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Phenotypic Characteristics of *Pasteurella Multocida* Isolated From Commercial Chickens Affected By Fowl Cholera in Jos, Nigeria

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ABSTRACT

This study was conducted to isolate and study the phenotypic characteristics *Pasteurella multocida* organism recovered from commercial chicken with cases of fowl cholera in Jos, Nigeria. A total of 2000 samples consisting of bone marrow, heart, liver, lung and spleen (400 each) were collected from 400 clinically sick chickens 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 and Microbact test. Disk diffusion method was employed to test for the sensitivity of the 12 P. *multocida* isolates confirmed by biochemical and Microbact test. The 12 isolates of P. *multocida* were tested for their sensitivity against 15 different antibiotics. Drug sensitivity test revealed that ciprofloxacin, streptomycin and gentamicin were (100%) highly effective against the 12 P. *multocida* isolates. High resistance of P. *multocida* was recorded for ampicillin 91.7%, amoxicillin/clavulanic acid 83.3% and trimethoprim/sulfamethoxazole 66.7%. It was concluded that biochemical characterization, Microbact test and antibiotic susceptibility test are essential for quick diagnosis and the selection of appropriate antibiotic agents for the treatment of fowl cholera.

Key words: Characteristics, Chickens, Cholera, Fowl, Jos, Nigeria, *Pasteurella*

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 (OIE, 2008). Fowl cholera is caused by *Pasteurella multocida* which is a gram negative, bipolar, non-motile, non-spore forming rod-shaped bacterium. The *P. multocida* is responsible for fowl cholera in birds but 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 to the host animal, specie, time, geographical origin, and antimicrobial pre-treatment of the animal (Caprioli, 2000). 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; White et al., 2002).

Microbact is an analytical profile index consisting of 12 biochemicals and 12 sugars impregnated into micro wells. The test is analyzed based on identification package V2.03 (Windows ^{Tm}). Percentage probabilities of isolates above 75% were considered positive (Mugg and Hill, 1981). Despite the importance of *P. multocida* as a causative agent of fowl cholera, there is inadequate information regarding the biochemical variations, Microbact test profile and drug sensitivity pattern. The current investigation therefore seeks to document the results of phenotypic characteristics of *Pasteurella multocida* isolated from commercial chickens in Jos, Nigeria.

MATERIAL AND METHODS

Collection of samples

Poultry clinics and laboratory 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

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November, 2013 and October, 2014 (8 chickens/week) (Portney and Watkins, 2007).

Sampling locations

Sampling of clinically sick chickens was done. 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 commercial chickens submitted for diagnosis in these three sampling points. A total of 2000 samples consisting of bone marrow, heart, liver, lung and spleen (400 each) were collected from 400 clinically sick chickens for the isolation of *P. multocida*. 133 clinically sick chickens each were sampled at Plateau State Veterinary Hospital and ECWA Veterinary Clinic, while 134 were sampled at Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom, Jos, Nigeria.

Transportation of samples

The samples collected were transported on ice to the Bacteriology Unit of the Central Diagnostic Laboratory, NVRI, Vom, Jos for culture and microbiological examination as described by Cowan and Steel (2004).

Culture and isolation of organism

Each sampled organ was seared with spatula and incised with a small sterile scapel blade and forceps. Swabs from these organs were inoculated directly onto selective medium, such as Casein Sucrose Yeast (CSY) agar, blood agar and incubated aerobically at 37^0C for 24 hours. *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 hours 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 *Pasteurella 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 Cowan and Steel (2004).

Microbact test

All the 12 *P. multocida* isolates recovered by biochemical test were further subjected to Macrobact test according to the manufacturer's instructions (Oxoid $^{(R)}$ United Kingdom). Macrobact 24E system is a commercial microsystem for the identification common clinical isolates of enterobacteriaceae miscellaneous Gram negative bacilli. It consists of dehydrated subtrates distributed in the wells of microtitre trays. After overnight incubation at 37^0C, suitable reagents were added and color changes of the different test were recorded. The results were transcribed into a code and organisms were identified by the use of a computer based profile register according to Mugg and Hill, (1981).

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. Antimicrobial agents that were tested include: 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 (30μg), 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 5 ml of sterile Muller Hinton medium and incubated at 37^0 C for 5 min. The turbidity of each isolate in the homogenous suspension was measured in a Nephelometer (Shanghai Yuefeng instrument, Model-SD 2-5, China) to get a 0.5 Mac Farland standard which correspond to 1×107colony forming unit. Each isolate, consisting of a 24 hours 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 37^0C for 24 hours. The resistance profile of *P. multocida* was assessed as described by Shivachandra et al. (2004). Isolate resistant to at least three different antibiotic classes 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).

RESULTS

Biochemical tests

Of the 2000 clinical samples analyzed, 12 (0.6%) *Pasteurella multocida* isolates were confirmed by biochemical test. Biochemical profiles of *P. multocida* indicated that 100% were Gram negative rods, indole positive and all reduced nitrate to nitrite. Variable

reactions were observed; non hydrogen sulphide production (88.9%), while 11.1% were negative; non lactose fermenters (83.7%) and 16.3% negative; ornithine decarboxylase positive (63.9%), 36.3% were negative; non urease production (91.7%), 8.3% produced urease and oxidase production (69.4%), while 30.6% were found to negative (Table 1).

Microbact test

Microbact test identified 12 *P. multocida* isolates with cut-off point of 75% above and probability index of <1/100,000,000. All isolates were found to ferment glucose, mannitol, ortho-nitrophenyl-β-galactoside (ONGP) and indole positive (Table 2).

Antimicrobial susceptibility of isolates

All 12 avian *P. multocida* isolates that were tested for sensitivity against a panel of 15 antimicrobial agents exhibited absolute 100% susceptibility to streptomycin, gentamicin and ciprofloxacin; followed by oxytetracycline and pefloxacin (91.7% each); furasol, enrofloxacin and chloramphenicol had 83.3% each. High resistance was shown to Ampicillin 91.7% and Amoxicillin/clavulanic acid 83.3% (Table 3).

Table 1. Morphobiological and biochemical characterization of twelve *Pasteurella multocida* specie isolated from chickens with fowl cholera in Jos, Nigeria.

Test	Reaction	Percentage rxt
Gram stain	-ve	100
Urease production	-ve	91.7
Oxidase production	+ve	69.4
Nitrate reduction	+ve	100
Indole reaction	+ve	100
Catalase reaction	+ve	50
Hemolysis	-ve	87.8
Hydrogen sulphide (H_2S)	-ve	88.9
Ornithine Decarboxylase	+ve	63.9
Lactose fermentation	-ve	83.7
Maltose fermentation	-ve	86.1
Mannose fermentation	+ve	83.3
Glucose fermentation	+ve	72.2

rxt – reaction; -ve – Negative; +ve - Possitive

Table 2. Identification of *Pasteurella multocida* isolated from chickens with fowl cholera using Microbact Gram negative bacilli (GNB) 24E kit

Serial number	*Pasteurella Multocida* Isolate	Octal number	Percentage Identification	Probability index
1	1	537736600	99.19	<1/100,000,000
2	12	576611410	99.75	<1/100,000,000
3	57	577611421	99.97	<1/100,000,000
4	72	567610666	99.70	<1/100,000,000
5	122	546600400	98.76	<1/100,000,000
6	150	577623550	98.77	<1/100,000,000
7	200	577623600	99.99	<1/100,000,000
8	207	577721421	96.03	<1/100,000,000
9	231	566723400	99.79	<1/100,000,000
10	236	577711421	99.99	<1/100,000,000
11	258	576723400	99.79	<1/100,000,000
12	354	576623420	83.26	<1/100,000,000

Table 3. Antimicrobial susceptibility profile of 12 *Pasteurella multocida* isolated from chickens with fowl cholera in Jos, Nigeria.

Antibiotic agent	Disc concentration	No. of strains	No. of strains	Percentage Sensitivity	Percentage Resistant
Chloramphenicol	30	2	10	83.3	16.7
Enrofloxacin	10	2	10	83.3	16.7
Ampicillin	10	11	1	8.3	91.7
Amoxicillin/Clavulanic	30	10	2	16.7	83.3
Gentamicin	10	-	12	100	0.0
Oxytetracycline	10	1	11	91.7	8.3
Erythromycin	10	7	5	41.7	58.3
Steptomycin	10	-	12	100	0.0
Trimethoprim/Sul.[1]	30	8	4	33.3	66.7
Ciprofloxacin	10	-	12	100	0.0
Pefloxacin	10	1	11	91.7	8.3
Rocephin	25	3	9	75	25
Furasol	10	2	10	83.3	16.7
Tylosin	10	4	8	66.7	33.3
Anicillin	10	7	5	4.7	58.3

[1]Sul. = Sulfamethoxazole

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DISCUSSION

The findings of this study showed that all *Pasteurella multocida* isolates were Gram negative, indole positive and they reduced nitrate to nitrate; however, they were observed to differ in their abilities to ferment other biochemical reagents and sugars such as hydrogen sulphide, urease, oxidase, lactose and ornithine decarboxylase among others. This intra specie variation could presumably be due to certain intrinsic phenotypic variations that exist among *P. multocida* organisms, since test results are based on colour change due to enzymatic reactions. The implication of the remarkable variations observed in response of *P. multocida* within the same specie to known conventional tests used for their detection will pose a great difficulty in arriving at confirmatory test for this organism in the laboratory. This finding lend credence to the results of a previous studies conducted by Ekundayo et al. (2008), Blackall et al. (1998) and Butt et al. (2003) who observed that avian strains of *P. multocida* in particular have been found to exhibit considerable phenotypic variability. However, the findings of this study is at variance with report of Madsen et al. (1985) and Kamp et al. (1990) who documented that *P. multocida* isolates from avian species were indole negative; the possible reason for this remains to be determined.

Microbact test was also observed to be reliable as it identified all the twelve isolates *P. multocida* that were confirmed by biochemical test without ambiguity. With this result, Microbact test has proved to be a dependable test in the confirmation of *P. multocida*.

Antibiotic susceptibility of the 12 *P. multocida* isolates revealed that all the isolates showed variable responses to different antibiotic agents. This report is similar to the observation of Aye et al. (2001). The current findings as well as those of Shivachandra et al. (2004) indicated that ciprofloxacin, streptomycin and gentamicin were highly effective against the 12 *P. multocida* isolates followed by pefloxacin, oxytetracycline, furasol, enrofloxacin and chloramphenicol. The judicious use of these drugs by poultry practitioners will go a long way in controlling cases of fowl cholera in Jos. However, these findings differ remarkable from the reports of Gutierrez et al. (1993) in Spain and Yoshimura et al. (2001) in Japan who documented that aminoglycoside antibiotic such as streptomycin and gentamicin showed resistance activity against tested isolates of *P. multocida*. The present study has indicated that effective control of fowl cholera in Jos can be achieved using ciprofloxacin, gentamicin, streptomycin, enrofloxacin, pefloxacin, furasol and oxytetracycline.

On the other hand, high resistance of *P. multocida* isolates was recorded for ampicillin followed by amoxicillin / clavulanic acid, trimethoprim / sulfamethoxazole, erythromycin, anicillin, and tylosin in this study. Kulkarni et al. (1990) also recorded resistance to trimethoprim / sulfamethoxazole and ampicillin. The multidrug resistance shown by *P. multocida* isolates is presumably attributed to the extensive and pervasive use of antimicrobial agents by poultry farmers and veterinary practitioners. Similar report on the emergence of multidrug resistant strains of *P. multocida* have been described by Shivachandra et al. (2004).

The apparent inability of these conventional drugs to be effective against *P. multocida* isolates signifies a serious consequence to poultry farmers and clinicians because this will severely undermine the effective control of fowl cholera.

CONCLUSION

It was concluded that biochemical characterization, Microbact test and antibiotic susceptibility test are essential for quick diagnosis and the selection of appropriate antibiotic agents for the treatment of fowl cholera.

Recommendations

Therefore, it is 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 *P. multocida* pathogens. It is also recommended that ciprofloxacin, streptomycin and gentamicin should be the drugs of choice for treatment of fowl cholera in Jos, Nigeria.

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