

Original Research Article

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Isolation of Multidrug Resistant *Staphylococcus aureus* from Pyometra in Rabbit

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ABSTRACT

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Pyometra infections are commonly found in rabbit. In this study, a two-year-old rabbit with the history of heavy cervico-vaginal discharge was examined. Vaginal swab was collected aseptically and processed for bacterial identification. Gram positive cocci was identified in gram staining. *Staphylococcus aureus* was isolated on selective media (Baired Parker Agar) exhibited jet-black colonies with halo around and confirmed by biochemical tests such as catalase positive, oxidase negative and coagulase positive. Antibiogram assay of *Staphylococcus* strain revealed multidrug resistance for many antibiotics. Only quinolones and fluoroquinolones groups of antibiotic were found sensitive for *Staphylococcus aureus* infection.

Introduction

Rabbits are not only most commonly used lab animals for experimental studies but a domestic companion animal too. Both female and male rabbits can be affected by infections of the urogenital tract. Endometritis, orhitis and pyometra are disorders commonly found in rabbits. Many contagious infections of reproductive system has been reported which leads the abortion, sub-fertility and other

reproductive disorder (Jacques *et al.*, 1986). *Staphylococcus aureus* has been reported to be the most serious problem in these farms and this organism has been isolated from 70% of the infected animals (Segura *et al.*, 2007). It has been found the most prevalent bacteria in cases of pyometra and pneumonia (Segura *et al.*, 2007). Although development of the disease depends on the general resistance of the host and virulence of bacteria. *S. aureus* have been shown to be general factors

for the testicular inflammation and endometritis in rabbits which leads to decrease fertility. Another study showed that staphylococci based mastitis was one of the main factors of culling of adult rabbits in farms (Viana *et al.*, 2011). As the bacterial infection is one of the main causes for existence of the disease, the infections of uterus are treated with antibiotics. But due to continuous emergence of drug resistant bacterial strains the problem become persists. The incidences of multidrug resistance staphylococcus infections have been reported to be on upsurge (Goni *et al.*, 2004; Vancraeynest *et al.*, 2004). The indiscriminate use of antibiotics results emergence of resistant micro-organisms (Phillips *et al.*, 2004). In present study, a case was examined for type of bacterial infection in uterus and its resistance against different antibiotics.

Materials and Methods

Sample collection

A 2-year-old rabbit with the history of severe vaginal discharge was examined in the TVCC hospital. The rabbit did not show any other apparent illness. The sample of vaginal discharge of rabbit was collected aseptically with sterile cotton swab and sent to the Department of Veterinary Microbiology, College of Veterinary Sciences and AH MHOW for bacteriological examination of cervico-vaginal discharge.

Isolation and identification of bacteria

The sample was processed same day. It was inoculated in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hrs. Nutrient Agar (NA) was inoculated with the overnight (BHI) broth for primary isolation. The inoculated plates were incubated at 37°C for 24 hours. On the basis of colony characteristics on different media and gram staining it was subcultured on Baird Parker

Agar (BPA). The colony exhibited the black colonies on BP agar, which is the typical characteristic of *Staphylococcus aureus*. The isolate was further confirmed with the biochemical test such as catalase, oxidase and coagulase test.

Antibiotic sensitivity test

Antimicrobial sensitivity test of isolated *Staphylococcus aureus* was performed by the standard disc diffusion procedure as per CLSI standards (CLSI, 2014). The *Staphylococcus aureus* strain was tested on Muller Hinton agar against 25 µg antibiotics such as Ampicillin 25µg, Ampicillin/ sulbactam A/S, Amikacin 30µg, Amoxycillin/ clavulonic acid 30, Ceftriaxone 30, Cefotaxime/ clavulanic acid 30, Ciprofloxacin 5µg, Co-triamoxazole 25µg, Doxycycline hydrochloride 30µg, Enrofloxacin 10µg, Gentamycin 10µg, Levofloxacin 5µg, Nitrofurantoin 300µg, Norfloxacin 10, Ofloxacin 5µg, Penicillin G 10µg, Sulphafurazole 300µg, Tobramycin 10µg, Moxifloxacin 5µg, Cefuroxime 30µg, Oxytetracycline 30µg, Ceftazidime/ clavulonic acid 30µg, Gatifloxacin 10µg, Azithromycin 15µg and Cloramphenicol C 30µg.

Results and Discussion

It has been reported that there are age-dependent differences in the diseases affecting rabbits; gastrointestinal and respiratory diseases are the most common conditions affecting younger rabbits (Hinton, 1979; Vors, 1980), whereas respiratory and reproductive diseases are the principal causes of death in adult does (Rosell, 1996).

In present study, preliminary isolation and gram staining exhibited a gram-positive cocci bacteria (Fig. 1). The isolate found positive for catalase (Fig. 2) and negative for oxidase were suspected to be staphylococcus.

Fig.1 Gram positive cocci

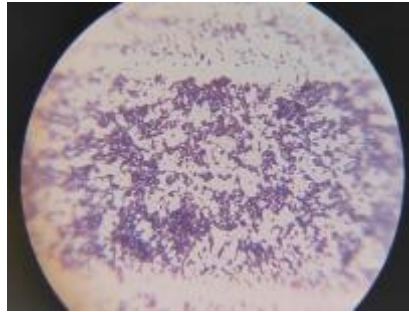


Fig.2 Catalase positive test

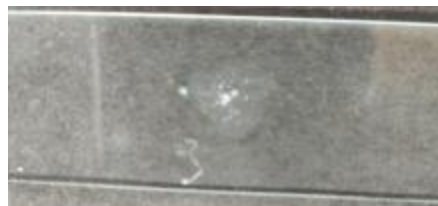
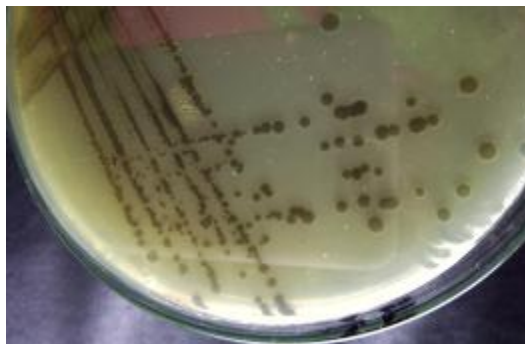


Fig.3 Black colonies of Baird Parker Agar



On the basis of coagulase test strain was identified as coagulase positive staphylococcus strain. On Baird Parker agar, staphylococcus strain exhibited black colonies (Fig. 3) with halo around which is typical characteristic of *Staphylococcus aureus* strain.

In rabbits, problems of staphylococcosis arise when *S. aureus* bacteria infect small dermal lesions and invade subcutaneous tissue (Okerman *et al.*, 1984) and dermatitis considered the main prominent signs in *S. aureus* infection in rabbits (Segura *et al.*, 2007). In a previous study, *S. aureus* infection

was sporadically observed in internal organ abscesses with predominance in lungs, liver and uterus (Bogaert *et al.*, 2003; Holliman and Girvan, 1986; Segura *et al.*, 2007).

In present study, AntibioGram of staphylococcus strain showed the sensitivity for only four antibiotics among 25 tested antibiotics. i.e. Ciprofloxacin, Enrofloxacin, Norfloxacin and Levofloxacin. Multi drug resistance strain of staphylococcus was identified. In a study, the percentage of antimicrobial-resistant strains was high (59%), with resistance to oxytetracycline and

Vancomycin (91.67%) being the most frequently observed (Goni *et al.*, 2004; Vancraeynest *et al.*, 2004). These high drug resistance levels by the rabbit isolates could be explained by the intensive use and misuse of antimicrobial drugs in human and veterinary medicine leads to the selection of resistant organisms (Smith and Coast, 2002; Cizman, 2003; Levy and Marshall, 2004). There is an additional concern over the potential transmission of resistant organisms and resistance genes from livestock to man through consumption and handling of foods derived from animals (Tollefson and Karp, 2004). Antibiotic resistance could be a factor of failure of curing the herd by different antibiotic administered; another factor could be pathogenic ability of *S. aureus* strains and the endemic pattern of the disease.

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