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**Molecular characterization of *Pasteurella multocida* isolated  
from rabbits**

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# **CONTENTS**

<b>Subject</b>	<b>Page</b>
<b>1- INTRODUCTION:</b> .....	<b>1</b>
<b>2- REVIEW OF LITERATURES:</b> .....	<b>6</b>
2-1. History of <i>Pasteurella multocida</i> microorganisms:.....	<b>6</b>
2-2. Bacteriological features of <i>Pasteurella multocida</i> : .....	<b>8</b>
2-2.1. Isolation and identification of <i>Pasteurella multocida</i> :.....	<b>8</b>
2-2.1.1. Morphology of <i>Pasteurella multocida</i> :	<b>8</b>
2-2.1.2. Growth conditions of <i>Pasteurella multocida</i> :.....	<b>12</b>
2-2.1.3. Cultivation of <i>Pasteurella multocida</i> :.....	<b>14</b>
2-2.1.4. Biochemical characters of <i>Pasteurella multocida</i> :.....	<b>20</b>
2-2.1.5. Incidence of isolation of <i>Pasteurella</i> by traditional methods :	<b>23</b>
2-3. Serological characterization of <i>Pasteurella multocida</i> isolated from diseased rabbits:.....	<b>25</b>
2-4. Molecular characterization of <i>Pasteurella multocida</i> isolates by conventional PCR assay:.....	<b>27</b>
2-4.1. Identification of ( <i>kmt1</i> ) gene of <i>Pasteurella multocida</i> by conventional PCR:.....	<b>27</b>
2-4.2. Detection of capsular type of <i>Pasteurella multocida</i> by multiplex PCR:.....	<b>30</b>
2-4.3. Incidence of isolation of <i>Pasteurella multocida</i> from diseased rabbits by PCR:.....	<b>32</b>
2-4.4. Detection of virulence associated genes of <i>Pasteurella multocida</i> by multiplex PCR:.....	<b>33</b>
2-4.5. Random Amplified Polymorphic DNA (RAPD) analysis of <i>Pasteurella multocida</i> isolates:.....	<b>34</b>
2-5. Antimicrobial susceptibility test of <i>Pasteurella multocida</i> :.....	<b>37</b>
<b>3- MATERIAL AND METHODS:</b> .....	<b>42</b>

3-1. Animals and Samples:.....	42
3-1.1. Animals:.....	42
3-1.2. Samples from diseased rabbits:.....	42
3-2. Material used for isolation and identification:.....	43
3-2.1. Media used for isolation and identification:.....	43
3-2.2. Stains used:.....	44
3-2.3. Reagents and chemicals for isolation and identification:.....	44
3-2.4. Equipment and tools used in isolation and identification of <i>Pasteurella multocida</i> :.....	45
3-3. Material used for molecular characterization of <i>Pasteurella multocida</i> by PCR:.....	46
3.3.1. Material used for detection of species specific gene and capsular type associated genes of <i>Pasteurella multocida</i> :.....	46
3. 3.2. Material used for used for detection of selected virulence genes of <i>Pasteurella multocida</i> :.....	48
3-3.3. Material used for (RAPD) PCR analysis:.....	49
3-4. Material used for antimicrobial sensitivity test:.....	52
3-4.1. Media used for antimicrobial sensitivity test:.....	52
3-4.2. Antimicrobial discs ( <b>Bioanalyse, Ankara,Turkey</b> ):.....	52
3-4.3. McFarland tube – concentration (0.5%):.....	53
<b>- METHODS:</b> .....	53
3-1. Collection of samples:.....	53
3-2. Preparation of samples:.....	54
3-3. Bacteriological examination:.....	54
3-4. Serological characterization of <i>Pasteurella multocida</i> isolated from diseased rabbits:.....	57

3-5. Methods used for molecular characterization of <i>Pasteurella multocida</i> by PCR:.....	<b>57</b>
3-6. Antimicrobial sensitivity testing of <i>Pasteurella multocida</i> isolated from diseased rabbits: .....	<b>66</b>
<b>4- RESULTS:</b> .....	<b>69</b>
4-1. Incidence of <i>Pasteurella</i> isolates from diseased rabbits by cultural methods:.....	<b>69</b>
4-2. Morphological and biochemical characters of <i>Pasteurella</i> isolates from diseased rabbits:.....	<b>70</b>
4-3. Incidence of <i>Pasteurella</i> isolates from rabbits in Sohag governorate by traditional methods:.....	<b>74</b>
4-4. Serological characterization of <i>Pasteurella</i> isolates from diseased rabbit	<b>75</b>
4-5. Identification of <i>Pasteurella</i> isolates by conventional PCR:.....	<b>76</b>
4-6. Capsular type of <i>Pasteurella multocida</i> isolates by multiplex PCR:.....	<b>78</b>
4-7. Incidence of <i>Pasteurella multocida</i> isolated from diseased rabbits in Sohag governorate by PCR: .....	<b>79</b>
4-8. Incidence of virulence associated genes of <i>Pasteurella multocida</i> isolated from rabbits by multiplex PCR:.....	<b>80</b>
4-9. Results of molecular studies on <i>Pasteurella multocida</i> strains isolated from rabbits by (RAPD) PCR:.....	<b>83</b>
4-10. Antimicrobial sensitivity testing of <i>Pasteurella multocida</i> isolates from diseased rabbits in Sohag governorate: .....	<b>88</b>
<b>5- DISCUSSION:</b> .....	<b>90</b>
<b>- CONCLUSION AND RECOMMENDATIONS:</b> .....	<b>107</b>
<b>6- SUMMARY:</b> .....	<b>109</b>
<b>7- REFERENCES:</b> .....	<b>112</b>
<b>- ARABIC SUMMARY:</b> .....	

## **LIST OF TABLES**

<b>No.</b>	<b>Title</b>	<b>Page</b>
<b>(1)</b>	Different tissue samples collected from rabbits at different localities in Sohag governorate:.....	<b>43</b>
<b>(2)</b>	Oligonucleotide primer sequence of species specific gene ( <i>kmt1</i> ) by conventional PCR:.....	<b>46</b>
<b>(3)</b>	Oligonucleotide primer sequence for identification of capsular associated genes of <i>Pasteurella multocida</i> by multiplex PCR:.....	<b>47</b>
<b>(4)</b>	Oligonucleotide primers sequences used for detection of selected virulence associated genes by multiplex PCR:.....	<b>48</b>
<b>(5)</b>	Oligonucleotide primers sequences used for detection of genetic diversity of <i>Pasteurella multocida</i> strains by (RAPD) markers:.....	<b>52</b>
<b>(6)</b>	Antimicrobial discs that used for antimicrobial sensitivity test of <i>Pasteurella multocida</i> strains:...	<b>53</b>
<b>(7)</b>	Preparation of conventional PCR master mix. for detection of ( <i>kmt1</i> ) gene:.....	<b>57</b>
<b>(8)</b>	Preparation of multiplex PCR master mix. for detection of capsular type associated genes:.....	<b>58</b>
<b>(9)</b>	Cycling conditions of different primers during conventional PCR for detection of ( <i>kmt1</i> ) gene:...	<b>58</b>
<b>(10)</b>	Cycling conditions of the different primers during multiplex PCR for detection of capsular type associated genes:.....	<b>59</b>
<b>(11)</b>	Setting up the thermal cycler for <i>Pasteurella multocida</i> virulence associated genes ( <i>pfhA</i> , <i>hgbB</i> , <i>tbpA</i> and <i>toxA</i> )	<b>62</b>
<b>(12)</b>	Setting up the thermal cycler for <i>Pasteurella multocida</i> virulence associated genes ( <i>sodA</i> and <i>PtfA</i> ):.....	<b>62</b>
<b>(13)</b>	Setting up the thermal cycler for <i>Pasteurella multocida</i> virulence associated genes ( <i>sodC</i> , <i>oma87</i> and <i>nanB</i> ):..	<b>63</b>

<b>(14)</b>	Preparation of (RAPD) PCR master mix. for detection of <i>Pasteurella multocida</i> genetic diversity:.....	<b>64</b>
<b>(15)</b>	Cycling conditions of different primers during (RAPD) PCR for detection of <i>Pasteurella multocida</i> genetic diversity:.....	<b>65</b>
<b>(16)</b>	Incidence of <i>Pasteurella</i> isolates from diseased rabbits by cultural methods:.....	<b>69</b>
<b>(17)</b>	Cultural and morphological characters of <i>Pasteurella</i> isolates:	<b>71</b>
<b>(18)</b>	Incidence of <i>Pasteurella</i> isolates from diseased rabbits by biochemical activity:.....	<b>71</b>
<b>(19)</b>	Biochemical characters of <i>Pasteurella</i> isolates from rabbits:	<b>72</b>
<b>(20)</b>	Incidence of <i>Pasteurella multocida</i> isolates from rabbits in Sohag governorate by traditional methods:.....	<b>75</b>
<b>(21)</b>	Serological characterization of <i>Pasteurella</i> isolates from diseased rabbits:.....	<b>76</b>
<b>(22)</b>	Identification of <i>Pasteurella</i> isolates by conventional PCR:..	<b>77</b>
<b>(23)</b>	Capsular type of <i>Pasteurella multocida</i> isolates by using multiplex PCR:.....	<b>78</b>
<b>(24)</b>	Incidence of <i>Pasteurella multocida</i> isolated from diseased rabbits in Sohag governorate by PCR:.....	<b>79</b>
<b>(25)</b>	Incidence of virulence associated genes of <i>Pasteurella multocida</i> isolated from rabbits by multiplex PCR:....	<b>80</b>
<b>(26)</b>	RAPD profiles of <i>Pasteurella multocida</i> genomic DNA isolates amplified by primer (OPA9) (GGGTAAC GCC):.....	<b>86</b>
<b>(27)</b>	RAPD profiles of <i>Pasteurella multocida</i> genomic DNA isolates amplified by primer (OPA1) (CAGGCC CTTC):.....	<b>87</b>
<b>(28)</b>	Antimicrobial sensitivity testing of <i>Pasteurella multocida</i> isolated from rabbits in Sohag governorate:.....	<b>89</b>

## **LIST OF FIGURES**

<b>No.</b>	<b>Title</b>	<b>Page</b>
<b>(1)</b>	Incidence of <i>Pasteurella</i> isolates from diseased rabbits by cultural methods:.....	<b>70</b>
<b>(2)</b>	Incidence of <i>Pasteurella</i> isolates from diseased rabbits by biochemical activity:.....	<b>72</b>
<b>(3)</b>	Gram negative, cocco-bacillary or rod shaped <i>Pasteurella multocida</i> by Gram stain (100x objects):.....	<b>73</b>
<b>(4)</b>	Bipolarity of <i>Pasteurella multocida</i> on blood smear from heart blood and stained by Giemsa stain (100x objects):.....	<b>73</b>
<b>(5)</b>	Colonial morphology of <i>Pasteurella multocida</i> isolates on blood agar appear as non-haemolytic small yellowish white colony (dew drop like colony):.....	<b>73</b>
<b>(6)</b>	Colonial morphology of <i>Pasteurella multocida</i> isolates on TSA as small mucoid colony (dew-drop appearance):.....	<b>74</b>
<b>(7)</b>	No growth of <i>Pasteurella multocida</i> on MacConkey agar:.....	<b>74</b>
<b>(8)</b>	Incidence of <i>Pasteurella multocida</i> isolates from rabbits in Sohag governorate by traditional methods:.....	<b>75</b>
<b>(9)</b>	Agarose gel showing amplified product of 460 bp for ( <i>kmt1</i> ) gene using specific primer by conventional PCR:	<b>77</b>
<b>(10)</b>	Agarose gel showing amplified product of 1044 for capsular associating gene of <i>Pasteurella multocida</i> by multiplex PCR:.....	<b>78</b>
<b>(11)</b>	Incidence of <i>Pasteurella multocida</i> isolated from diseased rabbits in Sohag governorate by PCR:.....	<b>79</b>

(12)	Agarose gel electrophoresis of ( <i>pfhA</i> ) (275 bp), ( <i>hgbB</i> ) (499 bp), ( <i>toxA</i> ) (846) and ( <i>tbp</i> ) (728) virulence associated genes for characterization of <i>Pasteurella multocida</i> by multiplex PCR:.....	81
(13)	Agarose gel electrophoresis of multiplex PCR of ( <i>sodA</i> ) (361 bp) and ( <i>ptfA</i> ) (488 bp) virulence genes for characterization of <i>Pasteurella multocida</i> by multiplex PCR:.....	82
(14)	Agarose gel electrophoresis of multiplex PCR of ( <i>sodC</i> ) (235 bp), ( <i>nanB</i> ) (544 bp) and ( <i>oma87</i> ) (948 bp) virulence associated genes for characterization of <i>Pasteurella multocida</i> by multiplex PCR:.....	83
(15)	RAPD profiles of <i>Pasteurella multocida</i> genomic DNA isolates amplified by using (OPA 9) primer:.....	85
(16)	Similarity matrix among (10) <i>Pasteurella multocida</i> isolates as estimated by using (OPA 9) primer:.....	85
(17)	RAPD profiles of <i>Pasteurella multocida</i> genomic DNA isolates amplified by using (OPA 9) primer:.....	86
(18)	Similarity matrix among (10) <i>Pasteurella multocida</i> isolates as estimated by using (OPA 1) primer:.....	87
(19)	Clustering dendrogram of <i>Pasteurella multocida</i> using (RAPD) data:.....	88
(20)	Antimicrobial sensitivity testing of <i>Pasteurella multocida</i> :.....	89



## **List of Abbreviations**

<b>Abbreviations</b>	<b>Word</b>
<b>AK</b>	Amikacin
<b>AM</b>	Ampicillin
<b>AX</b>	Amoxicillin
<b>BHI</b>	Brain heart infusion
<b>Bp</b>	Base pair.
<b>C</b>	Chloramphenicol
<b>CN</b>	Gentamicin
<b>CRO</b>	Ceftriaxone
<b>CSY</b>	Casein sucrose yeast
<b>DNA</b>	Deoxyribonucleic acid.
<b>DNTPs</b>	Deoxy nucleotide triphosphate solution
<b>E</b>	Erythromycine
<b>EUCAST</b>	European Committee on antimicrobial Susceptibility Testing
<b><i>HgbB</i></b>	Iron acquisition related factor-B gene.
<b>HS</b>	Hemorrhagic septicemia.
<b>H<sub>2</sub>S</b>	Hydrogen sulfide
<b>K</b>	Kanamycine
<b>LPS</b>	Lipopolysaccharide
<b>MDR</b>	multi-drug resistant
<b>MR</b>	Methyl red.
<b><i>NanB</i></b>	Neuraminidase-B gene.
<b>NB</b>	Nutrient broth.
<b>OIE</b>	Office International des Epizooties.
<b><i>oma87</i></b>	Outer membrane protein gene.

<b>OMPS</b>	Outer membrane proteins
<b>PCR</b>	Polymerase chain reaction.
<b><i>PfhA</i></b>	Filamentous hemagglutinin gene.
<b><i>PtfA</i></b>	Type IV fimbriae gene.
<b>RAPD</b>	Random Amplification of Polymorphic DNA
<b>SBA</b>	Sheep blood agar.
<b><i>SodA</i></b>	Superoxide dismutase gene.
<b><i>SodC</i></b>	Superoxide dismutase gene.
<b>SXT</b>	Trimethoprim/sulfamethoxazole
<b>T</b>	Oxytetracycline
<b>TAE</b>	Tris acetate EDTA
<b><i>TbpA</i></b>	Transferrin binding protein gene .
<b>TMB</b>	Tetra methylbenzidine.
<b><i>ToxA</i></b>	Dermonecrotic toxin gene.
<b>TSA</b>	Tryptic soy agar
<b>TSI</b>	Triple sugar iron agar.
<b>VP</b>	Voges-Proskauer 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 (*kmt1*) gene (species-specific gene for *Pasteurella multocida*) by conventional PCR and revealed that only 10 (50%) out of (20) isolates were positive to

(*kmt1*) 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 (*pfhA*, *hgbB*, *tbpA*, *toxA*, *sodA*, *ptfA*, *sodC*, *nanB* and *oma87*) 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 trimethoprim/ 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.