Egypt. J. Vet. Sci. **Vol. 54,** No. 2, pp. 287-295 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Identification and Antimicrobial Susceptibility of Uterine Bacterial Isolates From Mares with Endometritis in India

Aeknath Virendra[1], Sarita U. Gulavane[1], Zulfikar A. Ahmed[2], Varsha D. Thorat[3], Ravindra J. Chaudhari[1], Sandeep M. Gaikwad[1], Raju R. Shelar[1], Shailesh D. Ingole[4], Afroza Khanam[5], Firdous A. Khan[5]

[1]*Department of Animal Reproduction, Gynecology and Obstetrics, Mumbai Veterinary College, Mumbai, 400012, Maharashtra, India.*

[2]*Equus stud farm, Village Shirgoan, Talegoan Dhabade, Salumbra, Pune, 410506, Maharashtra, India.*

[3]*Department of Veterinary Microbiology, Mumbai Veterinary College, Mumbai, 400012, Maharashtra, India.*

[4]*Department of Veterinary Physiology, Mumbai Veterinary College, Mumbai, 400012, Maharashtra, India.*

[5]*Department of Large Animal Medicine and Surgery, School of Veterinary Medicine, St. George's University, Grenada, West Indies.*

BACKGROUND: Endometritis is a major cause of infertility in mares worldwide. In India, information on endometritis in mares is limited. **Objectives**: The purpose of this study was to identify uterine bacterial isolates from endometritis-affected mares in India and evaluate their antimicrobial susceptibility pattern. **Methods**: Endometritis was diagnosed based on history of infertility and findings from transrectal examination, visual inspection of low-volume uterine lavage samples, endometrial cytology and bacterial culture. Uterine lavage samples were collected from 15 mares with endometritis and subjected to bacterial culture and antimicrobial susceptibility test. **Results**: The most common bacterial isolates were *Escherichia coli* (*E. coli*, 40.0%), *Staphylococcus* (26.7%), *Streptococcus* (20.0%), and *Klebsiella* (13.3%) species. Most of the *E. coli* isolates were susceptible to gentamicin (83.3%), netilmicin sulphate (83.3%), tobramycin (83.3%), nitrofurantoin (83.3%), amikacin (66.7%), and ampicillin/sulbactam (66.7%). All of the *Staphylococcus* isolates were susceptible to gentamicin, amikacin, and ampicillin/sulbactam. All *Streptococcus* and *Klebsiella* isolates were susceptible to gentamicin, amikacin, and netilmicin sulphate. Furthermore, all *Klebsiella* isolates were susceptible to sparfloxacin, ampicillin/sulbactam, tobramycin, cefadroxil, and co-trimoxazole. Ampicillin and amoxicillin were the least effective antimicrobials with susceptibility percentages ranging between 0 to 33.3% for the various isolates. **Conclusions**: The results suggest that *E. coli* is the most common cause of equine endometritis in India. Based on the antimicrobial susceptibility patterns, it can be concluded that gentamicin, amikacin, netilmicin sulphate and ampicillin/sulbactam may be the best first-line antimicrobials for clinical application in equine endometritis cases in India while awaiting antimicrobial susceptibility test results.

Keywords: Antimicrobials, Bacteria, Endometritis, Horse, Uterus

Corresponding author: Aeknath Virendra, E- mail.: draeknathvirendra@gmail.com, Tel. +91-8989821354
Firdous A. Khan, Email: fkhan8@sgu.edu; ORCID ID: 0000-0002-1903-8784
(*Received* 01/11/2022; *accepted* 01/01/2023)
DOI. 10.21608/EJVS.2023.171432.1407

Introduction

Endometritis is an important problem facing the global equine industry. In a survey completed by equine practitioners, it was ranked as the third most common medical condition in horses [1]. Endometritis is a major cause of infertility in mares [2] and it results in significant losses to the equine breeders due to conception failures, early embryonic losses, and diagnostic and therapeutic expenses [3-6]. Although endometritis in mares has been attributed to a multitude of infectious and non-infectious causes [7], bacteria are considered the most common infectious cause of this condition. Bacterial endometritis has been reported in as high as 60% of barren mares [8]. The most common isolates reported in the literature were *Escherichia coli*, *Streptococcus* species (more commonly *Streptococcus equi* subspecies *zooepidemicus*), *Pseudomonas aeruginosa*, *Klebsiella* species and *Staphylococcus* species [9-14].

Treatment of bacterial endometritis in mares involves use of antibiotics, often in combination with other approaches such as uterine lavage and oxytocin to facilitate uterine clearance [2,7]. Selection of the first-line antimicrobial while awaiting results of antimicrobial susceptibility tests is usually based on clinician preferences and findings from previous antimicrobial susceptibility studies on equine uterine isolates. Antimicrobial susceptibility studies on equine uterine isolates have been conducted in different countries including Sweden [9], Italy [11], Egypt [12], and Spain [13]. However, to our knowledge there is no published information about bacterial causes of equine endometritis and their antimicrobial susceptibility in India. The present study was, therefore aimed at identifying uterine bacterial isolates from mares with endometritis in India and evaluating their antimicrobial susceptibility pattern.

Material and Methods

Animals

All animal handling and clinical procedures performed in this study were approved by the Institutional Ethics Committee for Veterinary Clinical Research. Forty-eight Thoroughbred mares from three different stud farms in and around Pune, India were used in this study. Fifteen out of the forty-eight mares had a history of infertility and were diagnosed to have endometritis on the basis of transrectal examinations indicating excessive intrauterine fluid accumulation (> 2 cm), turbid appearance of low-volume uterine lavage samples, inflammatory endometrial cytology (greater than 2 neutrophils per high power field), and positive bacterial culture [2,15].

Collection of low-volume uterine lavage samples

Mares were sampled during oestrus detected on the basis of behavioural signs and results of transrectal examinations. After restraining each mare in stocks, the tail was wrapped, and the perineum was cleaned using soap and water. A low-volume uterine lavage sample was transcervically collected from each mare using sterile water and a sterile lavage assembly.

Bacterial culture and characterization

The low volume uterine lavage samples were inoculated in BHI (Brain heart infusion) agar, MacConkey agar, EMB (Eosin-methylene blue) agar, Blood agar and Mannitol salt agar (MSA) plates using the streak plate method. The agar plates were then incubated at 37°C for 24 hours. The growth from agar plates was smeared on a clean glass slide followed by Gram staining to identify the type of bacteria. The pure culture was then inoculated in sterile BHI broth for overnight at 37°C followed by inoculation of the turbid broth on sterile BHI slant and kept at 37°C for 24 hours for obtaining isolates.

Various tests such as Methyl red (MR) test, Voges-Proskauer test (VP), Indole test, Nitrate reduction test, Urea hydrolysis test, Citrate reduction test, Catalase test, and oxidase test were performed for the biochemical characterization of the bacterial isolates. These tests included.

Antimicrobial susceptibility test (AST)

The isolated bacterial cultures from the BHI slants were inoculated in the Mueller Hinton (MH) broth and kept at 37°C for overnight. After inoculation density (~0.5 McFarland), incubation time and temperature standardization, antibiotic sensitivity of each bacterial isolate was tested on MH agar *via* the Kirby-Bauer disk diffusion method. The ICOSA universal I and ICOSA universal II discs (HiMedia Laboratories Private Limited, Mumbai, India) containing

Amikacin, Ampicillin, Ampicillin/Sulbactam, Amoxicillin, Azithromycin, Cefaclor, Cefadroxil, Cefoperazone, Cefotaxime, Ceftazidime, Ceftriaxone, Cefuroxime, Chloramphenicol, Ciprofloxacin, Clarithromycin, Cloxacillin, Co-Trimoxazole, Erythromycin, Gentamicin, Nalidixic acid, Netilmicin, Nitrofurantoin, Norfloxacin, Penicillin-G, Roxithromycin, Sparfloxacin, Tobramycin, and Vancomycin were used in this test. Bacterial growth inhibition around each antibiotic disc was then estimated, compared with standard chart and isolates were categorized as susceptible (sensitive) and non-susceptible (intermediate and resistant) for calculation of susceptibility percentage [16,17].

Statistical analyses

Data was analyzed using statistical software (IBM® SPSS® for Windows, Version 28.0. Armonk, NY: IBM Corp). Antibiotic susceptibility (in percentage) of each bacterial isolate was calculated and the associated 95% confidence intervals were determined using the Clopper-Pearson exact method.

Results

Among the 48 uterine lavage samples subjected to culture, bacterial growth was observed in 15 samples. Of the 15 isolates, 8 (53.0%) isolates were Gram-positive and 7 (47.0%) isolates were Gram-negative. The isolated bacterial species were *E. coli* (40.0%; 6/15), *Staphylococcus* (26.7%; 4/15), *Streptococcus* (20.0%; 3/15), and *Klebsiella* (13.3%; 2/15). Gram-negative rod-shaped *E. coli* showed pink colour (suggestive of lactose fermentation), smooth round opaque colonies on MacConkey agar and characteristic green metallic sheen on EMB agar. *Klebsiella* isolates were Gram-negative rods and produced characteristic highly mucoid colonies on MacConkey agar. The golden-yellow colonies on BHI agar, pink colour colonies on MSA and Gram-positive cocci in characteristic irregular

TABLE 1. Identification of the uterine bacterial isolates by biochemical tests

Name of the test	*Escherichia coli*	*Staphylococcus* spp.	*Streptococcus* spp.	*Klebsiella* spp.
Indole	Positive	Negative	Negative	Negative
Methyl red	Positive	Positive	Positive	Negative
Voges-Proskauer	Negative	Positive	Negative	Positive
Citrate reduction	Negative	Negative	Positive	Positive
Catalase	Positive	Positive	Negative	Positive
Oxidase	Negative	Negative	Negative	Negative
Urea hydrolysis	Negative	Positive	Negative	Positive
Nitrate reduction	Positive	Positive	Negative	Positive

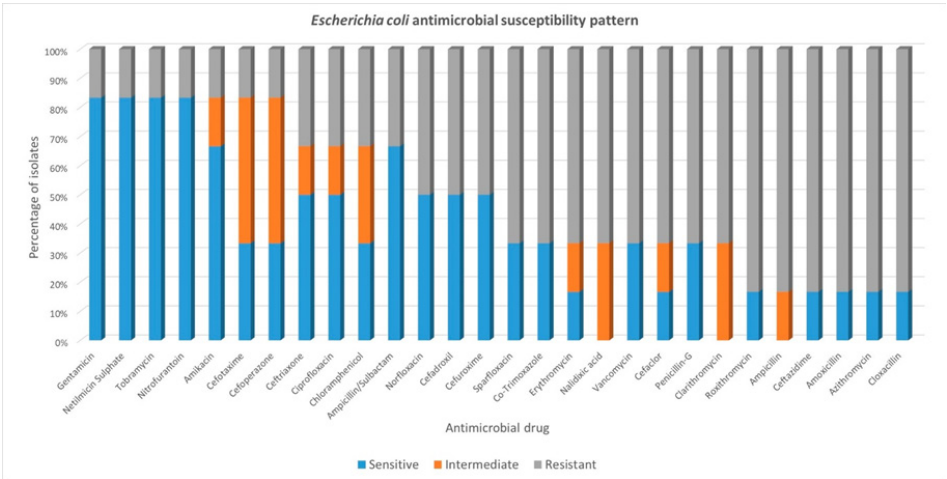

Fig. 1. Antimicrobial susceptibility pattern of *E. coli* isolates from mares with endometritis in India

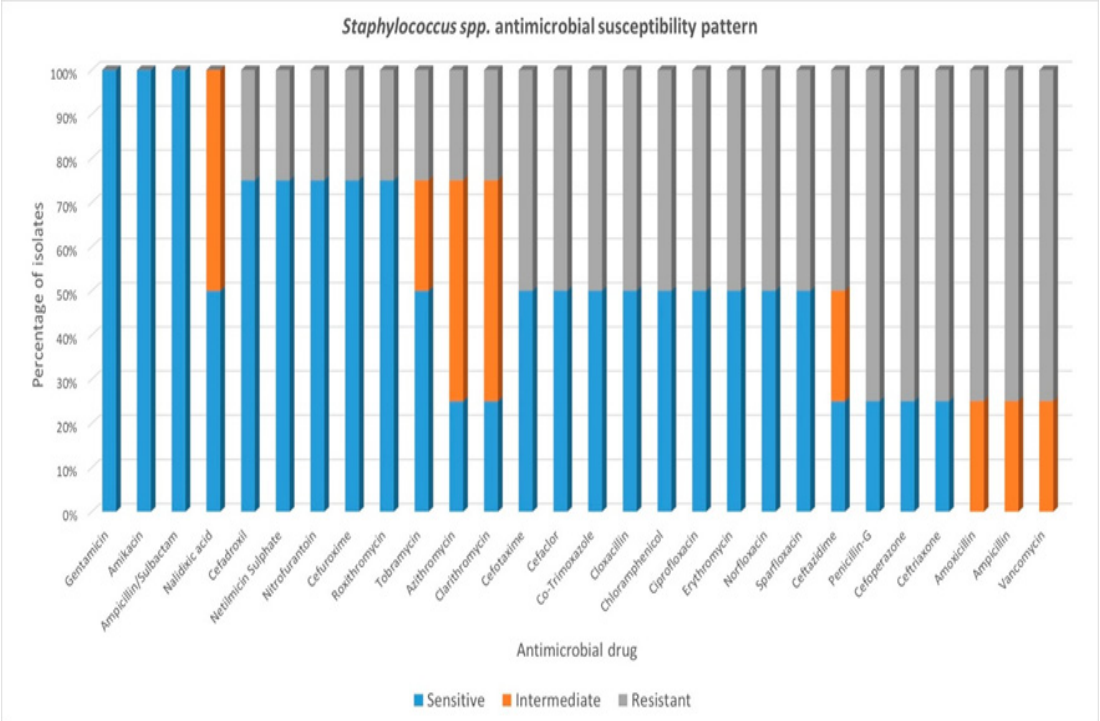

Fig. 2. Antimicrobial susceptibility pattern of *Staphylococcus* isolates from mares with endometritis in India

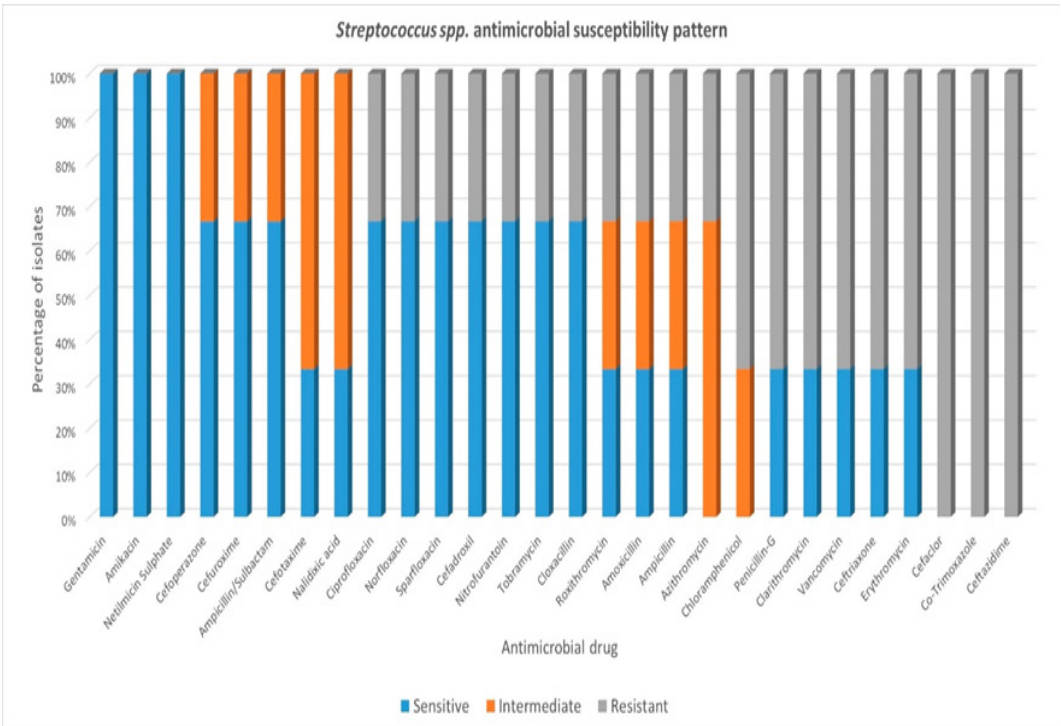

Fig. 3. Antimicrobial susceptibility pattern of *Streptococcus* isolates from mares with endometritis in India

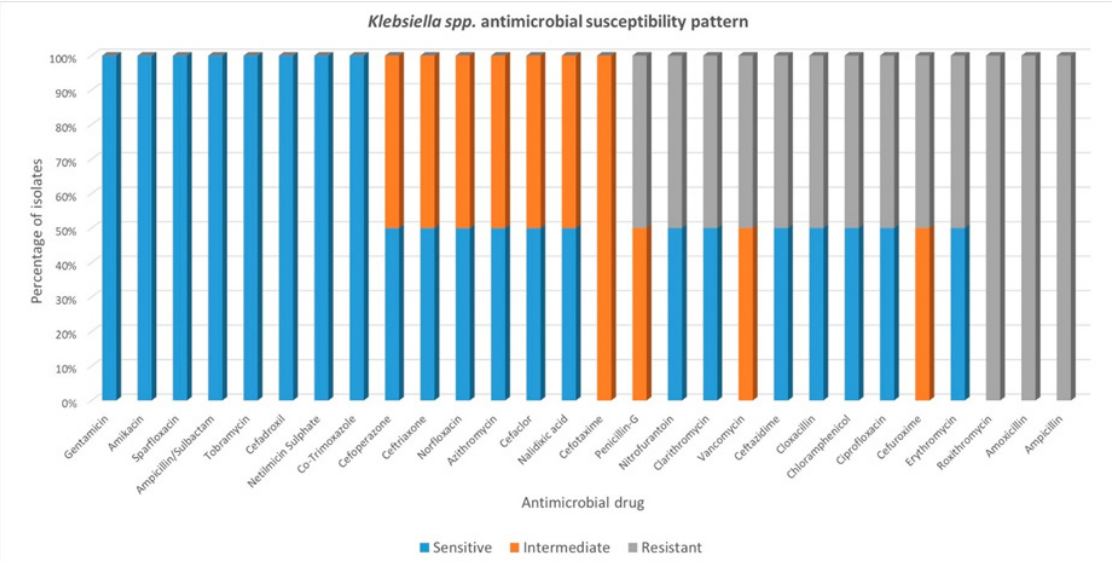

Fig. 4. Antimicrobial susceptibility pattern of *Klebsiella* isolates from mares with endometritis in India

grape fashion pointed towards *Staphylococcus*. While *Streptococcus* isolates reflected Gram-positive cocci arranged in long chains and showed pinpoint haemolytic colonies on blood agar. The identification of the different types of bacterial isolates was further confirmed by biochemical test results (Table 1).

The antimicrobial susceptibility patterns of *E. coli*, *Staphylococcus*, *Streptococcus*, and *Klebsiella* isolates from mares in the present study are shown in Figures 1 to 4, respectively.

The overall antimicrobial susceptibility patterns for all 15 isolates are shown in Figure 5. The percentages of the isolates susceptible to each antibiotic and the associated 95% confidence intervals are presented in Table 2. Most of the *E. coli* isolates were susceptible to gentamicin (83.3%), netilmicin sulphate (83.3%), tobramycin (83.3%), nitrofurantoin (83.3%), amikacin (66.7%), and ampicillin/sulbactam (66.7%). All of the *Staphylococcus* isolates were susceptible to gentamicin, amikacin, and ampicillin/sulbactam. Similar susceptibility percentage (100%) was observed for gentamicin, amikacin, and netilmicin sulphate against *Streptococcus* and *Klebsiella* isolates. Furthermore, all of the *Klebsiella* isolates were susceptible to sparfloxacin, ampicillin/sulbactam, tobramycin, cefadroxil, and co-trimoxazole. Ampicillin and amoxicillin were the least effective antimicrobials with susceptibility percentages ranging between 0 to 33.3% for the various isolates. Low combined susceptibility percentages (<50%) were observed for 17 of the 28 tested antibiotics (Table 2).

Discussion

The bacterial isolates detected in the present study were similar to the isolates commonly reported in previous studies conducted elsewhere [9,10-13,18]. Many of those studies reported *E. coli* as the most frequent bacterial isolate with frequencies varying between 27.9% to 50.0% [9,10,12,14,18]. Similar results were observed in the present study with *E. coli* being the predominant isolated bacterial species from mares with endometritis. In contrast to our findings and the other studies mentioned above, *Streptococcus* group C and *Staphylococcus* were reported as the most common bacterial isolates, respectively [11,13]. Disparities in frequently isolated bacteria from mares between different studies have been previously attributed to different geographic locations, diverse mare populations, and exposure to different antimicrobial agents [19]. The other three bacterial isolates detected in the present study (*Staphylococcus*, *Streptococcus*, and *Streptococcus* species) have been reported previously in mares with endometritis in various countries [9-13,18]. Although the present study provides useful preliminary information, further studies involving larger and wider sample sizes are required to get a better understanding of the microbial populations associated with equine endometritis in India.

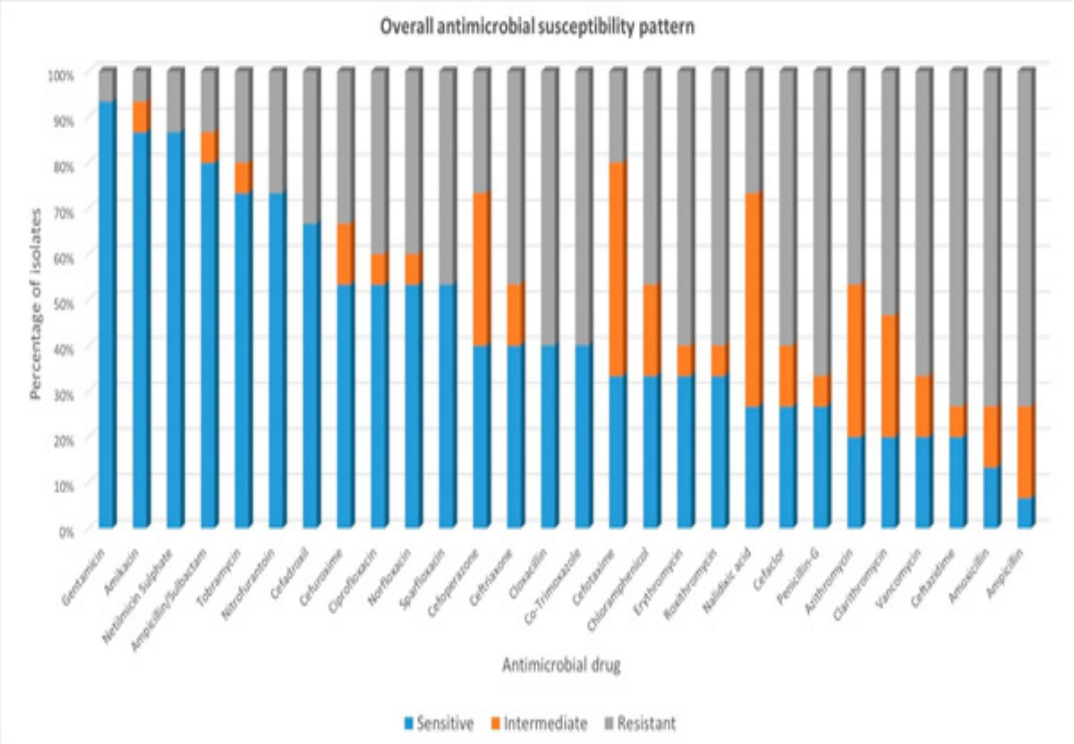

Fig. 5. Combined antimicrobial susceptibility pattern of all isolates from mares with endometritis in India

The antimicrobial susceptibility patterns observed in the present investigation were similar to those reported in some of the previous studies [9,13,18] in which gentamicin was found to be a very effective antimicrobial drug. In addition to gentamicin, amikacin, netilmicin sulphate and ampicillin sulbactam were associated with a high susceptibility percentage in our study. Pertinently, amikacin was also reported to be highly effective in two retrospective studies conducted in Italy [18] and Spain [13]. However, slightly different results were reported in a few other studies. Enrofloxacin and amoxicillin-clavulanic acid were reported to be highly effective in one study [11], whereas ciprofloxacin, levofloxacin, and enrofloxacin were shown to be highly effective in another study [12]. Based on the antimicrobial susceptibility patterns observed in the present study, it was concluded that gentamicin, amikacin, netilmicin sulphate and ampicillin/sulbactam may be the best first-line antimicrobials for clinical application in equine endometritis cases in India while awaiting antimicrobial susceptibility test results.

The high resistance rate of the uterine bacterial isolates to more than half of the antibiotics tested in this study is yet another confirmation of the wider antimicrobial resistance issue in India. Antimicrobial resistance is an ever-growing public health concern throughout the world, but the situation is significantly worse in developing countries such as India [20]. Reasons include a high burden of infectious diseases and unrestricted access to medications, resulting in increased and incorrect use of antimicrobial drugs [21,22]. The results of this study further underscore the need for antimicrobial stewardship in veterinary medicine in India.

Conclusions

The present study demonstrated that *E. coli* is the most common uterine bacterial isolate from mares with endometritis in India. Antimicrobial susceptibility testing of the bacteria isolated from the mares indicated that gentamicin, amikacin, netilmicin sulphate and ampicillin/sulbactam may be the best first-line antimicrobials for clinical application in equine endometritis cases in India. The overall high rate of antimicrobial resistance observed in the present study further emphasizes the need to judiciously use antimicrobials in veterinary practice in India.

TABLE 2. Antimicrobial susceptibility pattern of the uterine bacterial isolates from mares with endometritis (n=15)

Antibiotic	E. coli (n=6)	Staphylococcus spp. (n=4)	Streptococcus spp. (n=3)	Klebsiella spp. (n=2)	Overall (n=15)
Gentamicin	83.3 (35.9, 99.6)	100.0 (39.8, 100.0)	100.0 (29.2, 100.0)	100.0 (15.8, 100.0)	93.3 (68.1, 99.8)
Cefoperazone	33.3 (4.3, 77.7)	25.0 (0.6, 80.6)	66.7 (9.4, 99.2)	50.0 (1.3, 98.7)	40.0 (16.3, 67.7)
Chloramphenicol	33.3 (4.3, 77.7)	50.0 (6.8, 93.2)	0.0 (0.0, 70.8)	50.0 (1.3, 98.7)	33.3 (11.8, 61.6)
Ciprofloxacin	50.0 (11.8, 88.2)	50.0 (6.8, 93.2)	66.7 (9.4, 99.2)	50.0 (1.3, 98.7)	53.3 (26.6, 78.7)
Cefuroxime	50.0 (11.8, 88.2)	75.0 (19.4, 99.4)	66.7 (9.4, 99.2)	0.0 (0.0, 84.2)	53.3 (26.6, 78.7)
Roxithromycin	16.7 (0.40, 64.1)	75.0 (19.4, 99.4)	33.3 (0.80, 90.6)	0.0 (0.0, 84.2)	33.3 (11.8, 61.6)
Norfloxacin	50.0 (11.8, 88.2)	50.0 (6.8, 93.2)	66.7 (9.4, 99.2)	50.0 (1.3, 98.7)	53.3 (26.6, 78.7)
Ceftazidime	16.7 (0.40, 64.1)	25.0 (0.60, 80.6)	0.0 (0.0, 70.8)	50.0 (1.3, 98.7)	20.0 (4.3, 48.1)
Sparfloxacin	33.3 (4.3, 77.7)	50.0 (6.8, 93.2)	66.7 (9.4, 99.2)	100.0 (15.8, 100.0)	53.3 (26.6, 78.7)
Amikacin	66.7 (22.3, 95.7)	100.0 (39.8, 100.0)	100.0 (29.2, 100.0)	100.0 (15.8, 100.0)	86.7 (59.5, 98.3)
Penicillin-G	33.3 (4.3, 77.7)	25.0 (0.60, 80.6)	33.3 (0.80, 90.6)	0.0 (0.0, 84.2)	26.7 (7.8, 55.1)
Azithromycin	16.7 (0.40, 64.1)	25.0 (0.60, 80.6)	0.0 (0.0, 70.8)	50.0 (1.3, 98.7)	20.0 (4.3, 48.1)
Cefadroxil	50.0 (11.8, 88.2)	75.0 (19.4, 99.4)	66.7 (9.4, 99.2)	100.0 (15.8, 100.0)	66.7 (38.4, 88.2)
Cefotaxime	33.3 (4.3, 77.7)	50.0 (6.8, 93.2)	33.3 (0.8, 90.6)	0.0 (0.0, 84.2)	33.3 (11.8, 61.6)
Cefaclor	16.7 (0.40, 64.1)	50.0 (6.8, 93.2)	0.0 (0.0, 70.8)	50.0 (1.3, 98.7)	26.7 (7.8, 55.1)
Netilmicin Sulphate	83.3 (35.9, 99.6)	75.0 (19.4, 99.4)	100.0 (29.2, 100.0)	100.0 (15.8, 100.0)	86.7 (59.5, 98.3)
Co-Trimoxazole	33.3 (4.3, 77.7)	50.0 (6.8, 93.2)	0.0 (0.0, 70.8)	100.0 (15.8, 100.0)	40.0 (16.3, 67.7)
Clarithromycin	0.0 (0.0, 45.9)	25.0 (0.6, 80.6)	33.3 (0.8, 90.6)	50.0 (1.3, 98.7)	20.0 (4.3, 48.1)
Ampicillin/ Sulbactam	66.7 (22.3, 95.7)	100.0 (39.8, 100.0)	66.7 (9.4, 99.2)	100.0 (15.8, 100.0)	80.0 (51.9, 95.7)
Nalidixic acid	0.0 (0.0, 45.9)	50.0 (6.8, 93.2)	33.3 (0.8, 90.6)	50.0 (1.3, 98.7)	26.7 (7.8, 55.1)
Nitrofurantoin	83.3 (35.9, 99.6)	75.0 (19.4, 99.4)	66.7 (9.4, 99.2)	50.0 (1.3, 98.7)	73.3 (44.9, 92.2)
Tobramycin	83.3 (35.9, 99.6)	50.0 (6.8, 93.2)	66.7 (9.4, 99.2)	100.0 (15.8, 100.0)	73.3 (44.9, 92.2)
Vancomycin	33.3 (4.3, 77.7)	0.0 (0.0, 60.2)	33.3 (0.8, 90.6)	0.0 (0.0, 84.2)	20.0 (4.3, 48.1)
Ceftriaxone	50.0 (11.8, 88.2)	25.0 (0.6, 80.6)	33.3 (0.8, 90.6)	50.0 (1.3, 98.7)	40.0 (16.3, 67.7)
Cloxacillin	16.7 (0.4, 64.1)	50.0 (6.8, 93.2)	66.7 (9.4, 99.2)	50.0 (1.3, 98.7)	40.0 (16.3, 67.7)
Amoxicillin	16.7 (0.4, 64.1)	0.0 (0.0, 60.2)	33.3 (0.8, 90.6)	0.0 (0.0, 84.2)	13.3 (1.7, 40.5)
Ampicillin	0.0 (0.0, 45.9)	0.0 (0.0, 60.2)	33.3 (0.8, 90.6)	0.0 (0.0, 84.2)	6.7 (0.2, 31.9)
Erythromycin	16.7 (0.4, 64.1)	50.0 (6.8, 93.2)	33.3 (0.8, 90.6)	50.0 (1.3, 98.7)	33.3 (11.8, 61.6)

The percentages of the isolates susceptible to each antimicrobial are presented and the associated 95% confidence intervals are shown in the parentheses.

Acknowledgements

The authors are thankful to the Mumbai Veterinary College and Rashtriya Krishi Vikas Yojana (RKVY, National Agricultural Development Scheme) project for providing the research facilities and financial assistance to conduct the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Traub-Dargatz, J.L., Salman, M.D. and Voss, J.L. Medical problems of adult horses, as ranked by equine practitioners. *J. Am. Vet. Med. Assoc.*, **198**(10), 1745–1747 (1991).
2. LeBlanc, M.M. and Causey, R.C. Clinical and subclinical endometritis in the mare: both threats to fertility. *Reprod. Domest. Anim.*, **44**(Suppl. 3), 10–22 (2009).
3. Riddle, W.T., LeBlanc, M.M. and Stromberg, A.J. Relationships between uterine culture, cytology and pregnancy rates in a Thoroughbred practice. *Theriogenology,* **68**(3), 395–402 (2007).
4. LeBlanc, M.M. Advances in the diagnosis and treatment of chronic infectious and post-mating-induced endometritis in the mare. *Reprod. Domest. Anim.*, **45**(Suppl. 2), 21–27 (2010).
5. Christoffersen, M. and Troedsson, M. Inflammation and fertility in the mare. *Reprod. Domest. Anim.*, **52** (Suppl. 3), 14–20 (2017).
6. Morris, L.H.A., McCue, P.M. and Aurich, C. Equine endometritis: a review of challenges and new approaches. *Reproduction,* **160**(5), R95–R110 (2020).
7. Canisso, I.F., Segabinazzi, L.G.T.M. and Fedorka, C.E. Persistent Breeding-Induced Endometritis in Mares - a Multifaceted Challenge: From Clinical Aspects to Immunopathogenesis and Pathobiology. *Int. J. Mol. Sci.*, **21**(4), 1432 (2020).
8. Morris, L.H. and Allen, W.R. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. *Equine Vet. J.*, **34**(1), 51–60 (2002).
9. Albihn, A., Baverud, V. and Magnusson, U. Uterine microbiology and antimicrobial susceptibility in isolated bacteria from mares with fertility problems. *Acta. Vet. Scand.*, **44** (3-4), 121–129 (2003).
10. LeBlanc, M.M., Magsig, J. and Stromberg, A.J. Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. *Theriogenology*, **68**(3), 403–412 (2007).
11. Frontoso, R., De Carlo, E., Pasolini, M.P., van der Meulen, K., Pagnini, U., Iovane, G. and De Martino, L. Retrospective study of bacterial isolates and their antimicrobial susceptibilities in equine uteri during fertility problems. *Res. Vet. Sci.*, **84**(1), 1–6 (2008).
12. Barbary, H.A., Abo-ghonema, I.I., El-Bawab, I.E. and Fadel, M.S. Diagnosis and Treatment of Bacterial Endometritis in Arabian Mares. *Alex. J. Vet. Sci.*, **49**(2), 116–125 (2016).
13. Díaz-Bertrana, M.L., Deleuze, S., Pitti Rios, L., Yeste, M., Morales Fariña, I. and Rivera Del Alamo, M.M. Microbial Prevalence and Antimicrobial Sensitivity in Equine Endometritis in Field Conditions. *Animals*, **11**(5), 1476 (2021).
14. Nocera, F.P., Ambrosio, M., Conte, A., Di Palma, T., Castaldo, S., Pasolini, M.P., Fiorito, F. and De Martino, L. Importance of broth-enrichment culture in equine endometritis diagnosis. *New Microbiol.*, **44**(1), 19–23 (2021).
15. Katila, T. Evaluation of diagnostic methods in equine endometritis. *Reprod. Biol.*, **16**(3), 189–196 (2016).
16. Brown, D.F., Hope, R., Livermore, D.M., Brick, G., Broughton, K., George, R.C., Reynolds, R. and BSAC Working Parties on Resistance Surveillance. Non-susceptibility trends among enterococci and non-pneumococcal streptococci from bacteraemias in the UK and Ireland, 2001-06. *J. Antimicrob. Chemother.*, **62**(Suppl. 2), 75–85 (2008).
17. Koppe, U., von Laer, A., Kroll, L.E., Noll, I., Feig, M., Schneider, M., Claus, H., Eckmanns, T. and Abu Sin, M. Carbapenem non-susceptibility of *Klebsiella pneumoniae* isolates in hospitals from 2011 to 2016, data from the German Antimicrobial Resistance Surveillance (ARS). *Antimicrob. Resist. Infect. Control,* **7**, 71 (2018).

18. Pisello, L., Rampacci, E., Stefanetti, V., Beccati, F., Hyatt, D.R., Coletti, M. and Passamonti, F. Temporal efficacy of antimicrobials against aerobic bacteria isolated from equine endometritis: an Italian retrospective analysis (2010-2017). *Vet. Rec.*, **185**(19), 598 (2019).

19. Davis, H.A., Stanton, M.B., Thungrat, K. and Boothe, D.M. Uterine bacterial isolates from mares and their resistance to antimicrobials: 8,296 cases (2003-2008). *J. Am. Vet. Med. Assoc.,* **242**(7), 977–983 (2013).

20. Sharma, C., Rokana, N., Chandra, M., Singh, B.P., Gulhane, R.D., Gill, J.P.S., Ray, P., Puniya, A.K. and Panwar, H. Antimicrobial Resistance: Its Surveillance, Impact, and Alternative Management Strategies in Dairy Animals. *Front. Vet. Sci.*, **4**, 237 (2018).

21. Kumar, S.G., Adithan, C., Harish, B.N., Sujatha, S., Roy, G. and Malini, A. Antimicrobial resistance in India: A review. *J. Nat. Sci. Biol. Med.*, **4**(2), 286–291 (2013).

22. Kakkar, M., Walia, K., Vong, S., Chatterjee, P. and Sharma, A. Antibiotic resistance and its containment in India. *Br. Med. J.,* **358**, j2687 (2017).