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Multidrug Resistant *Pasteurella multocida* Strains Isolated from Chickens with Cases of Fowl Cholera in Jos, Nigeria

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Abstract: Antibiotic resistance is often encountered despite multiple antibiotics being used for the treatment of fowl cholera in Jos. This study was conducted to determine the antibiotic resistant profile of *Pasteurella multocida* isolated from chickens in Jos. A total of 2000 samples consisting of bone marrow, heart, liver, lung and spleen (400 each) were collected from 400 clinically sick chickens between November, 2010 and October, 2011 for the isolation of *P. multocida*. Swab from each sample was cultured on 7% defibrinated sheep blood, MacConkey and casein sucrose yeast agar. Presumptive colonies of *P. multocida* were subjected to biochemical characterization. Isolates identified by biochemical tests were further subjected to Microbact GNB 24E test. Disk diffusion method was employed to test the sensitivity of all the twelve *P. multocida* isolates confirmed by biochemical and Microbact GNB 24E test. The twelve pure isolates of *P. multocida were* tested for their sensitivity against fifteen different antibiotics. Drug sensitivity test conducted on *P. multocida* isolates showed that some of the isolates were resistant to penicillin 11 (73%), microlides 9 (60%), sulfanomides 8 (53.3%), cephalosporins 3 (20%) and other new groups of antibiotics 4 (27%). High resistance of *P. multocida* was recorded for ampicillin (91.7%) followed by amoxicillin/clavulanic acid (83.3%), trimethoprim/sulfamethoxazole (66.7%), erythromycin and anicillin (58.3%) each, while tylosin was (33.3%). This study revealed that there is an emergence of multidrug resistance in some *P. multocida* strains among chickens in Jos, Nigeria. It is therefore recommended that antibiotic sensitivity test should be incorporated on a routine bases as part of measure to control fowl cholera and minimize the emergence of *P. multocida* resistance.

Key words: *Pasteurella multocida*, multidrug, resistance, chickens, jos

INTRODUCTION

Fowl cholera is a contagious bacterial disease that affects both domestic and wild birds. Most outbreaks of fowl cholera affect chickens, turkeys, ducks and geese (Rimler and Glisson, 1997). This disease remains a significant obstacle to sustainable poultry production in most parts of tropical Asia and Africa. The fowl cholera usually occurs as a fulminating disease with massive bacteraemia, high morbidity and mortality (Office International Des Epizootics, 2008).

Fowl cholera is caused by *P. multocida* which is a gram negative, bipolar, non-motile, non-spore forming rod-shaped bacterium. *Pasteurella multocida* is responsible for fowl cholera in birds, atrophic rhinitis in swine, snuffles in rabbit, septicaemia haemorhagica ovis in goat, pneumonia in cattle and haemorhagic septicaemia in cattle and buffalo. *Pasteurella multocida* is not host specific (Rimler and Glisson, 1997; Arashima and Kumasaka, 2005).

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 (Arora *et al*., 2005). Antibiotic resistance of *P. multocida* isolates varies according the host animal, specie, time, geographical origin and antimicrobial pre-treatment of the animal (Caprioli *et al*., 2000). Multiple antibiotic resistance in pathogenic bacteria in food-producing animals and environmental sources is recognized as a global problem for public health (Bronzwaer *et al*., 2002 and White *et al*., 2002).

Despite the extensive use of multiple antibiotics for the treatment of fowl cholera in Jos, Nigeria, there is scanty information regarding the multiple drug resistance of the causative agent of this disease. The current study therefore seeks to document the results of multiple antibiotic resistance of *P. multocida* strains isolated from chickens affected by fowl cholera in Jos, Nigeria.

MATERIALS AND METHODS

Collection of samples: Veterinary Laboratory, Hospital and Clinic such as Central Diagnostic Laboratory of the

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National Veterinary Research Institute, Vom, Plateau State Veterinary Hospital and ECWA Veterinary Clinic were identified in Jos North and South Local Government Areas for sample collection.

Systematic random sampling method (one in five; every 5th bird on each visit) was applied for the selection of 400 clinically sick chickens between November, 2010 and October, 2011 (8 chickens/week for clinically sick chickens).

Sampling locations

Sampling of clinically sick chickens: Three sampling points such as Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom, Plateau State Veterinary Hospital and ECWA Veterinary Clinic were used for the collection of tissue samples from sick chickens submitted for diagnosis in these three sampling points. Tissue samples collected were heart blood, femur, lungs, spleen and liver (400 each from clinically sick chickens, giving a total of 2000 tissue samples). One hundred and thirty three clinically sick chickens each were sampled at Plateau State Veterinary Hospital and ECWA Veterinary Clinic, while one hundred and thirty four were sampled at Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom, Jos.

Transportation of samples: The samples collected were transported on ice to the Bacteriology Unit of the Central Diagnostic Laboratory, NVRI, Vom, Jos, Nigeria for culture and microbiological examination as described by Clinical and Laboratory Standard Institute (CLSI, 2009).

Culture and isolation of organism: Each sampled organ was seared with spatula and incised with a small sterile scalpel blade. Swabs from these organs were inoculated directly onto selective medium, such as Casein Sucrose Yeast (CSY) agar, blood agar and incubated aerobically at 37°C for 24 h. *Pasteurella multocida* colonies were subjected to Gram and methylene blue staining for cellular morphology. 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 h of incubation were subjected to further analysis. Cultural and morphological examinations were conducted as described by Cowan and Steel (2004). Capsular and bipolar organisms were further confirmed as *P. multocida* by biochemical tests according to CLSI (2009).

Biochemical characterization: *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 dulcitol, maltose, D-mannitol, D-sorbitol, D-sucrose, L-arabinose, 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 (2009).

Microbac test: All the twelve *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.

Antibiotic susceptibility test: Twelve isolates of *P. multocida* isolates confirmed by biochemical and Macrobact test were tested for their susceptibility against 15 conventional antibiotic agents commonly used for the treatment of fowl cholera in Nigeria. Antimicrobial agents tested were: Chloramphenicol (30 µg), enrofloxacin (10 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), gentamicin (10 µg), oxytetracycline (10 µg), erythromycin (10 µg), streptomycin (10 µg), trimetoprim/sulfamethoxazole (septrin) (30 µg), ciprofloxacin (10 µg), pefloxacin (10 µg), rocephin (25 µg), furasol (10 µg), tylosin (10 µg) and anicillin (10 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.* (1966). Thus; three colonies of *P. multocida* were made into homogenous suspension in 5ml of sterile Muller Hinton medium and incubated at 37°C for 5 min. 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 10^7 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.* (2004). 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 and matched with respective standard zone diameter to interpret the test culture as resistant, intermediate or sensitive according to the procedure of Bauer *et al.* (1966).

Statistical analysis: Data generated was entered into Microsoft excel, while descriptive statistical analysis was conducted using statistical package for social sciences SPSS (version 12.01).

RESULTS

Out of the two thousand clinical samples analyzed, 12 (0.6%) *Pasteurella multocida* isolates were confirmed by biochemical and Microbact test.

Antimicrobial susceptibility of isolates: Of the 12 avian *P. multocida* isolates tested for antimicrobial resistance, 8 (66.7%) isolates showed resistance to at least three and above of the antimicrobial compounds. The *P. multocida* isolates showed 11 (73%) resistance to penicillin. Resistance to microlides was prevalent in 9 (60%) of the isolates. Resistance to sulfanomides 8 (53.3%) was also observed. Only 3 (20%) of the 12 *P. multocida* isolates were resistant to cephalosporins and tetracyclines, while other new groups of antibiotics had 3 (27%). High resistance was shown to Ampicillin (91.7%) and Amoxicillin/clavulanic acid (83.3%) (Table 1 and 2). Figure 1 shows multiple antibiotic resistance pattern exhibited by *P. multocida* isolate I.

DISCUSSION

The use of antimicrobials has been greatly compromised due to the emergence of resistant microorganisms. In some instances, the extensive use of antibiotics has elicited varying degree of success depending on the kind of drug used (Rimler and Glisson, 1997). This study revealed that *P. multocida* isolates showed multiple resistances to sulfanomides, microlides, penicillin cephalosporins and other new groups of antibiotics. It was observed that most *P. multocida* isolates were resistant to ampicillin, amoxicillin/clavulanic acid, trimethoprim-sulfamethoxazole, erythromycin, anicillin and tylosin. Kulkarni *et al.* (1990) in India also recorded 73.7% resistance to trimethoprim/sulfamethoxazole and 77.8% to ampicillin. The apparent inability of these conventional drugs to be effective against *P. multocida* isolates portends grave consequence to poultry farmers and clinicians because this will severely undermine the effective control of fowl cholera. The high resistance of *P. multocida* isolates to ampicillin, amoxicillin/clavulanate acid, trimethoprim/sulfamethoxazole, erythromycin, anicillin and tylosin has highlighted that prevention and therapeutic effect on avian *P. multocida* strains in Jos, Nigeria should no longer be expected from these

Table 1: Antibiotic resistance profile of twelve *Pasteurella multocida* isolates tested against 15 antimicrobial agents

Pasteurella multocida isolates	Total number of drugs to which isolate was resistant	Antibiogram (resistant drugs)	Percentage resistance
1	8	CH, Cx, Am, Au, E, SXT, Ro, Ani	53.3
12	6	Cx, Am, Au, O, E, SXT	40
57	6	CH, Am, Au, Ani, E, SXT	40
72	5	Am, Au, Ani, E, SXT	33.3
122	3	Am, E, Ani	20
150	7	Am, Au, E, SXT, Fur, Tyl, Ani	46.7
200	2	Am, Au	13.3
207	3	Am, Au, SXT	20
231	5	AM, Au, SXT, Tyl, Ani	33.3
236	6	AM, Au, SXT, Pef, Ro, Ani	40
258	1	Tyl	6.7
354	5	Am, Au, E, Ro, Fu	33.3

KEY: CH: Chloramphenicol; SXT: Trimethoprim/sulfamethoxazole; CX: Enrofloxacin; Pef: Perfloxacin; CPX: Ciprofloxacin; Ro: Rocephin; AM: Ampicillin; Fur: Furasol; AU: Amoxicillin/clavulanate; Tyl: Tylosin; CN: Gentamicin; Ani: Anicillin; O: Oxytetracycline; S: Streptomycin; E: Erythromycin

Table 2: Resistance of *Pasteurella multocida* to different classes of antibiotics

P. multocida	Quino	Aminog	Tetracy	Sulph	Micro	Peni	Cepha	Others	Total	(%)
1	1	0	1	1	1	1	1	1	7	46.7
12	1	0	1	1	1	1	0	0	5	33.3
57	0	0	0	1	1	1	0	1	4	26.7
72	0	0	0	1	1	1	0	0	3	20.0
122	0	0	0	0	1	1	0	0	2	13.3
150	0	0	0	1	1	1	0	0	3	20.0
200	0	0	0	0	0	1	0	0	1	6.7
207	0	0	0	1	0	1	0	0	2	13.3
231	0	0	0	1	1	1	0	0	3	20.0
236	0	0	0	1	0	1	1	1	4	26.7
258	0	0	0	0	1	0	0	0	1	6.7
354	0	0	1	0	1	1	1	1	5	33.3
Total	2 (13.3%)	0 (0%)	3 (20%)	8 (53.3%)	9 (60%)	11 (73%)	3 (20%)	4 (27%)	40	266.7

Key: Quino: Quinolones; Micro: Microlides; Aminog: Aminoglycosides; Peni: Penicillin; Tetray: Tetracyclines; Cepha: Cephalosporins; Sulph: Sulphonamides

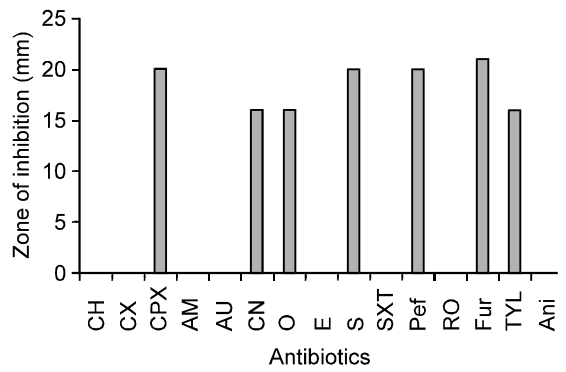

Fig. 1: Bar chart showing the resistant pattern of *Pasteurella multocida* isolate recovered from chicken in Jos, Nigeria.
KEY: CH: Chloramphenicol; SXT: Trimethoprime/ sulfamethoxazole; CX: Ennfloxacin; Pef: Perfloxacin; CPX: Ciprofloxacin; RO: Rocephin; AM: Amoxicillin/clavulanate potassium; Fur: Furasol; AU: Augumentin; Tyl: Tylosin; CN: Gentamicin; Ani: Anicillin; O: Oxytetracyclin; S: Streptomycin; E: Erythromicin

antibiotics. This may necessitate a longer duration of therapy or change of antibiotics. The consequences of these are reduction in the level of production, increase in the cost of production and a threat to availability of animal protein. 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. Arora *et al.* (2005) 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 emergence of resistant strains *P. multocida* could also be linked to conjugative R-plasmid which is commonly responsible for interspecies and inter-generic spread of multidrug resistance and transfer of such plasmid among pathogenic strains may give rise to epidemic spread of infection (Lee *et al.*, 2006). This might probably suggests that other avian microbial pathogens could be resistant to so many classes of antibiotics in Jos, Nigeria.

The present study also indicates that eight *P. multocida* isolates were resistant to a panel of antimicrobial agents, since the isolates were resistant to more than three classes of drugs. If the multi-drug resistance observed in this study continues unabated, soon there will be no effective antibiotics against fowl cholera. The antibiogram profiles obtained in the present study indicated that variable patterns of multidrug resistance existed among field isolates of *P. multocida* in Jos. Similar reports about the emergence of multidrug-resistant strains of *P. multocida* among different isolates have been documented by Shivachandra *et al.* (2004), Arora *et al.* (2005) and Zahoor and Siddique (2006).

It is therefore recommended that antibiotic sensitivity test should be incorporated on a routine bases as part of measure to control fowl cholera and minimize the emergence of resistance not only in target *P. multocida* pathogens but also in zoonotic bacteria, for the protection of public health.

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