Veterinary Biomedical & Clinical Journal

ISSN 2622-1012
Vet Bio Clin J. Vol. 6 No. 2 , December 2024 pp: 79-85
DOI: 10.21776/ub.VetBioClinJ.2024.006.02.4

Research Article

Antibiotic Sensitivity Profile of *Pasteurella multocida* in Cattle in Malang, Indonesia

Dodik Prasetyo[1], Indah Amalia Amri[2]*, Dian Siswanto[3], Rahayu Shafa Camila Sanjoyo[4], Monica Widyaningrum[4], Tanti Widya[4], Fidi Nur Eka Puji Dameanti[2]

[1] Veterinary Internal Medicine Laboratory FVM UB, Malang, East Java, Indonesia
[2] Veterinary Microbiology and Immunology Laboratory FVM UB, FVM UB, Malang, East Java, Indonesia
[3] Biology Departement, Faculty of Mathematics and Natural Science Veterinary, UB, Malang, East Java, Indonesia
[4] Bachelor of Veterinary Medicine Program, FVM UB, Malang, East Java, Indonesia
[*]*Email*: indahamaliaamri@ub.ac.id

Citation:
Prasetyo, D; Amri, I. A; Siswanto, D; Sanjoyo, R. S. S; Widyaningrum, M; Widya, T and Dameanti, F. N. E. P. (2024). Antibiotic Sensitivity Profile of Pasteurella multocida in Cattle in Malang, Indonesia. *Veterinary Biomedical and Clinical Journal*, 6(2), 79-85. https://doi.org/10.21776/ub.VetBioClinJ.2024.006.02.4

Article history:
Received: 13 October 2024
Accepted: 11 December 2024

Abstract: This study was conducted in Malang, Indonesia, to assess the prevalence and antibiotic susceptibility of *Pasteurella multocida* in cattle exhibiting clinical signs of respiratory disease. Nasal and tracheal swabs were collected from 50 cattle across multiple farms and tested for *P. multocida*. The isolates were identified through colony morphology, Gram staining, and biochemical tests, with 4 out of 50 samples (8%) testing positive for *P. multocida*. Antibiotic susceptibility was determined using the disk diffusion method, involving seven antibiotics commonly used in veterinary practice. Results showed that 75% of the isolates were sensitive to ceftriaxone, while amoxicillin-clavulanic acid was effective in 50% of cases. However, all isolates demonstrated resistance to erythromycin and tetracycline. These findings emphasize the importance of susceptibility testing to guide appropriate antibiotic therapy and prevent the development of resistance. Further research is necessary to monitor resistance patterns and improve treatment strategies for pasteurellosis in cattle.

Keywords: antibiotic susceptibility, cattle, Indonesia, Malang, pasteurellosis

INTRODUCTION

Pasteurella multocida is a significant pathogen in veterinary medicine, particularly affecting livestock such as cattle. This bacterium has been known for its role in various infections, which leads to substantial economic losses in the livestock industry due to decreased productivity and increased veterinary costs. Addressing these economic losses necessitates effective treatment strategies, which rely on the proper use of antibiotics. Understanding the antibiotic susceptibility profile of *P. multocida* is crucial for selecting appropriate antimicrobial therapies, especially in regions where antibiotic resistance has become increasingly prevalent (Sayed et al., 2023). In Malang, Indonesia, understanding the antibiotic sensitivity of *P. multocida* isolates from cattle is essential for improving therapeutic outcomes and managing infections effectively.

Research has shown that *P. multocida* isolates are sensitive to antibiotics such as enrofloxacin and gentamicin, while exhibiting resistance to penicillin G and ampicillin (Qandoos et al., 2022). This variability in antibiotic susceptibility is likely influenced by factors such as geographical location and antibiotic usage patterns. Also, Alemu et al. (2023) demonstrated that certain *P. multocida* isolates were 100% susceptible to ampicillin and gentamicin, which might have reflected regional differences in antibiotic resistance patterns, further emphasizing the complexity of resistance dynamics in different areas.

The emergence of multi-drug resistant strains of *P. multocida* poses a significant challenge in managing infections. Akhter et al., 2023 noted that the prevalence of multi-drug resistance in *P.*

multocida isolates from sheep in Bangladesh underscored the need for continuous monitoring of antibiotic susceptibility (Akhter et al, 2023). This situation is echoed by various studies that document the presence of resistance genes in *P. multocida*, complicating treatment options and necessitating the use of alternative antibiotics or combination therapies (Vu-Khac et al, 2020).

In Indonesia, limited data exist regarding the antibiotic susceptibility profile of *P. multocida* in cattle. This study aimed to fill that gap by providing a focused analysis of the antibiotic sensitivity of *P. multocida* isolates from cattle in Malang. By identifying effective antibiotics and understanding resistance patterns, this research will contribute to better management practices and therapeutic strategies in veterinary medicine.

METHOD

Sample Collection

The study was conducted in Malang, Indonesia. Nasal and tracheal swabs were collected from cattle exhibiting clinical signs of respiratory disease. A total of 50 cattle were sampled, ensuring a representative distribution across different farms from May 2024 to July 2024. The samples were collected using sterile swabs and immediately placed in transport media (Amies Transport Medium) to preserve the viability of the bacteria during transport to the laboratory (Mushtaq et al, 2023).

Isolation and Identification of *Pasteurella multocida*

In the laboratory microbiology, nasal and tracheal swabs were aseptically inoculated onto Blood Agar Plates to promote the selective growth of *P. multocida*. The inoculated plates were incubated at 37°C for 24-48 hours in a 5% CO2-enriched atmosphere under anaerobic conditions. For comparison, the swabs were also inoculated onto MacConkey agar (MCA) plates to evaluate the ability of the bacteria to grow on selective media. Colony identification was based on morphological characteristics, including the appearance of small, grayish colonies emitting a distinct mousy odor and (Desem et al, 2023).

Gram Staining and Biochemical Testing

Suspected *P. multocida* colonies were first subjected to Gram staining to determine their Gram-negative nature. The bacteria appeared as small, coccobacillary or rod-shaped Gram-negative organisms. Following Gram staining, a series of biochemical tests were conducted to confirm the identity of the isolates. These tests included the oxidase test, catalase test, and fermentation of sugars such as glucose (Gharibi et al, 2017).

Antibiotic Susceptibility Testing

Antibiotic susceptibility of the confirmed *P. multocida* isolates was assessed using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of antibiotics commonly used in veterinary medicine was tested for, including; Ceftriaxone 30µg (CRO), Ciprofloxacin 5µg (CIP), Amoxicillin-Clavulanic Acid 30µg (AMC), Enrofloxacin 5µg (ENR), Gentamicin 10µg (CN), Erythromycin (E), and Tetracycline 30µg (TE). The zones of inhibition were measured and interpreted to determine the susceptibility or resistance of the isolates (CLSI, 2020).

Data Analysis

The data obtained from the isolation, identification, and antibiotic susceptibility testing of *P. multocida* isolates were analyzed descriptively.

RESULT AND DISCUSSION

Pasteurella multocida is a Gram-negative bacterium a significant pathogen in both livestock and humans. In cattle, it is primarily associated with diseases such as the bovine respiratory disease complex (BRDC) and hemorrhagic septicemia, which may lead to severe economic losses in the livestock industry (Ara et al, 2016). The bacterium is part of the normal flora of the upper respiratory tract in many animals, including cattle, and may become pathogenic under certain conditions, particularly when the host is stressed or immunocompromised (Abed et al., 2020).

The results presented in Table 1 provide a concise overview of the identification of *Pasteurella multocida* from the samples collected in this study. Out of 50 cattle showing clinical signs of respiratory disease in Malang, 4 samples (8%) tested positive for *P. multocida*. This finding aligns with previous studies reporting varying prevalence rates, indicating that environmental and management factors significantly influence the occurrence of *P. multocida*. This result is consistent with a study by Besung et al. (2016) in Bali, which also reported a low prevalence (5%) in cattle, with 15 positive samples out of a total of 300 samples. Despite these findings, the low prevalence observed in this study does not align with field observations, where many cattle were clinically suspected of being infected with *P. multocida*. This discrepancy may be due to limitations in sampling techniques, timing of sample collection, or the sensitivity of the diagnostic methods employed.

Table 1. Identification of *P.multocida*

Test	Result/Indication	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Blood Agar	Small, grayish colonies, mousy odor	+	+	+	+
MacConkey Agar	No growth	+	+	+	+
Gram Stain	Gram-negative, coccobacillary	+	+	+	+
Oxidase Test	Positive	+	+	+	+
Catalase Test	Positive	+	+	+	+
Glucose Fermentation	Acid without gas	+	+	+	+

The positive samples were confirmed by the distinct morphological characteristics of the colonies, which appeared as small, grayish colonies with a characteristic mousy odor on blood agar. No growth was observed on MacConkey agar, consistent with *P. multocida* known inability to grow on selective media such as MacConkey agar. Gram staining revealed Gram-negative, coccobacillary or rod-shaped bacteria, and biochemical tests further confirmed the identity of the isolates based on their ability to ferment glucose without gas production, a key indicator of *P. multocida* (Gharibi et al, 2017).

Antibiotic resistance is a growing concern in the treatment of *P. multocida* infections. The increasing prevalence of multidrug-resistant strains is attributed to several factors, including the overuse of antibiotics in veterinary medicine and the use of antibiotic additives in animal feed (Hasnan et al., 2022). Studies have shown that *P. multocida* isolates from cattle often exhibit resistance to multiple antibiotics, complicating treatment options (Priyantha et al, 2021).

The antibiotic susceptibility profile of the four *P. multocida* isolates was assessed using various antibiotics, as shown in Table 2. The results demonstrate variable susceptibility patterns across the isolates, with some showing resistance (R), intermediate (I) and full sensitivity (S) to particular antibiotics.

Table 2. Antibiotic Susceptibility Testing for *P.multocida* with zone of inhibition (mm) for seven antibiotics

Isolate	Zone Inhibition (mm)						
	CRO	**CIP**	**AMC**	**ENR**	**CN**	**E**	**TE**
1	29 (S)	29 (S)	24 (S)	24 (S)	19 (S)	6.5 (R)	9 (R)
2	21.5 (I)	24 (I)	19 (S)	24 (S)	19 (S)	9 (R)	4 (R)
3	24 (S)	24 (I)	16.5 (I)	19 (I)	14 (R)	4 (R)	4 (R)
4	24 (S)	24 (I)	16.5 (I)	19 (I)	14 (R)	4 (R)	4 (R)

*CRO: Ceftriaxone; CIP: Ciprofloxacin; AMC: Amoxycilin clavulanic acid; ENR: Enrofloxacin; CN: Gentamicin; E: Erythromicin; TE: Tetracycline

The study found that 75% of *P. multocida* isolates were sensitive to ceftriaxone (CRO), with 25% showing intermediate sensitivity. This suggests that ceftriaxone remains a strong option for treatment, although some cases may require caution, higher doses, or alternative therapies to manage infections effectively. Notably, ceftriaxone (CRO) demonstrated a high sensitivity rate, with 100% of isolates being sensitive according to some studies (Venu et al, 2023).

Similarly, amoxicillin-clavulanic acid (AMC) was effective for 50% of the isolates, while the remaining 50% displayed intermediate sensitivity, indicating that while this antibiotic combination could be useful, it might have required careful monitoring to ensure treatment success in some cases. In terms of fluoroquinolones, only 25% of the isolates were sensitive to ciprofloxacin (CIP), with 75% showing intermediate sensitivity. This finding suggested reduced efficacy for ciprofloxacin, which might still have been effective but less reliable in this population. Similarly, enrofloxacin (ENR) displayed a 50% sensitivity rate, with the other 50% showing intermediate sensitivity, reflecting its potential use but also limitations in some cases. Gentamicin (CN) showed more concerning results, with only 50% of the isolates being sensitive, while the other 50% were resistant. This finding is consistent with previous research that identified resistance genes, such as aphA1, strB, and aacA4, which confer resistance to aminoglycosides, including gentamicin, in *P. multocida* isolates (Wang et al., 2017). This indicate that gentamicin may have limited effectiveness against *P. multocida* in this population, and resistance might pose a challenge in certain treatment scenarios. The study also highlighted significant resistance to erythromycin (E) and tetracycline (TE), with 100% of the isolates resistant to both antibiotics. These results indicate that neither erythromycin nor tetracycline were suitable for treating *P. multocida* infections in this study, necessitating the use of other, more effective antibiotic options.

This study has some limitations that need to be acknowledged when evaluating the findings. The sample size used was rather limited, comprising only 50 cattle and only four isolates of *P. multocida*. A larger sample size will be necessary to yield more representative findings regarding the prevalence and antibiotic resistance profile of *P. multocida* in the region. Secondly, geographical factors

impacted the results of this study. The data gathered pertains exclusively to cattle from the Malang region, and thus, the results specifically reflect the situation in this area. Consequently, additional research with a broader geographical scope and larger sample sizes was recommended to achieve a more comprehensive understanding of the antibiotic resistance patterns of *P. multocida* throughout Indonesia.

CONCLUSION

In the present study, while several antibiotics, including ceftriaxone and amoxicillin-clavulanic acid, were found to be effective against *P. multocida* isolates, the emergence of resistance to commonly used antibiotics such as erythromycin and tetracycline highlights the growing challenge of multidrug resistance. The variability in sensitivity to fluoroquinolones and gentamicin further emphasizes the need for careful selection of antibiotics. These findings support judicious use of antibiotics and stress the importance of performing susceptibility testing to avoid treatment failures and ensure the effective management of pasteurellosis in cattle.

DECLARATION ON THE USE OF AI IN THE WRITING PROCESS

The authors of this document affirm that no generative artificial intelligence (AI) or AI-assisted tools were employed to produce content, concepts, or hypotheses during the composition of this work. AI has been exclusively utilized to enhance readability and refine language under strict human supervision and control. Subsequent to the application of AI technologies, the authors reviewed and modified the manuscript to ensure accuracy and coherence. The authors acknowledge AI's potential to generate ostensibly authoritative content that may be inaccurate, incomplete, or biased. In light of this, the authors ensured that the manuscript underwent thorough human revision and evaluation. In accordance with Elsevier's Authorship Policy, the authors confirm that no AI- or AI-assisted technologies have been credited as an author or co-author of this manuscript. The authors fully acknowledge that authorship entails responsibilities and tasks that can only be attributed to and executed by humans, and that they have adhered to these guidelines in the preparation of this document.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support provided by the Directorate of Research and Community Service (DRPM), Universitas Brawijaya, through the PDP (Penelitian Dasar Pemula) 2024

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

ETHICAL STANDARDS

This study received ethical approval from the Komisi Etik Penelitian (KEP) Universitas Brawijaya, Malang, Indonesia and and the authors have taken permission from the farm owners to publish the data.

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