Egypt. J. Vet. Sci. **Vol. 55,** No. 1, pp. 209-222 (2024)

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

Antimicrobial Activity of Hydrogen Peroxide against Endometritis-Causing Microorganisms in Mares

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EARLY and accurate detection and isolation of the pathogens associated with endometritis in mares are crucial to initiating correct treatment in time, inhibiting bacterial resistance development, and optimizing fertility. This study aimed at the diagnosis of endometritis in mares through ultrasound examination followed by the isolation and identification of the causative microorganisms. In addition, the determination of the antibiotic susceptibility of the isolated microorganisms was carried out. Moreover, the *in-vitro* antibacterial efficiency of hydrogen peroxide (H_2O_2) against the isolated pathogens was assessed. Out of the examined 60 samples, 34 (56.7%), 30 (50.0%), 9 (15.0%), and 9 (15.0%) were confirmed positive for the presence of *Pseudomonas aeruginosa*, *Escherichia coli, Serratia fonticola,* and *Klebsiella pneumoniae subsp. ozaenae,* respectively. Of the *P. aeruginosa* isolates, 76.5% showed intermediate resistance to gentamicin. *Escherichia coli* showed 3.3%, 43.3%, 46.7%, 16.7%, 13.3%, 13.3%, and 26.7% resistance against cefazolin, gentamicin, amoxicillin/clavulanic acid, cefotaxime, ciprofloxacin, chloramphenicol, and tetracycline, respectively. *Serratia fonticola* isolates showed 100.0% resistance to each of gentamicin and amoxicillin/clavulanic acid while it showed 44.4% resistance to cefazolin and 66.7% resistance to each of cefotaxime, ciprofloxacin, chloramphenicol, and tetracycline. *Klebsiella pneumoniae subsp. ozaenae* showed 22.2% and 44.4% intermediate resistance against amoxicillin/clavulanic acid and cefazolin, respectively. Concerning the hydrogen peroxide, the mean inhibition zones' diameters (mm) were 54.67, 48.33, 46.33, and 49.67 against *Pseudomonas aeruginosa, Escherichia coli, Serratia fonticola,* and *Klebsiella pneumoniae subsp. ozaenae*, respectively. This study demonstrated the very high *in-vitro* bacteriostatic and bactericidal efficiency of H_2O_2 at a concentration of 0.047% against bacteria-associated endometritis.

Keywords: Endometritis, Antimicrobial-Resistance, *Escherichia coli*, Hydrogen Peroxide, *Pseudomonas*.

Introduction

Equine endometritis, a persistent inflammation of the endometrium, is blamed as one of the serious causes of subfertility in mares [1]. Endometritis may be acute or chronic, infectious or non-infectious [2]. Generally, endometritis is considered a therapeutic challenge for those who are working in the equine breeding industry [1]. This disease is contributing to substantial economic losses in the purebred equine breeding field due to the failure of the mares to conceive

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(Received 14/08/2023, accepted 03/09/2023)
DOI: 10.21608/EJVS.2023.229286.1560

besides the early embryonic death, especially if accompanied by misdiagnosis, subsequent treatment failure, and the development of antibiotic-resistant bacteria [3].

Mares having a normal immunological defense response can clean the inflammatory byproducts and microbial contamination (bacteria, fungi, excess sperm, seminal plasma, and inflammatory products) from the uterine lumen within 48 hours after breeding [4]. However, mares having either; inadequate innate immunity, venereal pathogens, delayed uterine clearance, defective vestibule-vaginal seal, poor conformation of the perineum, and cervical stenosis, will fail to eliminate the inflammatory and microbial byproducts within 48 hours after breeding [5]. This can easily develop chronic infectious endometritis, which can lead to a hostile environment for the sperm or the embryos, making the uterus an unfavorable environment to the conceptus. Ultrasound examination of the uterus can help in the early identification of any intrauterine endometritis fluid, edema, and inflammation [6].

Endometritis has been previously handled with a multi-modal approach like antibiotics, uterine lavage, anti-inflammatory drugs, and ecbolic. However, treatment of endometritis is commonly implemented by using indiscriminate antibiotics as routine work without prior antimicrobial susceptibility testing, which could potentially influence the development of antibiotic-resistant bacterial strains, which in turn can lead to a lack of response to the usual antibiotics [4]. Considering the antibiotic-resistant bacteria development, there is a necessity to create substitutional non-traditional therapies for the treatment of infectious endometritis in mares and at the same time can be used as disinfecting agents for the mares' surrounding environment. Hydrogen peroxide (H_2O_2) is an environmentally friendly oxidant compound, that constitutes a significant influence on the chemical industry such as inorganic or organic chemical synthesis and paper pulp bleaching, and on environmental treatment such as water treatment and medical disinfection [7]. Moreover, H_2O_2 is commonly used as a disinfection agent sanitizing medical equipment in hospitals [8]. This study aimed at the identification of endometritis in mares through ultrasound examination followed by isolation, and identification of the causative microorganisms. In addition, the antibiotic susceptibility of the isolated microorganisms as well as the *in-vitro* antimicrobial efficiency of H_2O_2 against the isolated pathogens were investigated.

Material and Methods

Sample collection

Mares with a history of repeat breeder and non-responsive antibiotic treatment were clinically confirmed to be affected with endometritis by the trans-rectal ultrasonographic detection of endometrial fluid (SONOVET R3, Samsung, Madison, South Korea). A total of 60 samples; 37 uterine swabs, 10 vaginal swabs, 4 cervical swabs, 6 uterine swabs of aborted mares, and 3 stomach content of aborted fetuses were collected with the aid of double-guarded swabs (Minitube, Germany) into 5 ml Brain Heart Infusion broth (CM1135B, Oxoid, UK). The swabs were transferred to an insulated sterile ice box and transported to the Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, Cairo, Egypt where they were analyzed immediately for the presence of microorganisms.

Isolation and identification of microorganisms causing endometritis

For the isolation and identification of *pseudomonas*, samples were grown on Pseudomonas Agar Base media supplemented with Pseudomonas CFC Supplement (CM0559 + SR0103, Oxoid, UK) and were handled according to a previous study [9]. *Enterobacteriaceae* were isolated using Violet Red Bile Glucose agar (VRBGA, CM1082, Oxoid, UK) [10]. Typical colonies of the family *Enterobacteriaceae* were confirmed by the API system strips (bioMérieux, France) according to the manufacturer's instructions. For the isolation and identification of campylobacters, samples were manipulated according to [11].

Antimicrobial susceptibility of the bacterial isolates

The isolates were tested for their antimicrobial resistance profiles using the Kirby-Bauer disk diffusion method based on the National Committee for the Clinical and Laboratory Standards Institute (CLSI) [12]. The experiments were done using Mueller-Hinton agar (CM0337, Oxoid, UK) and antibiotic-impregnated disks (Oxoid, UK). In case of *Pseudomonas aeruginosa*, ceftazidime (CAZ) (30 µg), gentamicin (CN) (10 µg), amikacin (AK) (30 µg), aztreonam (ATM) (30 µg), cefepime (FEP) (30 µg), ciprofloxacin (CIP) (5 µg), imipenem (IPM) (10 µg), and piperacillin

tazobactam (TZP) (100/10 µg) were used. With other microorganisms, gentamicin (CN) (10 µg), amikacin (AK) (30 µg), amoxicillin/clavulanic Acid (AMC) (30 µg), cefepime (FEP) (30 µg), cefoxitin (FOX) (30 µg), cefotaxime (CTX) (30 µg), ciprofloxacin (CIP) (5 µg), imipenem (IPM) (10µg), ceftazidimE (CAZ) (30µg), chloramphenicol (C) (30µg), and tetracycline (TE) (30µg) were used. After application of the antimicrobial-impregnated disks onto the seeded Mueller-Hinton agar plates, the plates were incubated overnight at 37°C and the inhibition zone diameters were measured in millimeters and interpreted according to guidelines of the CLSI [12].

The antibacterial effect of hydrogen peroxide against the bacterial isolates

Agar well diffusion method of H_2O_2

Using the agar well diffusion method, the H_2O_2 30% solution (7722-84-1, Sigma-Aldrich Chemie GmbH) was tested for its antibacterial effectiveness against the bacterial isolates recovered in this study. Muller-Hinton agar plates were prepared with a uniform thickness of approximately 4 mm, and the agar was allowed to be set at ambient temperature to solidify, and then wells of 6 mm diameter were formed using a sterilized borer. A fresh culture of the bacterial cells (100 µl, 1.5×10^8 CFU/ml) was swabbed on the surface of the agar plates. The wells were then filled with 100 µl of 30% H_2O_2. The plates were prepared in triplicates and then left for 30 min at room temperature to allow the diffusion of the H_2O_2. After incubation at the previously mentioned appropriate time and temperature, the diameter of the transparent zones of inhibition was measured [13].

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination of hydrogen peroxide

The antimicrobial efficiency of the H_2O_2 solution was evaluated against the recovered isolates via the determination of the minimum bactericidal concentration (MBC) value, minimum inhibitory concentration (MIC) value, and MBC/MIC ratio using the 96-well broth micro-dilution plate method. A tenfold dilution was prepared from the stock solution of the 30% H_2O_2 to obtain a 3% solution. Then, two-fold serial dilutions were prepared using the Mueller-Hinton broth (MH, CM0405B, Oxoid, UK) to prepare the following dilutions (1.5%, 0.75%, 0.375%, 0.188%, 0.094%, 0.047%, 0.023%, 0.012%, and 0.006%). To each well containing vertically the appropriate 50 µl of H_2O_2 dilutions, 50 µl of the adjusted bacterial inoculum concentration of 5×10^5 CFU/ml was added horizontally. Each plate included growth and sterility control wells; the growth control wells contained MH broth medium with the tested bacterial concentrations, and the sterility control wells contained only MH broth medium. The plates were then covered to avoid dehydration. The plates were prepared in triplicates and then maintained in an incubator at 37°C for 18 to 20 hours. The lowest concentration of H_2O_2 that inhibited bacterial growth was considered the MIC. After the MIC determination, aliquots of 100 µl from all wells that showed no bacterial growth were streaked onto Brain Heart Infusion agar (BHI, CM1136, Oxoid, UK) plates and incubated at 37°C for 20 hours. The lowest concentration of the H_2O_2 that kills 100% of the initial bacterial population and shows no colonial growth on the BHI agar after 20 hours of incubation at 37°C was recorded as the minimum bactericidal concentration [14].

Statistical analysis

The statistical analyses were carried out using IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 25 (2017) for Windows [15]. Results were statistically analyzed using the simple frequency table and descriptive statistics (mean, 95% confidence interval for mean, median, std. deviation, and std. error).

Results

Bacterial isolation and identification

The results represented in Table 1 and Figure 1 reveal that *Pseudomonas aeruginosa* was isolated only from the uterine swabs (91.9%). Meanwhile, the isolation rates of *Escherichia coli* were 35.1%, 100.0%, 100.0%, and 50.0% from the uterine swabs, vaginal swabs, cervical swabs, and uterine swabs of the aborted mares respectively. *Serratia fonticola* were isolated from the uterine swabs (8.1%) and the uterine swabs of aborted mares (100.0%). *Klebsiella pneumoniae subsp. ozaenae* were isolated only from the uterine swabs (24.3%). *Campylobacter* species were not detected at all. Out of the examined 60 samples, 34 (56.7%), 30 (50.0%), 9 (15.0%), and 9 (15.0%) samples were confirmed positive for the presence of *P. aeruginosa*, *E. coli, S. fonticola,* and *K. pneumoniae subsp. ozaenae* respectively.

Antimicrobial susceptibility of the isolated bacterial species

Table 2 and Figure 2 show the antimicrobial susceptibility patterns of the isolated *Pseudomonas aeruginosa*. It is clear that all *P. aeruginosa* isolates were sensitive to all tested antimicrobials except 76.5% of the isolates showed intermediate resistance to gentamicin. Table 3 and Figure 3 show the antimicrobial susceptibility patterns of the other isolated microorganisms (*Escherichia coli*, *Serratia fonticola*, and *Klebsiella pneumoniae subsp. ozaenae*). It is clear that all the isolated *E. coli*, *S. fonticola*, and *K. pneumoniae subsp. ozaenae* were 100% sensitive to each of amikacin, cefepime, cefoxitin, and imipenem and ceftazidime. On the other hand, *E. coli* showed 3.3%, 43.3%, 46.7%, 16.7%, 13.3%, 13.3%, and 26.7% resistance against cefazolin, gentamicin, amoxicillin/clavulanic acid, cefotaxime, ciprofloxacin, chloramphenicol, and tetracycline, respectively. *Serratia fonticola* isolates showed 100.0% resistance to each of gentamicin and amoxicillin/clavulanic acid while it showed 44.4% resistance to cefazolin and 66.7% resistance to each of cefotaxime, ciprofloxacin, chloramphenicol, and tetracycline. *Klebsiella pneumoniae subsp. ozaenae* isolates showed 100% sensitivity to each of gentamicin, cefotaxime, ciprofloxacin, chloramphenicol, and tetracycline while it showed 22.2% and 44.4% intermediate resistance against amoxicillin/clavulanic acid and cefazolin, respectively.

Antibacterial effect of hydrogen peroxide against the bacterial isolates recovered from mares' endometritis

Agar well diffusion method

Data shown in Table 4 and Figure 4 reveal that the mean inhibition zone diameters (mm) of H_2O_2 were 54.67, 48.33, 46.33, and 49.67 against *Pseudomonas aeruginosa, Escherichia coli, Serratia fonticola,* and *Klebsiella pneumoniae subsp. ozaenae*, respectively.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of hydrogen peroxide against the bacterial isolates

Data shown in Figure 5 reveal that the mean MIC and MBC values of the H_2O_2 against the isolated bacteria were at 0.047% which has both bacteriostatic and bactericidal activities and the MBC/MIC ratios of the H_2O_2 was 1 for all tested microorganisms.

Discussion

Infectious bacterial endometritis can occur at breeding due to the entrance of pathogenic or non-pathogenic bacteria into the uterus [6]. Commensal bacteria on the external genitalia of mares or stallions can be carried forward into the uterus when the mares are naturally mated or artificially inseminated. Non–pathogenic commensal bacteria could become pathogenic when introduced into the uterus [5]. Generally, it is estimated that 60% of mares who are incapable of being pregnant, suffer from bacterial endometritis [4]. Early and accurate detection and isolation of pathogens associated with endometritis in mares, are crucial to initiating correct treatment in time, inhibiting bacterial resistance development, and optimizing fertility. The application of the ultrasound examination, double-guarded swabs, microbiological examination of uterine samples, and antibiotic susceptibility testing of the isolates might enhance the accuracy of the diagnostic procedures [3]. Out of 60 samples examined in the current study, 34 (56.7%), 30 (50.0%), 9 (15.0%), and 9 (15.0%) were confirmed positive for the presence of *P. aeruginosa*, *E. coli, S. fonticola,* and *K. pneumoniae subsp. ozaenae,* respectively. A previous study recorded 30.5% positive bacterial cultures of the examined uterine swabs from mares suffering endometritis, and the most commonly isolated bacteria were *E. coli* (10.6%), *K. pneumoniae* (8.8%), *P. aeruginosa* (10.1%), *β-hemolytic Streptococcus* (36.5%), and *Aeromonas hydrophila* (4.1%) [5]. In another investigation on equine endometritis, 58% of the examined samples yielded positive bacterial cultures, and the most frequently isolated bacteria were α-haemolytic *Streptococcus* (27%), *E. coli* (27%), *β-haemolytic Streptococcus* (26.1%), and *Staphylococcus* species (19.1%) [3]. In addition, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Corynebacterium* species, and *Streptococcus zooepidemicus* were the most common causative bacteria affecting the uterus of mares suffering from infertility [16]. The most common bacterial pathogens recovered from equine endometritis were *E. coli*, *Aspergillus* sp., and *Streptococcus* species [2].

In the present study, 56.7% and 15.0% of the examined samples were confirmed positive for the presence of *P. aeruginosa* and *K. pneumoniae subsp. ozaenae*, respectively. *Pseudomonas aeruginosa* is commonly recognized as one of the main causes of endometritis in mares due to its ability to produce a biochemically complex biofilm [3]. A previous study showed a high incidence (40%) of *P. aeruginosa* and *K. pneumoniae* from cases of endometritis in

mares [17]. With increasing the environmental temperature and humidity, especially in summer months, both *P. aeruginosa* and *K. pneumoniae* incidences were found to be increased. On the other hand, *P. aeruginosa* is a known common contaminant of water pipes and tap water exits, and the regular cleaning of mares by these infected water sources could increase the rate of *P. aeruginosa* infection in mares. In addition, wood shavings were recognized as a source of both *K. pneumoniae* and *P. aeruginosa* and were incriminated in genital infections in horses that are bedded on them [5]. *Klebsiella* species and *P. aeruginosa* were isolated from mares that had not responded to the treatments of endometritis and that may be due to the mares' pendulous form of their uteri which made them incapable of ejecting out the contaminants [16].

In the current study, 50% of the examined samples were confirmed positive for the presence of *E. coli*. Similar studies reported that equine endometritis is one of the major problems in horses, and the most commonly isolated bacteria were *E. coli* and β-haemolytic *Streptococcus* [6, 18, 19]. *Escherichia coli* (26.58%) isolates were isolated from mares suffering from endometritis [17]. The high incidence of *E. coli* infections noticed in this study and previous studies could be due to environmental causes and breeding seasons as this study was conducted in the summer months. Indeed, it was reported that *E. coli* levels increase in the summer months [5]. In addition, *E. coli* was the most frequent microorganism isolated from endometritis in mares. This could be due to the elevated level of *E. coli* infection in the paddocks or foaling boxes in which mares are isolated to give birth. In addition, this elevated level of *E. coli* could also be due to morphological abnormalities, especially in aged mares like vestibule-vaginal junction which lead to the accumulation of pathogens and air in the vagina and uterus after foaling or when the cervix is relaxed. In this case, coliform colonization of the endometrium usually occurs [16].

The prevalence of the isolated bacteria from endometritis cases in mares may vary according to many factors. The different study areas, equine breeds, breeding management, geographic locations, nature of infection, the interaction between different bacterial agents, sampling technique, sample transport condition, conservation of the sample, doing of enrichment step, culture media, microbiological analysis, conditions of incubation, previous treatment with different antimicrobial drugs, biofilm formation by some microorganisms like *P. aeruginosa*, and increased endometrial fibrosis, have been the most reported factors affecting the isolation rate of bacteria from endometritis [3]. However, the enrichment step in BHI broth might have a neutralizing/reactivating effect on a few of the aforementioned issues that may avoid bacterial growth following direct smearing [3]. Further, environmental factors like temperature, humidity, and using stallions for both semen collection and mating in the same breeding season can increase the transmission of such pathogens [5].

In the current study, 76.5% of *P. aeruginosa* isolates were intermediate resistant to gentamicin. *Escherichia coli* showed 3.3%, 43.3%, 46.7%, 16.7%, 13.3%, 13.3%, and 26.7% resistance against cefazolin, gentamicin, amoxicillin/clavulanic acid, cefotaxime, ciprofloxacin, chloramphenicol, and tetracycline, respectively. *Serratia fonticola* isolates showed 100% resistance to each of gentamicin and amoxicillin/clavulanic acid while it showed 44.4% resistance to cefazolin and 66.7% resistance to each of cefotaxime, ciprofloxacin, chloramphenicol, and tetracycline. *Klebsiella pneumoniae subsp. ozaenae* showed 22.2% and 44.4% intermediate resistance against amoxicillin/clavulanic acid and cefazolin, respectively. In a previous similar study, all the bacterial isolates were at minimum resistant to one tested antibiotic, while *P. aeruginosa* and *K. pneumoniae* isolates showed the highest resistance to most of the tested antibiotics as they were resistant to 6 and 5 of the 12 tested antibiotics, respectively [4]. However, in another study, the isolated *K. pneumoniae* from uterine infections in mares were sensitive to all tested antibiotics [21]. In another study conducted on mares suffering from endometritis, all the bacterial isolates showed 36.4% susceptibility to trimethoprim-sulphonamide, and 76.1% to amikacin, while 76.5% of the *E. coli* isolates were susceptible to ceftiofur [5]. *Escherichia coli* and *Streptococcus zooepidemicus* isolates were sensitive to procaine penicillin G while were resistant to ceftiofur [19]. In another study, 96% of *E. coli* isolates were sensitive to ceftiofur while other isolates were resistant to penicillin [22]. An observed decrease in resistance of *E. coli* to streptomycin (from 76% in 2012 to 33% in 2016) was observed while the resistance to amoxicillin was 40%. Furthermore, 10% of *E. coli* strains were resistant to fluoroquinolones and/or cephalosporins. The decrease in resistance level

of *E. coli* to those antibiotics could be due to their limited use following their critical regulations by the French government and WHO authorities since they were designated as 'critically important' [21]. However, the resistance of *E. coli* to cephalosporins (fourth-generation and third-generation) may be related to the production of carbapenemase, extended-spectrum β-lactamase (ESBL), or cephalosporinase [21]. Generally, the susceptibility of the bacterial species towards tested antibiotics can increase or decrease over time, regulated by the intrinsic effect of the used antibiotics and the naturally created bacterial resistance ability in the bacterial pathogen [5].

The emergence of antibiotic-resistant bacteria has become a major problem worldwide. Hence, looking for new and alternative drugs against multidrug-resistant pathogens is important for the treatment of infectious diseases [23]. H_2O_2 is an environmentally friendly strong oxidizing antimicrobial chemical agent, which decomposed into O and H_2O by catalase. It has effective bactericidal and bacteriostatic activity against a wide range of microorganisms due to the generation of long lifespan reactive oxygen species (ROS) that can easily kill bacteria by oxidative stress [24]. According to the agar well diffusion method conducted in the current study, the mean inhibition zone diameters (mm) of H_2O_2 were 54.67, 48.33, 46.33, and 49.67 against *P. aeruginosa, E. coli, S. fonticola,* and *K. pneumoniae subsp. ozaenae*, respectively. The generation of hydroxyl free radicals by H_2O_2 acts as a biocidal agent that can harm fundamental cell structures of bacterial cells such as proteins, DNA, and lipids, displaying strong bactericidal effects [25]. The supplementation of bacterial cultures with H_2O_2 caused the degradation of the bacterial DNA [8]. Furthermore, compared with other disinfectants, H_2O_2 is a safe, odorless, colorless, non-carcinogenic, and environmentally friendly broad-spectrum disinfectant that can kill all types of pathogens [26]. The intrauterine treatment with H_2O_2 can effectively be used to improve cure and conception rate in cases of equine endometritis [27]. Weak solutions of H_2O_2 were beneficial in acute endometritis when exudates were found in the uterine lumen. Additionally, H_2O_2 caused disruption of the performed biofilms over the endometrium [27, 28].

In the current study, the mean MIC and MBC values of the H_2O_2 against the isolated bacteria were at a concentration of 0.047%, which showed both bacteriostatic and bactericidal activities. These results are in partial agreement with the similar studies. The MIC and MLC (minimum lethal concentration) values of H_2O_2 were in the range of 0.007% and 0.109%, respectively [25]. Additionally, the effective concentration of H_2O_2 was noticed at 0.014% against *S. aureus*, while it was 0.028% for both *Salmonella Typhimurium* and *E. coli* O157:H7 [29]. A high bacteriostatic and bactericidal efficiency of 7% H_2O_2 solution against a wide range of MDR (multidrug resistance) microorganisms, grown on multiple surface materials and equipment was recorded [25]. The main benefit of H_2O_2 is that its derivatives are oxygen and water which are consistent with organic food. Indeed, UV treatment (time= 30 or 60 min) of organic food with immersion in 3% H_2O_2 can remarkably inhibit salmonella [30]. Additionally, H_2O_2 is recognized as safe and has been used as a broad antimicrobial agent at a 3 % concentration [30]. H_2O_2 at a concentration of 3% has been commonly used to clean wounds and kill pyogenic microorganisms [24]. At high concentrations, ranging from 3 to 30%, the H_2O_2 antibacterial efficiency was recorded against several microorganisms including *Pseudomonas* species, *Streptococcus* species, and *Staphylococcus* species, where the bacterial cell death occurred due to irreversible oxidative damage to the DNA, proteins, enzymes, and bacterial cell membrane. Moreover, H_2O_2 concentrations higher than 50 mM are required for chromosomal DNA degradation and bacterial cell death [8]. Complete killing or inhibition of bacterial growth was reported at the H_2O_2 concentration of 0.1 mM [31]. The *E. coli* strains were inhibited by the exogenous H_2O_2 reaching MIC 90 at 1.25 mM/10^7cfu/ml [8]. Interestingly, the treatment of *E. coli* by H_2O_2 resulted in higher bacterial inactivation. The exposure of *E. coli*, for 10 min to H_2O_2 (1.20 mM) resulted in log10 bacterial cell reductions lower than 1.06 [32]. The H_2O_2 caused DNA degrading activity in *E. coli* cells at 2.5 mM (2.5 μmoles/10^7cfu/ml) [8]. Finally, a detailed study is required to be conducted to explore the *in-vivo* effect of H_2O_2 in the treatment of endometritis in mares.

Conclusion

Routine swabbing and culturing of mares' genitalia are of great importance, especially at the beginning of the breeding season. The exposure of animals to antibiotics should be regulated and kept at a minimum to limit their consumption.

This study demonstrated the very high *in-vitro* bacteriostatic and bactericidal efficiency of hydrogen peroxide at a concentration of 0.047% against bacteria-associated endometritis. Hydrogen peroxide could be used as a highly effective disinfectant for controlling a broad range of bacteria. A detailed study is required to be conducted to explore the *in-vivo* effect of H_2O_2 in the treatment of endometritis in mares.

Acknowledgement

Not applicable.

Conflict of Interest

There is no conflict of interest

Ethical approval

Ethical approval was obtained from the Animal Care and Use Committee of the National Research Center and Medical Research Ethics Committee with approval number NRC-19-143.

Funding statement

Not applicable.

TABLE 1. Incidences of different bacterial species among mares' endometritis samples.

	Samples											
	Uterine swabs (37)		Vaginal swabs (10)		Cervical swabs (4)		Uterine swabs of aborted mares (6)		Stomach content of aborted fetuses (3)		Total	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
Pseudomonas aeruginosa	34	91.9%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	34	56.7%
Escherichia coli	13	35.1%	10	100.0%	4	100.0%	3	50.0%	0	0.0%	30	50.0%
Serratia fonticola	3	8.1%	0	0.0%	0	0.0%	6	100.0%	0	0.0%	9	15.0%
Klebsiella pneumoniae subsp. ozaenae	9	24.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	9	15.0%
Campylobacter species	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

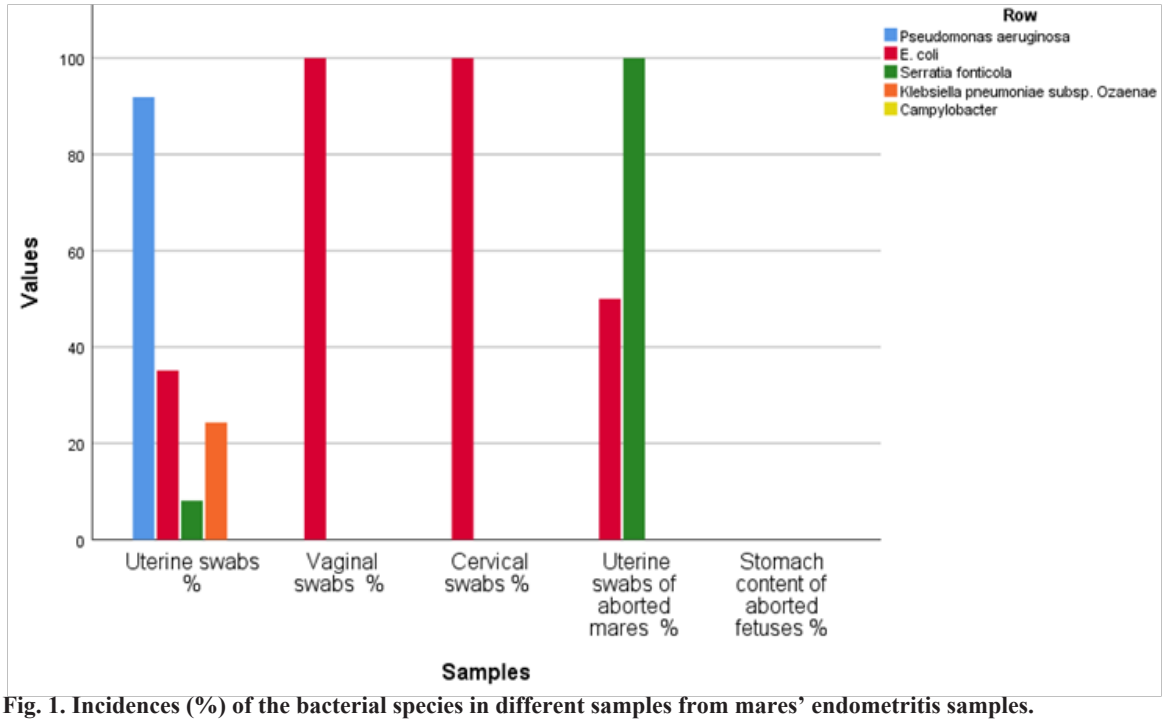

Fig. 1. Incidences (%) of the bacterial species in different samples from mares' endometritis samples.

TABLE 2. Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates

Antibacterial agent	Interpretation	Number	%
Ceftazidime (CAZ) (30 µg)	S	34	100.0%
	I	0	0.0%
	R	0	0.0%
Gentamicin (CN) (10 µg)	S	8	23.5%
	I	26	76.5%
	R	0	0.0%
Amikacin (AK) (30 µg)	S	34	100.0%
	I	0	0.0%
	R	0	0.0%
Aztreonam (ATM) (30 µg)	S	34	100.0%
	I	0	0.0%
	R	0	0.0%
Cefepime (FEP) (30 µg)	S	34	100.0%
	I	0	0.0%
	R	0	0.0%
Ciprofloxacin (CIP) (5 µg)	S	34	100.0%
	I	0	0.0%
	R	0	0.0%
Imipenem (IPM) (10 µg)	S	34	100.0%
	I	0	0.0%
	R	0	0.0%
Piperacillin Tazobactam (TZP) (100/10 µg)	S	34	100.0%
	I	0	0.0%
	R	0	0.0%

S: Sensitive I: Intermediate R: Resistant

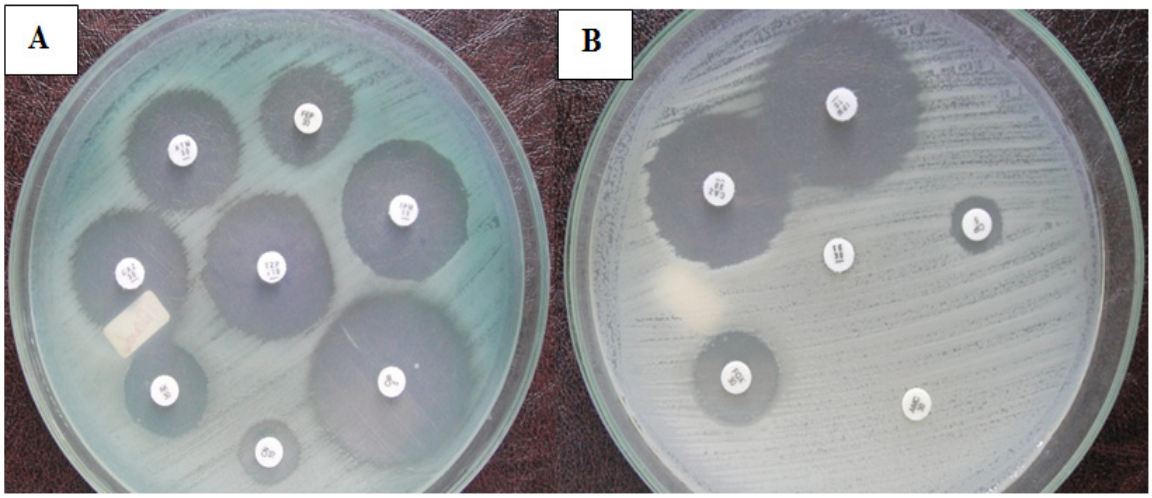

Fig. 2. Antimicrobial susceptibility of (A) *Pseudomonas aeruginosa* and (B) *Escherichia coli* .

TABLE 3. Antimicrobial susceptibility of *Escherichia coli*, *Serratia fonticola*, and *Klebsiella pneumoniae subsp. ozaenae.*

		Microorganisms					
		Escherichia coli		*Serratia fonticola*		*Klebsiella pneumoniae subsp. ozaenae*	
		Number	%	Number	%	Number	%
Cefazolin (KZ) (30 µg)	S	20	66.7%	0	0.0%	5	55.6%
	I	9	30.0%	5	55.6%	4	44.4%
	R	1	3.3%	4	44.4%	0	0.0%
Gentamicin (CN) (10 µg)	S	17	56.7%	0	0.0%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	13	43.3%	9	100.0%	0	0.0%
Amikacin (AK) (30 µg)	S	30	100.0%	9	100.0%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	0	0.0%	0	0.0%	0	0.0%
Amoxacillin/Clavulanic Acid (AMC) (30 µg)	S	9	30.0%	0	0.0%	7	77.8%
	I	7	23.3%	0	0.0%	2	22.2%
	R	14	46.7%	9	100.0%	0	0.0%
Cefepime (FEP) (30 µg)	S	30	100.0%	9	100.0%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	0	0.0%	0	0.0%	0	0.0%
Cefoxitin (FOX) (30 µg)	S	30	100.0%	9	100.0%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	0	0.0%	0	0.0%	0	0.0%
Cefotaxime (CTX) (30 µg)	S	23	76.7%	0	0.0%	9	100.0%
	I	2	6.7%	3	33.3%	0	0.0%
	R	5	16.7%	6	66.7%	0	0.0%
Ciprofloxacin (CIP) (5 µg)	S	26	86.7%	3	33.3%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	4	13.3%	6	66.7%	0	0.0%
Imipenem (IPM) (10µg)	S	30	100.0%	9	100.0%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	0	0.0%	0	0.0%	0	0.0%
CeftazidimE (CAZ) (30µg)	S	30	100.0%	9	100.0%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	0	0.0%	0	0.0%	0	0.0%
Chloramphenicol (C) (30µg)	S	26	86.7%	3	33.3%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	4	13.3%	6	66.7%	0	0.0%
Tetracycline (TE) (30µg)	S	22	73.3%	3	33.3%	9	100.0%
	I	0	0.0%	0	0.0%	0	0.0%
	R	8	26.7%	6	66.7%	0	0.0%

S: Sensitive I: Intermediate R: Resistant

TABLE 4. Antibacterial activities of H_2O_2 against bacterial isolates using agar well diffusion method.

Microorganisms	Inhibition zone diameter (mm)		Statistic	Std. Error
Pseudomonas aeruginosa	Mean		54.67	.667
	95% Confidence Interval for Mean	Lower Bound	51.80	
		Upper Bound	57.54	
	Median		54.00	
	Std. Deviation		1.155	
Escherichia coli	Mean		48.33	1.667
	95% Confidence Interval for Mean	Lower Bound	41.16	
		Upper Bound	55.50	
	Median		50.00	
	Std. Deviation		2.887	
Serratia fonticola	Mean		46.33	.667
	95% Confidence Interval for Mean	Lower Bound	43.46	
		Upper Bound	49.20	
	Median		47.00	
	Std. Deviation		1.155	
Klebsiella pneumoniae subsp. ozaenae	Mean		49.67	2.667
	95% Confidence Interval for Mean	Lower Bound	38.19	
		Upper Bound	61.14	
	Median		47.00	
	Std. Deviation		4.619	

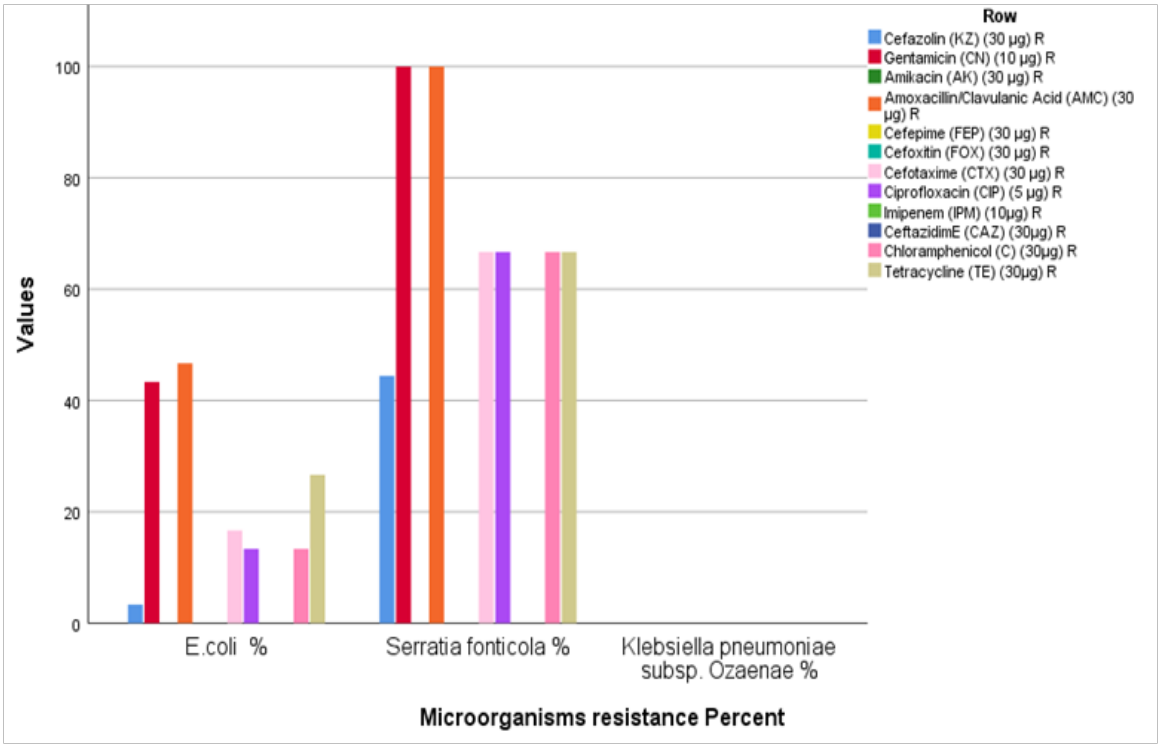

Fig. 3. Percentages of resistance in *Escherichia coli, Serratia fonticola,* and *Klebsiella pneumoniae subsp. ozaenae.*

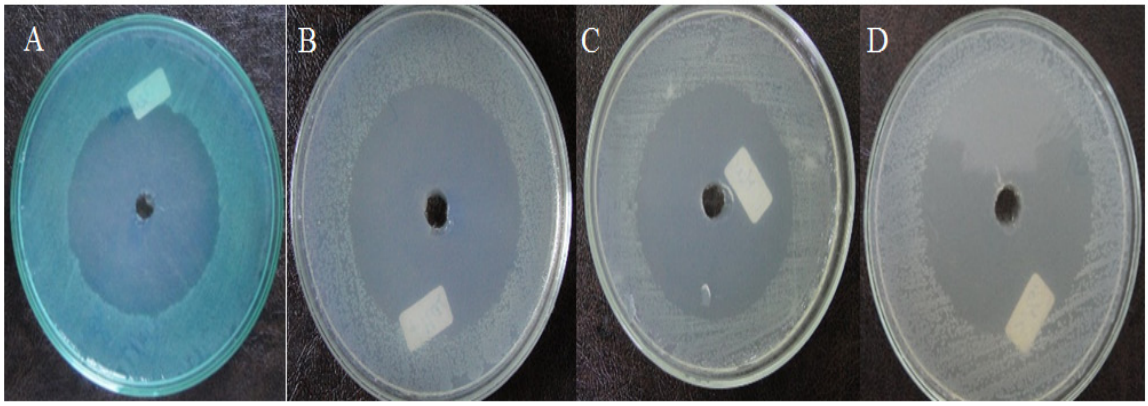

Fig. 4. Agar well diffusion method of H_2O_2 against the isolated bacteria; (A) *Pseudomonas aeruginosa,* (B) *Escherichia coli,* (C) *Serratia fonticola,* (D) *Klebsiella pneumoniae subsp. ozaenae.*

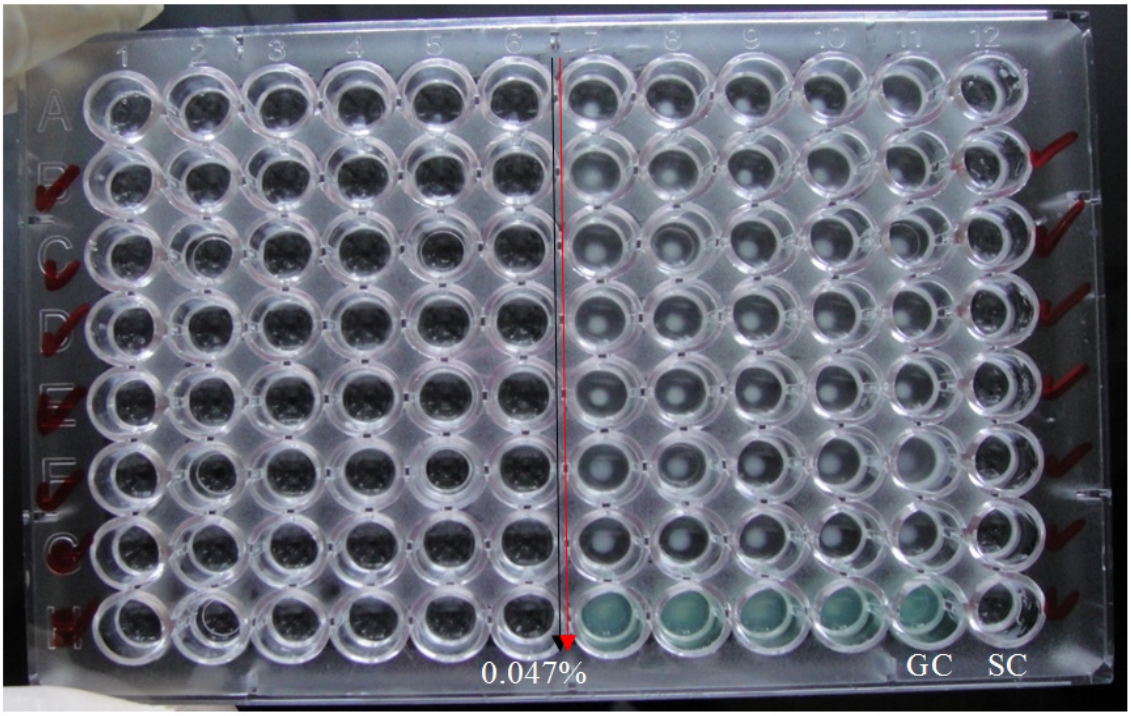

Fig. 5. The MICs and MBC of H_2O_2 against the isolated bacteria using the microtiter plate method. Columns titled 1-10 contain two-fold serial dilutions of H_2O_2. Rows titled A-H; contain *Escherichia coli, Serratia fonticola, Escherichia coli, Serratia fonticola, Serratia fonticola, Klebsiella pneumoniae subsp. ozaenae, Klebsiella pneumoniae subsp. ozaenae,* and *Pseudomonas aeruginosa,* respectively. Column 11 (GC) contains the growth control wells, and column 12 (SC) contains the sterility control wells. Red and black arrows indicate the MICs and MBCs respectively.

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النشاط المضاد للميكروبات للهيدروجين بيروكسيد ضد الكائنات الحية الدقيقة المسببه لالتهاب بطانة الرحم في الأفراس

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يعد العزل المبكر والدقيق لتشخيص مسببات مرض التهاب بطانة الرحم في الأفراس أمرًا بالغ الأهمية للمساعده على بدء العلاج الصحيح في الوقت المناسب، ولمنع تطور مقاومة البكتيريا، ولتحسين الخصوبة في الأفراس. تهدف هذه الدراسة إلى تشخيص مرض التهاب بطانة الرحم في الأفراس من خلال فحص الموجات فوق الصوتية متبوعًا بعزل وتشخيص الكائنات الحية الدقيقة المسببة للمرض. بالإضافة إلى ذلك، تهدف ايضا هذه الدراسة الى تحديد مدى قابلية او مقاومه الكائنات الدقيقة المعزولة للمضادات الحيوية. أخيرًا، تحليل مدى الكفاءة المضاده للبكتيريا للهيدروجين بيروكسيد ضد الكائنات الحية الدقيقة المعزولة والمسببة لمرض التهاب بطانة الرحم في الأفراس. وكانت النتائج كالتالى: من بين ال ٦٠ عينة من الأفراس المصابة بمرض التهاب بطانة الرحم و التى تم تشخيصها بالسونار، تم تأكيد وجود ٣٤ (٥٦,٧٪) و ٣٠ (٥٠,٠٪) و ٩ (١٥,٠٪) و ٩ (١٥,٠٪) عينه إيجابية لوجود بكتريا الزائفة الزنجارية (*Pseudomonas aeruginosa*)، بكتريا العصيات القولونية (*Escherichia coli*)، بكتريا سراتيه فونتيكولا (*Serratia fonticola*)، بكتريا الكليبسيلا الرئوية اوزونيه (*Klebsiella pneumoniae subsp. ozaenae*) على التوالي. وفى اختبار الحساسيه للمضادات الحيويه، أظهرت ٧٦,٥٪ من عترات الزائفة الزنجارية مقاومة متوسطة للجنتاميسين. فى حين أظهرت عترات بكتريا العصيات القولونية مقاومة ٣,٣٪ و ٤٣,٣٪ و ٤٦,٧٪ و ١٦,٧٪ و ١٣,٣٪ و ١٣,٣٪ و ٢٦,٧٪ ضد السيفازولين والجنتاميسين والأموكسيسيلين/حمض الكلافيولانيك والسيفوتاكسيم والسيبروفلوكساسين والكلورامفينيكول والتتراسيكلين على التوالي. أظهرت عترات بكتريا سراتيه فونتيكولا مقاومة بنسبة ١٠٠,٠٪ لكل من الجنتاميسين والأموكسيسيلين/حمض الكلافيولانيك، بينما أظهرت مقاومة ٤٤,٤٪ للسيفازولين ومقاومة ٦٦,٧٪ لكل من سيفوتاكسيم وسيبروفلوكساسين وكلورامفينيكول وتتراسيكلين. أظهرت عترات بكتريا الكليبسيلا الرئوية اوزونيه مقاومة متوسطه بنسبة ٢٢,٢٪ و ٤٤,٤٪ ضد الأموكسيسيلين/حمض الكلافيولانيك و السيفازولين على التوالي. بالنسبة لتحليل كفاءة الهيدروجين بيروكسيد ضد الكائنات الحية الدقيقة المعزولة والمسببة لمرض التهاب بطانة الرحم في الأفراس كانت النتائج كالتالى: أظهر الهيدروجين بيروكسيد مناطق تثبيط للبكتيريا المعزولة وكان متوسط القطر (مم) تقدر ب ٥٤,٦٧ و ٤٨,٣٣ و ٤٦,٣٣ و ٤٩,٦٧ ضد بكتريا الزائفة الزنجارية، بكتريا العصيات القولونية، بكتريا سراتيه فونتيكولا، بكتريا الكليبسيلا الرئوية اوزونيه على التوالي. هذه الدراسة أظهرت كفاءة عالية جدًا للهيدروجين بيروكسيد مضاده للبكتيريا ضد الكائنات الحية الدقيقة المعزولة والمسببة لمرض التهاب بطانة الرحم في الأفراس، وهذه الكفاءه كانت مثبطه للبكتيريا وايضا قاتله للبكتيريا عند تركيز ٠,٠٤٧٪.

الكلمات الدالة: التهاب بطانة الرحم , مقاومة مضادات الميكروبات، بكتريا العصيات القولونية ، الهيدروجين بيروكسيد ، بكتريا الزائفة الزنجارية.