American Journal of Biomedical and Life Sciences
2021; 9(4): 197-208
http://www.sciencepublishinggroup.com/j/ajbls
doi: 10.11648/j.ajbls.20210904.14
ISSN: 2330-8818 (Print); ISSN: 2330-880X (Online)

Prevalence and Antimicrobial Susceptibility Profiles of *Pasteurella multocida* in Village Chickens (*Gallus gallus domesticus*) in Maiduguri, Borno State, Nigeria

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To cite this article:
Jallailudeen Rabana Lawal, Amina Ibrahim, Muazu Ayuba, Umar Isa Ibrahim. Prevalence and Antimicrobial Susceptibility Profiles of *Pasteurella multocida* in Village Chickens (*Gallus gallus domesticus*) in Maiduguri, Borno State, Nigeria. *American Journal of Biomedical and Life Sciences*. Vol. 9, No. 4, 2021, pp. 197-208. doi: 10.11648/j.ajbls.20210904.14

Received: June 23, 2021; **Accepted**: July 10, 2021; **Published**: August 27, 2021

Abstract: *Pasteurella multocida* is a highly contagious bacterial pathogen that causes cholera in chickens and water fowls. From September 2019 to February 2021, 600 samples, consisting of tracheal and cloacal swabs (300 samples each), were obtained from 300 seemingly healthy village chickens from households and live bird markets to evaluate the prevalence of *P. multocida*, test for its antibiotic susceptibility profile and multiple drug resistance patterns. Trachea and cloacal swabs collected were cultured on sheep blood agar and MacConkey agar, isolation and identification was based on morphological characteristics. Prevalence was higher in chickens sampled from live birds' markets (27.0%) than those from households (16.3%), and higher in hens (13.3%) than in cocks (8.7%). Pure culture colonies were characterized using biochemical test and isolates identified by biochemical characterization were further subjected to Microbact GNB 24E test. Twenty three pure isolates of *P. multocida* were recovered, eighteen found in the trachea, and five in the cloaca, with an overall prevalence of 21.7%. Disk diffusion approach was used to assess in vitro susceptibility of isolates to 18 different antimicrobial agents. Isolates demonstrated multidrug-resistant to 15 of the antimicrobial compounds used. Antibiogram showed isolates to be extremely susceptible to ciprofloxacin, nitrofurantoin, and neomycin, and total resistant to erythromycin, amoxicillin/clavulinate, cefuroxime, ampicillin, enrofloxacin, tylosin, and furasol. Isolation of *Pasteurella multocida* from healthy village chickens, indicates they are carriers of the pathogen and that the bacterium has multidrug resistance. To control fowl cholera, it is also recommended that field veterinarians conduct sensitivity tests prior to administering antibiotics.

Keywords: Pasteurella Multocida, Antimicrobial Susceptibility, Antibiotic Resistance, Village Chickens

1. Introduction

Poultry production forms an integral part of many rural communities in developing countries of the world [2, 6]. Several studies carried out in many developing countries concerning the benefits derived from poultry productions have confirmed its significance to the livelihood of mankind [28, 30, 76]. It has been reported that poultry production provides comparatively greater financial profit and income in form of cash from the sales of live birds and eggs than cattle, small ruminants or pigs, alleviates poverty and where used as food (meat and eggs) it provides high quality animal protein which reduces malnutrition, improves nutritional status and serves as food security for the rural dwellers [76].

Village poultry production system is predominantly practiced by many poultry farmers in developing countries including Africa which usually involves mixed poultry species farming managed under the semi-intensive or extensive husbandry systems [27, 31]. These birds are allowed to scavenge freely around the vicinities of the communities for feed and other needs and are seldom provided with nutritious feed supplementation, housing or given special attention in terms of health care [2, 51]. Unfortunately, this practice yield low and poor quality poultry products as well as predispose the birds to various types of disease [5, 27]. Infectious diseases are considered as

one of the most important causes of economic loss in poultry industries in developing countries including Nigeria [24]. Bacterial diseases has been reported to be highly prevalence and considered a major threat to successful poultry production in Nigeria [29, 40]. Among the bacterial diseases, fowl cholera is a major threat to the poultry industry that hampers the profitable poultry production [52]. Fowl cholera is considered to be one of the most contagious and economically important bacterial diseases of poultry particularly chicken, turkeys, ducks and geese caused by *Pasteurella multocida* and remains an important havoc for the poultry production in developing countries [67]. *Pasteurella multocida* is a zoonotic bacterium that can infect a wide range of species, such as mammals and poultry [43, 53, 63, 75]. *Pasteurella multocida* is one of the members of Pasteurellaceae family which is a Gram-negative, non-motile, cocco-bacillus in shape, capsulated, non-spore forming bacterium occurring singly, in pairs or occasionally as chains or filaments belonging to the Pasteurellaceae family [4, 8, 17, 37]. *P. multocida* strains are classified into serogroups A, B, D, E and F based on capsular antigens, and further classified into 16 serotypes (1 to 16) primarily based on lipopolysaccharide antigens [38, 52]. Fowl cholera is caused by *P. multocida* type A:1, A:3 and type D in Asian countries [58]. Among the different serogroups, serotype A:1 strains causes 80% mortality, in contrast 20% mortality caused by type D strains of fowl cholera in chickens [46]. Pathogenicity or virulence of *P. multocida* is variable and complex, depending on the host species, strain, variation within the strain or host, and conditions of contact between two birds [59].

Fowl cholera may occur either as per-acute, acute or chronic forms, and the clinical signs vary depending on the form of the disease. However, *Pasteurella multocida* inhabits as commensal of the respiratory tract of many avian species and produces disease when the birds are under stress [32]. Symptoms of fowl cholera is characterized by mucous oral and nasal discharge, facial edema, blackening of comb and wattles, ataxia, back ward retraction of head, ruffled feathers, rise in temperature, off feed and water, diarrhea and an increased respiratory rate, dull, depression with high morbidity (up to 50%) [36]. Laying flocks are mostly affected by fowl cholerae because of their more susceptibility to the disease as compared with younger chickens [72].

Carrier birds play a major role in the transmission of fowl cholera [19, 50]. Also, research shows that transmission can occur by bird-to-bird contact via aerosolized bacteria as well as through ingestion of bacteria in contaminated environments [11].

Antibiotics are used to a large extent for the treatment of fowl cholera. However, prolong and pervasive use of antibiotics has resulted in *P. multocida* acquiring resistance to most of the commonly used antimicrobials [7]. Antibiotic resistance of *P. multocida* isolates varies according the host animal, specie, time, geographical origin and antimicrobial pre-treatment of the animal [16]. Multiple antibiotic resistances in pathogenic bacteria in food-producing animals and environmental sources are recognized as a global problem for public health [15, 74]. Despite the increase in antibiotic resistance found in *P. multocida* and the extensive use of multiple antibiotics for the treatment of fowl cholera in Nigeria, there is no information regarding the prevalence, antimicrobial susceptibility and multiple drug resistance pattern of the causative agent of this disease in Maiduguri, Borno State, Nigeria. The aim of this present study was to determine the prevalence of Pasteurellosis among seemingly healthy village chickens, isolate and identify *Pasteurella multocida* isolates, determine its antimicrobial susceptibility profile, and record its multiple antibiotic resistance pattern.

2. Materials and Methods

2.1. Study Area

The present study was conducted in Maiduguri, the capital and largest city of Borno State Nigeria (Figure 1). The study area lies within the semi-arid (Sahel savannah) zone of the North-Eastern part of Nigeria [1]. Maiduguri is located on latitude 11° 48^{1}N an d 11° 52^{1} N and Latitude 13° 02^{1} East and 13° 12^{1} East, at about 350 m (1161 ft) above sea level with an ambient temperature range of 32 to 45°C. The relative humidity is generally low throughout the state, ranging from as low as 13% in the driest months of February and March to the highest values of 70-80% in the rainy season months of July and August [73].

Figure 1. *Map of Borno State showing the studied area (asterisk). Source: Google map.*

2.2. Study Period

Following the consent of the village chicken owners throughout the study locations, sampling took place from September 2019 to February 2021. Sampling locations were visited on alternating days during the sampling period.

2.3. Sample Population

During the sample collection periods, tracheal and cloacal swabs were aseptically collected from apparently healthy village chickens from households and those brought for sales/dressing at major live bird markets within the study

area. Village chicken flock sizes ranged from 10 to 60 per household, with birds reared exclusively under a mixed poultry extensive management system, and village chickens sampled from live bird markets were guaranteed to have come from scavenging conditions.

2.4. Sample Size Determination

Since the exact prevalence of *Pasterella multocida* (Fowl cholera) infection in village chickens in the study area was unknown, the desired sample size for the study was calculated using the equation described by Thrusfield [70]. To maximize the sample size, it was assumed that the expected prevalence was 50%, absolute precision was 5% and the confidence interval level was set to be 95% as shown below:

$$\mathrm{n} = \frac{1.962 \times pq(1 - pexp)}{I_2}$$

Where, n=the required sample size, p=expected prevalence, q=1 – p; and I=absolute precision, that is the largest acceptable differences between the true and the estimated prevalence. As a result, 300 study populations were selected for the sampling area.

One hundred and fifty (150) village chickens were sampled from fifteen (15) households in three (3) study locations (at least 10 chickens from each household) within the study area to determine the carrier status and prevalence of Pasteurella multocida in apparently healthy village chickens in the study area, *viz:* Unimaid Staff quarters, Gwange and Fori wards. Information concerning type of management system used in the rearing of village chickens as well as the level of biosecurity around chickens' housing were observed and recored. More so, 150 village chickens were also sampled from three live birds' markets, *viz:* Monday market, Custom and Tashan Bama markets. The markets that were sampled were all located within the study area, and they typically accept live birds for slaughter and sales from households and communities. Age, which appears to influence outcomes in prevalence studies, was not included in this study due to challenges in gaining agreement for sample from chicken owners in the study area. The total sample collected was 600 because dual samples of tracheal and cloacal swabs were obtained from each sampled village chicken.

2.5. Transportation of Samples

Swab sticks were used to collect tracheal and cloacal swabs. Both swab samples were properly labelled and transported in transport containers to the Department of Veterinary Medicine Research Laboratory for culturing and microbiological processing, as per the Clinical and Laboratory Standard Institute's guidelines [20].

2.6. Isolation, Cultural and Identification of Pasteurella Multocida

P. multocida organisms were cultured according to the standard method described by Cowan [21]. Each swab sample was inoculated separately by streaking into different selective medium such as Sheep Blood agar (SBA), Nutrient agar (NA), MacConkey agar (Biomark) and Nutrient broth (NB) for screening of the samples. The inoculated media were incubated at 37°C for 24 hrs in an incubator (Royalcare England. DNP 9022A) for the appearance of characteristic colony. Based on the colony characteristics, subsequent selective subculture was done to obtain pure culture of *P. multocida*. The isolated pure culture was subjected for Gram staining and methylene blue-staining for cellular morphological identification of the bacteria.

All cultures showing Gram negative, with bipolar coccobacilli characteristics were cultured on MacConkey agar and incubated under the same condition as stated above. Isolates that do not grow on MacConkey after 48 hours of incubation were subjected to further analysis. Cultural and morphological examinations were conducted as described by Cowan and Steel [22]. *Pasteurella multocida* isolates were selected based on the cultural characteristics on blood agar. The appearance of a zone of erythrocyte lysis around or under bacterial colonies indicated hemolysis on sheep blood agar. The morphological appearance was also determined. Capsular and bipolar organisms were further confirmed as *P. multocida* by biochemical tests some of which are: motility, catalase, oxidase, H_2S production, nitrate, urease, indole, methyl red and citrate tests according to CLSI [20].

2.7. Biochemical Characterization

For biochemical characteristics, different tests such as Methyl red (MR), Indole production, catalase, oxidase, and sugar fermentation tests were done. *Pasteurella multocida* obtained from various samples were sub-cultured on specialized media and subjected to comprehensive phenotypic characterization. Presumptive isolates of *P. multocida* were further subjected to Gram reaction. Field isolates of the organism were identified on the basis of sugar fermentation reaction, such as maltose, D-mannitol, D-sorbitol, D-sucrose, D-glucose, D-xylose; and other specific biochemical tests like triple sugar iron agar slant (TSI), indole, catalase, oxidase, nitrate reduction, motility, ornithine decarboxylase and urease, according to CLSI [20]. NA slants were used to maintain stock culture. For the maintenance, the *P. multocida* bacteria were inoculated in slant by streaking and were incubated at 37°C overnight. Finally, sterile mineral oil was overlaid and kept the slant at room temperature for future use.

2.8. Microbac Test

All the twenty three *P. multocida* isolates identified by biochemical test were further subjected to Macrobact GNB 24E kit test, Oxoid®, United Kingdom, according to the manufacturer's instructions.

2.9. Antimicrobials Susceptibility Test

In vitro antimicrobials susceptibility testing of the

identified *Pasteurella multocida* isolates was performed using disc diffusion test (Oxoid, UK). The organisms were standardized using McFarland standard at the absorbance of 450nm. Each isolate was then inoculated onto Mueller-Hinton agar medium (Oxoid, UK), then 15 minutes later, the antimicrobial discs were applied. Twenty three (23) isolates of *P. multocida* isolates confirmed by biochemical and Macrobact test were tested for their susceptibility against the conventional antibiotic agents commonly used for the treatment of fowl cholera in Nigeria. The following antimicrobial agents were tested: Neomycin (NEO 10 µg), Ceftazidime (CAF 30 µg), Cefuroxime (CRX 30 µg), Gentamicin (GEN 10 µg), Ciprofloxacin (CPR 10 µg), Ofloxacin (OFL 10 µg), Trimetoprim/Sulfamethoxazole (Septrin) (TRI/SUL 30 µg), Chloramphenicol (CHL 30 µg), Nitrofurantoin (NIT 300 µg), Ampicillin (AMP 10 µg), Amoxicillin/Clavulinate (AUG 30 µg), Enrofloxacin (ENR 10µg), Streptomycin (STR 10 µg), Furasol (FUR 10 µg), Tylosin (TLY 10 µg), Oxytetracycline (Oxy 10 µg), Perfloxacin (PER 10 µg), and Rocephin (ROC 25 µg). The antibiogram of all the isolates was determined on Muller Hinton medium supplemented with 5% defibrinated sheep blood according to the disc diffusion method by Bauer *et al*. [9]. Following the application of antimicrobial discs, the plates were incubated at 37 °C for 24 h in an incubator (Royalcare England, DNP 9022A). The turbidity of each isolate in the homogenous suspension was measured in a Nephelometer to get a 0.5 Mac Faland standard which correspond to 1 x 107 colony forming unit. Each isolate, consisting of a 24 h-old culture was spread evenly on plates. The culture was allowed to absorb onto the plate for about 10 min. Subsequently, each antimicrobial disc was picked with a sterile forcep and placed on the plate containing the medium at an appropriate distance from each other. The plates were later incubated at 37°C for 24 h. The resistance profile of *P. multocida* was assessed as described by Shivachandra *et al*. [66]. Isolate resistant to at least three different classes of antibiotic was classified as multidrug resistant. The diameter of the zone of inhibition of each antibiotic was measured (millimetres) and matched with respective internationally accepted standard zone diameter to interpret the test culture as resistant, intermediate or sensitive according to the procedure of Quinn e*t al*. [57].

2.10. Data Analysis

Data generated were entered into Microsoft office Excel spread sheet, Risk Ratios (RR) and 95% CI on the Relative Risk (RR) were calculated using the Chi-Square or Fisher's exact test to determine strength and significance of associations between the sexes of chickens and infection as well as location of sample collection and infection of chickens with *Pasteurella multocida*. The prevalence of *Pasteurella multocida* among the sampled population was calculated using frequencies and percentages in GraphPad prism® version 5.01 for windows (GraphPad Software, Inc., San Diego, California, USA) computer based program. The observed prevalence and 95% confidence intervals (CI) were evaluated and "*P"* values equals to or less than 0.05 were regarded significant.

3. Results

The diagnosis of *Pasteurella multocida* in tracheal and cloacal swab samples collected from both sexes of village chickens (*Galllus gallus domesticus*) from households that rears large population of village chickens mixed with other village poultry species and from live birds markets within Maiduguri were based on the microscopic / phenotypic characteristics exhibited by the colonies on nutrient agar plates and their biochemical reactions. Three hundred (300) apparently healthy village chickens were sampled from which a total of six hundred (600) swabs samples were collected comprising 300 tracheal swabs and 300 cloacal swabs samples. Out of the total swabs samples collected, *Pasteurella multocida* was isolated and identified in pure culture from 65/300 infected chickens with an overall carrier status and isolation rate of 21.7% (17.4% – 26.7% at 95% Confidence Interval) as shown in Table 1.

Table 1. *Prevalence and Carrier Status of Pasteurella multocida Carrier Status in Village Chickens (Gallus gallus domesticus) in Maiduguri, Borno State, Nigeria.*

Number of chicken examined	Number of chickens infected	Prevalence (%)	95% CI LL – UL
300	65	21.7	17.4 – 26.7

Key: CI=Confidence Interval; LL – UL=Lower limit – Upper limit

The isolation of *Pasteurella multocida* from swab samples collected from village chickens based on study location of sample collections revealed high carrier status of the bacterium in sample collected from live birds' markets 81/300 (27.0%) compared to samples collected from households 49/300 (16.3%) with prevalent rates of 27.0% (22.3% – 32.3% at 95% Confidence Interval) and 16.3% (12.6% – 20.9% at 95% Confidence Interval) respectively. The result of the carrier status of *Pasteurella multocida* isolation from samples collected from live birds markets shows higher prevalence of the bacterium isolate in samples collected from Monday market 42/100 (42.0%) compared to Customs market 24/100 (24.0%) and Tashan Bama market 15/100 (5.0%) at a prevalence rates of 14.0% (10.5% – 18.4% at 95% Confidence Interval), 8% (5.4% – 11.6% at 95% Confidence Interval) and 5.0% (3.1% – 8.1% at 95% Confidence Interval) respectively. However, considering samples collected from households, swab samples collected from village chickens in Gwange ward 25/100 (25.0%) had the highest carrier status frequency of *Pasteurella multocida*

followed by village chickens from UniMaid staff quarters 14/100 (14.0%) and Fori ward 10/100 (10.0%) at a prevalence rates of 8.3% (5.7% – 12.0% at 95% Confidence Interval), 4.7% (2.8% – 7.7% at 95% Confidence Interval) and 3.3% (1.8% – 6.0% at 95% Confidence Interval) respectively as shown in Table 2.

Table 2. *Prevalence and Carrier Status of Pasteurella multocida in village chickens (Gallus gallus domesticus) according to sampling locations in Maiduguri Borno State, Nigeria.*

Sample location	Study location	No. of sample collected	No. of sample affected (%)	Prevalence %	95% CI LL – UL
Live birds market	Monday Market	100	42 (42.0)	14.0	10.5 – 18.4
	Custom Market	100	24 (24.0)	8.0	5.4 – 11.6
	Tashan Bama Market	100	15 (15.0)	5.0	3.1 – 8.1
Total		300	81 (27.0)	27.0	22.3 – 32.3
Household	Gwange Ward	100	25 (25.0)	8.3	5.7 – 12.0
	Unimaid Staff Quarters	100	14 (14.0)	4.7	2.8 – 7.7
	Fori Ward	100	10 (10.0)	3.3	1.8 – 6.0
Total		300	49 (16.3)	16.3	12.6 – 20.9

Key: CI=Confidence Interval; LL – UL=Lower limit – Upper limit

The prevalence and carrier status of *Pasteurella multocida* in village chickens (*Gallus gallus domesticus*) according to the type of samples collected revealed higher frequency of the bacterium in the tracheal swabs tested 51/300 (17.0%) compared to the cloacal swabs 14/300 (4.7%) at a prevalence rate of 17.0% (14.2% – 20.2% at 95% Confidence Interval) and 4.7% (3.3 – 6.7% at 95% Confidence Interval) respectively. Moreover, there was statistical significant difference (p–value < 0.0001 at 95% confidence interval; χ^2=52.331 and Relative risk=3.643) between the isolation of the bacterium in the tracheal swabs and the cloacal swabs. A total of 23 isolate of *Pasteurella multocida* were obtained from 300 village chickens examined during the present research, which represent an isolation rate of 3.8%. Of the 23 isolates, 18 were obtained from the trachea and 5 from the cloaca. Hence, the percentage isolation of *Pasteurella multocida* significantly (p< 0.0001) higher from the trachea (6.0%) isolates from 300 swab samples tested compared to the claoca (1.7%) isolates from 300 claocal swabs samples tested as shown in Table 3.

Table 3. *Prevalence of Pasteurella multocida Carrier Status of in village chickens (Gallus gallus domesticus) according to type of samples collected in Maiduguri Borno State, Nigeria.*

Number of sample collected	Type of sample collected	Number (%) of samples infected	Number (%) of *Pasteurella multocida* Isolates (N=300)	Prevalence (%)	95% CI LL – UL	χ^2	*P*-value	RR
300	Tracheal swabs	51 (17.0)	18 (6.0)	17.0[a]	14.2 – 20.2	52.331	<0.0001	3.643
300	Cloacal swabs	14 (4.7)	5 (1.7)	4.7[b]	3.3 – 6.7			
600	Overall	65 (10.8)	23 (3.8)	21.7	18.6 – 25.1			

Key: N=Number of chickens sampled; CI=Confidence Interval; LL – UL=Lower limit – Upper limit; χ^2=Chi-square; RR=Relative Risk

[a,b]Different superscripts indicate significant ($p < 0.05$) difference in prevalence

The carrier status and prevalence of *Pasteurella multocida* isolated from village chickens according to sex shows higher prevalence of the bacterium in the Hen (females) chickens 39/150 (26.0%) sampled compared to the Cocks (males) 26/150 (17.3%) at a prevalence rate of 13.0% (9.7% – 17.3% at 95% confidence interval) and 8.7% (6.0% – 12.4% at 95% confidence interval) respectively. There was no statistical significant difference (p=0.0926; χ^2=2.828; and Relative risk=0.6667) between the prevalence rate of *Pasteurella multocida* isolated from samples collected from the cocks and hens of the chickens as shown in Table 4.

Table 4. *Prevalence of Pasteurella multocida according to sex of village chicken (Gallus gallus domesticus) in Maiduguri Borno State, Nigeria.*

Risk factors	Variable	Number of chickens examined	Number (%) of chickens affected	Prevalence (%)	95% CI LL – UL	χ^2	*P*-value	RR
Sex	Cocks	150	26 (17.3)	8.7[a]	6.0 – 12.4	2.828	0.0926	0.6667
	Hen	150	39 (26.0)	13.0[a]	9.7 – 17.3			
Total		300	65 (21.7)	21.7	17.4 – 26.7			

Key: CI=Confidence Interval; LL – UL=Lower limit – Upper limit; χ^2=Chi-Square; RR=Relative Risk

[a,b]Different superscripts indicate significant ($p < 0.05$) difference in prevalence

The result of distribution of *Pasteurella multocida* isolates from village chickens in pure culture based on their hemolytic or non-hemolytic characteristics on sheep blood agar revealed that all the 18 isolates from the tracheal swabs and 5 from cloacal swabs were non-hemolytic bacterium as shown in Table 5.

Table 5. *Haemolytic characteristics of Pasteurella multocida isolates from village chickens (Gallus gallus domesticus) on Sheep blood agar.*

Type of sample collected	No. of *Pasteurella multocida* isolates tested	Type of *Pasteurella multocida* isolated	
		Non-haemolytic (%)	Haemolytic (%)
Tracheal swabs	18	18 (100.0)	0 (0.0)
Cloacal swabs	5	5 (100.0)	0 (0.0)
Total	23	23 (100.0)	0 (0.0)

Table 6. *Biochemical identification for Pasteurella multocida isolated from Village Chicken (Gallus gallus domesticus) in Maiduguri Borno State, Nigeria.*

Biochemical test	Number of isolates tested	Number of isolates positive
Oxidase	23	23
Citrate	23	23
Nitrate	23	23
Xylose	23	23
Inositol	23	0
Maltose	23	0
Urease	23	0
Indole	23	23
Catalase	23	23
Salicin	23	0
Raffinose	23	0
Galactose	23	23
D – glucose	23	23
Fructose	23	23
D – mannitol	23	23
Sucrose	23	23
Sorbitol	23	23

The result of the biochemical test for *Pasteurella multocida* isolates in pure culture from village chickens revealed that the 23 isolates shows positive reactions to test with Catalase, Oxidase, Citrate, Nitrate, Xylose and Indole, however demonstrate negative reactions to Inositol, Urease and Salicin. Moreover, the 23 isolates ferment Galactose, D-glucose, Fructose, D-mannitol, Sucrose and Sorbitol, however, the isolates shows negative reactions to Maltose and Raffinose as shown in Table 6.

The result of the antimicrobial sensitivity test shows that *Pasteurella multocida* isolates from this study has high susceptibility to Ciprofloxacin, Nitrofurantoin and Neomycin, moderate susceptibility to Gentamicin, Streptomycin, Chloramphenicol, Ofloxacin and Trimetoprim / Sulfamethoxazole (Septrin), moreover, the isolates has shown fair susceptibility to Perfloxacin, and Oxytetracycline. However, *Pasteurella multocida* isolates from this study has shown completely resistant to Erythromycin, Amoxicillin/Clavulinate, Cefuroxime, Ampicillin, Enrofloxacin, Tylosin and Furasol as shown in Table 7.

Table 7. *Antimicrobial susceptibility test for Pasteurella multocida isolated from Village Chicken (Gallus gallus domesticus) in Maiduguri Borno State, Nigeria*

Antibiotics	Degree of Antimicrobial susceptibility of isolates			
	1	2	3	4
Gentamicin (GEN 10 µg)		++		
Ciprofloxacin (CIP 10 µg)	+++			
Erythromycin (ERY 10 µg)				-ve
Streptomycin (STR 30 µg)		++		
Amoxicillin/Clavulinate (AMO/CLA 30 µg)				-ve
Cefuroxime (CEF 30 µg)				-ve
Neomycin (NEO 10 µg)	+++			
Perfloxacin (PER 10 µg)			+	
Chloramphenicol (CHL 30 µg)		++		
Ofloxacin (OFL 10 µg)		++		
Nitrofurantoin (NIT 300 µg)	+++			
Trimetoprim/Sulfamethoxazole (Septrin) (TRI/SUL 30 µg)		++		
Ampicillin (AMP 10 µg)				-ve
Enrofloxacin (ENR 10µg)				-ve
Tylosin (TLY 10 µg)				-ve
Oxytetracycline (OXY 10 µg)			+	
Furasol (FUR 10 µg)				-ve

Key: +++=Highly susceptible; ++=Moderately susceptible; +=Fairly susceptible; -ve=Completely resistant

Table 8 shows multi-drug resistance profile of 23 *Pasteurella multocida* isolates from village Chicken tested against 17 antimicrobial agents. Two (2) isolates showed resistance to eleven (11) of the antimicrobial compounds, three (3) isolates showed resistance to ten (10) of the antimicrobial compounds, three (3) isolates showed resistance to nine (9) of the antimicrobial compounds, four (4) isolates showed resistance to eight (8) of the antimicrobial compounds, five (5) isolates showed resistance to seven (7) of the antimicrobial compound, two (2) isolates showed resistance to six (6) of the antimicrobial compound, three (3) isolates showed resistance to five (5) of the antimicrobial compound, and one (1) isolate showed resistance to four (4) of the antimicrobial compounds.

Table 8. *Multi-drug resistance of profile of 23 Pasteurella multocida isolates from Village Chicken (Gallus gallus domesticus) in Maiduguri Borno State, Nigeria tested against 18 antimicrobial agents.*

S/No	*Pasteurella multocida* isolates	Antibiogram (resistant drugs)	Total number of resisted drugs
1.		ERY, AMP, CEF, AMO/CLA, TYL, ENR, FUR	7
2.		ERY, AMO/CLA, ENR, TYL, FUR, OXY, CHL, TRI/SUL, GEN, OFL, CEF	11
3.		AMP, AMO/CLA, TYL, OXY, ERY, TYL, ENR,	7
4.		CEF, PER, AMP, FUR, AMO/CLA, TYL, OXY, FUR, ERY, NIT	10
5.		AMP, CEF, TYL, ENR, CHL, ERY, FUR,	7
6.		ENR, AMP, AMO/CLA, TYL, FUR, OXY, ERY, CEF	8
7.		ERY, CEF, TYL, NIT, ENR, FUR	6
8.		CHL, TRI/SUL, ENR, AMP, TYL, ERY	6
9.		ENR, AMP, AMO/CLA, TYL, GEN, OXY, ERY, FUR, CEF	9
10.		CHL, TRI/SUL, ENR, FUR, AMP, AMO/CLA, TYL, NIT, CEF, ERY, OFL	11
11.		FUR, ENR, AMP, AMO/CLA, TYL, OXY, ERY, CEF	8
12.		AMO/CLA, ENR, PER, FUR, ERY	5
13.		ENR, CEF, AMP, FUR, AMO/CLA, TYL, OXY, ERY	8
14.		ENR, AMP, AMO/CLA, ERY,	4
15.		TRI/SUL, ENR, PER, AMP, FUR, AMO/CLA, ERY	7
16.		CHL, AMP, AMO/CLA, TYL, ENR, ERY, CEF	7
17.		TRI/SUL, ENR, AMP, FUR, AMO/CLA, TYL, GEN, OXY, ERY, CEF	10
18.		CHL, TRI/SUL, ENR, PER, AMO/CLA, TYL, GEN, OXY, ERY	9
19.		TRI/SUL, ENR, PER, AMP, FUR, TYL, OXY, ERY, NIT, CEF	10
20.		CEF, ENR, AMP, TYL, ERY	5
21.		PER, AMP, FUR, AMO/CLA, ENR, TYL, ERY, OFL, CEF	9
22.		ENR, AMP, AMO/CLA, FUR, ERY,	5
23.		ENR, CEF, AMP, TYL, FUR, OXY, ERY, OFL	8

KEY: CHL: Chloramphenicol; TRI/SUL: Trimethoprim/Sulfamethoxazole; ENR: Enrofloxacin; PER: Perfloxacin; CIP: Ciprofloxacin; DOX: Doxycycline; AMP: Ampicillin; FUR: Furasol; AMO/CLA: Amoxicillin/Clavulanate; TYL: Tylosin; GEN: Gentamicin; NEO: Neomycin; OXY: Oxytetracycline; STR: Streptomycin; ERY: Erythromycin; OFL: Ofloxacin; NIT: Nitrofurantoin; CEF: Cefuroxime

4. Discussion

Pasteurella multocida is a zoonotic bacterium that can infect a wide range of species, such as mammals and poultry. *Pasteurella multocida* type A is the etiologic agent of fowl cholera, a highly contagious and fatal disease of chickens [53]. The present study relied on diagnosis of the carrier status of *Pasteurella multocida* were based on the phenotypic characteristics exhibited by the colonies on nutrient agar plates and their biochemical reactions. This study has confirmed that apparently healthy village chickens reared under scavenging management system with other village poultry species, which is the most common husbandry system for the village chickens in many developing countries in Africa including Nigeria, may be healthy carriers of *Pasteurella multocida* in the study area. This finding is consistence with Moemen *et al*. [46] and Muhairwa *et al*. [48] who has also previously reported the occurrence of *P. multocida* in healthy village chickens after conducted systematic investigation. The isolates obtained in the present study have demonstrated biochemical characteristics that were consistent with those of as *P. multocida* subsp. *Multocida* and *P. multocida* subsp. *gallicida*. Contrary to this, Muhairwa *et al*. [47] reported an addition *P. multocida* subsp. *septica* from healthy chickens. The overall prevalence rate of 21.7% recorded in this research is considered apparently high among scavenging physically healthy village chicken flocks in the study area. This prevalence rate was found to be higher than 13.04% and 11.42% reported by Hossain *et al.* [35] and Panna *et al*. [53] respectively but however lower than 59.72% reported by Belal [10] in backyard poultry. The variation in the prevalence rate of *P. multocida* reported from various study might be associated to the number of samples tested, method employed in isolation, differences in age and breeds of the chickens as well as difference in management and husbandry system of chickens sampled. The present research was conducted to determine the carrier status of the bacterium in apparently healthy village chicken flocks under mixed poultry production system. The bacterium have been previously isolated from apparently healthy chicken, ducks [39, 44, 53], clinically sick chickens [3, 23, 36, 38, 39, 71], as well as sheep, swine cattle, cats and dogs [18, 13, 19, 62]. Previous studies has shown higher prevalence of *Pasteurella multocida* in apparently healthy scavenging village chickens compared to broilers and layers of the exotic breed [33, 36, 44, 71]. Isolation of *Pasteurella multocida* from apparently healthy village chickens may pose a serious threat of transmission of infection to commercial poultry farms especially small scale in the study area.

From the result of the present study, *Pasteurella multocida* was more frequently isolated from swab samples collected from village chickens at live birds' markets 27.0% compared to chickens sampled from households 16.3% in the study area. This finding may not be unexpected, because there are no discriminations of health status or screening for diseases before mixing of different poultry species in live birds markets. This finding and observation of the present research buttress previous report of Persson and Bojesen [56] who have also reported high prevalent rate of emerging bacterial diseases in free range chickens in a similar study and have attributed it to poor bio-security. Moreover, the stress of

transportation and handling of birds in live birds' markets may further suppress the immunity of the birds to succumb to the bacterium infection.

In the present research, *Pasteurella multocida* were recovered from both the tracheal and cloacal swabs, but more frequently from the tracheal swabs. This finding supports the report of Dashe *et al.* [23], Laban *et al*. [39], Lee *et al*. [41], Mbuthia *et al*. [44] and Victor *et al*. [71]. The result of the present research work is also in agreement with other researchers who have reported more frequent isolation of bacteria from the oral cavity compared to the cloacal orifice, and has associated their findings to the fact that the bacteria are common part of the normal flora of the upper respiratory tract [12, 49, 54, 55, 68]. The bacterium has also been isolated predominantly from of organs of the respiratory system such as the lungs and trachea [36, 39, 45].

The finding of the present research showed that the carrier status and prevalence of *Pasteurella multocida* isolated from village chickens according to sex was higher in the hen (females) chickens sampled compared to the cocks (males). Although, there was no statistical significant difference between the prevalence rates of *Pasteurella multocida* isolated from the cocks and hens of the chickens. This might be attributed to the fact that the female chickens are usually reared for breeding and kept for longer period of time while the males are usually sold out for meat and sacrifices. However, the none significant difference between the prevalence rates of the bacterium and sexes of chickens on the other hand signifies that both sexes of chickens shares equal chance of getting infected when exposed to the bacterium. The finding of the present research supports the result of Laban *et al*. [39], who have reported higher prevalence of the bacterium in laying birds in a similar study.

The result of the present research shows that all the *Pasteurella multocida* isolates from village chickens in pure culture were found to be non-hemolytic on sheep blood agar and neither did it grow on McConkey's agar nor Eosine methylene blue agar. These findings were in agreement with several other studies which reported that isolates of *Pasteurella multocida* were non-hemolytic on blood agar and do not grow on Eosine methylene blue agar, Salmonella-shigella agar and McConkey's agar [4, 8, 37, 39, 43, 53, 65]. This indicated that the non-hemolytic *Pasteurella multocida* is the most naturally abundant strain of the bacterium among scavenging village chickens in the study area. In the present research, the selected isolates of *Pasteurella multocida* were found to grow well in sheep blood agar media, as described by Mbuthia *et al*. [44] and Panna *et al*. [53]. All the isolated organism in this study were found to be gram-negative cocco-bacillary shape in Gram staining method, and bipolar characteristics in Leishman's staining method, which was consistent with the findings of Akhtar [4], Ashraf *et al.* [8], Ievy *et al.* [37], and Panna *et al*. [53]. All the organisms were found to be non-motile when examined under microscope by hanging drop technique which supports the finding of Laban *et al*. [39] and Panna *et al*. [53].

The finding of the present research shows that isolates of *Pasteurella multocida* isolated in pure culture from village chickens shows positive reactions to biochemical test with catalase by producing bubbles, oxidase by changing its color which agrees with findings of Ievy *et al*. [37] and Panna *et al*. [53]. The isolates also shows positive reactions to biochemical test with citrate, nitrate, xylose and indole, however demonstrate negative reactions to inositol, urease and salicin. Moreover, the isolates of *Pasteurella multocida* isolated in this present research ferment galactose, D-glucose, Fructose, D-mannitol, Sucrose and Sorbitol, however, the isolates shows negative reactions to maltose and raffinose. These fermented sugars producing acid without gas formation. These findings of biochemical reactions were consistent with the findings of Akhtar [4], Ashraf *et al.* [8], Christensen *et al.* [17], Ievy *et al.* [37], Manasa [43], Panna *et al*. [53], Shivachandra *et al.* [64], Tabatabai [69] for *Pasteurella multocida*.

The antimicrobial sensitivity of the *Pasteurella multocida* isolates in this present research showed the bacterium to be high susceptible to ciprofloxacin, nitrofurantoin and neomycin, moderate susceptible to gentamicin, streptomycin, chloramphenicol, ofloxacin and trimetoprim / sulfamethoxazole (septrin), moreover, the isolates has shown fair susceptibility to perfloxacin, oxytetracycline and doxycycline. However, *Pasteurella multocida* isolates from this present research has shown completely resistant to erythromycin, amoxicillin/clavulinate, cefuroxime, ampicillin, enrofloxacin, tylosin and furasol, which has highlighted that prophylactic and therapeutic effect of these compound on avian *Pasteurella multocida* strains in the present study area should no longer be expected from these antibiotics. Moreover, if the multi-drug resistance observed in this study continues persistent, soon there will be no effective antibiotics against fowl cholera. These findings are closely associated and comparable to results reported from previous similar studies [7, 23, 25, 34, 36, 46, 66, 71]. Antibiotics are the most used veterinary products for the management of *P. multocida* infections in animals. The main antibiotic classes approved for treatment of respiratory diseases include first-generation antibiotics, but also critically important antibiotics such as fluoroquinolones [26], which are also used in humans. The finding of the present research has indicated that ciprofloxacin, nitrofurantoin and neomycin may provide a more promising and reliable therapy for fowl cholera in the study area. The antibiogram profiles obtained in the present research indicated that variable patterns of multidrug resistance existed among field isolates of *P. multocida* from apparently healthy village chickens in Maiduguri, Nigeria. The multidrug resistance of *P. multocida* is presumably attributed to the use of antibiotics as additives in poultry feed, extensive and pervasive use of antimicrobial agents by poultry farmers and Veterinary practitioners which is in agreement with Dashe *et al*. [23] and Victor *et al*. [71]. Arora *et al*. [7] also recorded that injudicious use of antibiotics in poultry has contributed remarkably in the resistance of *P. multocida.* Another possible reason for the multiple resistance of *P. multocida* could be attributed to the proliferation of fake or sub-standard drug in Nigeria. The

reason for multidrug antimicrobial resistance in village chickens maybe alarming, since these classes of birds are not routinely treated with conventional antibiotics as it is been done in commercial or exotic poultry farming business. However, antimicrobial resistance in *Pasteurella multocida* has been linked to conjugative small plasmids referred to as the R-plasmid which is commonly responsible for interspecies spread of multidrug resistance [25]. The coexistence and spread of these small plasmids has resulted in *P. multocida* isolates that are multidrug resistant [42, 60]. This might probably suggests that other avian bacterial pathogens could be resistant to so many classes of antibiotics in Maiduguri, Nigeria. Similar reports about the emergence of multidrug resistant strains of *P. multocida* among different isolates have been documented by Arora *et al*. [7], Dashe *et al*. [23], Shivachandra *et al*. [66], Victor *et al*. [71], and Zahoor and Siddique [77].

The finding of the present research revealed that all twenty three *Pasteurella multocida* isolates showed multiple resistances to eleven classes of the antimicrobial compounds tested. This finding consistent with similar researches conducted by Dashe *et al*. [23], Laban *et al*. [39] and Victor *et al*. [71] which have also reported episode of multiple antimicrobial resistance from *Pasteurella multocida* isolates in their various studies.

5. Conclusion

Isolation and identification of *Pasteurella multocida* was successfully done from tracheal and cloacal swabs from naturally infected village chickens which indicated that they are natural carriers of the pathogen. This showed that the bacterium is prevalent among the apparently healthy village chickens that are reared amongst other village poultry species in the study area and carrier status was found to be at a prevalence rate of 21.7%. The occurrence of the bacterium in swabs samples collected from apparently healthy village chickens is attributed to natural infection probably from contaminated environment since there was no previous report of serious outbreak of disease caused by the bacterium in the study area. Twenty three isolates of *P. multocida* were isolated from apparently healthy village chickens. The unhygienic scavenging nature of village chickens might be considered as the most predisposing factor of the diseases transmission and infection among extensively reared village chickens. Also the indiscriminate mixing of several poultry species in live birds local markets might also contribute to the horizontal transmission of the organism. The in-vitro antimicrobial susceptibility of the isolated bacterium has demonstrated multidrug resistance, but high susceptible to ciprofloxacin, nitrofurantoin and neomycin, this suggested that the bacterium can be most effective treated with this antimicrobial chemotherapy.

6. Recommendation

1. Village chickens are natural carriers of *Pasteurella multocida,* therefore, commercial poultry farmers should be educated about the economy significance of the disease.
2. The isolation and identification of *Pasteurella multocida* should be attempted in other village poultry species in the study area or in uncertain clinical disease of village chickens.
3. To control disease transmission to susceptible birds, strict biosecurity measures should be observed in all levels of poultry production systems.
4. Molecular researches involving genotypic characterization of *Pasteurella multocida* in several poultry species and other geographical location should be conducted in Northern Nigeria.

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