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Antimicrobial resistance and virulence genes profiling of *proteus* species from poultry farms in Lafia, Nigeria

MC Owoseni[1], O Oyigye[1], B Sani[1], J Lamin[1,2] and A Chere[1]
Department of Microbiology[1], Federal University of Lafia, and Dalhatu-Araf Specialist Hospital[2], Lafia, Nasarawa State, Nigeria

Abstract
Background: The poultry industry is important in boosting food security in a population; however, the poultry environment and products can serve as channels of antimicrobial resistant pathogens via the food chain portending a health risk to humans and the environment. This study investigated the prevalence, antimicrobial resistance and virulence of *Proteus* species from the feed, drinking water and eggshells of four selected poultry farms in Lafia, Nasarawa State.
Methodology: Farm samples (n =216) comprising feed (64), drinking water (64) and swabs from eggshells (88) were collected and processed for isolation of *Proteus* species using standard bacteriological methods. The antibiotics susceptibilities of isolates to ten (10) commercial antibiotics and carriage of three (3) virulence genes (*rsbA, ureC* and *luxS*) were investigated using disc diffusion test and Polymerase Chain Reaction, respectively. Statistical significance difference among the farms, sample types and Proteus species were calculated using one-way ANOVA.
Results: Of the total samples studied, 34.26% (74/216) were positive for *Proteus* species. *Proteus* species were more prevalent in drinking water samples (37.84%; 28/74) and feed samples (33.78%; 25/74) and least prevalent in eggshells (28.38%; 21/74). *Proteus* species (n= 74) comprised *P. mirabilis* 78% (58/74) and *Proteus vulgaris* 22% (16/74) with *P. mirabilis* more predominant than *P. vulgaris* in all the four farms sampled. The prevalence rate of *Proteus* species was not statistically significantly different ($p \geq 0.05$) among the farms, sample types, and species. Isolates were 100% susceptible to Amikacin and exhibited the highest resistance (25.7%) to tetracycline. Molecular characterization of the virulence genes of *Proteus* species revealed the presence of *luxS* genes in *P. vulgaris* and *rsbA* and *ureC* genes in *P. mirabilis* and *P. vulgaris*.
Conclusions: The overall prevalence rate of Proteus species was low (34.26%) in the samples collected and majority of the isolates were susceptible to the antibiotics tested. Nonetheless, the level of resistance to the antibiotics tested and carriage of virulence genes is indicative of a significant health risk to the consumers from transmission of *Proteus* species via the food chain.

Keywords: *Food safety, Proteus species, virulence genes, antimicrobial resistance, poultry farms*

Résumé
Contexte : L'industrie avicole est importante pour renforcer la sécurité alimentaire d'une population ; cependant, l'environnement et les produits de la volaille peuvent servir de canaux d'agents pathogènes résistants aux antimicrobiens via la chaîne alimentaire, ce qui laisse présager un risque pour la santé des humains et de l'environnement. Cette étude a examiné la prévalence, la résistance aux antimicrobiens et la virulence des espèces de Proteus dans les aliments, l'eau potable et les coquilles d'œufs de quatre fermes avicoles sélectionnées à Lafia, dans l'État de Nasarawa.
Méthodologie : Des échantillons de ferme (n =216) comprenant des aliments (64), de l'eau potable (64) et des écouvillons de coquilles d'œufs (88) ont été collectés et traités pour l'isolement des espèces de Proteus à l'aide de méthodes bactériologiques standard. Les sensibilités aux antibiotiques des isolats à dix (10) antibiotiques commerciaux et le portage de trois (3) gènes de virulence (rsbA, ureC et luxS) ont été étudiées à l'aide du test de diffusion sur disque et de la réaction en chaîne par polymérase, respectivement. La différence de signification statistique entre les fermes, les types d'échantillons et les espèces de Proteus a été calculée à l'aide de l'ANOVA à un facteur.
Résultats : Sur le total des échantillons étudiés, 34,26 % (74/216) étaient positifs pour l'espèce Proteus. Les espèces de Proteus étaient plus fréquentes dans les échantillons d'eau potable (37,84 % ; 28/74) et les échantillons d'aliments (33,78 % ; 25/74) et moins présentes dans les coquilles d'œufs (28,38 % ; 21/74). Les espèces de Proteus (n=74) comprenaient

Correspondence: Dr. Christiana Owoseni, Department of Microbiology. Federal University of Lafia, PMB 146, Nasarawa State, Nigeria. E-mail:moji.owoseni@gmail.com.

P. mirabilis 78 % (58/74) et Proteus vulgaris 22 % (16/74) avec P. mirabilis plus prédominant que P. vulgaris dans les quatre fermes échantillonnées. Le taux de prévalence des espèces de Proteus n'était pas statistiquement significativement différent (p 0,05) entre les fermes, les types d'échantillons et les espèces. Les isolats étaient 100 % sensibles à l'amikacine et présentaient la résistance la plus élevée (25,7 %) à la tétracycline. La caractérisation moléculaire des gènes de virulence des espèces de Proteus a révélé la présence de gènes luxS chez P. vulgaris et de gènes rsbA et ureC chez P. mirabilis et P. vulgaris.

Conclusions : Le taux de prévalence global des espèces de Proteus était faible (34,26 %) dans les échantillons collectés et la majorité des isolats étaient sensibles aux antibiotiques testés. Néanmoins, le niveau de résistance aux antibiotiques testés et le port de gènes de virulence sont révélateurs d'un risque sanitaire important pour les consommateurs lié à la transmission des espèces de Proteus via la chaîne alimentaire.

Mots clés : *Sécurité alimentaire, Espèces Proteus, gènes de virulence, résistance aux antimicrobiens, élevages avicoles*

Introduction

The poultry industry is a major component of the Nigerian economy, providing a source of income to farmers and leading the production of high-quality protein for the fast-growing population due to the affordability and acceptability of their products [1]. However, the incidence of microbial infections from infected birds with clinical diseases in the poultry farm environment and products is a concern to public health and food safety and may cripple economic gains derived from this industry [2,3].

Proteus poses a significant challenge to both humans and animals worldwide due to its multiple resistant forms and is prevalent in several food and animals including poultry [4]. With several foodborne poisoning attributed to *Proteus* and he rising incidence of *Proteus* induced foodborne infections, it is pertinent that control programs and prophylactic measures be developed to prevent and combat outbreaks of foodborne infections/poisoning from poultry and poultry products [5]. Poultry and other poultry products are believed to be the primary vehicle for the transmission of *Proteus* [6]. Poultry might have *Proteus* strains in their droppings and on their bodies (feathers, feet and beaks), even when they appear healthy and clean. The *Proteus* species can stick to cages, coops, feed and water dishes, hay, plants and soil in the area where the birds live; also eggshells may become contaminated with *Proteus* from poultry droppings or the area where they are laid [7] . From a public health perspective, the number of eggs and animals infected by *Proteus* is a risk factor for human disease or infection [8].

Human cases of *Proteus* are typically acquired through the consumption of contaminated food. *Proteus* usually spread through the faecal-oral route (contamination of hands or objects with bacteria shed in the stool) [9]. *Proteus* in chicken droppings might be transmitted to vulnerable workers while handling infected chicken directly or through faecal-contaminated poultry products [10, 11]. Generally, there are two possible routes of egg contamination by *Proteus*. Eggs can be contaminated by penetration of the bacterium through the eggshell from the colonised gut or from contaminated faeces during or after horizontal transmission. Horizontal transmission occurs following ingestion of food or water already contaminated with faeces of clinically infected birds or carriers, presence of dead chickens, poultry farm attendants and contaminated feeds [12]. The second possible route is by direct contamination of the yolk, albumen, eggshell membranes or eggshells before oviposition, originating from the infection of reproductive organs with *Proteus* (vertical transmission) [13,14].

Consumption of raw/undercooked eggs has consistently been identified as the primary risk factor for *Proteus mirabilis* [15]. *Proteus* is known to cause human urinary tract infection (UTI), nosocomial infection, wound infection [16, 17] and showed clear history of zoonosis in vast host range with the emergence of multidrug resistance (MDR) in recent years [9]. Multidrug-resistant *Proteus* may be transmitted among poultry farm workers who may transmit the pathogen in the surrounding environment. Humans use many classes of antimicrobial agents used in animals, and there is a potential selection and spread of antimicrobial-resistant bacteria or genes from animals to humans through the food chain [18]. The indiscriminate use of antibiotics or congeners has created enormous pressure for the selection of antimicrobial resistance among bacterial pathogens worldwide, including *Proteus* strains found in poultry products and poultry environment [19].

The virulence potential of *Proteus* in connection to its pathogenicity has been investigated in animal husbandry including poultry. Virulence of the *Proteus* species is caused by several factors which are regulated and expressed by virulence genes encoded in operons [20, 21]. These virulence genes increase the pathogenicity of *Proteus* species among which include urease which is the most important enzyme for kidney and bladder stone formation [22] and enables it to produce an environment in which it can survive [23]. The *luxS* gene is involved in the

synthesis of autoinducer 2 (AI-2) secreted by bacteria and used to communicate both the cell density and the metabolic potential of the environment [24]. Swarming behaviour of *P. mirabilis* mediated by *rsbA* gene has been associated with biofilm formation and extracellular polysaccharide formation [25]. Therefore, there is imperative that continuous surveillance of poultry farms be established to monitor possible transmission routes of microbial pathogens from farms via the food chain.,

This study investigated the prevalence, antimicrobial resistance and virulence gene profiles of *Proteus* species isolated from Poultry farm samples in Lafia, Nigeria.

Material and methods

Sample collection and bacterial isolation

A cross-sectional study was conducted for two months between May and June 2019, and a total number of 216 samples from four commercial poultry farms in Lafia, Nasarawa State were collected. The sample size was determined by using the prevalence of 16.67% reported by Esther Chat *et al.* [26] and the following the equation described by Naing *et al* [27] as

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where: n = sample size
P = prevalence from a previous study = 16.67% = 0.17.
Z = standard normal distribution at 95% confidence interval= 1.96.
D = absolute desired precision at 5% = 0.05.

Samples included poultry feed (n = 64), drinking water (n=64) and unbroken eggshells (n = 88) from four selected poultry farms in equal amounts during early morning hours on a weekly interval. Swab samples collected from surfaces of eggshells were inoculated into buffered peptone water (BPW) (Oxoid, UK) in screw-capped bottles, incubated at 37 ºC for 24 h and thereafter pre-enriched in Selenite-F broth. A loopful of culture from Selenite-F was sub-cultured by streaking onto Xylose lysine deoxycholate agar (XLD) and incubated at 37 ºC for 24h [28, 29]. One millilitre of poultry drinking trough was inoculated into 9ml of BPW and the mixture was incubated at 37°C for 24h. Thereafter, 1ml of the solution was transferred into 9ml Selenite-F broth and a loopful was streaked on XLD agar (Oxoid Basingstoke and Hampshire, UK) and incubated at 37 °C for 24 h.

Identification of presumptive isolates

Bacterial isolates were identified based on their morphological and biochemical identification [29, 30]. Isolates showing transparent colonies with black centre on XLD due to hydrogen sulphide production were selected as presumptive bacteria and subcultured on nutrient agar [29] Pure isolates were confirmed by their biochemical characteristics using urease, Triple sugar Iron test and citrate test. Afterwards, bacterial isolates identified as *Proteus* species were delineated into their various species by the indole test and stored on agar slants for further analysis.

Antimicrobial susceptibility and resistance profiling of Proteus species

The antimicrobial resistance profiles of *Proteus* species to ten antibiotics were determined by the disc diffusion method using standard procedures described by the Clinical Laboratory Standards Institute (CLSI) [31]. Ten commercial antibiotics disk (Oxoid, UK) which include: Amikacin (AMK) (30µg), Tetracycline (TET) (30µg), Ciprofloxacin (CPX) (5µg), Erythromycin (ERY) (15µg), Gentamycin (GEN) (10µg), Ampicillin (AMP) (10µg), Ofloxacin (OFX) (5µg), Ceftriaxone (CRO) (30µg), Levofloxacin (LVX) (5µg), and Penicillin (PEN) (10µg) were used.

Bacterial species were spread on Mueller Hinton agar and antibiotics discs were aseptically placed on the plates using sterile forceps. Plates were incubated for 24h at 37ºC. Thereafter, the diameters of the zones of inhibition were measured in millimetre (mm) and results were interpreted using the Clinical Laboratory Standards Institute (CLSI) interpretative charts [31].

Molecular detection of Virulence Genes of Proteus species

The molecular detection of virulence genes of *Proteus* species was carried out at Applied Biotech International Limited (ABINL), Abuja, Nigeria. Among the highly antibiotics resistant *Proteus* species to the tested antibiotics in this study, fifteen *Proteus* isolates were selected and characterized to detect the virulence genes *luxS, ureC* and *rsbA* genes.

DNA extraction

Genomic DNA was extracted using the boiling method. Briefly, 5 mL of bacterial isolates grown in Luria Bertani (LB) broth at 37 ºC for 8 h were centrifuged at 14000 rpm for 3 min. The cells were resuspended in 500 µl of double distilled water and heated at 95 ºC for 20 min in the heating chamber. The heated bacterial suspension was cooled on ice

and centrifuged at 14000 rpm for 3 min. The supernatant containing the DNA was transferred to a 1.5 ml microcentrifuge tubes and stored at "20 ºC for further use.

DNA Quantification

The extracted genomic DNA was quantified using the NanoDrop 1000 spectrophotometer by placing a drop (approximately 2 µl) on the sample space and analysed using the NanoDrop 1000 software.

Molecular characterisation of virulence genes of Proteus species

The virulence genes of *Proteus* species were characterised by Polymerase chain reaction technique. The following primer sets were used, (5' – TTGAAGGACGCGATCAGACC – 3') and (3' – ACTCTGCTGTCCTGTGGGTA- 5') which amplifies a 467 bp sequence of *rsbA* gene [32]. (5' – ACTCTGCTGTCCTGTGGGTA -3') and (3' – GTTATTCGTGATGGTATGGG-5') which amplifies the 317 bp sequence of *ureC* gene (Pathirana et al., 2018) and (5' – GTATGTCTGCACCTGCGGTA- 3') *and (*3' – TTTGAGTTTGTCTTCTGGTAGTGC- 5') which amplifies the 464 bp sequence of *luxS* genes (Abbas *et al.*, 2015). Briefly, gene amplification was carried out on thermal cycler (AB Biosystem, USA) at a final volume of 25 µl for 35 cycles. The PCR mix included X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, dNTPs, MgCl) and the primers at a concentration of 0.2M and 0.5 µl DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 59ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 20 minutes and visualised on a UV transilluminator.

Statistical analysis

All data generated were analysed using One-way ANOVA on IBM SPSS version 22. Statistical differences were calculated among the various farms, sample types and species at $p \leq 0.05$.

Results

Identification and prevalence of Proteus species

Presumptive isolates were identified based on their cultural and biochemical characteristics. Cultural characteristics included transparent colonies with black centre on Xylose Lysine Deoxycholate agar due to hydrogen sulphide production. A total of 74 isolates were confirmed positive on urease media with the production of pinkish-red colouration of the medium. Further examination using Triple sugar Iron test showed black butt from hydrogen sulphide production (H_2S) while motility test produced a cloudy and distinct line of inoculation. Citrate test was positive for *Proteus* species. *P. vulgaris* was indole positive while *P. mirabilis* was indole negative.

The prevalence rate among the samples (n=216) from the four poultry farms was 34.26% (74/216) (Table 1). About 23.86% (21/88) of eggshell swab samples were positive for *Proteus* while 39.06% (25/64) and 43.75% (28/64) of feed and drinking water were positive for *Proteus* species respectively among the four farms selected, farm A and D had the highest prevalence rate of 9.26% (20/216), followed by farm B and farm C with prevalence rates of 8.80% (19/216) and 6.94% (15/216) respectively. However, the prevalence of *Proteus* sp. did not vary significantly (p d" 0.05) among all the samples collected from the four commercial farms.

For the sample types, water from the trough were more contaminated with *Proteus* species 37.84% (28/74), followed by the feed 33.78% (25/74), then the eggshell 28.38% (21/74) as shown in Figure 1.

Table 1: Prevalence of *Proteus* species from sampled farm equipment and products from four commercial farms in Nasarawa State

Sample type	Prevalence of positive samples (%)				
	Farm A	Farm B	Farm C	Farm D	Total number of positive samples (%)
Eggshell swabs (n=88)	5 (22.73)	5 (22.73)	4 (18.18)	7 (31.82)	21/88 (23.86)
Feed (n=64)	7 (43.75)	7 (43.75)	5 (31.25)	6 (37.5)	25/64 (39.06)
Drinking water (n=64)	8 (50.00)	7 (43.75)	6 (37.5)	7 (43.75)	28/64 (43.75)
n=216	20 (9.26)	19 (8.80)	15 (6.94)	20 (9.26)	74/216(34.26)

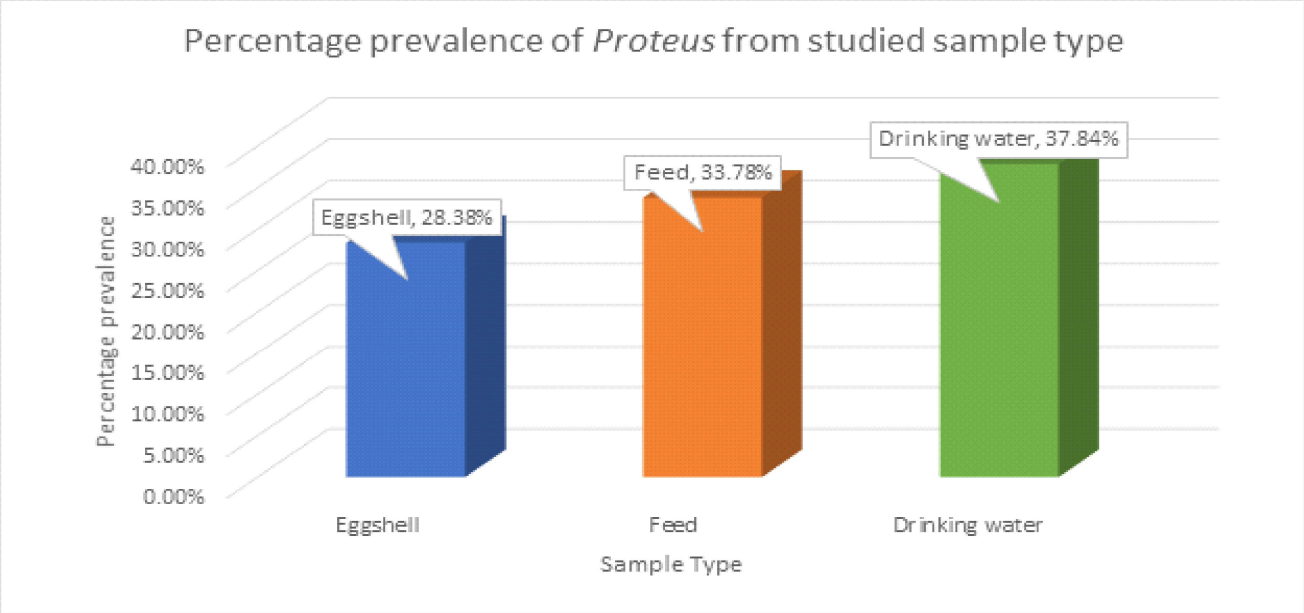

Fig. 1: Percentage prevalence of *Proteus* species from the samples studied (n=74)

Table 2 shows that *P. mirabilis* was more prevalent 78% (58/74) than *P. vulgaris* 21.6% (16/74) among all the samples from the four farms. The drinking water sample had the highest prevalence of *P. mirabilis* 82% (23/28), followed by feed sample 76% (19/25) and eggshell sample 76% (16/25) the feed and eggshell samples had the highest prevalence of *P. vulgaris* 24% (6/25 and 5/21 respectively) and the lowest prevalence of 18% (5/28) in drinking water.

Antimicrobial resistance profiling of Proteus species

The antimicrobial susceptibility profile of the 74 confirmed *Proteus* species was tested against a panel of 10 antibiotics (Table 3). *Proteus* species were susceptible to the antibiotics in varying amounts and susceptibility declined in the following order: Amikacin and Gentamicin (95.9%), Levofloxacin (93.2%), Ciprofloxacin and Penicillin (86.5%), Ofloxacin

Table 2. Prevalence of *P. mirabilis* and *P. vulgaris* from four commercial farms in Nasarawa State

Sample type	Farm A		Farm B		Farm C		Farm D		Cumulative Total across sampled farms	
	Pm (%)	*Pv (%)*	*Pm (%)*	*Pv (%)*	*Pm (%)*	*Pv (%)*	*Pm (%)*	*Pv (%)*	*Pm (%)*	*Pv (%)*
Egg shell	4/5 (80)	1/5 (20)	4/5 (80)	1/5 (20)	3/4 (75)	1/4 (25)	5/7 (71)	2/7 (29)	16/21 (76)	5/21 (24)
Feed	5/7 (71)	2/7 (29)	6/7 (86)	1/7 (14)	4/5 (80)	1/5 (20)	4/6 (67)	2/6 (33)	19/25 (76)	6/25 (24)
Drinking water	7/8 (88)	1/8 (12)	5/7 (71)	2/7 (29)	5/6 (83)	1/6 (17)	6/7 (86)	1/7 (14)	23/28 (82)	5/28 (18)
Total per *Proteus* sp	16/20 (80)	4/20 (20)	15/19 (79)	4/19 (21)	12/15 (80)	3/15 (20)	15/20 (75)	5/20 (25)	58/74 (78)	16/74 (22)

There was no statistical significant difference (p d 0.05) between and within the Proteus species, farms and sample type.
Key: *Pm: Proteus mirabilis; Pv: Proteus vulgaris*

The prevalence of *P. mirabilis* was in the order; farm A and C (80%), farms B (79%) and farm D (75%) while *P. vulgaris* was in the other farm D (25%), followed by farm B (21%) and farms A and C (20%).

(82.4%), Erythromycin (81.1%), Ampicillin (77.0%) and Tetracycline (68.9%). The resistance of *Proteus* to antibiotics was observed in the following order with the highest resistance recorded for tetracycline

Table 3. Antimicrobial resistance profile of *Proteus* species in the four commercial poultry farms in Nasarawa State

Antibiotics (µg)	Susceptible			Intermediate			Resistant		
	P. mirabilis (%)	*P. vulgaris* (%)	*Total*	*P. mirabilis* (%)	*P. vulgaris* (%)	*Total*	*P. mirabilis* (%)	*P. vulgaris* (%)	*Total*
Amikacin (30)	55 (94.8)	16 (100)	71(95.9)	3 (5.2)	0 (0)	3 (4.1)	0	0 (0)	0 (0)
Ciprofloxacin (5)	54 (93.1)	10 (62.5)	64(86.5)	3 (5.2)	5 (31.3)	8 (10.8)	0 (0)	2 (12.5)	3 (4.1)
Erythromycin (15)	51 (87.9)	9 (56.3)	60(81.1)	2 (3.4)	2 (12.5)	4 (5.4)	5 (8.6)	5 (31.3)	10 (13.5)
Tetracycline (30)	45 (77.6)	6 (37.5)	51(68.9)	4 (6.9)	0 (0)	4 (5.4)	9 (15.5)	10 (62.5)	19 (25.7)
Levofloxacin (5)	53 (91.4)	16 (100)	69(93.2)	4 (6.9)	1 (6.3)	5 (6.8)	1 (1.7)	0 (0)	1 (1.4)
Ofloxacin (5)	53 (91.4)	8 (50)	61(82.4)	3 (5.2)	6 (37.5)	9 (12.2)	3 (5.2)	1 (6.3)	4 (5.4)
Cefotaxime (30)	48 (82.3)	11 (68.8)	59(79.7)	4 (6.9)	2 (12.5)	6 (8.1)	6 (10.3)	3 (18.8)	9 (12.2)
Gentamicin (10)	57 (98.3)	14 (87.5)	71(95.9)	0 (0)	0 (0)	0 (0)	2 (3.4)	1 (6.3)	3 (4.1)
Ampicillin (10)	49 (84.5)	8 (50)	57(77.0)	6 (10.3)	4 (25.0)	10 (13.5)	3 (5.2)	4 (25.0)	7 (9.5)
Penicillin (10)	51 (87.9)	13 (81.3)	64(86.5)	5 (8.6)	1 (1.4)	6 (8.1)	2 (3.4)	2 (12.5)	4 (5.4)

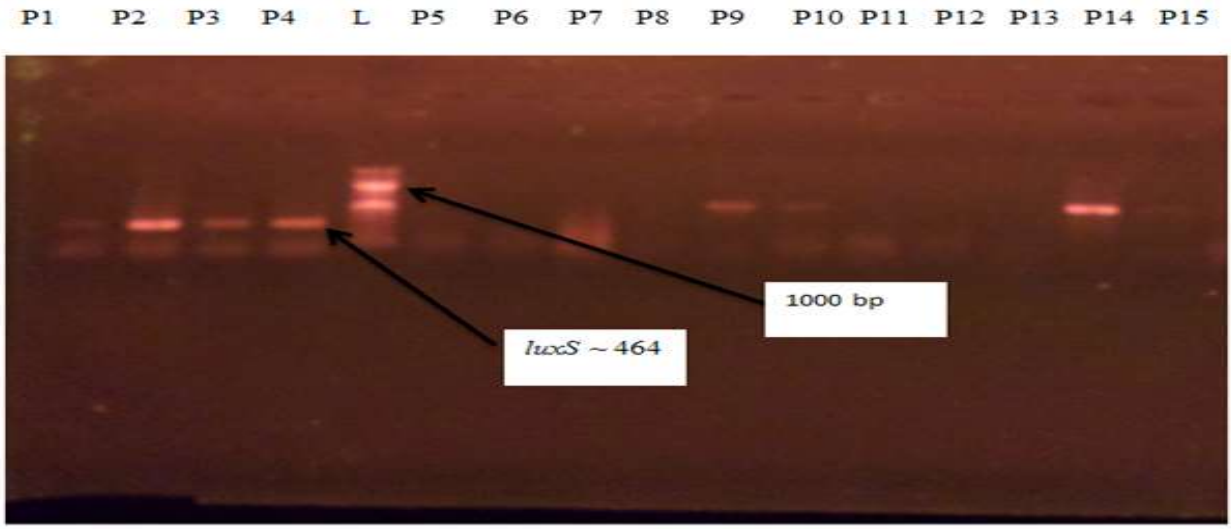

Fig. 2: *Agarose gel electrophoresis of the amplified luxS genes of the Proteus species. Lanes P1-P4 represent the luxS bands. Lane L represents the 1000 bp molecular ladder*
Key*: P1-P5 represent P. vulgaris; P6-P15 represent P. mirabilis.*

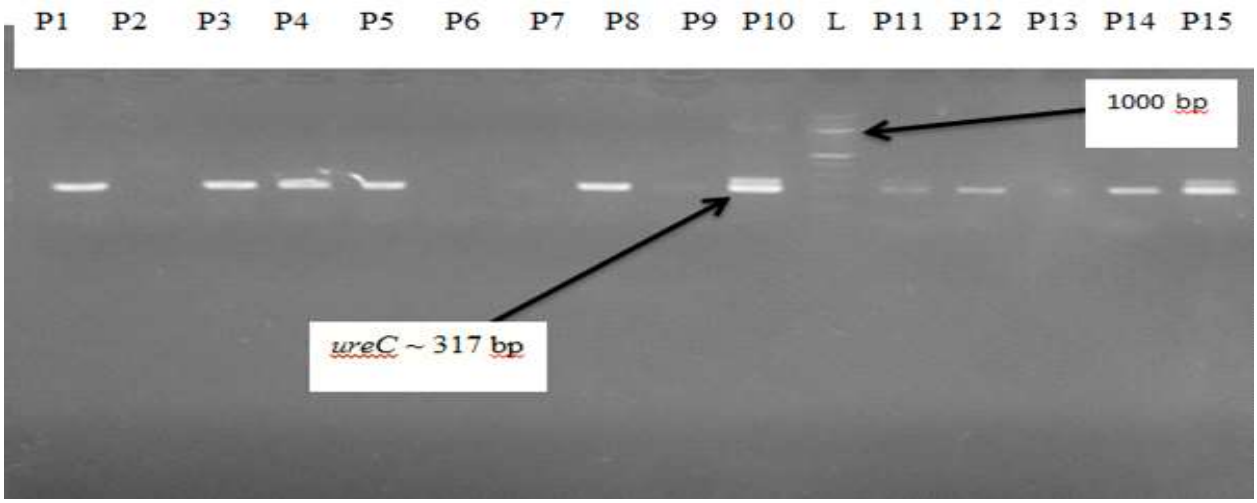

Fig. 3: Agarose gel electrophoresis of the amplified *ureC* genes of the *Proteus* species.
Lanes P1, P3-P5, P8, P10, P11, P12, P14 and P15 represent the *ureC* bands.
Lane L represents the 1000 bp molecular ladder, while other lanes show no band.
Key: P1-P5 represent *P. vulgaris;* P6-P15 represent *P. mirabilis.*

(25.7%) followed by Erythromycin (13.5%), Cefotaxime (12.2%), Ampicillin (9.5%), Ofloxacin and Penicillin (5.4%), Gentamicin and Ciprofloxacin (4.1%) and Levofloxacin (1.4%). No resistance was observed for Amikacin.

Among characterisation of virulence genes of bacterial species

Among the highly antibiotics resistant *Proteus* species to the tested antibiotics in this study, fifteen *Proteus* isolates were selected and characterized to detect the virulence genes *luxS, ureC* and *rsbA* genes. The *luxS* gene was detected in four *P.* vulgaris isolates (Figure 2). The *ureC* gene was detected in ten *Proteus* isolates (four isolates of *P. vulgaris* isolates and six *P. mirabilis* isolates) (Figure 3) while the *rsbA* gene was detected in one *P. vulgaris* and *P. mirabilis* isolates (Figure 4).

Discussion

The cross-sectional study examined the phenotypic resistance and virulence profile of *Proteus* species

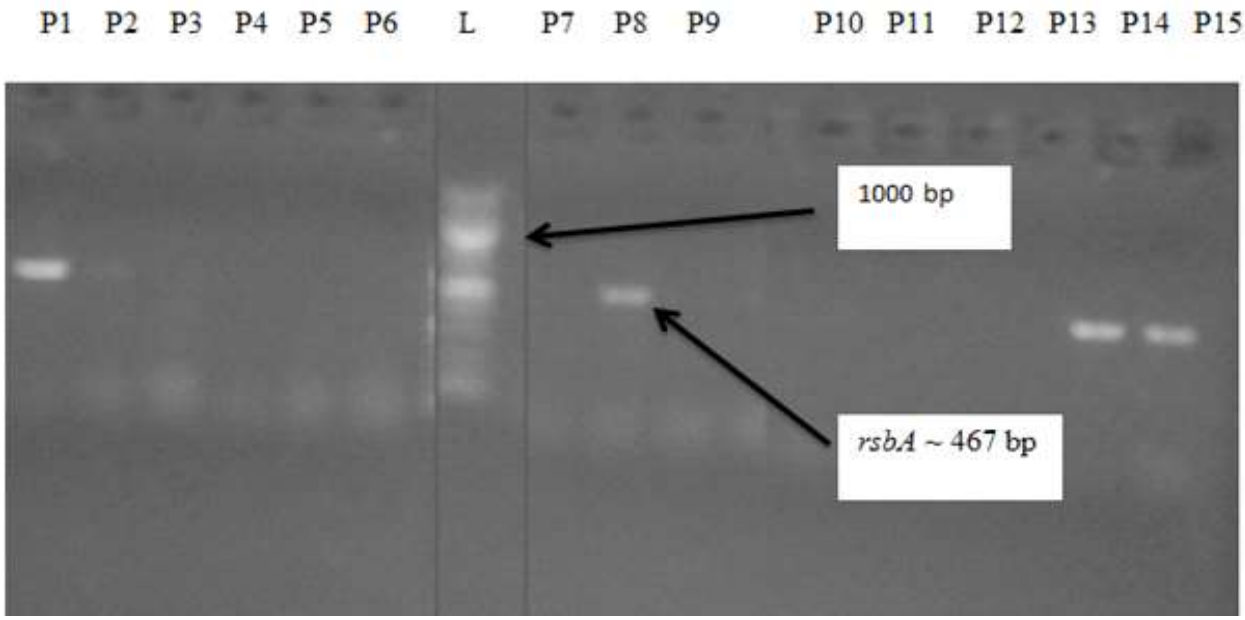

Fig. 4: *Agarose gel electrophoresis of the amplified rsbA genes of the Proteus species.*
Lanes P1 and P8 represent the rsbA bands.
Lane L represents the 1000 bp molecular ladder, while other lanes showed no band.
Key*: P1-P5 represent P. vulgaris; P6-P15 represent P. mirabilis.*

from eggshells, feed and drinking water samples collected from four commercial poultry farms in Lafia, Nasarawa State. The overall prevalence of *Proteus* species was approximately 34.3% (74 positive samples, n= 216) with drinking water having the highest number of positive samples and *P. mirabilis* was the predominant species found in all the samples.

Although the prevalence of *Proteus* species among all the samples from the four farms was not statistically significantly different ($p \geq 0.05$), the number of isolated *Proteus* species is still worrisome due to the ability of these bacterial pathogens to cause human infections such as pneumonia, septicaemia, central nervous system infection, food poisoning and urolithiasis [33]. Direct contact of humans with faecal droppings harbouring *Proteus* species can result in the transmission of the pathogens to immune-suppressed persons leading to urinary tract infections [20]. *P. mirabilis* has been frequently isolated from chicken eggs, cloacal swabs and environmental samples from poultry farms. Ubiebi [34] isolated *P. mirabilis* from poultry feeds in Delta State, Nigeria and *P. mirabilis* were among the isolates present in layers mash and broiler finisher samples obtained from ten poultry farms in Ekiti, Nigeria [35]. Enterobacteriaceae, including *P. mirabilis* and *P. vulgaris,* were frequently isolated from poultry birds [36].

Data generated from this study is of great public health importance as empirical evidence has demonstrated *Proteus* species' involvement in human diseases and as agents of animal infections and bacterial contamination of poultry products [19]. Additionally, the occurrence of *Proteus* species in these farms indicates defective biosafety measures in controlling rodents' infestations, insect vectors, wild birds and pet movements in the poultry pens.

The high prevalence of *Proteus mirabilis* (78%) reported in this study is comparably higher to the prevalence of 66% documented by Barbour *et al*. [33]. The highest prevalence of *Proteus* species, notably *P. mirabilis*, was recorded for farms A and C while farm D had the lowest *Proteus* species' prevalence. This result could be attributed to poor sanitary practices in the farms such as lack of control of the entry of rodents and wild birds into the poultry houses, poor cleaning and disinfection of pens, and the citing of the two farms in waterlogged areas. On the other hand, farm D was well fenced and good hygienic practices such as frequent cleaning of feed and water troughs were observed. Additionally, the entry of visitors into farm D is restricted in contrast to unrestricted visits to farms A-C.

Antibiotics resistant bacteria were detected in all the four farm samples. Meanwhile, a high susceptibility of *Proteus* to tetracycline (68.9%) and ampicillin (77.0%) was observed. The high susceptibility rate is in contrast to recent studies

demonstrating high resistance of members of the Enterobacteriaceae family to tetracycline and ampicillin. *Proteus mirabilis* was resistant to ampicillin-sulbactam at 70 – 79% [37]. *Proteus* species recovered from poultry farms in Ibadan were resistant at 24% to tetracycline Ayandiran *et al*. [38]. *E. coli* isolated from wastewater, abattoir and downstream rivers in Addis-Ababa was resistant to tetracycline and ampicillin at 72.2% and 100 %, respectively [39]. The 25.7% resistance to tetracycline in this study was low comparable to the result of Dadheech *et al*. [40] who recorded a 100% resistance of *Proteus* species isolated from chicken to tetracycline in Ajmer Region, India.

The isolates also exhibited varying resistance to the other antibiotics including erythromycin (13.5%) and cefotaxime (12.2%). Cefotaxime and erythromycin are common antibiotics used in cocktail combination of poultry medication. Cross resistance via mechanism of β-lactam resistance could also be responsible for this observed phenomenon. Antibiotics may be continuously administered as growth promoters to food-grade animals such as broilers and turkeys; therefore, the antibiotic selection pressure for resistance amongst bacteria is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria [41]. The interaction between the different components in a food chain or the environment further contributes to the spread of antibiotic resistance across species [42]. The most effective antibiotics against *Proteus* species were the aminoglycosides, amikacin and gentamicin, with 95.9% activity against *Proteus*. Similar findings reported by Kuznetsova et al., [36] showed a low resistance of *Proteus* to Amikacin and Gentamicin and total resistance to tetracycline. The resistance of *Proteus* species to ciprofloxacin (4.1%) was low in this study compared to the report of Kuznetsova *et al*. [36] who recorded a 36.8% resistance to ciprofloxacin. Ciprofloxacin in droppings of poultry birds collected in Bangladesh is in consonance with the result obtained in our study where *P. mirabilis* was sensitive to ciprofloxacin [19]. Okwonko *et al*. [43] found *P. mirabilis* to be 100% sensitive to streptomycin, an aminoglycoside in the same family with amikacin and gentamicin used in our study with a t sensitivity was 95.9%. Likewise, the resistant and susceptibility patterns of *P. mirabilis* to tetracycline and gentamicin respectively were established in poultry feeds purchased from Calabar, Nigeria [43]. This report suggests that the presence of *Proteus* in poultry feeds may be attributed to commercially sold feeds contaminated with *Proteus* and not necessarily from poultry workers directly infecting the feed. In agreement with similar studies, the aminoglycosides seem to be the drug of choice to treat *Proteus* infections. The availability and accessibility to antimicrobial compounds such as ciprofloxacin, streptomycin, gentamicin, erythromycin, tetracycline, and furazolidone among others in open markets to treat broiler/layer pose a challenge to reducing antimicrobial resistance in the poultry farms [44, 19]. The *ureC* gene was the predominant gene detected in this study. *ureC* gene is responsible for the elevation of urine pH, resulting in calculi formation and it plays a crucial role in the pathogenicity of *Proteus* species producing blockage in of indwelling urinary catheter, kidney stones and bladder stones [45,46]. Ram *et al*. [5] found *ureC* genes in the chicken cloacal swabs collected from a livestock farm complex in India. The frequency of occurrence of *ureC* genes (66.7%) among the *Proteus* species was higher than the other two genes. However, the frequency of 66.7% is lower than similar findings of [20] who isolated *Proteus* species from humans and pet turtles and reported 91.7% prevalence for *ureC* genes. Gene expression of *ureC* genes and other virulent genes in multidrug-resistant *P. mirabilis* isolated from diarrhetic animals in North-East China were associated with biofilm formation [47]. The *rsbA* gene was amplified in both *P. mirabilis and P. vulgaris* with a 26.7% frequency which is in contrast to the report of [32], who reported that *rsbA* gene could not be amplified in *P. mirabilis and P. vulgaris*. Swarming behaviour of *P. mirabilis* is mediated by *rsbA* gene, which may function as a protein sensor of environmental conditions [48]. The *rsb*A gene is also responsible for biofilm formation and extracellular polysaccharide formation [48]. The *lux*S genes were detected in all the *Proteus* species. *luxS* genes production has been implicated in biofilm formation by members of the Enterobacteriaceae, including *Proteus* species [24].

Conclusions

The prevalence of *Protus* species in four poultry farms sampled in this study was 34.26 %. Predominance of product species in two farms was associated with uncontrolled movement of persons within the farm and unhygienic conditions of the environment. Hygienic practices are necessary to ensure low prevalence and spread of *Proteus* from the farms to the table. Despite the high level of susceptibility of Proteus species to the test antibiotics in this study, the level of antibiotics resistance exhibited by *Proteus* is a potential health risk to public health. To minimise the spread of antimicrobial

resistance arising from poultry farms, it is vital to regulate the use of antimicrobial agents as growth promoters for poultry birds. P. vulgaris contained a higher level of virulent genes *ureC*, *luxS* and *rsbA* while *P. mirabilis* only contained the *ureC* gene. The presence of Proteus species in poultry farms carrying virulence genes is indicative of potential health risk associated with food supply in the studies area. This data is indicative of increased need for monitoring and surveillance of food production industry including poultry farms to minimize possible transmission of pathogenic microorganisms via the food chain. These measures will enable the check of pathogenic bacteria, including *Proteus* from poultry farms.

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