Faculty of Veterinary Medicine –Qena **South Valley University**

Department of Microbiology

Molecular characterization of *Pasteurella multocida* isolated from rabbits

Thesis presented by

Eman Hassan Mahrous Mohmmed

(B.V.Sc, Assiut University, 2008)

(M.Sc, South Valley University, 2017)

Submitted for the degree of

Ph.D. Degree in Veterinary Medicine

(Microbiology- Bacteriology)

Under supervision of

Prof. Mohamed Wael Abd Al–Azeem Mohamed

Professor of Microbiology and Dean of Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Prof. Faisal Abd El-latif Wasel

Professor of Microbiology, Department of Microbiology

Animal Health Research Institute. (Sohag Branch).

Dr. Waleed Khedr Mohamed younis

Lecturer of Microbiology, Department of Microbiology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

2022

CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

Abbreviations	Word
AK	Amikacin
AM	Ampicillin
AX	Amoxicillin
BHI	Brain heart infusion
Bp	Base pair.
C	Chloramphenicol
CN	Gentamicin
CRO	Ceftriaxone
CSY	Casein sucrose yeast
DNA	Deoxyribonucleic acid.
DNTPs	Deoxy nucleotide triphosphate solution
E	Erythromycine
EUCAST	European Committee on antimicrobial Susceptibility Testing
***Hgb*B**	Iron acquisition related factor-B gene.
HS	Hemorrhagic septicemia.
H2S	Hydrogen sulfide
K	Kanamycine
LPS	Lipopolysaccharide
MDR	multi-drug resistant
MR	Methyl red.
NanB	Neuraminidase-B gene.
NB	Nutrient broth.
OIE	Office International des Epizooties.
***oma*87**	Outer membrane protein gene.

OMPS	Outer membrane proteins
PCR	Polymerase chain reaction.
***Pfh*A**	Filamentous hemagglutinin gene.
***Ptf*A**	Type IV fimbriae gene.
RAPD	Random Amplification of Polymorphic DNA
SBA	Sheep blood agar.
***Sod*A**	Superoxide dismutase gene.
***Sod*C**	Superoxide dismutase gene.
SXT	Trimethoprime/sulfamethoxazole
T	Oxytetracycline
TAE	Tris acetate EDTA
***Tbp*A**	Transferrin binding protein gene .
TMB	Tetra methylbenzidine.
***Tox*A**	Dermonecrotic toxin gene.
TSA	Tryptic soy agar
TSI	Triple sugar iron agar.
VP	Voges-Proskuer test

SUMMARY

All samples were submitted for bacteriological examination, Recovery of Pasteurella isolates of diseased rabbits from lung, liver and heart were (23) (46%), (11) (22%), (13) (26%) respectively, with total incidence of (47) (31.3%) by cultural methods.

Also the isolated organisms were identified on basis of traditional phenotypic procedure as colonial, cellular morphology and microscopical examination then streaking on MacConkey agar and blood agar containing 5% sheep blood, as a result of that morphological character of *Pasteurella multocida* were on 5% sheep blood agar appeared as non-haemolytic small yellowish white colony (dew drop like colony), Approximately 1 mm in diameter while on tryptic soya agar appear very small white colony, by Gram stain, it appeared as Gram-negative, non-motile, non-spore forming and coccobacilli shaped bacteria with bipolarity by Giemsa stain.

Biochemical reactions proved that (20) (42.5%) out of (47) (31.3%) isolates had the typical biochemical properties of *Pasteurella multocida*.

Incidence of *Pasteurella multocida* isolates from diseased rabbits were (20) (13.3%) from total examined samples by traditional methods.

Serotyping of the selected (20) Pasteurella isolates by using latex agglutination test and revealed that (8) (40%) isolates belonged to somatic serotype (1, 3, 12) where serotype (1) was the most prevalent one with a percentage of 4 (20%) followed by serotype (3) with a percentage of (10%) and then serotype (12) with a percentage of (10%) finally (12) with a percentage (60%) isolates were un typed.

The recovered (20) isolates were submitted for molecular identification of (*kmt*1) gene (species-specific gene for *Pasteurella multocida*) by conventional PCR and revealed that only 10 (50%) out of (20) isolates were positive to

(*kmt*1) gene so confirmed to be *Pasteurella multocida*, Moreover, these isolates submitted for multiplex PCR and revealed that all (10) (100%) isolates belonged to serogroup (A) by multiplex PCR.

All the recovered isolates were further subjected to multiplex PCR screening of some common virulence genes and revealed that (*pfh*A, *hgb*B, *tbp*A, *tox*A, *sod*A, *ptf*A, *sod*C, *nan*B and *oma*87) with percentage of (30%, 60%, 0%, 0%, 70%, 90%, 90%, 90% and 100%), respectively.

Detection of genetic diversity and similarities between *Pasteurella multocida* strains by using (RAPD) markers that were (OPA1, OPA9),

The number of DNA fragments produced by (OPA9) primer differed from one strain to another. It ranged from (2-6) fragments for each strain with a total number of (35) fragments, length of bands ranged from 210 bp to 1700 bp, the number of DNA fragments produced by (OPA1) primer. It ranged from (4-6) fragments for each strain with a total number of (48) fragments, length of bands ranged from 260 to 1550 bp.

The amplification products showed high polymorphic bands in (10) *Pasteurella multocida*. It comprised 14% monomorphic and 86% polymorphic bands by using primer (OPA9) Monomorphic bands that emerged showed no variation in all samples, for example at the primers (OPA1) (260, 600, 710, 950 bp).

One of the most striking observations was the absence of unique bands between *Pasteurella multocida* genotypes.

The dendrogram clustered the ten *Pasteurella multocida* genotypes into two clusters that is estimation of similarity coefficients and dendrogram developments based on (RAPD) data have been used by different authors to evaluate the genetic diversity among *Pasteurella multocida* strains.

The present study showed that no relationship existed between (RAPD) pattern and serotypes.

In the same direction the recorded data documented the existence of genotypic differences among strains of the same serotype.

Concerning to similarity matrix the obtained data proved that this similarity ranged from 73 % to 96 %.

From the previous results the isolates, No. (5, 10) and (1, 3) were considered the most conserved ones among locally isolated *Pasteurella multocida* strains. The most surprising results were a high diversity percent between isolates, No.2 and No.8 because the isolate, No.2 was serotyped as, A: 3 while the isolate, No.8 was A: 1, these results urged that the serotype A: 3 and serotype A: 1 of low degree of DNA similarities should be represented in the *Pasteurella multocida* vaccine of rabbits.

Antimicrobial susceptibility testing of the recovered isolates by using (Baur disc diffusion method) revealed that almost strains were multidrug resistant (MDR) with a predominance of resistance to oxytetracycline and erythromycine (100% each) following by kanamycin and ceftriaxone (80% each) while they were sensitive to gentamycine (90%) following by trimethoprime/ sulfamethoxazole (80%).

These findings evidenced that molecular method as PCR could confirm the identity of *Pasteurella multocida* and provide rapid and reliable characterization than serological methods. (RAPD) (PCR) is very important technique for detection of genetic diversity of *Pasteurella multocida*, Also indicated that rabbits are potential sources of pathogenic *Pasteurella multocida* strains harboring virulence genes and have multidrug resistance strains. Therefore, it is evident that there is an urgent need for the judicious use of antibiotics in rabbit's treatment systems to successfully mitigate the propagation of drug resistance across *Pasteurella multocida* species.