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Antibiotic sensitivity test on pathogens causing reproductive tract infection in thoroughbred mares

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Abstract

Repeat breeding is one of the main problems associated with antibiotic resistance. The current study was to identify the bacterial infection and ascertain the degree of sensitivity of the isolated bacteria against a panel of antimicrobial agents. In a breeding season, 45 mares (42%) were positive for culture and 62 mares (58%) were negative for any pathogen. A total of 45 bacterial isolates were done antibiotic sensitivity test. They were 31 numbers of *Escherichia coli* and 8 numbers of *Streptococcus* spp. beta haemolytic group and 6 numbers of *Staphylococcus* spp. Ofloxacin showed higher sensitivity towards *E. coli* with 55%, Cefotaxime showed higher sensitivity towards *Streptococcus* spp. beta haemolytic group with 76% and Amoxicillin+clavulanate and Gentamicin showed 100% sensitivity towards *Staphylococcus* spp. The same organisms showed different sensitivity with different mares. Mainly *E. coli* was highly variable. Hence, this study suggests that antibiotic sensitivity test is highly recommended in *E. coli* infection.

Keywords: Thoroughbred mares, cervical swabbing, antibiotic resistance, *Escherichia coli*

Introduction

Endometritis in mares had been known to inflict major losses in the broodmare practice, as its prevalence ranges from 25 to 60% in barren mares. A timely diagnosis and an efficacious treatment in the breeding season would be essential for a successful breeding outcome^[1]. The diagnosis of endometritis could be critical in veterinarian's attempts to treat infertility. Clinical examination, trans-rectal palpation, ultrasonography of the reproductive tract, vaginal speculum examination, uterine culture and endometrial biopsy were the diagnostic modalities^[2]. Antibiotics could often be selected based on data from earlier studies, if the treatment had to be performed earlier without a microbiological diagnosis. However, the bacterial species isolated, as well as their susceptibility to antibiotics, could vary over time as well as from one population of horses to another^[3, 4]. In a previous study, *Streptococcus equi* spp. *Zooepidemicus* and *Escherichia coli* were the most common isolated organisms that accounted for 50-80% of bacterial endometritis. Other pathogens were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Corynebacterium* spp., *Actinobacter* spp., *Proteus* spp., *Citrobacter* spp., *Enterobacteria cloaca*, *Serratia* and *Streptococcus*^[5, 6].

Materials and Methods

Study population

This study was conducted in a thoroughbred stud farm located in South India. Cervical swabs were taken when the mares were in heat. The mares which showed positive for cervical swab culture were taken for this study. Out of 107 mares, 45 mares (42%) were positive for culture and 62 mares (58%) were negative for any pathogen. These 45 samples were taken for further study.

Collection of samples and Isolation of organism

Perineum was washed thoroughly with 0.1% Povidone Iodine solution in cotton and dried. Sterilized vaginal speculum (figure.1) was inserted into the vagina and sterile swab stick was introduced into cervix approximately 5 cm deep. Swabs were collected by rotating them clockwise and anti-clockwise to obtain adequate sample.

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Within one hour of sampling, swabs were streaked on sheep blood agar and McConkey agar. These agar plates were used for isolating, cultivating and determining hemolytic reactions of pathogenic micro-organism. Plates were inverted and incubated at 37 °C and examined 24 hours and 48 hours.

Antibiotic sensitivity test

Isolated organisms were taken in a sterile swab stick that was wet with sterile water. Then the swab was smeared in the agar plates three times in different directions and corner of the plate was also streaked fully. Aseptically antibiotic discs were placed on the medium and pressed gently on to the surface to get full contact with the medium. Thumb forceps was used to carry the discs after heating in the spirit lamp. The plates were inverted, incubated at 37 °C and examined after 24 hours.

Blood agar was used for *Staphylococcus* spp. and *Streptococcus* spp. beta haemolytic group. Nutrient agar was used for *E. coli* (figure. 5). But sometimes McConkey agar or blood agar was used for *E. coli* based on the medium availability due to COVID-19 (figure. 6).

A total of seven antibiotics were used for sensitivity analysis. All the antibiotics were procured from HiMedia Laboratories Pvt. Ltd. (Table. 1)

Table 1: Antibiotic drugs with concentration

S. No	Antibiotics	Abbreviation	Concentration
1	Amoxicillin + Clavulanate	AMC	20/10mcg
2	Amikacin	AK	30mcg
3	Azithromycin	AZM	30mcg
4	Cefotaxime	CTX	30mcg
5	Gentamicin	GEN	10mcg
6	Ofloxacin	OF	5mcg
7	Tetracycline	TE	30mcg

The susceptibility of the microorganisms to these different antibiotics was noted as Sensitive (S), Intermediate (I) and Resistant (R). Sensitivity results were made based on Zone Size Interpretative Chart (as per CLSI & EUCAST) [10].

Results and Discussion

A total of 45 bacterial isolates were done antibiotic sensitivity test. They were 31 numbers of *E. coli* (figure. 2) and eight numbers of *Streptococcus* spp. beta haemolytic group (figure. 3) and six numbers of *Staphylococcus* spp. (figure. 4).

Sensitivity to *E. coli*

Ofloxacin showed higher sensitivity towards *E. coli* with 55% followed by Azithromycin, Gentamicin and Amikacin with 45%, 42% and 35% respectively. In one study, Enrofloxacin was the only drug that was sensitive to all *E. coli* cultures [7] and in another study Gentamicin was sensitive to *E. coli* with 73.5% [8]. It is very high when compared to our study.

Tetracycline showed higher resistance towards *E. coli* with 68% followed by Cefotaxime, Amoxicillin+clavulanate and Amikacin with 65%, 48% and 39% respectively.

Sensitivity to *Streptococcus* spp. beta haemolytic group

Cefotaxime showed higher sensitivity towards *Streptococcus* spp. beta haemolytic group with 76% followed by Amoxicillin+clavulanate and Azithromycin had same sensitivity of 63%. Earlier reports stated that beta haemolytic *Streptococcal* were susceptible to ampicillin and beta –lactam antibiotics [7] and Amoxicillin/Clavulanic acid showed highest

susceptibility to *Streptococcus* spp. with 82.7% [8]. In our study, Amoxicillin+clavulanate were sensitive to 63% of the isolates.

Tetracycline showed higher resistance towards *Streptococcus* spp. beta haemolytic group with 76% followed by Amikacin and Gentamicin with 62% and 25% respectively. The same author also reported that beta haemolytic *Streptococcal* were resistant to Gentamicin and Tetracycline [7].

Sensitivity to *Staphylococcus* spp.

Amoxicillin+clavulanate and Gentamicin showed 100% sensitivity towards *Staphylococcus* spp. followed by Azithromycin and Cefotaxime with 83% and 67% respectively. But in a previous study, 96.2% of the *Staphylococcus aureus* was susceptible to Amoxicillin+Clavulanate [8]. This is lower when compared to our study.

Amikacin showed 100% resistance towards *Staphylococcus* spp. followed by Ofloxacin and Tetracycline with 33% resistance. But Amikacin had the broad spectrum activity against uterine pathogens and had excellent activity against *Staphylococcus aureus* in early report [9]. This variation maybe due to different geographical locations.



Fig 1: Cervical swabbing after the application of vaginal speculum

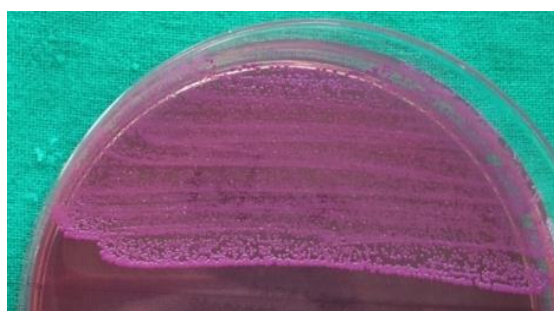
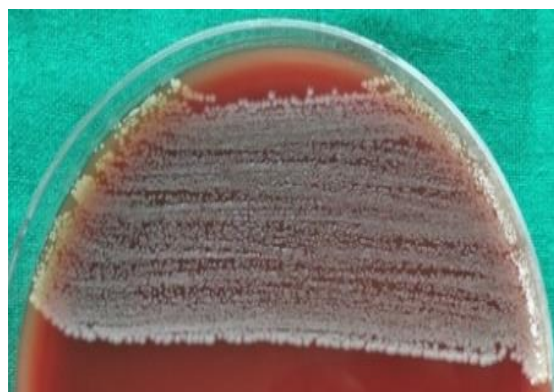


Fig 2: *E. coli* on blood agar agar(above) and McConkey (below)

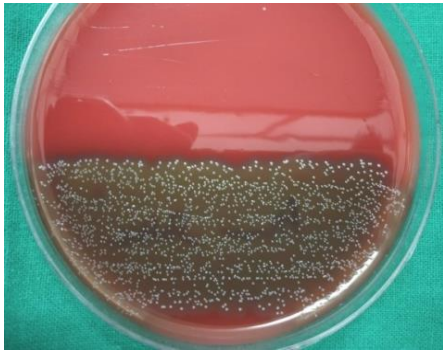


Fig 3: Beta hemolytic *Streptococci* spp. On Blood agar

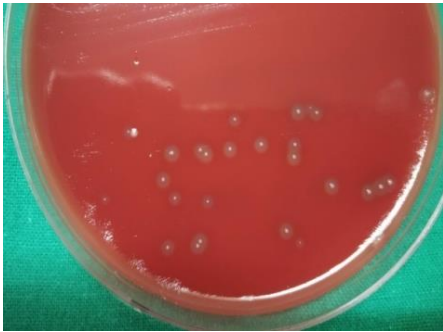


Fig 4: *Staphylococcus* spp. on Blood agar



Fig 5: ABST results in Nutrient agar



Fig 6: ABST results in McConkey agar

Mainly *E. coli* is highly variable. Tetracycline is resistant to all the three pathogens in our study. This study suggests that antibiotic sensitivity test is essential to prevent repeat breeding associated with antibiotic resistance.

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Conclusion

This study shows that only *Staphylococcus* spp. show 100 percent sensitivity to Amoxicillin+Clavulanate and Gentamicin with all the mares. But *Streptococcus* spp. beta haemolytic group and *E. coli* are variable to antibiotics.