# Prevalence of Antibiotic-Resistant *Escherichia coli* and *Salmonella spp.* in Cloacal Samples from Poultry Farms in Santa Maria, Bulacan, Philippines

Rene M. Sandoval, Jr.[1], Rienna Y. Soliven[1], Sophia Elena S. Tan[1], Joryn Chrystel R. Lim[1], Dustine Danae V. Villa[1], Arabela Lianne A. Antonio[1], Xieline Midgie F. Barrera[1], Mark Raymund G. Nava[1]

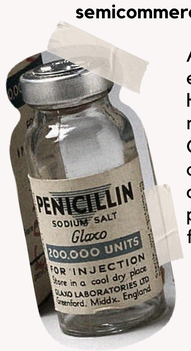
[1] *Department of Medical Technology, Faculty of Pharmacy, University of Santo Tomas, Manila, Philippines*

## INTRODUCTION

### Antibiotic Resistance in Foodborne Pathogens

Since its discovery in 1928, antibiotics have been crucial in treating bacterial infections. However, their misuse and easy accessibility have led to the development of **antibacterial resistance** (**ABR**), hindering healthcare. ABR occurs when bacteria become resistant to antibiotics, reducing their effectiveness. In the US, ABR leads to 35,000 deaths annually, and globally, it's associated with 1.27 million direct deaths and 4.95 million deaths overall. Low to middle-income countries like the Philippines face a greater burden and risk of transmission due to unsanitary food practices and improper waste disposal, among others. In line with the growing issue of ABR and in support of WHO's Health for All initiative, the researchers have focused on determining the **presence of multi-drug resistant bacteria in the poultry industry.**

Chicken production in the country has been increasing to meet rising demand. In 2022, total chicken production reached 1.87 million metric tons, a 7.0% growth from 2021. **Santa Maria, Bulacan, with over 50 commercial and semicommercial farms, dominates the industry.**




A study on antibiotic use in livestock farming revealed that farmers rely on antibiotics for their effectiveness, convenience, and low cost in maintaining herd health and productivity (Busch et al., 2020). However, even small doses of antibiotics in livestock contribute to the development of antibiotic resistance in harmful bacteria.

Completely banning antibiotic use in livestock is not feasible due to its impact on animal health, welfare, and food prices. A part of the solution to the issue is to identify and raise awareness about the root cause and severity of the problem, particularly among livestock owners. This study aims to assess the prevalence of antibiotic-resistant *Escherichia coli* and *Salmonella spp.* in cloacal samples from poultry farms in Santa Maria, Bulacan, Philippines.

By contributing to existing knowledge on ABR, this study may help in the evaluation of the effectiveness of current strategies and encourage policymakers to make necessary policy adjustments.

## METHODOLOGY

**Research Design:** **Quantitative Experimental** Research

**Study Locale:** **Santa Maria, Bulacan**, one of the top producers of poultry products in the country

**Inclusion Criteria:** **Commercial poultry farms** in Santa Maria, Bulacan utilizing antibiotics as growth enhancers or prophylaxis

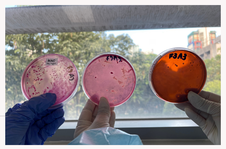
**Sample Size:** **Five broiler farms** (10% of the total registered farms in the study area)

**Data Gathering**

**Livestock Farms Ocular Visits**

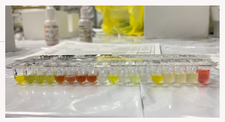
**Farm Profiling**
Semi-structured interviews with farm owners

**Collection of Cloacal Swabs**



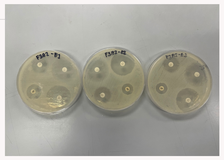
**Bacterial Isolation and Culture**
MacConkey, EMB, and SSA

**Presumptive Identification**
Biochemical Tests using LIA, TSI, SIM, Urease, and Citrate agar



**Confirmatory Identification**
using Remel RapID ONE System

**Kirby-Bauer Disk Diffusion**
Antibiotic Susceptibility Testing for *Escherichia coli* and *Shigella spp.*



**Statistical Analysis**
Fisher's Exact Test

## CONCLUSION

The study revealed a significant threat of ABR in the poultry farms in Santa Maria, Bulacan. A majority of isolates were identified as *Shigella sonnei.*, (54%) and *E. coli* (18%). *E. coli* isolates were notably resistant to Gentamicin and Ciprofloxacin, while *Shigella sonnei* isolates showed resistance to Ciprofloxacin, Chloramphenicol, and Ampicillin. Areas for further research include investigating the sudden increase in *Shigella sonnei* isolates, expanding the research locale, incorporating CLSI standards for agricultural products in conjunction with CLSI clinical standards to assess the development of antibiotic resistance in farms before its spread to humans, and conducting qualitative research to identify drivers of emerging antibiotic resistance.

## RESULTS & DISCUSSION

### More than half of the total bacterial isolates were multidrug-resistant.

54% of bacterial isolates from the cloacal swab samples were identified as *Shigella sonnei*, while only 18% were identified as *Escherichia coli*. Since *Salmonella spp.* were not isolated, the incidental finding *Shigella sonnei* were tested instead.. Antibiotic disks of four different drug classes were used in AST for *E. coli*, and a different set was used for *Shigella sonnei*. 2020 CLSI Performance Standard for AST was followed for the conduct and interpretation of the tests.

To ensure the accuracy of the Antibiotic Susceptibility Tests, Kirby Bauer Disc Diffusion was done in **triplicate** per isolate. Results showed that **more than half of both *E. coli* and *Shigella sonnei* isolates exhibited multidrug resistance**. The Fisher's Exact Test (p-value < 0.05) was further performed to compare the susceptibility to two different antibiotic disks.

**Table 1. Prevalence of Multidrug-Resistant Bacterial Isolates**

| | No. of Isolates Resistant to More Than One Antibiotic Disk | Total No. of Isolates | MDR (%) |
|---|---|---|---|
| *Escherichia coli* | 4 | 7 | 57.1 |
| *Shigella spp.* | 10 | 15 | 66.7 |
| **Total** | **14** | **22** | **63.6** |

**Table 1** shows the prevalence of multidrug resistant (MDR) isolates. 57.1% of *E. coli* isolates are MDR, while 66.7% of *Shigella spp.* isolates are MDR.

**Table 2. Results of Antibiotic Susceptibility Testing against *Escherichia coli***

| Name of Antibiotic | Disk Content (ug) | Resistant | | Intermediate/ Susceptible | | Total Isolates | |
|---|---|---|---|---|---|---|---|
| | | Total No. | Percentage (%) | Total No. | Percentage (%) | Total No. | Percentage (%) |
| **Colistin** | 10 | 0 | 0 | 7 | 100 | 7 | 100 |
| **Gentamicin** | 10 | 5 | 71 | 2 | 29 | 7 | 100 |
| **Imipenem** | 10 | 0 | 0 | 7 | 100 | 7 | 100 |
| **Ciprofloxacin** | 5 | 5 | 71 | 2 | 29 | 7 | 100 |

**Table 2** shows the result of KBDD test per individual antibiotic against *E. coli*. 71% of the isolates are resistant to Gentamicin and Ciprofloxacin as shown by their ZOI of less than 12 and 21 mm respectively. No resistance was shown against the stronger antibiotics (ZOI ≥ 12 for Colistin and 20 mm for Imipenem).

**Table 3. Results of Antibiotic Susceptibility Testing against *Shigella sonnei***

| Name of Antibiotic | Disk Content (ug) | Resistant | | Intermediate/ Susceptible | | Total Isolates | |
|---|---|---|---|---|---|---|---|
| | | Total No. | Percentage (%) | Total No. | Percentage (%) | Total No. | Percentage (%) |
| **Azithromycin** | 15 | 2 | 13 | 13 | 87 | 15 | 100 |
| **Ciprofloxacin** | 5 | 9 | 60 | 6 | 40 | 15 | 100 |
| **Chloramphenicol** | 30 | 10 | 67 | 5 | 33 | 15 | 100 |
| **Ampicillin** | 10 | 10 | 67 | 5 | 33 | 15 | 100 |

**Table 3** shows the result of KBDD test per individual antibiotic against *Shigella sonnei*. 67% of the isolates are resistant to Ampicillin and Chloramphenicol (ZOI ≤ 13 and 12 mm respectively). This was followed by Ciprofloxacin (60% resistant, ZOI ≤ 21 mm) and Azithromycin (13% resistant, ZOI ≤ 12 mm)

The broad use of antibiotics for various purposes (metaphylaxis, growth enhancement), discontinuous or irregular administration of livestock antibiotics, lack of veterinary consultations, and dispensation of doses higher than what is indicated may contribute to the prevalence of MDR bacteria isolated from the samples. This may be curbed through proper food handling, waste disposal, and stringent hygiene practices among all who are involved in the production of poultry and other food products.

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