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Research paper

Prevalence and antibiotic resistance of *Pasteurella multocida* isolated from chicken in Ado-Ekiti metropolis

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Abstract

Pasteurella multocida is a poultry bacterial pathogen causing fowl cholera in chicken. The prevalence and antibiotic susceptibility of *P. multocida* isolates from freshly dead chicken were determined. Ninety seven (97) freshly dead chicken from 23 different farms were analyzed for the presence of *P. multocida*. Swabs of the trachea and the liver of the necropsied chicken were activated on buffered peptone water and later cultured on blood agar and MacConkey agar. Pure culture of organisms were subjected to cultural and biochemical characterization. In vitro susceptibility of the pure isolates of *P. multocida* against 12 antimicrobial agents was determined using disk diffusion method. Twelve isolates of *P. multocida* were recovered from the chicken, with a prevalence of 12.4%. Nine of the isolates were recovered from the trachea and three from the liver. All the 12 isolates recovered from the birds were multi-resistant to the antibiotics used in this research. The antibiogram showed that all the isolates resisted ampicillin, amoxicillin/clavulinate, doxycycline and tylosine. Nitrofuratoin and gentamycin had the best antimicrobial activity with 25% and 50% resistance respectively. The resistance of other antibiotics are: Ofloxacin 75%, Ciprofloxacin 83.3%, Enrofloxacin 75%, Furasol 66.7%, Ceftazidime 91.7% and Cefuroxime 66.7%. This result showed that there is an emergence of multi- resistance in *P. multocida,* therefore it is important to carry out sensitivity test before administration of antibiotics in order to control fowl cholera.

Keywords: *Antibiotics; Fowl Cholera; Pasteurella multocida; Resistance.*

1. Introduction

Fowl cholera is a highly contagious disease caused by *Pasteurella multocida*. This organism affects a broad host range of birds both domestic and wild birds. It causes high mortality that incur significant economic losses in commercial and backyard poultry production [15]. It spreads by contamination of feed or water, by oral or nasal discharges from infected birds. Migratory birds have been reported as a major source of fowl cholera [8].

Although, vaccines are given to birds against fowl cholera, yet fowl cholera has remain one of the main causes of loss in poultry [12]. *P. multocida* is also responsible for atrophic rhinitis in swine, snuffles in rabbit, septicaemia haemorhagica ovis in goat, pneumonia in cattle and haemorhagic septicaemia in cattle and buffalo, showing that *P. multocida* is not host specific [1], [18]. 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 [2].

Antibiotic resistance of *P. multocida* isolates varies according to the host animal, specie, time, geographical origin and antimicrobial pre-treatment of the animal [7]. Multi-resistance pathogenic bacteria in food-producing animals and environmental sources is recognized as a global problem for public health [6], [21]. Multiple antibiotics are often recommended for the treatment of fowl cholera in Nigeria [10]. However, there is little information about multiple drug resistance of *P. multocida* as well as the prevalence of the pathogen in poultry; therefore this research is to document the results of multi-resistance *P. multocida* in Ekiti States, South Western Nigeria.

2. Methodology

Samples were collected from twenty three farms between January and June 2015, transported to the microbiology laboratory within two hours. The samples (ninety seven freshly dead chicken) were necropsied, swabs were collected aseptically from the trachea and the liver for bacteria isolation.

2.1. Bacteriology

The swabs collected from both the liver and the trachea were activated in buffered peptone water for 5 hours at 37^{o}C. A loop full of the activated organisms in the buffered peptone water were inoculated onto MacConkey agar (Biomark) and sheep blood agar by streaking. The plates were incubated at 37°C for 24 hours in an incubator (Royalcare England. DNP 9022A). The appearance of a zone of erythrocyte lysis around or under bacterial colonies indicated hemolysis on sheep blood agar.

2.2. Cultural and biochemical characterization

Pasteurella multocida isolates were selected based on the cultural characteristics on blood agar. The morphological appearance was also determined. Further confirmation was done by biochemical

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tests some of which are: motility, catalase, oxidase, H_2S production, nitrate, urease, indole, methyl red, Voges-Proskauer and citrate use tests [9].

2.3. Antimicrobial drug sensitivity test

In vitro susceptibility of the identified *Pasteurella multocida* isolates against antimicrobial agents was determined by the standard disk diffusion procedure. The organisms were standardized using McFarland standard at the absorbance of 450nm. The samples were inoculated on Muller-Hinton agar. The following antimicrobial agents were tested: Ceftazidime (CAZ 30 µg), Cefuroxime (CRX 30 µg), Gentamicin (GEN 10 µg), Ciprofloxacin (CPR 5 µg), Ofloxacin (OFL 5 µg), Nitrofurantoin (NIT 300 µg), Ampicillin (AMP 10 µg), Amoxicillin/Clavulinate (AUG 30 µg), Enrofloxacin (ENR10µg), Furasol (FUR 10 µg), Tylosin (TLY 10 µg) and Doxycycline (DOX10 µg). Following the application of antimicrobial discs, the plates were incubated at 37 ℃ for 24 h in an incubator (Royalcare England. DNP 9022A). The diameters of the zones of inhibition were measured (millimetres) and were compared to internationally accepted standard to determine the susceptibility or resistance of the isolate [17].

3. Results

Pasteurella multocida were detected in 12 of the 97 cases investigated with a prevalence of 12.4%. Extended phenotypic and biochemical characterization confirmed the isolates as *P. multocida*. The *P. multocida* isolates produced small, round, grayish, smooth, mucoid glistening and dewdrop-like colonies on blood agar plates and were Gram-negative coccobacilli. The strains did not grow on McConkey agar and were non-haemolytic on blood agar. Biochemical testing showed that all strains were urease negative, oxidase, citrate, indole and catalase positive. All the strains fermented galactose, fructose, D-glucose, D-mannitol and sucrose, while no reaction was recorded for inositol, raffinose and salicin.
The observation made on the samples revealed that most of the *P. multocida* isolated from the chicken were resistant to most group of antibiotic as shown in table 1.

Table 1: Percentage (%) Antibiotic Resistant of *Pasteurella multocida* Isolated from Chicken Trachea and Liver.

Isolates	AMP	AUG	OFL	TLY	CPR	ENR	DOX	FUR	GEN	NIT	CAZ	CRX
Trachea	100	100	66.7	100	88.9	77.8	100	66.7	33.3	22.2	88.9	55.6
n=9	(9)	(9)	(6)	(9)	(8)	(7)	(9)	(6)	(3)	(2)	(8)	(5)
Liver	100	100	100	100	66.7	66.7	100	66.7	100	33.3	100	100
n=3	(3)	(3)	(3)	(3)	(2)	(2)	(3)	(2)	(3)	(1)	(3)	(3)
Total	100	100	75	100	83.3	75	100	66.7	50	25	91.7	66.7
n=12	(12)	(12)	(9)	(12)	(10)	(9)	(12)	(8)	(6)	(3)	(11)	(8)

Key: Ampicillin (AMP), Amoxicillin/Clavulinate (AUG), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CPR), Enrofloxacin (ENR), Doxycycline (DOX), Furasol (FUR), Gentamicin (GEN), Nitrofurantoin (NIT), Ceftazidime (CAZ) and Cefuroxime (CRX).

Nitrofurantoin was found to be the most effective antibiotic with a resistance of (3) 25%, while the organism form (12) 100% resistance to ampicillin, amoxicillin/clavulinate, doxycycline and tylosine. Table 2 showed the antibiotic resistance pattern of the *P. multocida* isolates.

Table 2: Antibiotic Resistance Pattern of *P. Multocida* Isolated from Chicken Trachea and Liver.

Antibiotics	No of isolates	Percentage resistance (%)
AMP, AUG, OFL, TLY, CPR, ENR, DOX, FUR	4	33.3
ENR, DOX, FUR, TYL	7	58.3
AMP, AUG, CRX, CAZ	8	66.7
GEN, CRX, NIT, CAZ	1	8.3
TYL, AMP, DOX, AUG	12	100
CAZ, CRX, GEN	5	41.7
FUR, ENR, CPR	6	50
NIT, OFL	2	16.7

Key: Ampicillin (AMP), Amoxicillin/Clavulinate (AUG), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CPR), Enrofloxacin (ENR), Doxycycline (DOX), Furasol (FUR), Gentamicin (GEN), Nitrofurantoin (NIT), Ceftazidime (CAZ) and Cefuroxime (CRX).

4. Discussion

The occurrence of fowl cholera in commercial poultry birds had been reported as the major concern in the poultry industry by other workers [13], [14], and [16]. In this study, 12.4% isolation rate of *P. multocida* doubles the level reported by Mbuthia *et al*. [14] who recorded a rate of 6.2%. This difference may be due to the number of samples, method of isolation, presence of stress and age of birds sampled. Isolation of *P. multocida* in the trachea and liver of chicken was earlier reported by Dashe *et al.* [10].
The antibiotic resistance of the *P. multocida* in this study showed a high resistance to the tested antibiotics compare to reports of previous studies; Everlon *et al*. [11] reported antibiotic resistance of *P. multocida* to range between 1.5 and 5.2% in isolates from chicken while Dashe *et al.* [10] reported resistance ranging between 6.7 and 46.7%. In other various reports [2], [15], [20] it was found that the resistance level is not as high as found in this research. This may have been the result of misuse of antibiotics by most of the farmers in the territory even before they report to the clinic. Generally, farmers report cases in their farms after they have tried all the possible means, which often include heavy usage of antibiotics before making any clinical report.

The high resistance of *P. multocida* isolates to ampicillin, amoxicillin/clavulanate, doxycycline and tylosin has highlighted that prevention and therapeutic effect on avian *P. multocida* strains in Ekiti, Nigeria should no longer be expected from these antibiotics. 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 [4], [10]. Arora *et al*. [2] 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 [10].
The result observed in the resistance of isolates of *P. multocida* from freshly dead chicken is similar to what was reported for *Escherichia coli* and *Salmonella* sp. Atere *et al*. [5] and Atere [3] reported a high resistant level in the *E. coli* and *Salmonella* isolates respectively, which was attributed to misuse of antibiotics before clinical reports. Antimicrobial resistance in *P. multocida* has been linked to small plasmids [11]. The coexistence and spread of these small plasmids has resulted in *P. multocida* isolates that are multi-resistant [19]. In this study, nitrofuratoin and gentamycin showed the highest sensitivity, this may have been based on the fact that these antibiotics are not readily used in poultry.

5. Conclusion

With the increase in antibiotic resistance found in *P. multocida*, it is therefore recommended that antibiotic sensitivity test should be carried out before treatment; this will go a long way in the control of fowl cholera. It is also of great importance to encourage farmers to consult veterinary clinics (services), avoid self-prescription and carry out laboratory tests before any administration.

References

[1] Arashima, Y. and Kumasaka, K. (2005). Pasteurellosis as zoonosis. Int. Med., 44: 692-693. http://dx.doi.org/10.2169/internalmedicine.44.692.

[2] Arora, A. K., Virmani, S. K. J. and Oberoi, M. S. (2005). Isolation, characterization and antibiogram of *Pasteurella multocida* isolates from different animal species. Indian J. Anim. Sci., 75: 749-752.

[3] Atere, A. V. (2016). Multidrug resistant *Salmonella* sp isolated from chicken. International Journal of Biological research, 4 (1) 64-66.

[4] Atere, V. A, Bamikole, A. M. and Ajurojo, O. A. (2015) Antibiotic Susceptibility of Bacteria Isolated from Poultry Feeds Sold in Ado Ekiti, Nigeria. Journal of Advancement in Medical and Life Sciences V3I2.

[5] Atere, V. A., Bamikole, A. M., Ajurojo, O. A. and Alo, O. S. (2015). Antimicrobial Resistance Pattern of Pathogenic *Escherichia coli* Isolated from Chicken Liver and Trachea. Journal of Advancement in Medical and Life Sciences. V3I3.

[6] Bronzwaer, S. L., Cars, O., Buchholz, U. Molstad, S., Goettsch, W., Veldhuijzen, K. I., Kool, J. L., Sprenger, M. J. and Degener, J. E. (2002). European study on the relationship between antimicrobial use and antimicrobial resistance. Emerging Infectious Diseases, 8: 278-282. http://dx.doi.org/10.3201/eid0803.010192.

[7] Caprioli, A., L., Busani, J. L. and Helmuth, R. (2000). Monitoring of antibiotic resistance in bacteria of animal origin: Epidemiological and microbiological methodologies. Int. J. Antimicrobial Agents, 14: 295- 301. http://dx.doi.org/10.1016/S0924-8579(00)00140-0.

[8] Christensen, J. P., Dietz, H. H. and Bisgaard, M. (1998). Phenotypic and genotypic characters of isolates of *Pasteurella multocida* obtained from backyard poultry and from two outbreaks of avian cholera in avifauna in Denmark. Avian Pathology. 27: 373-381. http://dx.doi.org/10.1080/03079459808419354.

[9] Cowan, S. T. and Steel, K. J. (1970). Manual for the Identification of Medical Bacteria. 1st ed. Cambridge University Press, London, UK.

[10] Dashe, Y. D., Raji, M. A., Abdu, P. A., Oladele, B. S. and Sugun, M. Y. (2013). Multidrug Resistant *Pasteurella multocida* Strains Isolated from Chickens with Cases of Fowl Cholera in Jos, Nigeria. Int. J. Poult. Sci., 12 (10): 596-600. http://dx.doi.org/10.3923/ijps.2013.596.600.

[11] Everlon, C. R., Patrick, J. B., Renato, P. M. and Fernando, A. Á. (2013) Identification and antimicrobial susceptibility patterns of *Pasteurella multocida* isolated from chickens and Japanese quails in Brazil. Brazilian Journal of Microbiology 44 (1): 161-164. http://dx.doi.org/10.1590/S1517-83822013000100023.

[12] Glisson, J. R., Hofacre, C. L. and Christensen, J. P. (2003). Avian pasteurellosis. In: Saif, Y. M., Barnes, H. J., Glison, J. R., Fadly, A. M., McDougald, L. R. and Swayne, D. E. (Eds), Diseases of Poultry, 11th Ed. Iowa State University Press, Ames, Iowa, USA. Pp. 658-676.

[13] Kumar A. A., Shivachandra S. B., Biswas A., Singh V. P., Singh V. P. and Srivastava S. K. (2004). Prevalent serotypes of *Pasteurella multocida* isolated from different animal and avian species in India. Vet Res Comm, 28 (8): 657-667. http://dx.doi.org/10.1023/B:VERC.0000045959.36513.e9.

[14] Mbuthia G., Njagi L. W., Nyaga P. N., Bebora L. C., Minga U., Kamundia J. and Olsen J.E. 2008. *Pasteurella multocida* in scavenging family chickens and ducks: carrier status, age susceptibility and transmission between species. Avian Pathol, 37 (1): 51-57. http://dx.doi.org/10.1080/03079450701784891.

[15] Moemen, A. M., Mohamed-Wael, A. M., Ahmed, I. A., Awad, A. I. and Mohamed, S. A. (2012). *Pasteurella multocida* in backyard chickens in Upper Egypt: incidence with polymerase chain reaction analysis for capsule type, virulence in chicken embryos and antimicrobial resistance. Veterinaria Italiana, 48 (1): 77-86.

[16] Muhairwa, A. P., Mtambo, M. M. A., Christensen, J. P. and Bisgaard, M. (2001). Occurrence of *Pasteurella multocida* and related species in village free ranging chickens and their animal contacts in Tanzania. Vet Microbiol, 78: 139-153. http://dx.doi.org/10.1016/S0378-1135(00)00296-0.

[17] Quinn, P. J., Carter, M. E., Markey, B. and Carter, G. R. (1994). Clinical veterinary Microbiology. Wolf, London, Pp. 95–102.

[18] Rimler, R. B. and Glisson, J. R. (1997). Fowl cholera. In: B.W. Calnek. H. J., Barnes, E.W. Beard., L.R. McDougald and Y.M. Saif (Eds.), Diseases of Poultry 10th Edn., Ames, Iowa State University Press, pp: 143-159.

[19] San Millan, A., Escudero, J. A., Gutierrez, B., Hidalgo, L., Garcia, N., Montserrat, L., Dominguez, L., Zorn, G. B. (2009). Multi-resistance in *Pasteurella multocida* is mediated by coexistence of small plasmids. Antimicrob Agents Chemother 53:3399-3404. http://dx.doi.org/10.1128/AAC.01522-08.

[20] Shivachandra, S. B., Kumar, A. A., Biswas, A., Ramakrishnan, M. A., Singh, V. P. and Srivastava, S. K. (2004). Antibiotic sensitivity patterns among India strains of avian *Pasteurella multocida*. Trop. Anim.Health Product. 36: 743-750. http://dx.doi.org/10.1023/B:TROP.0000045950.35070.7f.

[21] White, D. G., Zhao, S., Simjee, S., Wagner, D. D. and McDermott, P .F. (2002). Antimicrobial resistance of foodborne pathogens. Microbial. Infect. 4: 405-412. http://dx.doi.org/10.1016/S1286-4579(02)01554-X.