

ISOLATION, IDENTIFICATION AND PATHOGENICITY OF SOME BACTERIAL AGENTS ISOLATED FROM RABBITS

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Abstract

Two hundreds and eighty four field cases of diseased rabbits either living ailing (135) or dead (149) were examined for the presence of bacterial infections. Most of the examined rabbits showed polybacterial infections. *Pasteurella multocida*, *E.coli*, *Bordetella bronchiseptica*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* were isolated from the examined samples in the ratios of 38.98%, 24.92% ,16.61%, 10.54%, 5.11%, 1.92% and 1.92%, respectively. *E.coli* isolates were serotyped as O2 , O78, O119, O114 and untypable which all found to be rhamnose negative. Pathogenicity tests of the isolated bacterial agents were studied . Symptoms, mortality rates and postmortem lesions were recorded and discussed in details. Antibioqram of the isolated bacterial species revealed serotypic variation in drug sensitivity to various antibiotics. Fluoroquinolones antibiotics were shown to be the most effective followed by chloramphenicol, sulphamethoxalin-trimethoprim and gentamycin. Fluoroquinolone are potentially good treatment for most bacterial infections in rabbits.

INTRODUCTION

Domestic rabbits are considered as one of the most important farm animals. Rabbits are reared for many purposes. Meat production is considered as the most important purpose for rearing rabbits. Rabbits were also reared for production of high quality Angora wool and as laboratory tools for many uses

Bacterial agents were incriminated in many serious diseased conditions of rabbits which are associated with substantial morbidity and mortality. Pasteurellosis caused by *Pasteurella multocida* is the most important disease of rabbits respiratory tract which are associated with great losses in both farm rabbits and those raised for laboratory research purposes (Spilki, 2002). Diarrhea caused by *Escherichia coli* is considered a major problem for rabbit breeding (Blanco *et al.*, 1997 and Penteado *et al.*, 2002). Also, *Salmonella spp.*, *Staphylococcus spp.* and *Pseudomonas spp.* were isolated from diseased rabbits (Rougier *et al.*,2006).

The aim of this work is to identify and to evaluate the prevalence of pathogenic bacteria responsible for diseased conditions in rabbits with references to their pathogenicity and susceptibility to different antibiotics.

MATERIALS AND METHODS

Selection of animals

To determine the prevalence of different bacterial agents associated with diseased conditions of rabbits, a total of 135 living ailing and 149 dead diseased rabbits of different ages and from different farms were submitted to Poultry Diseases Department, Animal Health Research Institute, Dokki, Giza, Egypt, and the case history, the clinical signs, and the postmortem examinations were recorded.

Collected samples

Samples were collected from rabbits either living ailing or freshly dead under complete aseptic conditions. The collected samples included: fecal swabs, nasal swabs, lungs, heart blood, livers, kidneys and bone marrows (Table 1)

Bacteriological examination

All samples collected for bacteriological examinations were inoculated in peptone buffer, brain heart infusion broth (oxid) and selenite-F broth (oxid) and incubated at 37°C for 24 hours. Direct cultivation of different samples on sheep blood agar, nutrient agar and MacConkey agar plates were incubated at 37°C for 24 hours. A loopful from each broth culture was inoculated onto blood agar, MacConkey agar (oxid) and XLD agar (oxid) plates and incubated at 37°C for 24 hours. Isolated colonies were picked up and identified morphologically, microscopically and biochemically according to Holt *et al.* (1996).

For primary detection of *Pasteurella multocida*, smears were taken from lungs, livers, and heart blood at autopsy and were stained with Giemsa and Gram stains for detection of Gram negative bipolar coccobacilli of *P. multocida*. For each pasteurella isolate, two mice were inoculated intra-peritoneal (I/P) with 0.5 ml of overnight incubated broth culture of *Pasteurella multocida*. From dead mice, heart blood was smeared and stained with crystal violet for detection of pasteurella bipolarity and inoculated on blood agar plates for re-isolation of the microorganism.

Salmonella isolates were serologically identified and serotyped according to Kauffman-White scheme (Kauffman, 1974) using polyvalent and monovalent O and H salmonella antisera. *E. coli* isolates were serotyped according to Blanco *et al.* (1992) using available O (O1, O2, O8, O78, O114 and O119) antisera.

Antibiotic susceptibility tests

Disc diffusion antimicrobial in-vitro susceptibility testing of the isolated bacterial agents to various chemotherapeutics was performed using discs diffusion technique according to NCCL method(NCCL,2002)

Pathogenicity tests of different isolated bacteria

A total of eighty, 4-6 weeks Boscat rabbits were used for pathogenicity testing of different bacterial species isolated from diseased rabbits proven to be free from pathogenic bacterial infections . The rabbits were divided equally into eight groups (ten rabbits per group).

Rabbits in group (1) were kept as negative control non-infected group inoculated I/P with 0.5 ml of sterile saline solution . Rabbits in groups 4 and 5 were infected intra-nasal with 6×10^8 cfu of *Bordetella bronchiseptica* and *Klebsiella pneumoniae*, respectively for two successive days. Rabbits in groups 3, 6 were inoculated orally with 6×10^8 CFU of *E. coli* and *Salmonella typhimurium*, respectively, while rabbits in groups 2, 7and 8 were inoculated intra-peritoneally with 6×10^8 CFU of *P. multocida* , *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates, respectively . The rabbits in all groups were kept under observation for two weeks after infection during which clinical signs and mortality rates were recorded. Postmortem lesions were recorded from dead rabbits accompanied with re-isolation of the experimentally infected bacteria in each group.

RESULTS AND DISCUSSION

Bacteriological examination of the samples collected from different cases of diseased rabbits revealed that most of the examined cases showed polybacterial infections.

The examined rabbits were suffering from respiratory disorders (nasal discharge, sneezing, cough), diarrhea, sudden death and high mortality. These results agreed with those obtained by Popovic and Trailovic(1988) who reported that enteritis complex, pasteurellosis and continuous staphylococcosis to be the most common causes of losses in rabbit farms.

The identification of the isolated bacterial agents revealed the isolation of *Pasteurella multocida*, *E. coli*, *Bordetella bronchiseptica*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. (Table 2).

Pasteurella multocida isolates constituted the major bacterial agents isolated from diseased rabbits (38.98%) which agreed with Nada,1994 who obtained incidence

of 7.9% *Pasteurella multocida* isolates from diseased and apparently healthy rabbits. The difference in the percentage of isolation may be attributed to the concerning the investigation only on diseased rabbits. Single infection with *Pasteurella multocida* constituted 28.7% of the isolates, while, 71.3 % of *Pasteurella multocida* isolation was mixed with other bacterial agents, mostly as *E.coli*, *Bordetella bronchiseptica*, *Klebsiella pneumoniae* and occasionally accompanied with *Staphylococcus spp.* or *Pseudomonas spp.*

These results agreed greatly with those obtained by Mahmoud *et al.*,(2005) who isolated *Pasteurella multocida* mixed with *E.coli*, *Bordetella bronchiseptica*, and *Diplococcus pneumoniae* from cases of rabbits suffering from respiratory manifestations and diarrhea . Also, Rougier *et al.* (2006) recorded the isolation of *Pasteurella multocida* strains without any other pathogens in 11.6% of the examined rabbit cases suffering from upper respiratory tract infection. They observed the combination of *Bordetella bronchiseptica* and *Pasteurella multocida* in 28.9% of examined rabbits and this dual infection with *Pseudomonas spp.* in 1.7% of the cases, *Staphylococcus spp.* in 5.8% or both strains in 0.8% .

E.coli isolates constituted 24.92% of the total isolates and were mostly isolated from cases suffering from diarrhea and gastrointestinal disturbances. The isolation of *E.coli* strains from cases of diarrheic rabbits was reported by many authors (Blanco *et al.*,1997, Abd-El-Rahman *et al.*, 2005 and Hatab and Moustafa,2007). On serotyping of the isolated *E.coli* (Table 3) they were found to be belonging to O2, O78, O119 and O114, while, 29 (37.2%) were untypable and all the isolates were rhamnose negative. Similar results were obtained by Blanco *et al.* (1997) who isolated 19 different O serogroups from cases of diarrheic rabbits included O2 and O119. However, rhamnose negative strains of *E coli* were usually isolated from cases of severe diarrhea in rabbits.

Salmonella isolates constituted 1.92% of the total isolates , and serotyped as *Salmonella typhimurium*. However, salmonellosis is uncommon disease of rabbits, but may produce epizootics of high morbidity and mortality and the disease in rabbits most often caused by *Salmonella typhimurium* and *Salmonella enteritidis* (Abd-El-Rahman *et al.*, 2005).

The results of the pathogenicity of the isolated bacterial agents (Table 4) revealed that *Pasteurella multocida* isolates were highly pathogenic for rabbits (80% mortalities) associated with symptoms of severe septicemia and re-isolation of the infected microorganisms from the all the internal organs of the dead rabbits. Similar results were obtained by Delong and Manning (1994). On the other hand, *Bordetella bronchiseptica* isolates were found to be low pathogenic (20% mortalities) for rabbits.

The infected rabbits showed symptoms of snuffles-like syndrome associated with serous rhinitis, and the postmortem lesions of the dead rabbits revealed bronchopneumonia and septicemia. *B. bronchiseptica* secretes a dermo-necrotising toxin which is responsible for nasal turbinate atrophy and may play a role in pneumonia and other respiratory infections(Quinn *et al.*,1994).

K. pneumoniae proved to be low pathogenic for rabbits (10% mortalities). The infected rabbits showed symptoms of pyrexia, sneezing and nasal discharges. The postmortem lesions of the dead rabbits revealed bronchopneumonia, pleuritis and congestion of the lungs. The microorganism was re-isolated from lungs and heart blood of dead animal. Similar results were obtained by Dhand *et al.*(2001) who recorded an outbreak of acute pneumonia in angora rabbits due to *K. pneumoniae* infection.

E.coli isolates produced 40% mortalities in infected rabbits. The infected animals showed symptoms of severe diarrhea and weakness. The postmortem lesions revealed severe enteritis with watery contents of the intestines . These results were in agreement with those recorded by Entessar *et al.* (2000) .

Salmonella isolates were found to be highly pathogenic for rabbits (50% mortalities). The infection was accompanied with pyrexia and severe diarrhea and the postmortem lesions showed severe enteritis ,vascular congestion of many visceral organs included: liver ,heart , spleen and kidneys with the presence of necrotic foci . This type of microorganism was isolated from all the internal organs of dead rabbits. Harwood(1989) reported the occurrence of an outbreak caused by *S. typhimurium* in breeding does which spread to young stocks resulting in 50% mortalities.

Staphylococcus aureus was found to be pathogenic for rabbits causing 40% mortalities . The post mortem lesions showed septicemia accompanied by disseminated abscesses in the parenchymatous organs. *Staphylococcus aureus* produces extra-cellular toxins, which are lethal for rabbits, in addition to the production of protein A, which non-specifically binds the Fc portion of IgG and inhibits the opsonization and phagocytosis, and blocks complement –mediated bactericidal mechanism (Delong and Manning, 1994).

Pseudomonas aeruginosa infection resulted in 40% mortality with postmortem lesions of numerous abscesses with blue green discoloration in the infected tissues and severe septicemia.

Results of antibiotic sensitivity tests of different isolated bacterial strains (Table 5) revealed serotypic variation in drug sensitivity pattern, but fluoroquinolone antibiotics (ciprofloxacin and enrofloxacin)were shown to be the most effective antibiotics for all the tested bacterial strains followed by chloramphenicol,

sulphamethoxazole - trimethoprim and gentamycin. These results agreed with those obtained by many authors(Nada 1994, Mahmoud *et al.*, 2005 and Rougier *et al.*,2006).

Conclusively, the results obtained in this investigation revealed that diseased conditions in rabbits were usually caused by mixed infection with different bacterial agents. Poor environmental conditions probably served as predisposing stress factors that may result in outbreaks among rabbit colonies. Also, the early diagnosis of bacterial infections and the use of the effective suitable antibiotics at the therapeutic dose is important to control the disease as early as possible. In this study, fluoroquinolones were shown to be potentially good treatment for most bacterial diseases of rabbits.

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Table 1. Incidence and types of examined samples collected from field rabbits cases.

| No. of examined cases | No. of examined cases | No. of samples |
|-----------------------|-----------------------|----------------|
| Living ailing | | |
| • Fecal swabs | 75 | 75 |
| • nasal swabs | 60 | 60 |
| • | | |
| Dead | 85 | |
| • Lungs | 85 | 85 |
| • heart blood | 85 | |
| • livers | 85 | 64 |
| • Bone marrows | 85 | |
| total | 539 | 284 |

Table 2. Incidence and types of bacterial agents isolated from diseased rabbits.

| Bacterial Isolate | Mixed infection (%) | Single infection (%) | Total No. (%) |
|----------------------------------|---------------------|----------------------|---------------|
| <i>Pasteurella multocida</i> | 87 (71.3%)* | 35(28.7%)* | 122(38.98%)** |
| <i>E. coli</i> | 56 (71.8%)* | 22(28.2%)* | 78 (24.92%)** |
| <i>Bordetella bronchiseptica</i> | 52 (100%)* | 0(0%)* | 52 (16.61%)** |
| <i>Klebsiella pneumoniae</i> | 33(100%)* | 0(0%)* | 33 (10.54%)** |
| <i>Staphylococcus aureus</i> | 16(100%)* | 0(0%)* | 16 (5.11%)** |
| <i>Salmonella typhimurium</i> | 6(100%)* | 0(0%)* | 6 (1.92%)** |
| <i>Pseudomonas aeruginosa</i> | 6(100%)* | 0(0%)* | 6 (1.92%)** |
| total | 256(81.8%)** | 57(18.2%)** | 313 (100%) |

percentage according to total number of the isolated species.

**percentage according to total number of isolates.

Table 3. serotyping of *E. coli* strains isolated from diseased rabbits.

| (o) serotype | No. of isolation | % of isolation | Rhamnose (+) | Rhamnose (-) |
|--------------|------------------|----------------|--------------|--------------|
| 02 | 19 | 24.4* | 0/19 | 19/19 |
| 078 | 14 | 17.9* | 0/14 | 14/14 |
| 0119 | 9 | 11.5* | 0/9 | 9/9 |
| 0114 | 7 | 9.0* | 0/7 | 7/7 |
| untyped | 29 | 37.2* | 0/29 | 29/29 |
| total | 78 | 100 | 0/78 | 78/78 |

* percentage according to total no. of *E. coli* isolates.

Table 4. Pathogenicity tests of bacterial agents isolated from rabbits

| Gp. No. | Bacterial Agent | No. Of Infected Rabbits | Dose Of Infection | Mode Of Infection | Mortality | |
|---------|--------------------------|-------------------------|--------------------|-------------------|-----------|----|
| | | | | | NO | % |
| 1 | Control negative | 10 | 0 | I/P | 0/1010 | 0 |
| 2 | <i>P. multocida</i> | 10 | 6x 10 ⁸ | I/P | 8/1010 | 80 |
| 3 | <i>E. coli</i> | 10 | 6x10 ⁸ | Orally | 4/1010 | 40 |
| 4 | <i>B. bronchiseptica</i> | 10 | 6x 10 ⁸ | I/N | 2/1010 | 20 |
| 5 | <i>K. pneumoniae</i> | 10 | 6x10 ⁸ | I/N | 1/1010 | 10 |
| 6 | <i>S. typhimurium</i> | 10 | 6x10 ⁸ | Orally | 5/1010 | 50 |
| 7 | <i>Staph. aureus</i> | 10 | 6x10 ⁸ | I/P | 4/1010 | 40 |
| 8 | <i>Ps. aeruginosa</i> | 10 | 6x10 ⁸ | I/P | 6/1010 | 60 |

CFU=colony forming unit

I/P=intra-pentoneal

I/N=intra-nasal

Table 5. Antibiotic sensitivity tests of different bacterial agents isolated from rabbits

| Bacterial isolate | percentage of antibiotic sensitive isolates | | | | | | | | | |
|--------------------------|---|-----|-----|-------|------|------|------|-----|-----|-----|
| | AMP | AML | C | CIP | CT | ENR | GM | OT | P | SXT |
| <i>p. multocida</i> | 64% | 70% | 92% | 98% | 60% | 96% | 86% | 46% | 70% | 90% |
| <i>E. coli</i> | 24% | 24% | 94% | 100% | 76% | 98% | 62% | 24% | 30% | 86% |
| <i>B. bronchiseptica</i> | 46% | 53% | 93% | 100% | 66% | 96% | 73% | 63% | 70% | 83% |
| <i>K. pneumoniae</i> | 0% | 0% | 85% | 100% | 50% | 95% | 90% | 25% | 10% | 75% |
| <i>Staph. Aureus</i> | 0% | 0% | 83% | 100% | 50% | 100% | 66% | 0% | 0% | 83% |
| <i>Ps. aeruginosa</i> | 75% | 56% | 25% | 100% | 12% | 93% | 93% | 62% | 75% | 56% |
| <i>Ps. aeruginosa</i> | 0% | 0% | 0% | 83.3% | 100% | 33% | 100% | 66% | 0% | 0% |

MP=ampicillin AML=amoxicillin C=chloramphenicol CIP=ciprofloxacin CT=colistin sulphate

ENR=enrofloxacin

GM=gentamycin OT=oxytetracycline P=penicillin SXT= sulphamthoxazole-trimethoprim

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عزل و تصنيف و ضراوة بعض المسببات البكتيرية المرضية المعزولة من الأرناب

جيهان مصطفى بدر

قسم بحوث وتشخيص أمراض الدواجن - معهد بحوث صحة الحيوان - مركز البحوث الزراعية -
وزارة الزراعة - الدقى - الجيزة

تم فحص ٢٨٤ حالة من الأرناب المريضة ، شملت ١٣٥ حالة مرضية و ١٤٩ حالة نفوق ،
و ذلك لبيان المسببات البكتيرية للحالات المرضية المختلفة. و قد أثبت الفحص البكتيرى إصابة
معظم الحالات المرضية بأكثر من نوع من المسببات المرضية لكل منها . و قد تم عزل كل من
باستريلا مالتوسيدا ، الميكروب القولونى ، البورديتيلا برونكوسيتيكا ، الكليسيبيلا نيمونى ،
المكورات العنقودية الذهبية ، السالمونيلا تيفيميوريم و السودوموناس ايروجينوزا من العينات التي تم
فحصها بنسب عزل مقدارها ٣٨,٩٨% ، ٢٤,٩٢% ، ١٦,٦١% ، ١٠,٥٤% ، ٥,١١% ،
١,٩٢% و ١,٩٢% على التوالي . و قد تم تصنيف عترات الميكروب القولونى المعزولة سيرولوجيا
إلى O114, O119, O78, O2 و غير مصنفة ، ووجدت كلها سلبية لأختبار التخمر لسكر الرامنوز .
تم إجراء إختبارات الضراوة للعترات المعزولة و تمت مناقشة الأعراض و نسب النفوق و الصفة
التشريحية بالتفصيل .

تم إجراء إختبارات الحساسية للميكروبات المعزولة من العترات المختلفة ، حيث ثبت
أختلاف حساسية الميكروبات المختلفة بأختلاف أنواع البكتيريا المعزولة و لكن معظمها أثبت حساسية
كبيرة لمجموعة الفلوروكينولونات من المضادات الحيوية المستخدمة يليها الكلورامفينيكول و
السلفاميثوكسازول-تراى ميثوبريم ثم الجنتاميسين . مما يثبت كفاءة الفلوروكينولونات فى علاج
حالات العدوى بالمسببات البكتيرية المرضية فى الأرناب.