



## Prevalence and antibiotics profiles of *Plesiomonas shigelloides* isolated from faecal and soil samples of selected poultry farms in Akoko North, Ondo State, Nigeria

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### Abstract

#### Keywords:

*Plesiomonas shigelloides*, Poultry, Faeces, Antibiotic resistance, Akoko

*This study examines the frequency of Plesiomonas shigelloides in soil and faecal samples from poultry farms. Samples were taken from a chicken farm in each of the towns of Ikare-Akoko and Oke-Agbe. Twenty eight samples in all were collected, tested using the serial dilution method for Plesiomonas shigelloides, and grown on Inositol Brilliant Green Bile Agar. To determine the effectiveness of the tested antibiotic against the isolate, an antimicrobial susceptibility test was conducted after observing the colony on a plate. The study shows that faecal samples have higher microbial count compare to soil samples. It further reveals that soil and faecal samples obtained from Ikare Akoko has the highest colony count of  $2.8 \times 10^5$  cfu/g and  $5.3 \times 10^5$  cfu/g respectively. Isolates were subjected to antibiotic such as cefotaxime (30µg), gentamicin (10µg), Meropenem (10µg), ofloxacin (5µg), tetracycline (30µg) and trimethoprim/sulfamethoxazole (1.25µg/23.27µg). The antimicrobial susceptibility test shows that isolate obtained from Ikare-Akoko were susceptible to cefotaxime (30µg), gentamicin (10µg), ofloxacin (5µg), tetracycline (30µg) and trimethoprim/sulfamethoxazole (1.25µg/23.27µg) but were resistant to Meropenem (10µg). However, isolates obtained from soil and faecal samples in Oke Agbe were susceptible to cefotaxime (30µg), gentamicin (10µg), ofloxacin (5µg) and trimethoprim/sulfamethoxazole but resistant to Meropenem (10µg) and few shows resistance to tetracycline (30µg). The finding of this study are that Plesiomonas shigelloides is prevalent in soil and poultry faeces samples in Ikare Akoko and Oke-Agbe, which may have an impact on public health and lead to food- and water-borne gastroenteritis outbreaks.*

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### INTRODUCTION

The soil around high-production farms is frequently microbially contaminated; in particular, arable land and pastures contaminated with sick animals' excrement play a significant role in pathogen transfer (Trawiska *et al.*, 2016). The progressive process of soil formation involves biological and physicochemical weathering over a long period. Microbes are crucial for the development of soil

aggregates and soil stability, which give soil its fertility and productivity. Additionally, certain microorganisms are introduced into the soil by human and animal activities in a variety of ways. By adding sewage sludge to agricultural fields or using bacteria as biodegradative agents, human activity directly introduces bacteria. Animal excrement or bird poop introduces germs to the environment (Wei *et al.*, 2013). Production of chicken is regarded as

one of the livestock subsectors with the highest growth, and this one of the fastest-growing livestock subsectors is poultry production, which has contributed to several health problems for both individuals who deal with poultry and those who consume chicken products (Mottet and Tempio 2017). In fact, most people frequently have direct or indirect interaction with poultry. The biggest risk is handling and killing live, infected chickens, although transmission can also happen when contact is made with droppings, feathers, organs, and blood (FAO 2020). It has been debatable whether to classify *Plesiomonas shigelloides*, a group of gram-negative, thermotolerant, motile, and pleomorphic microbes; it was originally categorized in the family Vibrionaceae but is now categorized as a member of the Enterobacteriaceae family (Janda and Abbott 2021). They typically range from 0.6 to 6.0 micrometers in length and width respectively and are motile with polar flagella, do not produce spores, and are facultatively anaerobic (Ciznar *et al.*, 2006). Consuming contaminated chicken items or coming into contact with them directly or indirectly can get you sick. Bacterial pathogens are the principal contributors to human intestinal illnesses from this source. There is a new, well-known threat to human health antibiotic-resistant bacteria are created as a result of the improper use of antibiotics in the production of chicken. This study helps to assess the prevalence and antibiotic signatures of the *Plesiomonas shigelloides* obtained from the faecal and soil samples of the chosen farms in this study.

## MATERIALS AND METHODS

### Methods

#### Sample collection and cultural Isolation of *Plesiomonas shigelloides* from soil and faeces

Soil and faecal samples were collected from two poultry farms located at Oke-Agbe and Ikare Akoko respectively. Faecal samples of birds were collected into two sterile universal bottles and were properly labelled using marker a to avoid mixing up the samples. Also, soil samples were collected from the same location into a sterile zip lock and was as well labelled to avoid mix-ups. The samples were transported to the laboratory for analysis. One gram of poultry bird faeces was weighed into a sterile beaker containing 100 ml of sterile distilled water. The beaker was shaken properly and 1 ml of the mixture was pipetted into a test tube containing 9 ml of sterile distilled water to make tenfold serial dilutions, dilutions, and the dilution continues upto  $10^{-5}$  dilution factor. One millilitres from the  $10^{-4}$  and  $10^{-5}$  dilution factors were aseptically pipette into a sterile Petri dish and cultured (American Public

Health Association, 2005). Inositol Brilliant Green Bile agar which has been sterilized by autoclaving and cooled to about 45 °C was poured aseptically into the Petri dishes containing the dilutions and allowed to solidify. The plates were incubated in an inverted position in an incubator for 24 hours. Pink colonies on the plates were counted and recorded as presumptive *P. shigelloides* isolates and the results were multiplied using appropriate diluents, counts were expressed in colony-forming unit per ml of sample. Furthermore, some selected pink colonies were later streaked out on nutrient agar to purify them and also subjected to oxidase enzyme production test using oxidase strips (PHE, 2015). Recovered presumptive isolates were later stored on glycerol stocks (-4 °C) for further analysis. All oxidase positive isolates were further confirmed using Microbact TM 24E Identification Kit afterward by following manufacturer instructions as earlier described by Osunla *et al.* (2021).

### Antibiotics Susceptibility Test

Confirmed *Plesiomonas shigelloides* isolates were examined for antimicrobial profiles against diverse antimicrobial agents using the Kirby Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2017). Six antimicrobial disks (Davies, Diagnostics, South Africa) belonging to six antimicrobial classes were selected, including ampicillin Cefotaxime (30µg), Gentamicin (10µg), Meropenem (10µg), Ofloxacin (5µg), Tetracycline (30µg) and Trimethoprim/Sulfamethoxazole (1.25 µg/23.75 µg).

## RESULTS AND DISCUSSIONS

Table 1 shows the colony count for samples retrieved in Ikare Akoko, it revealed that the soil faecal samples obtained from poultry farm in Ikare Akoko have the highest colony counts of  $2.8 \times 10^5$  and  $4.8 \times 10^5$  CFU/g respectively. As shown in Table. Table 2 also reveals that soil has the highest colony count of  $2.4 \times 10^5$  CFU/g and  $4.8 \times 10^6$  CFU/g in faecal sample obtained. Table 3 shows the result of the biochemical test for the identification of isolates using the Microbat 24E kit. Figure 1 shows the antibiotic susceptibility pattern for the isolates of samples collected in Ikare-Akoko and Oke-Agbe when tested against Cefotaxine, Gentamicin, Meropenem, Ofloxacin, Tetracycline and Trimethoprim.

**Table 1: Colony Count for Samples Collected in Ikare- Akoko**

Samples	$10^{-4}$	(CFU/g)	$10^{-5}$	(CFU/g)
Soil A1	24	$2.4 \times 10^5$	22	$2.2 \times 10^6$
Soil A2	21	$2.1 \times 10^5$	18	$1.8 \times 10^6$
Soil B1	28	$2.8 \times 10^5$	26	$2.6 \times 10^6$
Soil B2	25	$2.5 \times 10^5$	21	$2.1 \times 10^6$
Faecal A1	46	$4.6 \times 10^5$	49	$4.9 \times 10^6$
Faecal A2	48	$4.8 \times 10^5$	41	$4.1 \times 10^6$
Faecal B1	53	$5.3 \times 10^5$	46	$4.6 \times 10^6$
Faecal B2	42	$4.2 \times 10^5$	43	$4.3 \times 10^6$

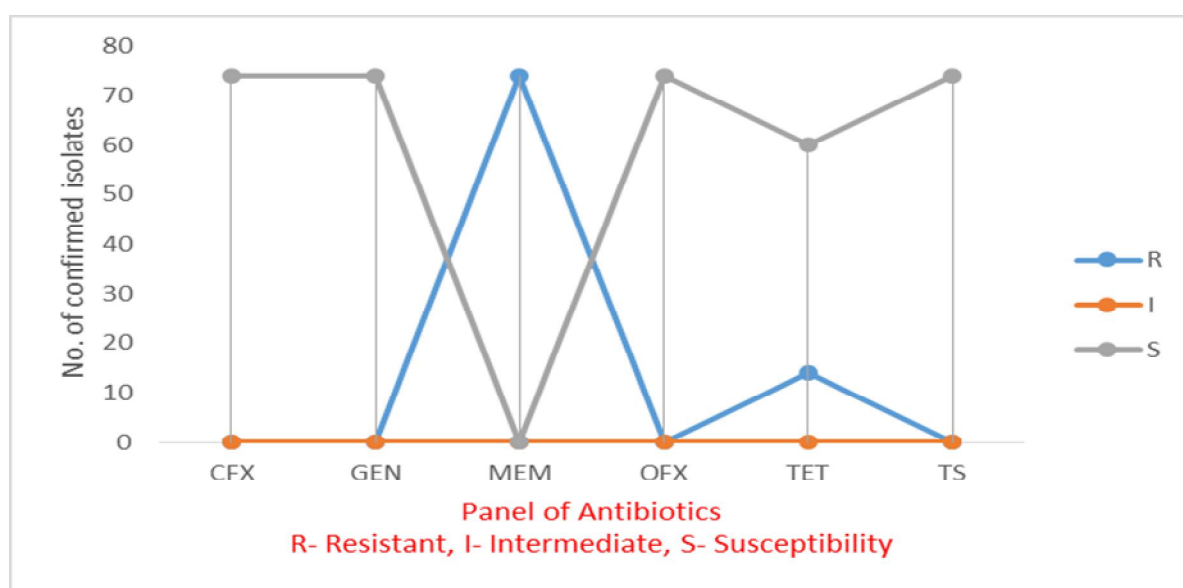
**Table 2: Colony Count for Samples Collected in Oke-Agbe**

Samples	$10^{-4}$	(CFU/g)	$10^{-5}$	(CFU/g)
Soil A1	15	$1.5 \times 10^5$	16	$1.6 \times 10^6$
Soil A2	18	$1.8 \times 10^5$	18	$1.8 \times 10^6$
Soil B1	21	$2.1 \times 10^5$	22	$2.2 \times 10^6$
Soil B2	24	$2.4 \times 10^5$	12	$1.2 \times 10^6$
Faecal A1	48	$4.8 \times 10^5$	31	$3.1 \times 10^6$
Faecal A2	42	$4.2 \times 10^5$	24	$2.4 \times 10^6$
Faecal B1	44	$4.4 \times 10^5$	38	$3.8 \times 10^6$
Faecal B2	48	$4.8 \times 10^5$	32	$3.2 \times 10^6$

**Table 3: Biochemical Identification of Isolates Using Microbact Identification Kit**

Isolates	Test result code	Probable Identity	% Probability
GOK 1	615613003	<i>Plesiomonas shigelloides</i>	98.56
GOK 2	605611003	<i>Plesiomonas shigelloides</i>	99.99
GOK 3	715201003	<i>Plesiomonas shigelloides</i>	99.91
REK 4	604603043	<i>Plesiomonas shigelloides</i>	99.99
REK 5	715201003	<i>Plesiomonas shigelloides</i>	99.99

KEY: GOK Represents isolates from Oke-Agbe; REK Represents isolates from Ikare-Akoko



**Figure 1:** Antibiotic Susceptibility Pattern for the Isolates of Samples Collected in Oke-Agbe Measured in Millimeter (mm)

**DISCUSSION**

These findings show that the soil sample had the highest microbial load of the microbe under investigation obtained from the poultry farm in Ikare-Akoko, while the faecal sample obtained from the poultry farm in Ikare Akoko showed the highest total count. Similar high microbial counts from poultry faeces were also reported in Sierra Leone by Mansaray et al. (2022). Further

assessment of the study reveals that faecal samples have higher microbial count compare to soil samples. This is in agreement with a previous study by Trawińska et al. (2006) who evaluated microbial contamination of the environment surrounding the poultry farm and observed the highest count of bacteria ( $5.9 \times 10^6$  cfu/g) in the soil samples. The Microbact TM gram-negative system is a standardized micro substrate system designed to simulate conventional biochemical substrates used

for the identification of Enterobacteriaceae. The overall prevalence of the recovered *Pleisomonas shigelloides* isolates from faecal and soil samples in poultry farms in the study area is relatively low at present, but may be of public health importance and can lead to an outbreak of bacterial zoonosis via the risk of contamination of poultry birds and products. The variation in the prevalence of *Pleisomonas shigelloides* is similar to what is documented in other settings such as Cameroon (Moffo *et al.*, 2021) and North West Ethiopia (Eyasu *et al.*, 2017) outlined, with prevalence within the range of about 45%. Low isolation of organism of almost 11% was recorded in free range birds in Nigeria in a study by Fuh *et al.* (2018). Variation in microbial loads as noticed in this study can be attributed to the age of the birds and the type of poultry sample used. *P. shigelloides* is a clinically significant bacteria, although little is known about its antimicrobial susceptibility profiles and pathogenic components in Nigeria. In immunocompetent hosts, the bacterium is suspected of causing sporadic and epidemic diarrhea, while in immunocompromised individuals, it is suspected of causing severe extra intestinal illness (Ramalivhana and Obi, 2009). Confirmed *Pleisomonas shigelloides* from both sampling sites were found to be 100% resistant to meropenem while few of the isolates recovered from Oke-Agbe were resistant to tetracycline. Susceptibility of *Pleisomonas shigelloides* to the panel of antibiotics used in this such as cefotaxime, gentamicin, ofloxacin and trimethoprim/sulfamethoxazole are widely reported across several studies. Antibiotics such as cefotaxime, gentamicin, ofloxacin and meropenem are considered critically important antibiotics for human use, and these antibiotics are under high prioritization based on their potential to transfer antibiotic resistance from pathogens of animals to humans (WHO, 2019). The level of resistance reported to meropenem in the present study (100%) is higher compared to other studies, where the resistance varied from 1.10 to 6 % (Adesiyani *et al.*, 2019; Ekundayo and Okoh, 2020). Similarly, the resistance to tetracycline reported in the present study (10%) is low compared to other studies, which varied from 19–51% (Adesiyani *et al.*, 2019; Ekundayo and Okoh, 2020). The study we conducted has certain advantages. To the best of our knowledge, this is the first investigation of *Pleisomonas shigelloides* prevalence and antibiotic resistance trends in poultry excrement from the Akoko metropolitan. Second, the single antibiotic discs used in this investigation were evaluated for antibiotic sensitivity using an antibiotic dispenser in accordance with CLSI recommendations. Using a laminar flow, all procedures for determining antibiotic sensitivity and isolating bacteria were carried out in a highly sterile laboratory setting.

The appropriate State and Federal parastatals should invest in doing comparable evaluations elsewhere in order to evaluate whether our findings are reproduced in susceptible areas of the country as this study's findings are based on one region of Ondo State. Establishing representative routine poultry surveillance units to track increases in antibiotic resistance is long overdue. Finally, in order to anticipate the likelihood that antibiotic resistance would spread to people, future studies from Nigeria should look for antibiotic resistant genes in poultry excrement.

## CONCLUSION

Antibiotic-resistant bacteria are present in poultry waste in the research location. If untreated poultry faecal droppings are utilized as manure for vegetable production, there is a serious risk that they will spread resistant *Pleisomonas* isolates to humans. The effectiveness of empirical treatment in these situations may be threatened by the widespread resistance to antibiotics used in human medicine. Additionally, there is a critical need for better environmental practices, specifically proper waste management, which could lower occurrences of antibiotic resistance. Antibiotic resistance patterns should be monitored by surveillance of poultry populations.

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