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Isolation, identification and antibiogram of Pasteurella multocida isolated from apparently healthy rabbits in Al-Bayda, Libya

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A B S T R A C T

The aim of this study was to investigate the prevalence of *Pasteurella multocida* in upper respiratory tract (nasal cavities) of apparently healthy rabbits. Twenty adult and young rabbits were sampled, using sterile cotton swabs for sample collection from the nasal cavities of test animals. Swabs were put into nutrient broth and incubated at 37° C for 24 hrs. nasal swabs from 14 of the 40 samples rabbits (35%) showed visible growth suggestive of *P. multocida*, eleven (78.6%) of the isolates were males, and three isolates (21.4%) were females, whereas 26 samples (65%) were negative. Presumptive identification of the isolates was performed according to conventional bacteriological methods of gross colonial morphology, Gram staining reaction, microscopic characteristics and biochemical tests. Results achieved in this study indicate that P. multocida is harbored in the upper respiratory tract of nearly third of the rabbit population in the laboratory animal unit of the faculty of veterinary Medicine, Omer Al- Muktar university. Antimicrobial sensitivity of *P. multocida* isolated were tested against seven antibacterial antibiotics. 100% of the isolates were found to be sensitivity to Neomycin and 71.4% were sensitivity to Tetracycline; Erythromycin and Streptomycin should sensitivity of 50% and 14.3%, respectively. One of the hand all isolates found to be resistant to Ofloxacin, Amoxicillin and Cephalexin.

Key words Antibiogram, Isolation, Identification, Pasteurella multocida, Rabbits.

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1. INTRODUCTION

Rabbit hemorrhagic disease (RHD) is rapidly fatal, with mortality rates of 70%–100% in adult rabbits. young rabbits (kits) are unaffected or sub clinically infected .This difference in disease susceptibility is poorly understood, but it may be due to changes in tissue-specific receptors that occur as young rabbits develop to adulthood (Mikami *et al*., 1999). The disease remains a significant obstacle to sustainable livestock production in most parts of tropical Asia

and Africa. It is caused by Pasteurella multocida a natural inhabitant of the mucosal surfaces of upper part of the respiratory tract of ruminants, and under predisposing environmental or management conditions which constitute stress for the animals such as transport (shipping fever), marketing, change of feed, climate or ventilation (Radostits *et al*., 2000). The disease is per acute, having a short clinical course, involving severe depression, pyrexia, sub mandibular edema, and dyspnea, followed by recumbency and death (Horadagoda *et al*., 2001). Pasteur first isolated the causative agent of fowl cholera, *P. multocida*, in 1880 (Robinson, 1944). Reclassification of the members of the genus Pasteurella in 1985 (Mutters *et al*., 1985) resulted in the description of at least 11 species, including *P. multocida* (which comprises three subspecies) and *P. dagmatis* (previously known as Pasteurella ‘‘gas’’ and Pasteurella new species 1). Pasteurella is thought to live in the nasal cavity of many rabbits. It can reside here for many months or years without ever causing any clinical signs of disease. However, rabbits can develop health problems caused by Pasteurella, especially if they are unwell or stressed. *P . multocida* is a well-known cause of morbidity and mortality in rabbits. The predominant syndrome is upper respiratory disease or "snuffles." *P. multocida* is often endemic in rabbit colonies and the acquisition of infection in young rabbits is correlated to the prevalence in adult rabbits (DiGiacomo *et al*., 1983).

2. MATERIALS AND METHODS

2.1. Collection of samples

Using sterile colon swabs (Bach, Italy), 40 nasal swabs were collected from 40 apparently healthy rabbits, housed in the laboratory animal unit of the Faculty of Veterinary Medicine, Omar AL- Mukhtar University. Rabbits were of different ages (22 were adults and 18 young's). Twenty-eight rabbits were males and twelve were females. Animals were allowed free access to feed and water and were moving freely. Each swabs was rotated inside the two nares of each rabbits and considered one sample. Samples were transported in immediately after collection to the laboratory for investigation.

2.2.Bacteriological examination:

2.2.1 Isolation of bacteria:

Collected samples were inoculated directly into nutrient broth and incubated for 24 hours at 37°C, then subcultured onto nutrient agar, MacConkey’s agar and Blood agar, and incubated aerobically at 37°C for 24 – 48 hours as described by Cruickshank, Duduid, Marmion, and Swain (1975).

2.2.2 Identification of isolated bacteria:

2.2.2.1. Morphological characters:

Pure cultures from each isolates were identified morphologically according to their culture characters, staining reactions, shape, size and arrangement.

2.2.2.2. Identification of P .multocida:

Suspected colonies were subcultured onto selective and non-selective agar to ensure that possible contaminants, the colonies of Pasteurella from each plate were collected for presumptive identification according to their morphological characteristics and biochemical tests. Isolated bacteria were identified according to Barrow and Feltham (1993) using the following test, growth on MacConkey’s agar plates, Nutrient agar, EMB agar, Salmonella-Shigella agar , EMB agar and Blood agar, motility test in soft agar, indole

production test, Urease activity into Christensen's urea medium, Catalase test and growth onto Simmons citrate agar medium were studied. The biochemical reactions were recorded finally, at least 48 hrs post incubation at 37°, while growth onto Simmons citrate agar medium and Christensen's urea medium was incubated at 37°C and readings were recorded after every 24 hrs.

2.3.Antibiogram of isolated P. multocida:
By application of disc diffusion technique according to Finegold and Martin (1982) using Muller-Hinton broth for preparation of standardized inoculums and Muller-Hinton agar as test plate and different antibiotic discs. The sensitivity of the isolates was tested for the following antibiotic discs : streptomycin (S), Amoxicillin (AMC) , Tetracycline, Neomycin (N), Ofloxacin (OX), Cephalexin (CX) and Erythromycin (E) (Table 1). And finally classified as resistant, intermediate, or susceptible according to Oxoid (1982) and Koneman *et al* (1983).

Table (1): Antimicrobials and concentrations used to test sensitivity of *P. multocida* isolates

Antimicrobial	Disc Code	Disc Concentration
Streptomycin	S	10 µg
Amoxicillin	AMC	3 µg
Tetracycline	T	10 µg
Neomycin	N	30 µg
Ofloxacin	OX	1 µg
Cephalexin	CX	1 µg
Erythromycin	E	15 µg

3. RESULTS

A total of fourteen (35%) *P. multocida* isolates could be isolated from all the sample showed visible growth suggestive of *P. multocida*, eleven (78.6%) of the isolates were males, and three isolates (21.4%) were females, whereas 26 samples (65%) were negative. All the fourteen isolates showed typical cultural characteristics of dew drop, mucoid, non-haemolytic colonies in blood agar. No growth was observed in MacConkey agar and Salmonella-Shigella media. Whitish, opaque and circular on Nutrient agar, Small, circular, convex, glistening colonies, no metallic sheen onto EMB agar. Grams staining of the smears revealed characteristic. Gram negative, cocco-bacillary or short rod shaped and generally arranged single or in pairs with bipolar appearance was observed after Gram staining of fresh culture of the organisms. The isolates subjected to biochemical tests It was observed to be Catalase test Positive as bubbling appeared in the tube due to production of H2S gas. Indole test was also positive, as the oily ring appeared on the surface of the media after Kovacs reagent had been added. *P. multocida* was found Citrate negative as it did not require citrate as its sole source of carbon., non-motile and non-haemolytic on blood agar as shown in Table (2 and 3).

Table (2): Cultural and staining characteristics of *P. multocida* isolated from Rabbits

Colony characteristics					Microscopic characteristics
Nutrient agar media	EMB agar media	MacConkey agar media	Blood agar media	SS agar	Gram negative, coccobacillary bipolar
Whitish, opaque, circular, translucent appearance	Small, circular, convex, glistening colonies, no metallic sheen	No colony appears	Whitish, opaque, circular, translucent appearance and no hemolysis	No growth	

Table (3): Biochemical properties of *P. multocida* isolated from Rabbits

Biochemical property	Reaction	Inference
Catalase production	+ve	Active bubbling observed
Haemolysis	-ve	No haemolysis on blood agar observed
Indole	+ve	red or red-violet color in the surface
Citrate production	-ve	Green colony observed
Urease production	-ve	Yellow colony observed
Motility	-ve	No motility
Growth on MacConkey media	-ve	No growth on MacConkey media observed
Growth on SS agar	-ve	No growth on Salmonella-Shigella media observed

Antimicrobial sensitivity testing

The results of antimicrobial sensitivity testing by disc diffusion method of seven selected antimicrobial agents are presented in (Table 4). All isolates strains were found to be resistant to Ofloxacin (1oo%), Amoxicillin, and Cephalexin; while others were highly sensitivity to Neomycin (100%), and as isolates were found sensitive to Tetracycline (71.4%). moreover, moderate sensitivity to Erythromycin 50%. The lowest sensitivity (14.3 %) was observed for Streptomycin.

Table (4): Antimicrobial sensitivity test of fourteen isolates of *P. multocida*

Antibiotics	Sensitive		Intermediate		Resistant	
	No	%	No	%	No	%
Neomycin	14	100.0	0	0.0	0	0.0
Tetracycline	10	71.4	0	0.0	4	28.6
Erythromycin	7	50.0	6	42.9	1	7.1
Streptomycin	2	14.3	7	50.0	5	35.7
Ofloxacin	0	0.0	0	0.0	14	100.0
Cephalexin	0	0.0	0	0.0	14	100.0
Amoxicillin	0	0.0	0	0.0	14	100.0

4. DISCUSION

The carriage of *p. multocida* as a commensal in the nasopharyngeal region of many wild and domestic animals, including rabbits is well authenticated in the literature. Rabbits are recognized carriers of *P. multocida* in their upper respiratory tract; however, under conditions of stress and predisposition, such as the presence of concomitant viral infections and adverse environmental stressors, *P. multocida* may spontaneously convert from commensal to pathogen, causing pneumonia in rabbits. In this study, the prevalence of P. *multocida* isolated from the nares of apparently healthy rabbits was 35%. These finding are comparable to those of Mohammed *et al*., (2013); Reisinger *et al*., (1959), who reported the isolation of P. *multocida* from rabbits of various ages. However, Balakrishnan *et al*., (2012) and Fahmy *et al*., (1985) recorded *P. multocida* species incidence in 100% of their samples, a percentage much higher than that of our results. Variation in results may be attributed to differences in rabbits' breeds, housing, rearing and management condition. All the positive isolates showed typical cultural characteristics of dew drop, mucoid. These colonies failed to grow on MacConkey's agar plates, SS agar, non-haemolytic on blood agar media and negative for pigment production. The addition blood agar media was the best media for *P. multocida* isolation in pure culture and with little contamination (Asran *et al*., 2016). The colonies examined microscopically revealed that all examined isolates were a Gram-negative, non-motile, non-spore forming and rod "coccobacilli" shaped or coccoid bacterium and generally arranged single or in pairs with bipolar. These findings are in accordance with Purushothaman, Jayathangaraj, Prabhakar, and Prabhakar (2008). The 14 isolates produced positive reactions with indole, and catalase testes while negative for urease and citrate utilization testes and these in agreement with (El-Dirbi, 1992; Quinn *et al*., 1994; Petersen *et al*., 2001; Asran *et al*., 2016). We also checked the antibiotic sensitivity of *Pasteurella multocida* by disc diffusion method. The antibiotics used: streptomycin, Amoxicillin, Tetracycline, Neomycin, Ofloxacin, Cephalexin and Erythromycin and *p. multocida* was found resistant to Ofloxacin, Amoxicillin, and Cephalexin and was more sensitive to Tetracycline. moderate sensitive to Erythromycin. The lowest sensitivity was observed for Streptomycin. Similar observations for Streptomycin and Erythromycin (Saad Eldin and Reda, 2016) have also been reported. Balakrishnan *et al*. (2012) observed the acquired resistance of *P. multocida* isolates to Ofloxacin. Kumar *et al*. (2009) found Neomycin the most effective antibiotic against *P. multocida*. Similarly, Shayegh *et al*. (2009) *et al.* (2009) reported 100% sensitivity of the bacteria to Neomycin. Amoxicillin were not very effective. These observations are in accordance to Jabeen *et al*. (2013).

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6. REFERANCES

Asran, E. A., Khalil, S. A., & Hegaz, A. E.-H. M. (2016). Identification and Molecular Analysis of

Pasteurella Multocida Isolated from Rabbits. Alexandria Journal for Veterinary Sciences, 48(1), 34-41.

Balakrishnan, G., Roy, P., Nagarajan, K., Selvaraj, J., & Manohar, B. M. (2012). Isolation, identification and antibiogram of, pasteurella, multocida isolates of, rabbits suffering from pasteurellosis. International Journal for Agro Veterinary and Medical Sciences, 6(1), 58-61.

Barrow, G. L., & Feltham, R. K. (1993). Cowanaud Steels. Manual For The Identification Of Medical Bacteria, 3th Ed. University Press Cambridge.

Cruickshank, R., Duduid, J. P., Marmion, B. P., & Swain, R. H. A. (1975). Medical Microbiology: 12th Ed. Vol. 11, Churchill Livingestone, Edinburgh, London And New York.

DiGiacomo, R., Garlinghouse, J. L., & Van, J. H. G. (1983). Natural history of infection with Pasteurella multocida in rabbits. Journal of the American Veterinary Medical Association, 183(11), 1172-1175.

El-Dirbi, E. (1992). Bacteriological studies on pasteurella infection in rabbits in Kalyoubia Governorate [Egypt]. (M. V. Sci.), Faculty of Veterinary Medicine, Zagazig University-Banha Branch.

Fahmy, M., Anisa, M., & Moustafa, A. (1985). Pathological Studies On Rabbit Diseases. Zagazig Veterinary Journal., 12, 285-304.

Finegold, S. M., & Martin, W. T. (1982). Diagnostic Microbiology 6 Th.Dethe C. V. Mosby Company, Usa.

Horadagoda, N., Hodgson, J., Moon, G., Wijewardana, T., & Eckersall, P. (2001). Role of endotoxin in the pathogenesis of haemorrhagic septicaemia in the buffalo. Microbial pathogenesis, 30(3), 171-178.

Jabeen, A., Khattak, M., Munir, S., Jamal, Q., & Hussain, M. (2013). Antibiotic susceptibility and molecular analysis of bacterial pathogen Pasteurella multocida isolated from cattle. Journal of Applied Pharmaceutical Science, 3(4), 106-110.

Koneman, E. W., Allens, S. D., Dowell, V. R., & Sommers, H. M. (1983). Colour Atlas And Textbook And Diagnostic Microbiology 2 Nd Ed. Lipincott Company, New York. Landon.

Kumar, P., Singh, V., Agrawal, R., & Singh, S. (2009). Identification of Pasteurella multocida isolates of ruminant origin using polymerase chain reaction and their antibiogram study. Tropical animal health and production, 41(4), 573-578.

Mikami, O., Park, J., Kimura, T., Ochiai, K., & Itakura, C. (1999). Hepatic lesions in young rabbits experimentally infected with rabbit haemorrhagic disease virus. Research in veterinary science, 66(3), 237-242.

Mohammed, H. A., Eid, A., & El-Bakrey, R. M. (2013). A Review of Rabbit Diseases in Egypt. WARTAZOA. Indonesian Bulletin of Animal and Veterinary Sciences, 23(4), 185-194.

Mutters, R., Ihm, P., Pohl, S., Frederiksen, W., & Mannheim, W. (1985). Reclassification of the genus Pasteurella Trevisan 1887

on the basis of deoxyribonucleic acid homology, with proposals for the new species Pasteurella dagmatis, Pasteurella canis, Pasteurella stomatis, Pasteurella anatis, and Pasteurella langaa. International Journal of Systematic and Evolutionary Microbiology, 35(3), 309-322.

Oxoid, M. (1982). The Oxoid Culture Media, Ingredients And Other Laboratory Service 5 Th En-, Oxoid Limited Hampshire, R. G. 240 P. W, England.

Petersen, K. D., Christensen, J. P., Permin, A., & Bisgaard, M. (2001). Virulence of Pasteurella multocida subsp. multocida isolated from outbreaks of fowl cholera in wild birds for domestic poultry and game birds. Avian pathology, 30(1), 27-31.

Purushothaman, V., Jayathangaraj, M., Prabhakar, T., & Prabhakar, P. (2008). Incidence of avian pasteurellosis in wild geese in captivity. Tamil Nadu Journal of Veterinary and Animal Sciences, 4 (5), 195 - 197.

Quinn, P., Carter, M., Markey, B., & Carter, G. (1994). Pasteurella species. Clinical Veterinary Microbiology. London: Wolfe Publishing, Mosby-Year Book Europe, 254-258.

Radostits, O., Gay, C., Blood, D., & Hinchcliff, K. (2000). Veterinary Medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th ed. WB Saunders. Philadelphia, London.

Reisinger, R., Heddleston, K., & Manthei, C. (1959). A myxovirus (SF-4) associated with shipping fever of cattle. Journal of the American Veterinary Medical Association, 135(3), 147-152.

Robinson, R. (1944). Human Infection With Pasteurellaseptica. British Medical Journal, 2, 725.

Saad Eldin, W. F. S., & Reda, L. M. (2016). Epidemiological prevalence of Pasteurella multocida in ducks. Japanese Journal of Veterinary Research, 64 (Suppl. 2), S251-S255.

Shayegh, J., Mikaili, P., Sharaf, J. D., & Rastgu, A. (2009). Antimicrobial resistance evaluation of Iranian ovine and bovine Pasteurella multocida. Journal of Animal and Veterinary Advances, 8(9), 1753-1756.