Investigation of Antibiotic Susceptibility Patterns in Bacteria Isolated from a Male Sheep Castration Surgery

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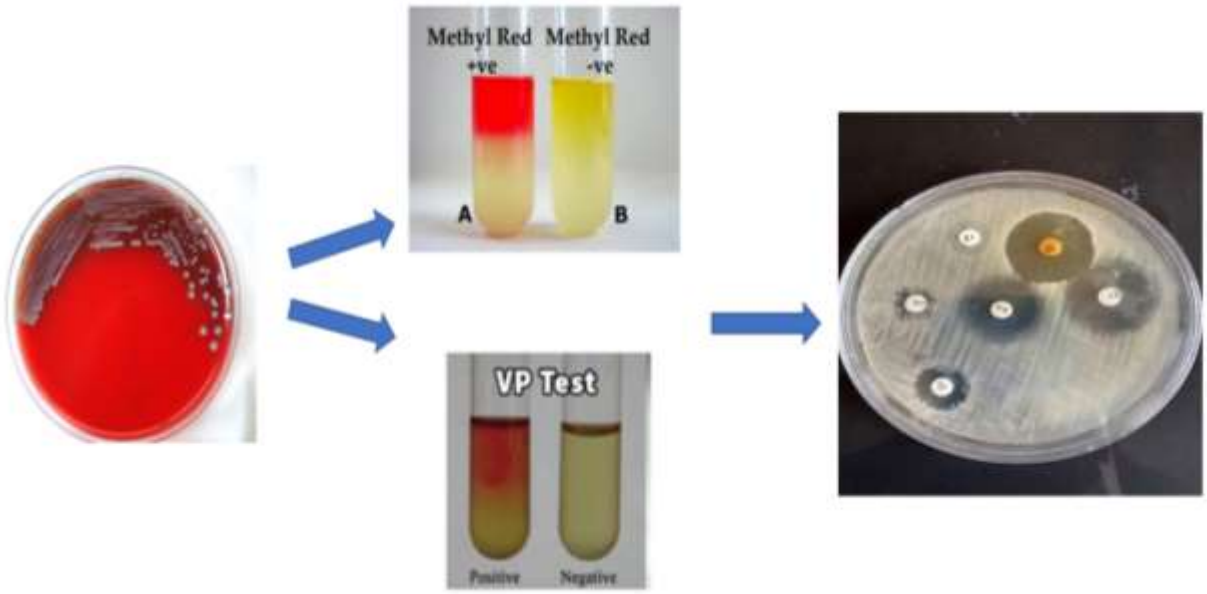
Abstract

There are different antibiotic resistance profiles among *Yersinia* spp. This pathogenic bacterium causes yersiniosis worldwide, requiring testing the organism's susceptibility in the local environment. An antibiotic susceptibility profile of a *Yersinia* spp. isolated from a castration surgical site were analyzed to provide insight into selecting appropriate antibiotics to treat *Yersinia* spp. infections while addressing antibiotic resistance issues effectively. The surgical site was swabbed before castration and cultures were performed. Samples of the surgical site were taken after the procedure, cultured, and then incubated. CLSI 2020 guidelines were followed for interpreting antibiotic susceptibility tests. The Kirby-Bauer disk diffusion method was applied to understand antibiotic susceptibility and resistance patterns better, and a zone of inhibition measurement was used to determine the zone of inhibition. Staining and microscopic examination of swab samples after surgery revealed a single colony of gram-negative bacteria. Laboratory tests confirmed that the isolated Gram-negative bacilli were indeed *Yersinia* spp. Methyl Red and Voges-Proskauer tests showed negative results, while Citrate utilization testing

demonstrated a positive effect. A positive impact was obtained for *Yersinia* spp. in the glucose fermentation test. Specifically, nitrofurantoin showed a significant zone of inhibition of over 17 mm, and gentamicin showed a more than 27 mm zone. However, resistance to ampicillin (11 mm), ceftriaxone, and cefazolin was observed. Due to the observed resistance to antibiotics, our results indicate that nitrofurantoin and gentamicin are likely to be the best options for treating *Yersinia* spp., in contrast to ampicillin, cefazolin, and ceftriaxone, which may be unsuitable because of resistance.

Keywords: Ampicillin, Antibiotic susceptibility, Bacterial identification, Drug resistance, *Yersinia* spp

Graphical abstract



1. Introduction

There is no doubt that antibiotic resistance is one of the most pressing public health crises facing both the human and animal populations in the modern world. *Yersinia* spp., specifically *Yersinia enterocolitica*, is an example of an organism that develops resistance to a wide range of antibiotics (1, 2). The extensive application of antibiotics indiscriminately in veterinary medicine contributes to antibiotic resistance. Despite the inherent risks of bacterial contamination, it is still common practice to use these devices in surgical procedures aimed at sterilizing animals (3, 4).

The emergence of *Yersinia*'s antibiotic-resistant strains has substantial consequences beyond geographic boundaries (5). This resilient strain challenges the effectiveness of treatment methods and animal health practices. Addressing antibiotic resistance in *Yersinia* spp., and related pathogens is imperative due to animal and human health interdependence. The problem of bacterial resistance in veterinary medicine can further complicate public health concerns.

It is vital to investigate *Yersinia*'s spp. susceptibility patterns to a wide range of antibiotics. A critical step towards safeguarding public health is understanding how bacteria respond to various antibiotics. The current study aims to determine which antibiotics are effective against *Yersinia* spp. when they adapt to antibiotics. This study includes insights into how veterinary medicine can make better decisions regarding treating sterilization procedures for better outcomes.

2. Methods and materials

2.1. Sampling

Samples were collected from the surgical site before preparation for castration surgery in a sheep. Before starting the surgery, the site was thoroughly cleaned with alcohol chlorhexidine and betadine iodine. The surgical site and suture location were swabbed following ceftriaxone administration and the completion of the procedure. After 24 hours of incubation of samples, following linear culture, crystal violet, iodine, and fuchsin stains were applied to determine the colony type.

As soon as bacteria were identified through Gram staining, they were cultured linearly within their specific media. To cultivate Gram-negative bacteria, including *Yersinia*, the EMB medium was used, along with Simon's citrate, TCI, SIM, and MRVP. A 24-hour incubator temperature of 37°C was used to promote bacteria growth and stability after being cultured in their specialized media. Testing with Methyl Red was performed using an MR medium. A VP test was conducted using an MRVP medium divided into two parts. SIM medium, which is semisolid, is then used for further investigation (6).

2.2. Antibiotic susceptibility test

Gram-negative bacteria were cultured on Mueller-Hinton agar medium once removed from the refrigerator. By inserting six antibiotic disks, this study examined the susceptibility of several different antibiotics, including nitrofurantoin, gentamicin, cefazolin, tetracycline, ceftriaxone, and ampicillin. The antibiotics chosen for this study were relevant to veterinary medicine since veterinarians may use them postoperatively. Sterilization was achieved by disinfecting the disks with alcohol and incubating them in the culture medium. At 37°C, the plates were incubated for 24 hours. The inhibition zones were measured with a ruler after 24 hours of incubation. Antibiotic susceptibility testing was performed using a standard established by the Clinical and Laboratory Standards Institute (CLSI) (7, 8).

3. Results

3.1. Sampling culture findings

Among the colonies sampled before surgery, gram-positive cocci, Gram-positive bacilli, and gram-negative bacilli were found. After surgery, one gram-negative colony was found in swab samples after staining and microscopic examination. An extensive series of biochemical tests determined the bacteria's identity. Testing the bacteria for Methyl Red (MR) determined that they did not produce acidic end products when they metabolized glucose. There was no evidence of acetoin production by the bacteria in Voges-Proskauer tests (VP). This suggests that enteric bacteria do not produce acetoin. During the Citrate utilization test, it was determined that the bacterium could utilize citrate alone as a carbon source. The bacteria showed positive results using a glucose fermentation test, indicating it can ferment glucose. A lack of these components prevented it from producing hydrogen sulfide (SH2), indole, or motility. These tests contributed to identifying *Yersinia* spp. as the pathogen in the case under investigation.

3.2. Antibiogram

The inhibition zones for each antibiotic were measured to determine the bacterium's susceptibility to antibiotics (Figure 1).

Figure 1. The antibiotic disks were inserted into the media, allowing six different antibiotics to be tested for *Yersinia* resistance. The antibiotics utilized were ceftriaxone, nitrofurantoin, gentamicin, cefazolin, ampicillin and Tetracycline.

In addition, this bacterium displayed an extensive inhibitory zone (more than 17 mm), which qualified it as highly sensitive to nitrofurantoin. There was also evidence to indicate that the bacteria are highly susceptible to gentamicin, based on the fact that the zone diameter of the zone was 27mm. Furthermore, tetracycline also showed an effect against the bacteria, displaying a zone diameter of 23 mm, indicating susceptibility. The zone diameter of the bacterium was only 11 mm despite its resistance to ampicillin. As the zone diameters were 17 and 10, it was evident that the bacterium was resistant to ceftriaxone and cefazolin (Table 1).

Table 1. Analyses of antibiotic sensitivity and resistance in *Yersinia* spp.

Antibiotic	Amount(µg)	Zone Size (mm)	Sensitive	Intermediate	Resistance
Nitrofurantoin	300	28	17≤	16-15	14≥
Gentamicin	10	27	15≤	13-14	12≥
Tetracycline	30	23	15≤	14-12	11≥
Ceftriaxone	30	17	23≤	22-20	19≥
Ampicillin	10	11	17≤	16-14	13≥
Cefazolin	30	10	23≤	22-20	19≥

4. Discussion

One of the critical pathogenic bacteria is *Yersinia* spp., a member of the Enterobacteriaceae family (9). There are several serotypes and biotypes of this bacteria. Among the factors contributing to the bacterium's pathogenicity are its ability to grow at temperatures between 0 and 44°C and the diversity of its surface antigens. The *Yersinia* spp. causes yersiniosis, a zoonotic disease affecting humans and animals (10). As a treatment, a preventative, and even a growth promoter, antibiotics are used in veterinary medicine to treat infections and prevent infectious diseases (11). Resistance to antibiotics is on the rise, affecting both bacterial populations and different hosts. Evidence suggests that horizontal gene transfer and mobile genetic elements are responsible for developing this resistance, which can reduce the effectiveness of antimicrobial agents in humans and animals (12).

According to a study conducted in Bulgaria, *Yersinia* spp., isolated from pork, is resistant to ampicillin, tetracycline, and nalidixic acid yet sensitive to chloramphenicol and gentamicin (13). In Egypt, another study found the highest resistance to ampicillin, cefazolin, and amoxicillin/clavulanic acid among the strains of *Yersinia* spp (14). Numerous studies have

observed a variety of antibiotic resistance patterns in *Yersinia* isolates (15, 16). As a result of these patterns, it is imperative to conduct local susceptibility testing in certain regions (17).

To successfully treat bacterial infections, it is crucial to choose effective antibiotics for dealing with bacteria, particularly highly pathogenic bacteria such as *Yersinia*. Based on the results of the current study, it appears that the isolate of *Yersinia* spp. from castration surgical sites was very susceptible to nitrofurantoin and gentamicin. Due to their close relationship, these antibiotics may effectively treat and eradicate *Yersinia* spp. infections. The treatment of *Yersinia* spp. infection should not involve antibiotics like ampicillin, ceftriaxone, or cefazolin due to their resistance to these drugs.

Limitations of the present study include the small sample size and the limited number of control groups. In this study, we only examined bacteria isolated from castration surgery of male sheep, which may limit the generalizability of the results. To increase the accuracy and generalizability of the results, the number of samples in future studies should be increased, and the number of control groups should be considered. Moreover, repeating the experiments under different conditions and using different laboratory methods can help improve the results' validity and accuracy. In addition, using animals from different breeds and conditions, especially in different geographical areas, can provide more information about antibiotic resistance patterns in various bacteria and help design more effective treatment strategies (Table 2).

Table 2. This table can be a helpful tool to present suggestions and challenges that researchers can address in future studies, ensuring more comprehensive and valid results.

Challenge	Description	Suggested Improvement

Sample Size	The current study had a small sample size, limiting the ability to generalize findings.	Increase the sample size to ensure more robust and reliable results.
Control Group Limitations	Only a limited number of control groups were used, which may not reflect the broader spectrum of cases.	Add more control groups with diverse conditions to improve the validity of comparisons.
Replication of Experiments	The experiments were not repeated under different conditions, affecting the results' reproducibility.	Repeat experiments across different settings and methodologies to confirm consistency and reliability.
Animal Diversity	The study focused on a single breed of sheep, limiting the representation of different animal variations.	Include animals from different breeds and geographical regions to obtain a wider range of data on antibiotic resistance.
Geographical Variation	The study did not account for geographical differences in bacterial resistance patterns.	Perform studies in multiple regions to observe geographical trends in antibiotic resistance.
Data Interpretation	The limitations may influence the results of the testing methods and protocols used.	Use various testing methods to confirm findings and ensure comprehensive data interpretation.
Statistical Significance	The study might not have fully explored the statistical	Perform deeper statistical analyses to explore the significance of

	significance across different groups.	observed differences in greater detail.

As a revolutionary system, it is highly recommended that nanotechnology be considered in overcoming the challenge of drug resistance (18). Nanotechnology has attracted much attention due to its potential to improve disease diagnosis, treatment, and prevention, especially in medicine and veterinary medicine (19). Important applications of nanotechnology include designing nanoparticles for precise drug targeting, faster identification of pathogens, and the creation of intelligent drug delivery systems (20). This technology can also help develop more accurate diagnostic tools and optimize treatments for drug-resistant diseases. Therefore, further research and investment in this area can promise significant advances in various scientific and medical fields.

Conclusion

Various bacterial strains exhibit different antibiotic resistance profiles, so tailored treatment must be considered. According to the findings of this study, nitrofurantoin, gentamicin, and ceftriaxone may also be more effective than ampicillin, cefazolin, and ceftriaxone against *Yersinia* spp.

Declarations and statements

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Conflict of interests

The authors declare no conflict of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. IR.IAU.BABOL.REC.1403.065.

Author contribution

Conceptualization: [MB], …; Methodology: [M.B., Z.A., N.Z.], …; Formal analysis and investigation: [All Authors], …; Writing - original draft preparation: [All Authors]; Writing - review and editing: [All Authors], …; Funding acquisition: [Self-funding], …; Supervision: [Mohaddeseh Babaei]. All authors checked and approved the final version of the manuscript for publication in the present journal.

Consent to participate

N/A

Consent for publication

N/A

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