

Isolation Of Bacterial Agents Causing High Mortality In Rabbits

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ABSTRACT

This study was carried out on 60 pet rabbits (40 diseased and 20 freshly dead), of different ages and sex from different localities in Sharkia Province. The bacteriological examination revealed the isolation of *Pasteurella multocida*, *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Citrobacter*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Streptococcus pyogenes* (36.7%, 26.7%, 16.7%, 13.3%, 10%, 8.3%, 6.7%, 5%, 5% respectively). The isolated bacteria were identified on the basis of traditional phenotypic procedures as colonial morphology, microscopical examination, biochemical reaction and serological identification. *P. multocida* was identified according to pathogenicity in mice and serotyping. The antibiogram revealed that the Enrofloxacin was the most effective agent against all bacterial strains, followed by gentamicin and trimethoprim-sulphmethoxazole. Meanwhile all isolates were resistant to chloromphenicol, Ampicillin, Erythromycin, Neomycin and flumquin.

INTRODUCTION

Rabbit industry, in Egypt, is recently progressing rapidly as they have an economic importance. They provide a protein which poor in fat, easily digestible, palatable and nutritious. Rabbits are characterized by high capacity of feed conversion and highly productive with short gestation and lactation periods. Moreover, they contribute to the production of fur, antibodies and antisera. They are used in different research aspects (1).

The rapid expansion of rabbit production is accompanied by several problems (2). It has been reported that some bacterial agents play a very important role in mortalities of rabbits all over the world which lead to a major financial losses (1).

Screening of samples for bacterial causes of deaths revealed the isolation of *Pasteurella multocida*, which is one of the most important pathogens of domestic rabbits. *Pasteurella multocida* was a normal saprophytic organism in the upper respiratory tract of rabbits and under stress factors such as crowding, poor sanitation, transportation, and high ammonia concentrations in the air often stimulate latent *P. multocida* to proliferate and cause Pasteurellosis (1). The disease may take many different forms, respiratory form, including pneumonia and

infection of the nasal passages and sinuses. Infections of the eye membranes, middle ear (otitis media) and genitourinary tract infection (3,4).

Enterobacteriaceae organisms, especially *E. coli* was the most prevalent isolated organism (5-8). The enteric diseases due to Enteropathogenic *E. coli* (EPEC) strains are considered a major cause of high mortality rate and growth retardation leading to substantial economic losses in rabbits, reduce productive activity and inducing pathological damage in different organs (intestine, liver, kidneys and heart) (9).

Salmonellosis is an uncommon disease of rabbit; it indicates unhygienic conditions or ingested contaminated food. It is primary a septicemic disease with a brief clinical course, but diarrhea and abortion may be observed in some rabbits (8). *Staphylococcus* is a common infection of rabbits caused by *Staph. aureus*, characterized by suppurative inflammation of any organ (1). Strepococcosis causes an acute septicemic syndrome in young rabbits (8). *Proteus mirabilis*, *Klebsiella* and *Citrobacter* could be isolated from caecum of rabbit with mucoid enteritis (10). *Pseudomonas aeruginosa* causes an exudative, moist dermatitis, septicemia, pneumonia and diarrhoea (11).

On the illumination of all mentioned data, this study was planned to isolate bacterial agents incriminated in rabbits mortalities, serotyping of these isolates, and determine the sensitivity of these isolates to different chemotherapeutic agents.

MATERIAL AND METHODS

Specimens

Sixty rabbits (40 diseased and 20 freshly dead) of different breeds (New-Zealand, Chanshela and Poskate), of both sex, at ages ranged from three weeks to four months were collected from both governmental and private farms in different localities at Sharkia Province.

Mice

Swiss-mice of 3 weeks old were obtained from Experimental Animal Unit. Vet. Med. Fac. Zag. Univ. Kept under good hygienic conditions and fed on suitable ration to be used in diagnostic experimental purpose.

Necropsy and sampling

Rabbits were examined clinically, and post mortem lesions were recorded. Specimens from affected organs (heart blood, lung, liver, and intestine) were taken from clinically diseased and freshly dead rabbits under aseptic techniques for bacteriological isolation. Heart blood smear and liver impression smears were collected for detection of Pasteurellosis.

Isolation and identification

Samples were inoculated into Nutrient broth, Rappaport broth, brain heart infusion broth and Brilliant green bile broth and incubated at 25°C and 37°C for 24 hours. Subculturing was carried out onto nutrient agar, Eosin-methylene blue (EMB) agar, MacConkeys agar, Brilliant green bile agar, Xylose-Lysine-deoxycholate agar, Mannitol salt agar, and 10% sheep blood agar. The inoculated plates were incubated at 37°C for 24 hours. Identification of bacterial species was assessed by observation of the colonial morphology, Gram staining and biochemical methods. Methods were as follows: catalase, nitrate reduction, H₂S production, indol

production, urease activity, methyl red production, Voges Proskauer test reaction, oxidase reaction, coagulase, motility, citrate, carbohydrate fermentation from glucose, trehalose, xylose, arabinose, fructose, galactose, maltose, mannose, sucrose, lactose and dulcitol, (12).

Animal inoculation test

Suspected *Pasteurella* organisms was confirmed by mice inoculation test (13). Mice were injected with 0.1 ml (1x10³ CFU/ml) of broth culture intraperitoneally from suspected *Pasteurella multocida* colonies. Heart blood and liver smears from died mice were stained with Giemsa and inoculated onto blood agar plates. The plates were incubated under aerobic conditions for 24-48 h at 37°C. Suspected *P. multocida* colonies were subcultured. Isolates were further identified by biochemical tests.

Serotyping of isolated organisms

E.coli isolates were serotyped using polyvalent and monovalent antisera using slide agglutination technique. *Salmonella* isolates were serotyped by using *Salmonella* somatic agglutination serum (polyvalent-o- group A-G) and *Salmonella* flagellar agglutination serum (polyvalent H.phase I and phase II) produced by Difco (Difco Lab.). (Animal Health Research Institute, El-Dokki, Giza, Egypt). Serotyping of *Pasteurella* isolates were carried out by Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

Antibiotic sensitivity test

Bacterial isolates were tested against enrofloxacin, rifamycin, gentamicin, oxtetracycline, doxycycline, flumequine, trimethoprim-sulfamethoxazole, streptomycin, ampicillin, erythromycin, neomycin and chlroamphenicol (Oxoid Lab.), using disc diffusion method (14). The inhibition zones were measured after 18 hours of growth and the recommendation given by the sensitivity discs manufacturer manual were taken in consideration.

RESULTS AND DISCUSSION

The symptoms were different due to different etiological agents but; the main clinical symptoms were depression, anorexia, exhaustion, rough fur, profuse watery diarrhea, bloated abdomen and recumbency. Some rabbits suffered from respiratory distress in the form of cough, sneezing, nasal and eye discharge, listlessness. Similar observation was previously recorded on same bacterial diseases causing early mortality in rabbits (1,8).

Post-mortem lesions observed were congestion of caracses, paint brush haemorrhage on the serosal surface of intestine. The small intestine and caecum were semi-empty and filled with mucoid contents mixed with gas bubbles and congestion of the visceral organs. In some cases pin-point necrotic foci on the surface of the liver were observed. Similar lesions were demonstrated in previous studies (1, 8). Rabbits suffering from respiratory symptoms were Congestion of mucous membrane of the nasal cavity with presence of mucopurulent nasal discharge. pneumonia, consolidation and abscesses with abundant yellowish white caseous pus filling most of the chest cavity. Similar observations were recorded by several investigators (8, 15-18).

Microscopical examination of blood samples and tissue smears of organs, concurrent with bacteriological examination of samples including morphological characters on plates, biochemical reactions which revealed detection of nine types of different Gram's positive as *Staph. aureus* and *S.pyogens* and Gram's negative bacterial isolates as *P.multocida*, *E.coli*, *Salmonella typhimurium*, *Pseud. aerogenosa*, *Citrobacter spp*, *Klebsiella pneumoniae* and *Proteous mirabilis*.

The frequency percentage of bacterial isolates from examined rabbits was shown in Table 1. The highest percentage of bacterial isolation recorded was *Pasteurella multocida* (36.7%) followed by *E. coli* (26.7%), *Staphylococcus aureus* (16.7%), *Salmonella typhimurium* (13.3%), *Streptococcus pyogenes* (10%), *Pseud. aerogenosa* (8.3%), *Citrobacter* (6.7%), *Klebsiella pneumoniae* (5%) and *Proteus*

mirabilis (5%). *Past. multocida*, *Kleb. pneumoniae*, *Staph. aureus*, *E. coli*, *Salmonella*, *Strept. pyogenes*, and *pseud. aerogenosa* were isolated from rabbit carcasses suffering from pneumonia with percentage 82.3%, 25.1%, 21.8%, 19.3%, 15.1%, 13.4%, and 7.8% respectively (19).

Table 1. Incidence of different bacterial pathogens isolated from 60 rabbits.

Bacterial isolates	Number of positive	Percentage %
<i>P.multocida</i>	22	36.7
<i>E.coli</i>	16	26.7
<i>Staph. aureus</i>	10	16.7
<i>Salm. typhimurium</i>	8	13.3
<i>S.pyogens</i>	6	10
<i>Pseud. aerogenosa</i>	5	8.3
<i>Citrobacter spp.</i>	4	6.7
<i>Kleb. pneumoniae</i>	3	5
<i>Proteous mirabilis</i>	3	5
Total	77	*

* N.B.: Mixed infection was recorded

E. coli (57.6%), *Past. Multocida* (33.9%) and *Salmonella spp.* (8.5%) were isolated from different breeds of rabbits at age of 1- 4 weeks (20). *Salmonella typhimurium* in pure culture or mixed with Streptococci or Pseudomonas could be isolated from diseased rabbits at age ranged from 2-3 months suffered from nasal discharge, bloody diarrhea, with 40% mortality rate (21). Bacteria associated with infections of upper respiratory tract of rabbits were *Staph. aureus*, *K. pneumoniae*, *B. bronchiseptica*, *E. coli*, *Pseudomonas spp.*, *Enterobacter spp.*, *Proteus spp.*, *Corynebacterium spp*, *Streptococcus spp.* and *Shigella spp.* (22). *E. coli* (17.7%), *Past.multocida* and *Salmonella spp.* with the same percent (14.6%), *Staph. Aureus* (16.7%), *K. pneumonia* (14%), *Streptococcus pyogenes* (13%) and *Pseudomonas spp* (9%) were associated with mortalities in young rabbits (23). *E. coli*, *Staph. aureus*, *K. pneumonia* and *clostridium* were isolated from rabbits with enteritis (9,24). These results were consistent

with previous recorded studies (8,25) which results revealed isolation of *E. coli*, *Staph. aureus*, *Past. multocida*, *Strept. pyogens*, *proteus spp.*, *K. pneumonia* and *Salmonella typhimurium* in percentage of 29.8% 8.5% 5.3% 3.8%, 3.7%, 1.5% and 0.25% respectively which were the main bacterial pathogens that were responsible for high mortalities in broiler rabbits. In contrast. *E.coli* was isolated in a percentage reached to 80% from rabbits suffered from diarrhoea, in addition to *proteus mirabilis* (27.3%), *Enterobacter* (22.3%), *K. pneumonia* (13.6%) and *Salmonella typhimurium* (13.7%) (26).

Serological identification of E.coli isolates

Results of *E.coli* serotyping revealed that identification of the following serotypes O128, O125, O119 and O55. Most of these serotypes were isolated from diseased rabbits. Results obtained were similar to previous several reports (1,20,25,26). Recently over 120 strains of *E. coli* were isolated from diarrheic rabbits with O126, O124, O125, O128 and O119 serotypes (27). Hatab and Moustafa (23) recorded that O126, O26 and O59 were

associated with newly weaned rabbit diarrhea. El Bakery (9) declared that several *E.coli* strains varying in virulence cause diarrhea in rabbits belonged to different serotypes O26 , O114 ,O44, O128 , O55 and O75 .

Serological identification Salmonella

Salmonella serotyping revealed that they belonged to *Salmonella typhmerium* .similar results were recorded (8,20). *Salmonella typhmerium* and *Salmonella enteritidis* were also isolate (23,26). In addition *Salmonella dublin* and *Salmonella gallinarum* were isolated from broiler rabbits (25).

Pathogenicity results

Mice inoculated with *Pasteurella multocida* suspension were died within 24-36 hrs post inoculation. Direct smear, from heart blood of dead mice were stained with Giemsa revealed the bipolar bacilli .*P.multocida* was reisolated from heart blood of freshly dead mice. These results agree with those previodsly reported (13,18).

Antimicrobial sensitivity test

Table 2. Antibiotic sensitivity test for the bacteria isolated from examined samples of rabbits.

Bacterial isolates / Antibiotic	Disc con	P. multocid	E. coli	Staph aureu	Sal. typhi	Str. pyog	Pseud aero	Citro	Kl. pneu	Prot.marb
Enrofloxacin	5 µg	S	S	S	S	S	S	S	S	S
Gentamicin	10 µg	S	S	S	R	S	R	Ms	S	R
Rifamycin,	5 µg	R	R	S	R	S	R	R	S	R
Trimethoprim	25 µg	S	S	S	R	S	R	S	S	Ms
oxitetracycline	30 µg	S	Ms	R	R	R	Ms	R	S	R
Streptomycin	10 µg	S	R	S	R	R	R	R	S	R
Doxycycline	30 µg	S	S	S	R	R	R	R	S	R
Flumequine	30 µg	R	S	R	S	R	S	Ms	R	Ms
Ampicillin	10 µg	Ms	R	S	R	S	Ms	S	R	Ms
Erythromycin	15 µg	Ms	R	S	R	R	R	R	S	R
Neomycin	30 µg	R	Ms	R	S	R	R	R	R	Ms
chlroamphenicol	30 µg	R	R	R	S	R	R	R	R	Ms

S: sensitive

R: resistant

Ms: moderately senesive

The extensive use of antibiotics as growth promoters and prophylactic agents for disease control in veterinary medicine has undoubtedly been responsible for large

numbers of bacteria that have become resistant to different antibiotics. Result of the antibiotic susceptibility pattern of the different bacterial isolates is shown in Table 2.

Results of susceptibility testing showed that most of the bacterial isolates were highly sensitive to Enrofloxacin followed by gentamicin and trimethoprim-sulphamethoxazole. Meanwhile all isolates were resistant to chloramphenicol, Ampicillin, Erythromycin, Neomycin and flumquin. Nearly similar results were reported by several investigators (8,23,25-27). From the above result it is appeared that *Pseud. Aerogenosa* is more resistant to most of the used antibiotics. Similar result was obtained which proved that *Pseudomonas spp.* isolated from rabbits were highly resistant to all antibiotics, but sensitive to bacteriocins (11).

Conclusion

From the present results it could be concluded that the bacterial agents (*Pasteurella multocida*, *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Citrobacter*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Streptococcus pyogenes*) have a highly fatal effect on rabbit populations. The mortalities in the rabbit farms were diagnosed from clinical signs, post mortem lesions, cultural isolation and identification of the organisms. Enrofloxacin is the most effective agent against all bacterial strains. Chemotherapy and reduction of stress by hygienic management in farm premises, provision of good nutrition, maintenance of adequate floor space, help prevention of infection.

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الملخص العربى

عزل بعض البكتيريا المسببة للوفيات العالية فى الارانب

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أجريت هذه الدراسة لتبين مدى انتشار الأمراض البكتيرية المسؤولة عن حدوث نسبة نفوق عالية بالأرانب وقد أجريت هذه الدراسة على عدد ٦٠ أرنب مصابة بأسهالات وأعراض تنفسية مختلفة فى اعمار واجناس وسلالات مختلفة من أماكن مختلفة فى محافظة الشرقية وكانت الأعراض الظاهرية للعينات المفحوصة تتمثل فى خمول عام وفقدان الشهية وأسهالات وبعض الأعراض التنفسية متمثلة فى الكحة والعطس وإفرازات من الأنف وأظهرت الصفة التشريحية عن وجود احتقان عام فى الأعضاء الداخلية وكذلك الأمعاء مع امتلائها بالغازات والسوائل ووجود تنكز فى الكبد واحتقان فى الجدار المبطن لتجويف الأنف ووجود مواد متجبنة فى التجويف الصدرى.

وقد أسفرت نتائج العزل البكتريولوجى عن وجود ميكروب الباستريلا مالتوسيدا و الاشيريشيا كولاي والميكروب العنقودى والسلمونيلا تيفيميوريم والسودوموناس ارجينوزا والستروباكتري و الكليبيسيلا نيمونى والبروتيس والأسترىبتوكوكس بيوجين بالنسب الاتية ٣٦,٧% و ٢٦,٧% و ١٦,٧% و ٣,١٣% و ١٠% و ٣,٨% و ٧,٦% و ٥% و ٥% على التوالى وقد تم تصنيف عترات الاشيريشيا كولاي سيرولوجيا الى O128, O125, O119, O55 .

باجراء اختبار الحساسية تبين أن معظم هذه الميكروبات كانت شديدة الحساسيه للانروفلوكساسين والجنتاميسين والتراميسوبريم وكانت مقاومة لكل من الأمبسلين و الكلوروميثانيكول و الأريثروميسين والنيوميسين والفليموكوين. وقد تم مناقشة النتائج والتوصيات الواجب إتباعها للمحافظة على الأرانب كثروة قومية.