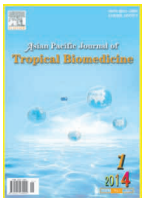
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(14)60200-8

Molecular analysis of *Pasteurella multocida* strains isolated from fowl cholera infection in backyard chickens

Mohamed-Wael Abdelazeem Mohamed[1], Moemen Abdel Azeem Mohamed Abdel Mageed[2*]

[1]*Department of Microbiology, Faculty of Veterinary medicine, South Valley University, Egypt*

[2]*Department of Poultry Diseases, Faculty of Veterinary Medicine, Assiut University, Egypt*

PEER REVIEW

Peer reviewer

Dr. Tolba Y. Abdel Motelib, Poultry Diseases Department, Col Vet Med, Assiut University, Assiut, Egypt.
Tel: 00208 8233 4699
E-mail: tolbassiut@yahoo.com

Comments

The study is up-to-dated and an interesting valuable research work in which authors have demonstrated the clonality of bacteria isolated from backyard chickens that improve the measures for prevention esp. for making vaccines and even in treatment.
Details on Page 12

ABSTRACT

Objective: To characterize *Pasteurella* isolated from backyard chickens using whole cell protein lysate profiles and random amplified polymorphic DNA (RAPD) techniques to show their genetic relationship because *Pasteurella multocida (P. multocida)* is an important cause of fatal infections in backyard chickens.

Methods: Twenty one *P. multocida* isolates were recovered previously from clinical cases of fowl cholera belonging to individual owners and phenotypically analyzed using biochemical tests and serotyping were used for the genetic characterization.

Results: Phylogenetic study based on both methods revealed that the recovered population of *P. multocida* isolated from backyard chickens differs markedly, constituting a well-separated cluster and appearance of 3 distinguishing lineages with greater discrimination shown by RAPD-PCR that resulted in two suclusters in cluster A and three subclusters in cluster B and were related greatly with capsular serogroups for the examined strains. The whole cell protein revealed the presence of dominant protein bands at approximately 41 and 61 kDa in all of the examined isolates that may be a virulent proteins share in the increasing of its pathogenicity. Clear distinctive bands ranged from 123 to 1 554 bp.

Conclusions: Based on the previous findings, there are three spreading clusters that may indicate the association of a small number of *P. multocida* variants with the majority of cases suggesting that certain clones of *P. multocida* are able to colonize the examined backyard chickens. Also, the ease and rapidity of RAPD-PCR support the use of this technique as alternative to the more labour-intensive SDS-PAGE system for strain differentiation and epidemiological studies of avian *P. multocida*. Further application of RAPD technology to the examination of avian cholera outbreaks in commercially available flocks may facilitate more effective management of this disease by providing the potential to investigate correlations of *P. multocida* genotypes, to identify affiliations between bird types and bacterial genotypes, and to elucidate the role of specific bird species in disease transmission.

KEYWORDS

Pasteurella multocida, Chickens,Variation, Molecular characterization

1. Introduction

Fowl cholera, caused by *Pasteurella multocida (P. multocida)*, occurs sporadically or enzootically in most countries of the world wherever intensive poultry production occurs, and is known as a bacterial disease with major economic importance due to its high mortality[1]. *P. multocida* is a heterogeneous species that pathogenicity of individual strains is highly variable and susceptibility to these bacterial strains varies considerably among avian

*Corresponding author: Moemen A. Mohamed, Department of Poultry Diseases, Faculty of Veterinary Medicine, Assiut University, Egypt.
Tel: 00208 8233 4699-171
E-mail: moemenassiut2002@yahoo.com

Foundation Project: Supported by Regular governmental annual fund every Fiscal year from Assiut university (Grant No. 9/020/08595/0).

Article history:
Received 1 Oct 2013
Received in revised form 14 Oct, 2nd revised form 20 Oct, 3rd revised form 25 Oct 2013
Accepted 18 Dec 2013
Available online 28 Jan 2014

species[2].

Generally, diagnosis of the disease in natural outbreaks largely depends on conventional methodologies comprising bacterial isolation and identification by serotyping and biochemical characterization, which reveal the presence of variable serogroups/types in different geographical regions[3]. However, it has been observed that conventional characterization is not sensitive enough to identify and differentiate each strain involved in natural infections[4,5].

The limitations of currently employed techniques have led to significant problems in understanding the disease outbreaks, origin and transmission of pathogens, the virulence characteristics of the organism as well as determining disease incidence and economic importance[5].

Alternatively, DNA-based methods have been applied for rapid identification and differentiation of avian strains of *P. multocida* originating from different regions[6,7].

A number of genotyping and genetic methods represent the major techniques for the characterization of *P. multocida*. Electrophoretic separation of whole cell and outer membrane proteins, or lipopolysaccharides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and DNA-based techniques such as polymerase chain reaction (PCR) based fingerprinting techniques based on either random amplification of polymorphic DNA (RAPD) by use of short and arbitrarily chosen DNA primers or detection of variable repetitive DNA regions, have been adapted to detect inter- or intra-strain variations which are rapid, specific and highly sensitive, and efficiently employed for differentiation of various strains of different micro-organisms from single/different outbreaks[4,6,8].

In addition, molecular techniques could also be an important tool to reveal epidemic patterns, trace sources of infection and aid the development of reasonable intervention strategies to reduce the presence and spread of *Pasteurella* infections in animals[9].

Due to the variability of *P. multocida* in general and lack of knowledge on isolates circulating in the upper Egypt, the aim of this work was to characterize the avian strains of *P. multocida* that recovered from cases of fowl cholera by comparative analysis of their whole cell protein (WCP) and RAPD profiles to gain deeper insight into the current *P. multocida* population to better understand the inter-strain relatedness, so as to be able to recommend epidemiology well-defined vaccines in the future.

2. Materials and methods

2.1. Bacterial strains and culture conditions

A total of 21 avian isolates of *P. multocida* were used in this study, including 12 strains serotype-1, 4 isolates serotype-3 and 5 ones untypable (Table 1).

Table 1
Distribution of the examined *P. multocida* isolates according to serotypes, place of isolation, SDS-PAGE and RAPD profiles.

No. of isolate	Serotype	Origin (place)	SDS-PAGE Cluster	RAPD-PCR Cluster
P46	A:1	Qena	1	-*
P47	A:1	Qena	1	1
P83	A:1	Qena	1	2
P87	A:1	Qena	1	1
P110	A:3	Qena	1	-
P120	A:3	Qena	1	1
P132	A:1	Qena	1	1
P145	A:1	Qena	1	1
P152	A:1	Qena	1	1
P265	A:1	Qena	1	1
P166	D	Qena	2	3
P50	D	Qena	2	-
P73	A:1	Qena	2	1
P108	A	Qena	2	2
P20	A:3	Qena	3	-
P259	A:3	Qena	3	-
P175	A	Sohag	2	2
P185	A:1	Sohag	1	1
P184	A:1	Sohag	1	1
P214	A:1	Aswan	1	-
P205	D	Aswan	3	-

The isolates were collected from cases of fowl cholera in chickens on various provinces around upper Egypt (Qena, Sohag and Aswan provinces). All of these isolates were previously characterized using carbohydrate fermentation profiles, serology and PCR typing[10]. These strains were stored at -80 °C in 80% (v/v) glycerol in brain heart infusion broth until further use, and before used they were cultivated on tryptic soy yeast extract (TSYE) agar, supplemented with 5% sheep blood, and incubated for 18 h at 37 °C and 7% CO_2.

2.2. SDS-PAGE analysis for whole cell protein

2.2.1. Preparation of isolates for protein extraction

Whole cell lysates were prepared by a method adapted from Lammeli[11]. Briefly, a colony was grown in trypticase soy broth for 24 h at 37 °C; cells were harvested by centrifugation (3 000 r/min for 5 min) at room temperature, washed at least three times with, and resuspend in, 10 mL of phosphate buffer solution (0.9 g NaCl, 0.02 g KCl, 0.02 g $KHPO_4$, 0.29 g $NaHPO_4$, distilled water to 100 mL, pH 7.2).

2.2.2. Gel Electrophoresis

Cells from 1.5 mL of the washed culture were harvested in a microfuge tube, resuspended in 100 μL of single strength SDS-PAGE sample lysis buffer (62.5 mmol/L Tris-HCl, 2% SDS,