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IN-VITRO SENSITIVITY OF SALMONELLA TYPHI ISOLATES FROM POULTRY FARMS TO SELECTED BRANDS OF CIPROFLOXACIN ON THE GHANAIAAN MARKET

Hayford Odoi ^{*1}, Vivian Etsiapa Boamah ² and Yaa B. O. Asubonteng ³

Department of Pharmaceutical Microbiology ¹, School of Pharmacy, University of Health and Allied Sciences, Ho, Volta region, Ghana.

Department of Pharmaceutics ², Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Komfo Anokye Teaching Hospital (KATH) ³, Kumasi, Ghana.

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**Correspondence to Author:
Odoi Hayford**

Department of Pharmaceutical Microbiology, School of Pharmacy, University of Health and Allied Sciences, Ho, Volta region, Ghana.

E-mail: hodoi@uhas.edu.gh

ABSTRACT: Poultry products are a major source of animal protein in Ghana. However, disease outbreaks have constantly threatened increased poultry production in some regions of the country. The most common source of human salmonellosis is food of poultry origin. The evolution of resistant strains of *Salmonella* in natural environments and animal houses presents a serious public health concern. Fluoroquinolones such as ciprofloxacin have remained effective agents in managing salmonellosis and other gastrointestinal infections over the years. However, despite growing evidence on evolving antibiotic resistance in *S. typhi*, few studies have assessed the susceptibility of *salmonella* species to commercially marketed ciprofloxacin brands. This study thus sought to determine the susceptibility of *S. typhi* isolated from 25 poultry farms in the Ashanti region to commercially available ciprofloxacin brands on the Ghanaian market. Antibiotic susceptibility tests were carried out using a modified Kirby Bauer disk diffusion assay and MIC determination by micro-broth dilution. Of the 50 *Salmonella typhi* isolates, 20% (n=10/50) were susceptible to pure ciprofloxacin, while 58% (29/50) of the isolates were resistant. *Salmonella typhi* isolated from poultry farm workers showed 12-40% susceptibility to all the brands of ciprofloxacin tested, but resistance ranged from 20-36%. Eighty-four (84) to 88% of the isolates from poultry litter were resistant, with only 8-16% of the isolates showing susceptibility to the commercially available brands of ciprofloxacin tested. Findings from this study indicate the need to also focus antibiotic resistance surveillance efforts on animal husbandry. This will enhance the identification of evolving antimicrobial-resistant strains and avoid irrational antimicrobial use in animal production.

INTRODUCTION: Poultry plays a significant role in providing protein in Ghana, with the annual poultry production in Ghana being estimated at 14,000 metric tonnes of meat and 200 million eggs ¹.

Poultry production has contributed socio-economically to rural livelihoods in all country regions ². Disease outbreaks have always threatened increased poultry production in Ghana ³.

This has made the use of antibiotics for prophylaxis and treatment of diseases common practice. Some of these antibiotics are directly incorporated in the feed and water of birds for purposes of growth promotion ⁴. However, there are concerns of residual antibiotic concentrations in poultry farms, which may select resistant strains of microorganisms ^{5, 6}.

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Salmonella serotype typhi (*S. typhi*), the etiologic agent of typhoid fever, causes an estimated 21.6 million cases and 600,000 deaths worldwide each year⁷. This highly adapted human specific pathogen has evolved remarkable mechanisms to ensure its survival and transmission^{8, 9}. Typhoid fever is a rare infection in developed countries¹⁰. However, in developing countries where safe water supply, environmental sanitation and food hygiene are not optimal, typhoid fever is still a major problem¹¹. In Ghana, it is estimated that typhoid fever cases account for 3.2% of all the infections recorded at hospitals¹². *S. typhi* is transmitted through food or water that has been contaminated with faeces from either acutely infected persons, persistent excretors, or chronic asymptomatic carriers^{13, 14}. The most common source of human salmonellosis is the food of poultry origin¹⁵. The widespread occurrence of *Salmonella* in the natural environment and animal houses has been a significant problem in public health¹⁶. It is known that salmonella can penetrate intact poultry eggs lying in faecally contaminated material and infect eggs during egg development before the shell is formed.

Attempts to control diseases caused by *Salmonella typhi* through the use of antibiotics have resulted in an increased prevalence of resistant strains¹⁷. Chloramphenicol, ampicillin, and trimethoprim / sulphamethoxazole have been used for treatment, but there are several reports of resistance to these agents¹⁸. Fluoroquinolones such as ciprofloxacin are currently the main choice for treatment¹⁹. However, there are growing concerns of increased resistance of *S. typhi* to ciprofloxacin as well^{20, 21}. In this study, the susceptibility of *S. typhi* isolated from poultry litter and farmhands to ten commercially available brands of ciprofloxacin in Ghana was investigated.

MATERIALS AND METHODS:

The Study Sites and Subjects: The study was conducted on fifty (50) *Salmonella typhi* isolates from 25 poultry farms in the Ashanti region of Ghana. 25 of the isolates were obtained from poultry litter samples and 25 from farmworkers from the respective poultry farms.

Isolation, Identification, and Confirmation of *Salmonella typhi*: All the samples collected from the poultry farms were each inoculated into 10ml

casein soya bean digest broth and incubated at 37 °C for 20 h. 1mL was transferred to 10 ml of tetrathionate bile brilliant green broth and incubated at 42 degrees Celsius for 20 h. The organisms were sub-cultured on Bismuth sulphite agar (Oxoid Ltd, Basingstoke, UK), Deoxycholate citrate agar (Oxoid Ltd, Basingstoke, UK), and Xylose lysine deoxycholate agar (Oxoid Ltd, Basingstoke, UK). Suspected colonies were then confirmed on Triple sugar iron agar using surface and deep inoculation²².

Antibiotic Susceptibility Testing using Disk

Diffusion Method: A modified Kirby-Bauer disk diffusion method²³ guided by recommendations of the Clinical and Laboratory Standards Institute was used to determine the *in-vitro* susceptibility of the isolates to standard ciprofloxacin (CIP-5, Oxoid Ltd, Basingstoke, UK) and ten (10) different brands of ciprofloxacin on the Ghanaian market. Sterile standard-sized paper discs soaked with a 5µg/ml concentration of each brand of ciprofloxacin were used. A loopful of a 24h culture of *Salmonella typhi* was suspended in sterile saline to 0.5 McFarland turbidity (corresponds to approximately 1.5 X 10⁸CFU/ml). A sterile swab was dipped into the standardized inoculum and used to inoculate the surface a solidified plate of Mueller-Hinton agar (Oxoid Ltd, Basingstoke, UK). Antibiotic discs prepared from the various brands of ciprofloxacin were placed on the surface of the inoculated agar plate using sterile forceps and incubated for 24h at 37°C. The diameter zones of inhibition for each brand of ciprofloxacin were measured.

Antibiotic Susceptibility Testing using the Broth

Dilution Technique: The MIC of pure ciprofloxacin (powder) was determined for all the 50 *Salmonella typhi* isolates using the broth microdilution method²⁴, and the susceptibility of the isolates were inferred using CLSI standards. Stock concentrations of 20µg/ml, 5µg/ml, and 2µg/ml of pure ciprofloxacin were prepared. A two-fold dilution of each antibiotic stock concentration was achieved in a 96 well microtitre plate using double-strength nutrient broth, sterile distilled water and a 20uL volume of a 24 h 0.5 McFarland turbidity standardized culture of each *Salmonella typhi* isolate adjusted to a total volume of 200uL. The microtiter plates were incubated at 37°C for 20 h.

The MIC was recorded as the least concentration that showed no visible bacterial growth which was detected by the absence of purple colour after the addition of 10 µL of 0.1% w/v 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to each well followed by incubation at 37°C for 30.

RESULTS AND DISCUSSION: A total of 50 *Salmonella typhi* strains were isolated from the 25 poultry farms in the Ashanti region of Ghana. The strains were isolated and identified based on their phenotypic and biochemical characteristics **Fig. 1**. The *S. typhi* isolates were classified as susceptible, intermediate, or resistant to the selected brands of ciprofloxacin depending on the diameter zones of inhibition and minimum inhibitory concentrations.

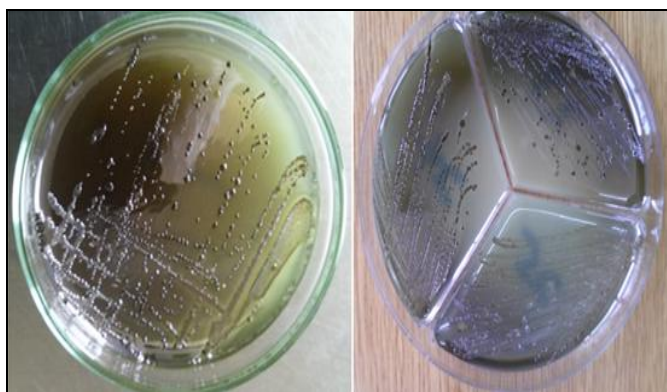


FIG. 1: PHOTOGRAPH OF SALMONELLA TYPHI WITH BLACK COLONIES AND METALLIC SHEEN AS SEEN ON BISMUTH SULPHITE AGAR

Of the 50 *Salmonella typhi* isolates, 20% (10/50) were susceptible to the pure ciprofloxacin (CIP-5µg/ml, Ernest Chemist, Ghana) while 58% (29/50) of the isolates were resistant **Fig. 2**.

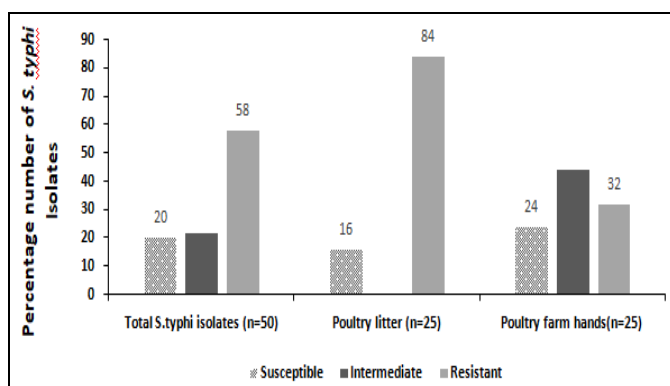


FIG. 2: SUSCEPTIBILITY OF SALMONELLA TYPHI ISOLATES FROM POULTRY LITTER AND POULTRY FARM WORKERS (FARM HANDS) TO PURE CIPROFLOXACIN (CIP-5µG/ML, ERNEST CHEMIST, GHANA)

These findings are not consistent with susceptibility reports by Saana *et al.*²⁵ who recorded 76.5% susceptibility to ciprofloxacin in 128 *S. typhi* strains isolated from hospitals in the Ashanti region of Ghana.

The susceptibility pattern of the *S. typhi* isolates to ciprofloxacin (20%) is also very low compared to findings from research conducted by Kasper²¹ and coworkers in Cambodia and Dimitrov *et al.*,²⁶ in Kuwait where *S. typhi* susceptibility to ciprofloxacin was 79% and 63.3% respectively. Sixteen percent (4/25) of the poultry litter isolates and 24% (6/25) of the isolates from the farmhands showed susceptibility to the pure ciprofloxacin used.

In 14 of the poultry farms investigated, *S. typhi* isolates obtained from poultry farmworkers were susceptible to pure ciprofloxacin while those from poultry litter were resistant. One poultry farm harboured resistant *S. typhi* strains in the farmhands but susceptible strains in the poultry litter, while seven (7) farms harboured resistant isolates from both poultry farm workers and litter. Three (3) poultry farms had isolated from both farmhands and poultry litter, being susceptible to pure ciprofloxacin. *Salmonella typhi* isolated from poultry farm workers showed 12-40% susceptibility to all the brands of ciprofloxacin tested, whereas resistance ranged from 20-36% **Fig. 2**.

Eighty-four (84) to 88% of the isolates from poultry litter were resistant to all the ciprofloxacin brands, with 8-16% of the isolates showing susceptibility to the commercially available brands ciprofloxacin **Fig. 4**. These findings generally indicate the low activity of some ciprofloxacin brands in Ghana against *S. typhi* isolates from poultry farms.

A study conducted by Annan-Prah *et al.*,²⁷ confirmed increased use of quinolone antibiotics in poultry production either as growth promoters or in prophylaxis.

This practice may lead to prolonged exposure of disease-causing organisms such as *Salmonella typhi* to minimal concentrations of quinolone antibiotics, which could develop resistance in the strains.

TABLE 1: SUSCEPTIBILITY OF S.TYPHI ISOLATES FROM POULTRY LITTER AND FARM HANDS TO TEN (10) BRANDS OF CIPROFLOXACIN (5µG/ML)

Brands of ciprofloxacin	Susceptible		Intermediate		Resistant	
	PL	FH	PL	FH	PL	FH
BRI	4	9	0	11	21	5
BR2	3	6	1	12	21	7
BR3	3	6	1	13	21	6
BR4	2	9	1	8	22	8
BR5	2	10	2	8	21	7
BR6	4	6	0	12	21	7
BR7	3	4	1	12	21	9
BR8	2	3	2	16	21	6
BR9	2	5	1	14	22	6
BR10	3	6	1	13	21	6
PCP (CIP-5µg/ml)	4	6	0	11	21	8

PL: Number of poultry litter isolates; FH: Number of isolates from poultry farm workers; BR1-BR10: Commercial brands of ciprofloxacin. PCP: Pure ciprofloxacin powder (Ernest Chemist, Ghana).

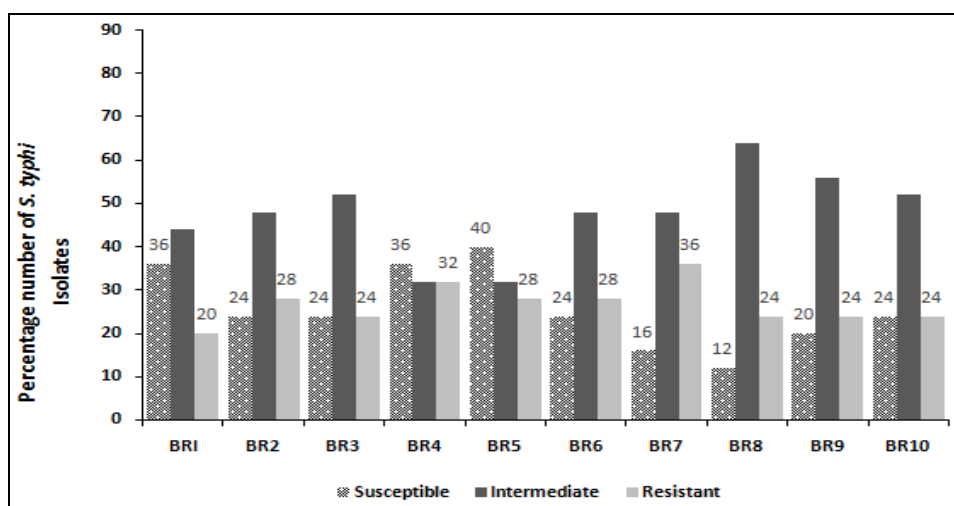


FIG. 3: SUSCEPTIBILITY OF S. TYPHI ISOLATES FROM POULTRY FARM WORKERS TO TEN (10) BRANDS OF CIPROFLOXACIN IN GHANA. BR1-BR10: COMMERCIAL BRANDS OF CIPROFLOXACIN

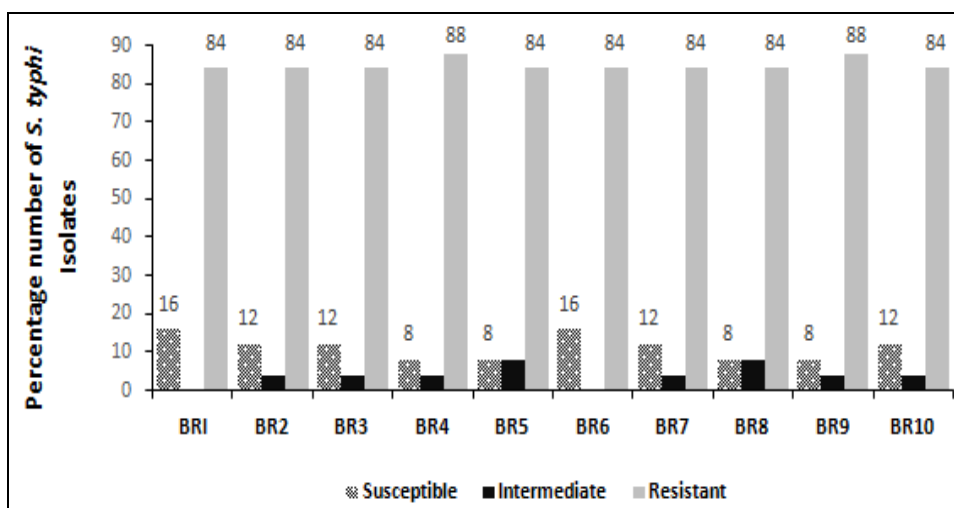


FIG. 4: SUSCEPTIBILITY OF S. TYPHI ISOLATES FROM POULTRY LITTER TO TEN (10) BRANDS OF CIPROFLOXACIN IN GHANA. BR1-BR10: COMMERCIAL BRANDS OF CIPROFLOXACIN

Farmhands exposed to these resistant strains of *S. typhias* an occupational hazard, may act as chronic carriers. Such individuals, therefore, should adhere

to wearing appropriate protective clothing. Most importantly, poultry farmers should observe withdrawal periods for poultry eggs and meat

during production. Wide variations in the activity profile of the various brands of ciprofloxacin call for enforced uniformity among antibiotic drug manufacturers and routine antibiotic resistance surveillance in animal husbandry

CONCLUSION: Findings from this study indicate poultry litter to be a high reservoir of resistant *S. typhi* strains. Also, all the *S. typhi* isolates showed less than 50% susceptibility to the commercial brands of ciprofloxacin in Ghana.

Therefore, there is the need for concerted efforts to ensure the appropriate use of antibiotics in animal husbandry to decrease residual antibiotic concentrations, which may impact antibiotic resistance evolution. Physicians should also consider the occupational background of patients to enhance a complete assessment of their level of risk of exposure to resistant bacteria strains.

This will help individualize treatment, leading to improving the patient's quality of life. Stakeholders like Veterinary Services, Food and Drugs Authority, Pharmaceutical Associations, Standards Board, Consumer Protection Agencies, and the Ministry of Health should enforce guidelines that enhance surveillance and promote rational antibiotic use in animal husbandry.

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Consent to publication: Not applicable

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