amoxicillin + clavulanic acid, CTX: cefotaxime, CXM: cefuroxime, CIP: ciprofloxacin, C: chloramphenicol, S: streptomycin, NA: nalidixic acid, GM: gentamicin, F/M: nitrofurantoin, TE: tetracycline; The values in the table indicate the count of isolates resistant to each antibiotic profile.

### *3.4. Antimicrobial Resistance Profiles in Poultry Sausages*

All 13 *E. coli* isolates from poultry sausages were resistant to tetracycline, with 92.3% also resistant to streptomycin. Resistance to ciprofloxacin and cefotaxime was observed in 53.8% of the isolates. Sensitivity above 50% was recorded for nitrofurantoin (76.9%), chloramphenicol (69.2%), gentamicin (53.8%), and cefuroxime (53.8%) (see Figure 3). Every isolate was resistant to at least one antibiotic, and 92.3% exhibited multidrug resistance. Among the *Salmonella* serotypes, Infantis showed intermediate sensitivity to ciprofloxacin and streptomycin and resistance to nalidixic acid. The Give serotype exhibited intermediate sensitivity to ciprofloxacin; one isolate was resistant to all other antibiotics, the other was sensitive. The Hadar serotype showed intermediate sensitivity to ciprofloxacin and chloramphenicol, but resistance to all other antibiotics. Table 4 lists the different resistance patterns.

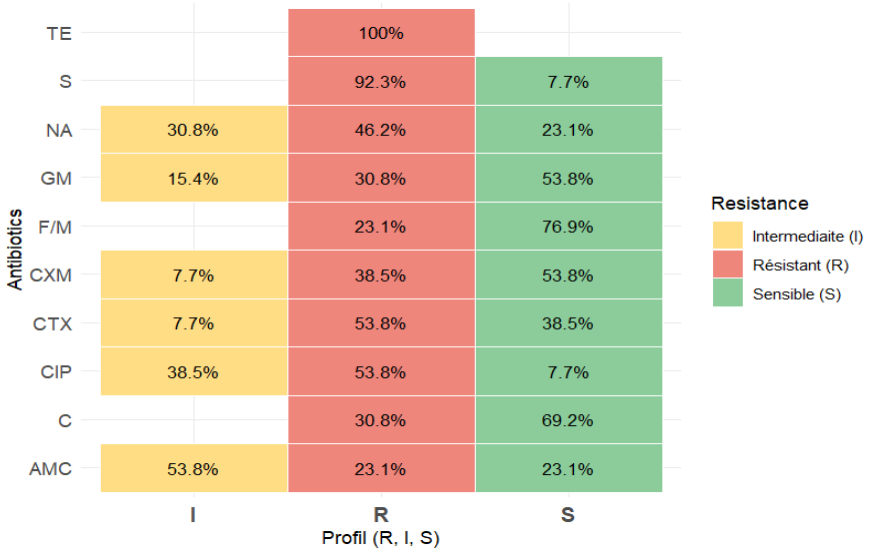


**Figure 3.** Antimicrobial resistance profiles of the *E. coli* isolates from poultry sausages. Legend: AMC: amoxicillin + clavulanic acid, CTX: cefotaxime, CXM: cefuroxime, CIP: ciprofloxacin, C: chloramphenicol, S: streptomycin, NA: nalidixic acid, GM: gentamicin, F/M: nitrofurantoin, TE: tetracycline; S: susceptible, I: intermediate, R: resistant.

### 3.5. Antimicrobial Resistance Profiles in the Clinical Escherichia coli and Salmonella spp. Isolates

Over half of the 56 *E. coli* isolates exhibited resistance to amoxicillin–clavulanic acid (78.6%), cefotaxime, cefuroxime, and streptomycin (85.7% each), gentamicin (83.9%), ciprofloxacin (89.3%), tetracycline (91.1%), and nalidixic acid (92.9%). Sensitivity was mainly observed to chloramphenicol (66.1%) and nitrofurantoin (69.6%) (see Figure 4). Multidrug resistance was identified in 96.4% of the isolates. Among the 25 *Salmonella* isolates from body fluids, resistance was highest to streptomycin (72%), chloramphenicol and ciprofloxacin (52% each), and tetracycline (56%). Most *Salmonella* isolates were sensitive to nalidixic acid (96%, gentamicin (92%), cefotaxime (84%), and nitrofurantoin (80%). Overall, 56% were multidrug-resistant, with only two isolates resistant solely to streptomycin (see Figure 5).

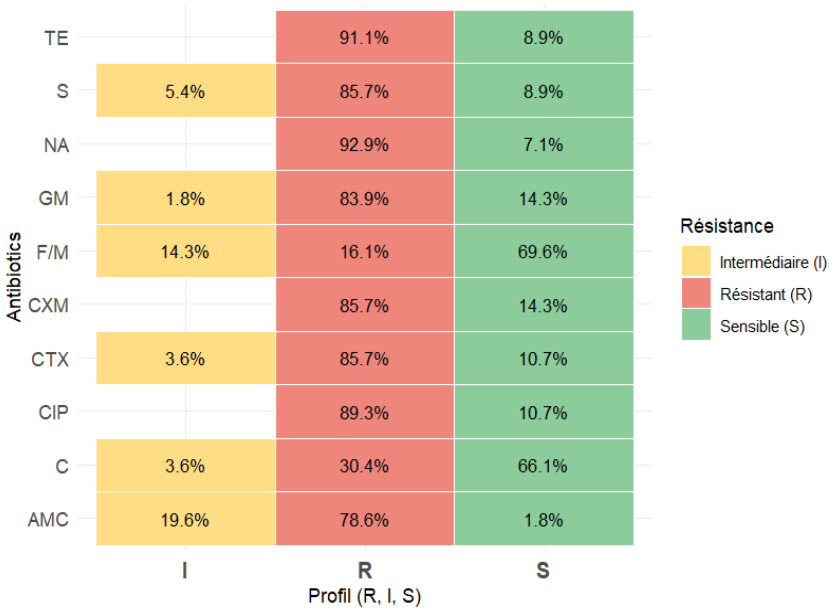


**Figure 4.** Antimicrobial resistance profiles of the *E. coli* isolates from clinical samples.

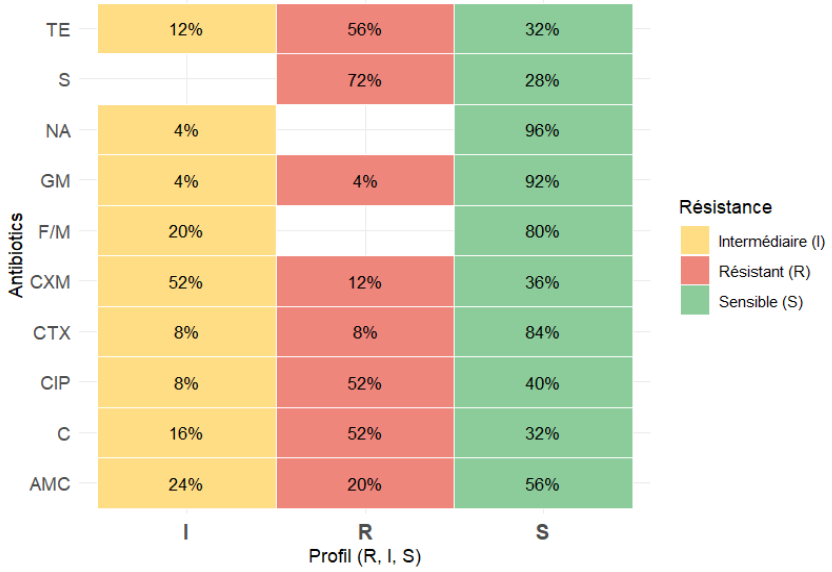


**Figure 5.** Antimicrobial resistance profile of the *Salmonella* spp. isolates from clinical samples. Legend: AMC: amoxicillin + clavulanic acid, CTX: cefotaxime, CXM: cefuroxime, CIP: ciprofloxacin, C: chloramphenicol, S: streptomycin, NA: nalidixic acid, GM: gentamicin, F/M: nitrofurantoin, TE: tetracycline; S: susceptible, I: intermediate, R: resistant.

## 4. Discussion

### *4.1. Prevalence Findings*

This study assesses the sanitary quality of table eggs and poultry sausages as reservoirs of foodborne bacteria. *Salmonella* is common in poultry products, frequently linked to human foodborne illness through undercooked meat and eggs [33]. Only 2.2% of the eggs tested positive for *Salmonella*, a rate lower than Ghana's (5.7%) and comparable to Korea's (2.3%) [34,35]. Unlike a previous Benin study [23], which detected no *Salmonella* in egg yolk, this study detected isolates in both eggshells and egg contents. Variations may be due to changes in contamination patterns, improved detection techniques, health management practices, rearing conditions, storage, sample size, or bacterial isolation methods. Of the three *Salmonella* serotypes detected, two were isolated from eggshells but not the contents, supporting the view that shell contamination does not necessarily reflect contamination within the contents. One serotype was recovered from egg contents, likely due to bacterial presence in the ovary or oviduct before shell formation [36]. The Muenster serotype found in egg contents has been reported in animal and human samples worldwide [37,38]. The Larochelle serotype from eggshells has also been documented in poultry and pig samples in Nigeria, Ethiopia, and Egypt [39,40]. For sausages, *Salmonella* prevalence was 5.6%, lower than the 33% reported in Morocco [41] but higher than the 3.9% found on Réunion Island [42,43] for pre-cooked sausages. This prevalence may indicate cross-contamination, inadequate storage, contamination of raw meat, or cold chain failures [44]. Non-compliance with sanitary standards in Benin's artisanal sausage workshops likely contributes. The presence of *Salmonella* in sausages may be due to increased heat resistance with the high fat content (around 69%), which protects bacteria during cooking [45]. The fat's amount and temperature influence the heat resistance of *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* [46].

Although serotypes such as Muenster, Larochelle, Give, and Hadar are infrequently reported, they have been sporadically associated with foodborne infections in different settings. Reports of *Salmonella* Muenster and Larochelle in human cases linked to various food products in Europe illustrate their potential public health relevance [47,48]. However, these observations are independent of the present study and do not imply any direct epidemiological or transmission link with the isolates described here. *Salmonella* Infantis is increasingly prevalent worldwide and remains a leading cause of human salmonellosis in the European Union, underscoring the importance of continued surveillance [49].

The overall *E. coli* prevalence in table eggs was 59.3%, exceeding the rates reported in India (11.7%) [50], Egypt (18%) [51], and Nigeria (54.4%) [52]. Contamination was primarily on the shell (88.4%) rather than the contents (11.6%). Similar shell-specific contamination has been reported in Nigeria (61%) and Pakistan (38.2%), whereas an Ethiopian study found 13.4% for shells and none in contents [53]. High eggshell prevalence is attributed to factors in production and marketing, including cloacal contamination, environmental exposure, insufficient protection, repeated handling, and poor storage. Floor-rearing with infrequently changed litter further increases the risk. No significant differences were found between farm and commercial eggs, suggesting upstream production factors are critical [54]. These findings emphasise the necessity of improved hygiene practices on farms to reduce the microbiological risk at the source. *E. coli* prevalence in poultry sausages (14.4%) aligns with studies by Abdel-Atty et al. [55] and Heybet et al. [56], which link *E. coli* in African sausages to diarrhoeal illness [57]. Contamination is often due to poor handling before and after cooking, leading to direct or indirect faecal and environmental exposure. Local sausage production, increasingly common in West Africa, including Benin, suffers from inadequate mastery and lacks regular sanitary inspections, favouring bacterial presence [58].

Additionally, cold chain disruptions, sometimes intentional to reduce electricity costs, promote bacterial growth, spoilage, and increase foodborne risk [59].

### *4.2. AMR Patterns Across Sources*

*Salmonella* isolates from table eggs and poultry sausages in Benin showed high multidrug resistance, consistent with reports from Egypt [60], the United States [61], and China [62]. Predominant resistance to nalidixic acid (6/7) and tetracycline (5/7) likely reflects widespread, often unregulated quinolones and cyclins use in local livestock [63]. Ciprofloxacin resistance was intermediate in several serotypes, raising concern given its role as the first-line therapy for invasive salmonellosis, and resistance may limit treatment options, especially where alternatives are scarce [64]. Two *Salmonella* Give isolates exhibited differing resistance, possibly reflecting variable on-farm antibiotic exposures. Similar resistance profiles between food and clinical *Salmonella* isolates, especially to streptomycin, tetracycline, and ciprofloxacin, may reflect comparable antibiotic selection pressures across animal and human settings [4]. *E. coli* isolates from eggs, sausages, and clinical samples commonly resisted antibiotics used in veterinary and human medicine. Tetracycline resistance was 88.4% for eggs, 100% for sausages, and 91.1% for clinical samples, comparable to findings from Zambia [65], India [66], Turkey [56], Kenya [67], and Nigeria [68]. Resistance to streptomycin, amoxicillin–clavulanic acid, cefuroxime, and ciprofloxacin was also widespread across the sources, reflecting patterns in other African and Asian countries [69,70]. Nonetheless, the isolates remained largely sensitive to nitrofurantoin and chloramphenicol in this study. In contrast, Okorie-Kanu et al. [52] reported resistance to nitrofurantoin among *E. coli* isolates in Nigeria, indicating that this antibiotic is still being used despite regulations. Similar resistance patterns in avian-derived foods and clinical isolates may indicate overlapping antibiotic usage and shared selective pressures rather than direct evidence of transmission.

### *4.3. Implications for Public Health and AMR Control*

Resistance to multiple antibiotics, including ciprofloxacin, cefotaxime, and gentamicin, was observed across eggshells, contents, sausages, and human isolates, indicating possible cross-transmission. The common AMC/S/TE resistance pattern in eggs implies antibiotic use for growth promotion [70]. Eggshells act as significant reservoirs of multidrug-resistant isolates, while sausage-specific fluoroquinolone resistance indicates selective pressure from food processing. Given these findings, enhanced antimicrobial resistance surveillance with phenotypic and genotypic integration is crucial. Limited data on antibiotic usage in Benin and widespread illegal distribution, with no national antimicrobial resistance monitoring system, exacerbate challenges. Animal products, such as milk, beef, and poultry, are often contaminated with antibiotic residues, sometimes exceeding the permitted limits [27].

### *4.4. Study Limitations*

Sampling was limited to convenience selection across five southwestern Benin departments, restricting national representativeness. The small local subsample limits robust origin-based comparisons. Reliance on phenotypic characterization precludes molecular source tracking and genotypic AMR confirmation. Microbial identification of food isolates relied on culture on chromogenic media and ISO-based screening (e.g., TBX for β-glucuronidase-positive *E. coli* and ISO 6579-1:2017 [29] enrichment for *Salmonella*), without additional biochemical panels or molecular confirmatory testing for *Escherichia coli*. As a result, some degree of misclassification cannot be excluded, particularly for atypical *E. coli* (β-glucuronidase-negative strains) or closely related Enterobacteriaceae with similar colony morphology, which may slightly over- or underestimate true prevalence and influence observed resistance distributions. Furthermore, the absence of molecular typing

and genotypic AMR characterization prevents source attribution, detection of specific virulence/AMR determinants, and comparison with international clonal lineages.

## 5. Conclusions

Table eggs and poultry sausages are widely consumed in Benin, but microbiological analyses expose flaws in hygiene, marketing practices, and sanitary oversight. Pathogens, including *Salmonella* (serotypes Hadar, Infantis, Muenster, Give, Larochelle, Subsp. I 3,10 eh:-) and *E. coli*, consistently contaminate these products. Isolates of *E. coli* and *Salmonella* from these foods, as well as from clinical samples, display multidrug resistance to the antibiotics commonly used in veterinary and human medicine, including fluoroquinolones, tetracyclines, penicillins, and cephalosporins. This poses a significant public health risk by restricting therapeutic options. Complex resistance patterns suggest ongoing selection for resistance to agents such as fluoroquinolones and cephalosporins, likely a result of unregulated therapeutic practices that compromise effective treatment of systemic infections. There is an urgent need for coordinated surveillance encompassing veterinary, food, and clinical domains. Additionally, awareness campaigns targeting stakeholders throughout the poultry value chain are essential, promoting alternatives to antibiotics, their rational use, and adherence to withdrawal periods before commercialisation.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/hygiene6010009/s1. Table S1: Measured inhibition zone diameters (mm) for *Escherichia coli* isolates from egg matrices and sausages. Table S2: Measured inhibition zone diameters (mm) for *Salmonella* spp. isolates from egg matrices and sausages. Table S3: Measured inhibition zone diameters (mm) for *Escherichia coli* and *Salmonella* spp. isolates from clinical isolates.

**Author Contributions:** Conceptualization, C.H., P.S., P.A., G.D. and N.K.; methodology, C.H., P.S. and N.K.; software, C.H.; validation, P.S., P.A., G.D. and N.K.; formal analysis, C.H., P.S., P.A., G.D. and N.K.; investigation, C.H.; resources, P.S., N.K. and G.D.; data curation, C.H.; writing—original draft preparation, C.H.; writing—review and editing, P.S., P.A., G.D. and N.K.; visualization, C.H.; supervision, P.S., P.A., G.D. and N.K.; project administration, P.S. and N.K.; funding acquisition, P.S. and N.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by ARES – CCD (Academy of Research and Higher Education of the Commission for Development Cooperation) through an exceptional doctoral scholarship; no specific grant number is applicable.

**Data Availability Statement:** The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

**Acknowledgments:** The authors would like to thank the ARES-CCD (Academy of Research and Higher Education of the Commission for Development Cooperation) for its support in the realization of this study through the financing of an exceptional doctoral scholarship.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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