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ANTIBIOTIC SUSCEPTIBILITY TEST OF MUCOID *ESCHERICHIA COLI* ISOLATES CAUSING DIARRHEA IN BALI CATTLE

Uji Sensitivitas Bakteri *Escherichia coli* Isolat Mukoid sebagai Penyebab Diare pada Sapi Bali terhadap Antibiotika

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Abstract

Mucoid *Escherichia coli* is a significant causative agent of diarrhoea in Bali cattle. This study aimed to determine the antibiotic susceptibility pattern of eleven mucoid *E. coli* isolates against four antibiotics: Streptomycin, Doxycycline, Trimethoprim/Sulfamethoxazole, and Bacitracin, using the Kirby-Bauer disk diffusion method. The results demonstrated that all isolates (100%) were susceptible to Doxycycline. In contrast, the isolates exhibited a high rate of resistance to Trimethoprim/Sulfamethoxazole (90.91%) and Bacitracin (100%). For Streptomycin, the majority of isolates (90.91%) displayed an intermediate phenotype, with a minority (9.09%) being susceptible. These findings conclusively recommend Doxycycline as the therapeutic agent of choice for diarrhoea in Bali cattle caused by mucoid *E. coli*. The use of antibiotics to which the isolates showed intermediate or resistant profiles is discouraged. Continuous surveillance of susceptibility patterns is essential to monitor evolving antibiotic resistance.

Keywords: Antibiotic, *E. coli*, sensitivity, susceptibility, resistance.

Abstrak

Escherichia coli mukoid merupakan agen penyebab diare yang signifikan pada sapi Bali. Penelitian ini bertujuan untuk menentukan pola kepekaan antibiotik dari sebelas isolat *E. coli* mukoid terhadap empat antibiotik: Streptomisin, Doksisiklin, Trimethoprim/Sulfamethoxazole, dan Basitrasin, menggunakan metode difusi cakram Kirby-Bauer. Hasil penelitian menunjukkan bahwa semua isolat (100%) peka terhadap Doksisiklin. Sebaliknya, isolat menunjukkan tingkat resistensi yang tinggi terhadap Trimethoprim/Sulfamethoxazole (90,91%) dan Basitrasin (100%). Untuk Streptomisin, sebagian besar isolat (90,91%) menunjukkan fenotipe intermediet, dan sebagian kecil (9,09%)

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peka. Temuan ini secara conclusif merekomendasikan Doksisiklin sebagai agen terapi pilihan untuk diare pada sapi Bali yang disebabkan oleh *E. coli* mukoid. Penggunaan antibiotik yang menunjukkan profil intermediet atau resisten tidak disarankan. Surveilans berkelanjutan terhadap pola kepekaan sangat penting untuk memantau evolusi resistensi antibiotik.

Kata kunci: Antibiotik, *E. coli*, sensitivitas, resistensi.

INRTRODUCTION

The Bali cattle (*Bos javanicus domesticus*) are an indigenous Indonesian cattle breed, now distributed across numerous regions of the country, and play a vital role in national meat provision (Besung, Watiniasih, Mahardika, Agustina, & Suwiti, 2019). A predominant health constraint encountered in Bali cattle is diarrheal disease, which can be caused by viral, parasitic, or bacterial agents. The primary bacterial causative agent of diarrhea in Bali cattle is pathogenic *Escherichia coli*, a condition known as colibacillosis (Horri, Suyanto, & Jusnita, 2023). However, it is noteworthy that *E. coli* residing in the bovine digestive tract is typically classified as normal flora. The pathogenesis of pathogenic *E. coli* is generally attributed to its ability to produce one or more potent cytotoxins, specifically known as Shiga-like toxins (Wang et al., 2024). The predominant clinical manifestation of colibacillosis is diarrhea. Transmission of colibacillosis in cattle typically occurs via the fecal-oral route, through either direct or indirect contact (Robi, Mossie, & Temteme, 2024).

Escherichia coli is a Gram-negative bacterium that naturally inhabits the gastrointestinal tract of humans and animals. However, several strains are pathogenic and can cause serious infections. In recent decades, *E. coli* has demonstrated increasing resistance to various classes of antibiotics, including β-lactams, fluoroquinolones, and aminoglycosides (Naidoo & Zishiri, 2025). This resistance is primarily driven by the irrational use of antibiotics in both clinical practice and the agricultural sector, which promotes the natural selection of resistant strains. A particularly concerning mechanism of resistance is the production of extended-spectrum beta-lactamases (ESBLs), enzymes that hydrolyze and inactivate broad-spectrum antibiotics such as cefotaxime and ceftazidime. The emergence of antibiotic-resistant *E. coli* not only complicates clinical management of infections but also elevates the risk of horizontal transfer of resistance genes to other bacteria, thereby posing a serious threat to global public health (Oliveira et al., 2024).

This study was conducted to perform an antibiotic susceptibility test of mucoid *E. coli* isolates, the causative agent of diarrhea in Bali cattle, against Streptomycin, Doxycycline, Trimethoprim/Sulfamethoxazole, and Bacitracin. The objective is to identify antibiotics that remain effective against this pathogen and to determine which ones should be avoided in treating infections caused by pathogenic mucoid *E. coli*. The findings aim to provide a basis for selecting appropriate therapeutic antibiotics, thereby facilitating effective treatment of cattle afflicted with diarrhea due to infection by these mucoid *E. coli* isolates.

RESEARCH METHODS

Ethical Approval for Animal Subjects

This study did not require formal ethical approval as it did not involve direct intervention or manipulation of the Bali cattle from which samples were collected. Sample collection was conducted solely via rectal swab, a procedure that does not cause significant pain, undue discomfort, or stress to the animals.

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Research Subject

The subjects of this research were eleven (11) mucoid *Escherichia coli* isolates. These were isolated from the feces of diarrheic Bali cattle. The samples originated from animals sold by farmers at the Beringkit Animal Market, Tabanan, Bali.

Research Design

This study employed an observational research design. The methodology involved observing and recording the measurement results of the inhibition zone diameters on Mueller-Hinton Agar (MHA) media.

Research Variables

The variables in this study consisted of independent, dependent, and control variables. The independent variable was the mucoid *Escherichia coli* isolate used as the test subject. The dependent variable was the diameter of the inhibition zone (clear zone) formed around the antibiotic disc, indicating the antibacterial effectiveness of the treatment. The control variables included several factors maintained constant throughout the experiment: cultural media pH, incubation temperature, incubation duration, and inoculum turbidity level (e.g., adjusted to a 0.5 McFarland standard). This was to ensure that the results obtained were influenced solely by the independent variable being tested.

Isolation and Identification of Mucoid *E. coli*

The isolation and identification of Escherichia coli were conducted using standard bacteriological procedures. Fresh fecal samples from diarrheic Bali cattle were collected using sterile rectal swabs and immediately transported to the laboratory in cool boxes. Samples were streaked onto selective media, Eosin Methylene Blue Agar (EMBA), and incubated aerobically at 37°C for 24 hours. Presumptive E. coli colonies were identified based on their characteristic metallic green sheen on EMBA. For further biochemical confirmation, Analytical Profile Index (API) 20E test strips (bioMérieux, France) were used according to the manufacturer's instructions. The isolates were also subjected to additional biochemical tests, including Indole production, Methyl Red, Voges-Proskauer, and Citrate utilization (IMViC), yielding typical E. coli profiles (++--). Mucoid isolates were specifically selected based on their sticky, viscous colonial morphology on agar plates.

The Antibiotic Susceptibility Testing of The Mucoid *E. coli* Isolates

The antibiotic susceptibility testing of the mucoid *E. coli* isolates was conducted using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA). Briefly, a bacterial suspension standardized to a 0.5 McFarland turbidity was uniformly lawn-cultured onto the MHA surface. Subsequently, antibiotic discs impregnated with fixed concentrations of Streptomycin, Doxycycline, Trimethoprim/Sulfamethoxazole, and Bacitracin were aseptically placed onto the inoculated agar. The plates were then incubated at 37°C for 18-24 hours. Following incubation, the diameters of the zones of inhibition surrounding each disc were measured in millimeters. These measured values were interpreted according to clinical breakpoint standards (such as those from CLSI or EUCAST) to categorize each isolate as Susceptible (S), Intermediate (I), or Resistant (R) to each antibiotic. The resulting profile, showing 100% susceptibility to Doxycycline and high resistance to Bacitracin and Trimethoprim/Sulfamethoxazole, provides a critical guide for effective antimicrobial therapy in cases of diarrhea in Bali cattle.

Data Analysis

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The analysis used was descriptive analysis. This was performed by measuring the inhibition zone diameters on MHA media. Following measurement, the zone diameters were interpreted according to established clinical and laboratory standards (e.g., CLSI or EUCAST guidelines).

RESULTS AND DISCUSSION

Results

In this study, antibiotic susceptibility testing was performed on eleven (11) mucoid *Escherichia coli* isolates. These isolates were obtained from the feces of diarrheic Bali cattle and were previously confirmed as *E. coli* based on biochemical identification using the Analytical Profile Index (APItm 20E) test conducted at the Veterinary Diseases Investigation Center, Denpasar (BBVet Denpasar). The morphological appearance of a representative mucoid *E. coli* isolate is shown in Figure 1. Subsequently, these isolates were subjected to susceptibility testing against several antibiotics: streptomycin, doxycycline, trimethoprim/sulfamethoxazole, and bacitracin. The results of the susceptibility test are presented in Figure 2.

The diameter of the inhibition zone (clear zone) around each antibiotic disc was measured. The susceptibility testing revealed that the mucoid *E. coli* isolates causing diarrhea in Bali cattle were susceptible (100%) to doxycycline and had a susceptibility rate of 9.09% to streptomycin. An intermediate resistance phenotype was observed for 90.91% of the isolates against streptomycin and for 9.09% against trimethoprim-sulfamethoxazole. However, the isolates were 100% resistant to bacitracin and 90.91% resistant to trimethoprim-sulfamethoxazole. The measured inhibition zone diameters for each antibiotic disc are displayed in Table 1 and were interpreted using the standard zone diameter breakpoints provided in Table 2.

Discussion

In environments characterized by prolonged multi-stress conditions, pathogenic bacteria can evolve various mechanisms to evade the host immune system, thereby facilitating infection. The capsule, a polymer layer excreted around the cell wall, serves critical functions for the bacterium. These include protection from phagocytic attack and facilitation of adaptation to its environment by enabling adherence to epithelial and endothelial cells. This polymer, which encapsulates the entire cell, is commonly found in natural pathogens and is integral to key bacterial processes. It aids in biofilm regulation, supports the establishment of long-term infections, diminishes the effectiveness of immune defenses, and protects the bacterium from antimicrobial agents. The presence of a capsule influences the bacterium's ability to interact with antibiotics and, in some cases, can affect the outcomes of antimicrobial susceptibility testing (AST). Consequently, encapsulated bacteria may appear less susceptible to antibiotics under laboratory conditions compared to *in vivo* settings, where the host immune system can help mitigate the protective effects of the capsule (Gao et al., 2024).

In certain instances, encapsulated bacteria may be inherently more resistant to specific antibiotic classes, particularly those that target cell wall synthesis or that require penetration of the outer cellular layer (Belay et al., 2024). For example, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* are encapsulated pathogens known to exhibit varying degrees of resistance to antibiotics such as penicillin, largely due to the capsule's role in impeding drug activity. Regarding the disk diffusion method for AST, the capsule can physically alter the inhibition zone around an antibiotic disk. By potentially reducing antibiotic diffusion through the matrix, the capsule may result in a smaller zone of inhibition compared to non-encapsulated bacterial strains (Zhang, Chen, Yu, Pan, & Liu, 2019).

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Antimicrobial susceptibility testing aims to determine whether a bacterium is susceptible or resistant to various antibiotic preparations. This is assessed by observing the zone of inhibition around antibiotic disks on Mueller-Hinton Agar (MHA) following 18-24 hours of incubation. While antibiotic therapy remains a cornerstone for treating gastrointestinal infections, its continuous use carries risks, notably the development of resistance in pathogenic microbes to specific antibiotics. Conducting AST on pathogenic bacteria is therefore essential for understanding their susceptibility patterns to guide effective disease treatment (Gajic et al., 2022).

The results indicate that the mucoid *E. coli* isolates were 100% susceptible to doxycycline. Doxycycline is a semi-synthetic tetracycline antibiotic. Tetracyclines possess broad-spectrum activity, inhibiting Gram-positive and Gram-negative bacteria, mycoplasma, chlamydia, rickettsia, and some protozoa. Doxycycline is effective in treating gastrointestinal infections, skin infections, and genitourinary tract infections. Bacitracin is typically used for the prophylactic treatment of local infections, burns, minor wounds, and gastrointestinal infections caused by Gram-positive bacteria. Furthermore, bacitracin is effective against bovine respiratory disease complex, particularly infections caused by *Pasteurella multocida* (Lai, Chen, Huang, Chuang, & Tang, 2016).

Streptomycin is a broad-spectrum aminoglycoside antibiotic used in multi-drug regimens for pulmonary tuberculosis. It is also active against some aerobic Gram-negative bacteria. Sulfamethoxazole is a bacteriostatic sulfonamide antibiotic that disrupts bacterial folate synthesis. It acts as a competitive antagonist of para-aminobenzoic acid (PABA). It is commonly administered in combination with trimethoprim, which inhibits the enzyme dihydrofolate reductase, thereby damaging bacterial nucleoprotein synthesis. The combination of these two drugs acts synergistically (Baran, Kwiatkowska, & Potocki, 2023).

Antibiotic susceptibility patterns are generally categorized into three groups: susceptible, intermediate, and resistant. The susceptible category indicates that the bacterium can be inhibited or killed by the antibiotic at standard therapeutic doses. Intermediate signifies that the antibiotic's inhibitory effect is at a borderline level, making therapeutic success dependent on the achievable drug concentration at the site of infection or the use of increased dosing. Resistant denotes that the bacterium is unaffected by concentrations of the antibiotic that are usually effective, rendering therapy with that agent likely unsuccessful (Rodloff, Bauer, Ewig, Kujath, & Müller, 2008). These patterns are typically determined through susceptibility testing methods like disk diffusion or by determining the Minimum Inhibitory Concentration (MIC), with results interpreted according to international standards such as those from CLSI or EUCAST. Understanding susceptibility patterns is crucial for selecting appropriate empirical therapy and for monitoring the evolution of antimicrobial resistance in bacterial populations (Gajic et al., 2022).

Antibiotic resistance occurs when antibiotics lose their efficacy in inhibiting bacterial growth due to the microorganism's acquired ability to counteract the antimicrobial agent's action. This phenomenon is also driven by the continuous and irrational use of antibiotics (Pulingam et al., 2022). Throughout biological history, contemporary bacteria are markedly different from their historical counterparts. This divergence is influenced by a confluence of cultural, genetic, physiological, ecological, and medical factors. Modern bacteria often harbor distinct plasmids and characteristics, a significant number of which have developed resistance to contemporary antibiotics. Antibiotic resistance propagates through genetic exchange between bacteria inhabiting humans, animals, and the environment (Landecker, 2016). Additionally, resistance can arise from mutations within the existing bacterial genome. Although mutations induced by external environmental factors contribute less to resistance, the influence of elements like

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water, soil, and other ecological conditions remains significant. Bacterial genetic variation is profoundly shaped by existing ecological diversity. Antibiotic-resistant bacteria diminish the pool of effective treatment options, rendering common bacterial infections increasingly difficult to treat (Pulingam et al., 2022). Furthermore, antibiotic resistance is associated with increased morbidity and mortality rates, as well as higher healthcare costs (Llor & Bjerrum, 2014).

CONCLUSION AND SUGGESTION

Conclusion

The results of this study demonstrate that the mucoid *E. coli* isolates exhibited a 100% susceptibility rate to doxycycline. In contrast, the isolates showed a high rate of resistance, being 100% resistant to bacitracin and 90.91% resistant to trimethoprim-sulfamethoxazole. Regarding streptomycin, the majority of isolates (90.91%) displayed an intermediate phenotype, with a small proportion (9.09%) being susceptible. Similarly, a minor subset of isolates (9.09%) showed an intermediate response to trimethoprim-sulfamethoxazole.

Suggestion

Based on these findings, doxycycline is recommended as the preferred antibiotic for the treatment of diarrhea in Bali cattle caused by these mucoid *E. coli* isolates, given their demonstrated full susceptibility. The use of antibiotics to which the isolates showed intermediate or resistant profiles (streptomycin, trimethoprim-sulfamethoxazole, and bacitracin) is not advised. To ensure continued efficacy of antimicrobial therapy, it is imperative to conduct periodic susceptibility testing to monitor and stay informed of the evolving susceptibility patterns of bacterial pathogens in the field.

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Table

Table 1. Inhibition Zone Diameters of Mucoid *E. coli* Isolates Against Streptomycin, Doxycycline, Trimethoprim/Sulfamethoxazole, and Bacitracin

E. coli isolate	Antibiotic Inhibition Zone Diameter (mm)			
	Streptomycin	Doxycycline	Bacitracin	Trimethoprim-Sulfamethoxazole
Isolate 1	12(I)	20(S)	0(R)	10(R)
Isolate 2	13(I)	20(S)	0(R)	10(R)
Isolate 3	12(I)	20(S)	0(R)	10(R)
Isolate 4	12(I)	20(S)	0(R)	10(R)
Isolate 5	12(I)	20(S)	0(R)	10(R)
Isolate 6	14(I)	20(S)	0(R)	0(R)
Isolate 7	13(I)	20(S)	0(R)	10(R)
Isolate 8	14(I)	18 (S)	0(R)	10(R)
Isolate 9	12(I)	20(S)	0(R)	10(R)
Isolate10	12(I)	20(S)	0(R)	10(R)
Isolate 11	15(S)	22(S)	0(R)	12(I)

Note: mm = millimetres; R = Resistant; I = Intermediate; S = Susceptible

Table 2. Standard Table of Inhibition Zone Diameters

Susceptibility Pattern	Antibiotic (mm)			
	Streptomycin	Doxycycline	Bacitracin	Trimethoprim-Sulfamethoxazole
Resistant	≤11	≤12	≤8	≤10
Intermediate	12-14	13-15	9-12	11-15
Susceptible	≥15	≥16	≥13	≥16

Figure

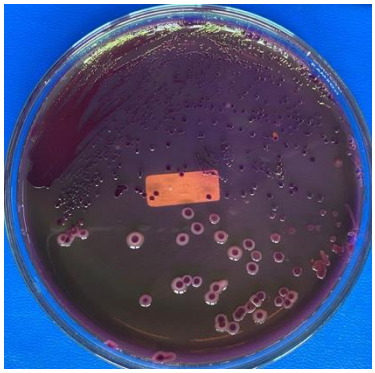

Figure 1. Bacterial Culture Results of Rectal Swab Samples from Diarrheic Bali Cattle on EMBA Media. The image shows Gram-negative bacterial colonies identified as mucoid *E. coli*.

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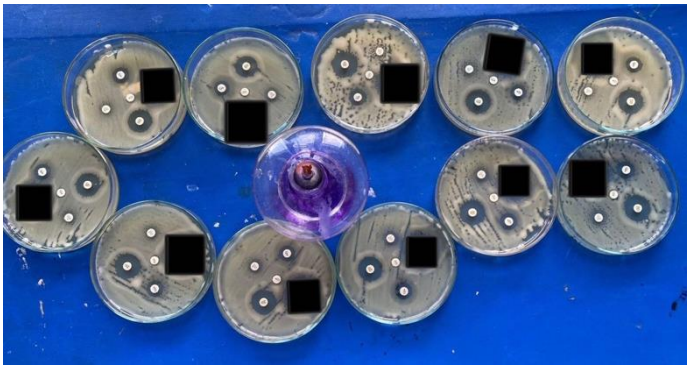

Figure 2. Antibiotic Susceptibility Test Results of Mucoid *E. coli* Isolates (Causative Agent of Diarrhea in Bali Cattle) on Mueller Hinton Agar Plates. The image shows (from left to right): Sulfamethoxazole/Trimethoprim, Streptomycin, Doxycycline, and Bacitracin (center).