Available online at https://journal.ipb.ac.id/index.php/tasj



# Multidrug-Resistant Methicillin-Resistant Coagulase-Negative Staphylococci and Staphylococcus aureus in Cattle and Goats from the East Coast of Peninsular Malaysia

# N. M. Mohamada,c, P. N. M. Zakaria, Z. Suhaili, S. A. Abu Bakar, & E. Aklilu, Aklilu,

<sup>a</sup>Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Malaysia <sup>b</sup>Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Terengganu, Malaysia <sup>e</sup>Centralised Laboratory Management Center, Universiti Sultan Zainal Abidin, Terengganu, Malaysia \*Corresponding author: zarizal@unisza.edu.my; erkihun@umk.edu.my (Received 12-12-2024; Revised 30-04-2025; Accepted 02-05-2025)

### **ABSTRACT**

This study investigates the prevalence and antimicrobial resistance profiles of methicillinresistant Staphylococcus aureus (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) in livestock from Kelantan, Terengganu, and Pahang, Malaysia. Nasopharyngeal swabs from 290 goats and 106 cattle were processed using an improved transport and enrichment method. Staphylococci were identified via PCR targeting the nucA and mecA genes, with antimicrobial susceptibility determined according to CLSI and EUCAST guidelines. Among 396 isolates, 55 (13.9%) were identified as S. aureus, including one MRSA isolate (0.25%), and methicillin resistance was detected in 31 CoNS isolates (7.8%), predominantly from goats. Fourteen of the MRCoNS isolates exhibited multidrug resistance to 3 to 7 antibiotic classes, with 47.2% of CoNS isolates being resistant to fusidic acid, raising concerns about zoonotic transmission and public health risks. The prevalence of staphylococcal colonization and methicillin resistance was higher in goats than in cattle, suggesting that environmental exposures, management practices, and antibiotic use contribute to the resistance patterns. The findings highlight the urgent need for enhanced biosecurity measures, prudent antibiotic use, and expanded surveillance to address antimicrobial resistance in livestock. A One Health approach that integrates human, animal, and environmental health is essential to mitigating the spread of resistance. This study provides baseline data to guide future research, interventions, and policies in reducing public health risks associated with MDR staphylococci in livestock.

antimicrobial resistance; coagulase-negative staphylococci; methicillin-resistant; **Keywords:** multidrug-resistant; Staphylococcus aureus

### **INTRODUCTION**

Staphylococcus aureus and coagulase-negative staphylococci (CoNS) are prevalent commensals and opportunistic pathogens in humans and animals, often associated with significant antimicrobial resistance (AMR) challenges (Guo et al., 2020; Terreni et al., 2021). The unregulated and widespread use of antibiotics in livestock farming has accelerated the emergence of multidrug-resistant (MDR) strains, such as methicillinresistant S. aureus (MRSA) and methicillin-resistant CoNS (MRCoNS) (Salam et al., 2023). These resistant pathogens pose serious threats not only to veterinary medicine but also to public health, as they can be transmitted from animals to humans through direct contact or contaminated animal products (Khairullah et al., 2023; Silva et al., 2022).

Several Asian studies have reported high MRSA and MRCoNS prevalence in livestock, particularly in regions with intensive farming practices (Kalai et al., 2021; Tanomsridachchai et al., 2021). However, limited data exist for the East Coast region of Malaysia, where cattle and goat farming constitutes a significant portion of the national livestock industry. Understanding the prevalence and antimicrobial resistance patterns in these livestock populations is crucial to mitigating the risk of zoonotic transmission and informing antibiotic stewardship strategies.

In Malaysia, MRSA has been previously identified in food-producing animals, particularly poultry and swine (Chai et al., 2020; Aklilu & Ying, 2020). However, data on MRCoNS prevalence in ruminants such as cattle and goats remains scarce. Additionally, most prior studies have relied on standard culture methods for staphylococcal isolation, which may not effectively capture resistant strains due to their variable growth requirements and selective pressures.

The mecA gene, encoding a modified penicillinbinding protein (PBP2a), is the primary determinant of methicillin resistance in staphylococci. While MRSA has been widely studied, the rising prevalence of MRCoNS—once considered less pathogenic—has emerged as a significant concern due to its role as reservoirs and transmitters of antimicrobial resistance genes (Silva *et al.*, 2022). Studies across Asia report alarmingly high rates of MRSA and MRCoNS in livestock, particularly in regions with intensive farming practices (Kalai *et al.*, 2021; Tanomsridachchai *et al.*, 2021).

In Malaysia, the East Coast states of Kelantan, Terengganu, and Pahang account for 44% of the national cattle population and 33% of the goat population (Jabatan Perkhidmatan Veterinar Malaysia, 2023), underscoring their importance to livestock production. However, limited data exist on the prevalence and resistance patterns of MRSA and MRCoNS in these regions, creating a significant knowledge gap. Addressing this gap is crucial for developing effective strategies to mitigate the spread of antimicrobial resistance in both veterinary and public health sectors.

This study provides the first known data on the prevalence and antimicrobial resistance profiles of MRSA and MRCoNS in cattle and goats from Kelantan, Terengganu, and Pahang. It employs an improved transport and enrichment method for staphylococcal isolation, which enhances bacterial recovery efficiency and minimizes contamination, contributing to more reliable resistance profiling.

This study aims to determine the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) in livestock from East Coast Malaysia. By examining isolates from cattle and goats, the research provides a comprehensive overview of the presence of these resistant strains in the region. Additionally, the study assesses the antimicrobial resistance profiles of these isolates, with a particular focus on multidrug resistance (MDR) patterns, which pose significant challenges in both veterinary and human medicine. Lastly, the study explores potential factors influencing antimicrobial resistance, including livestock species, farming practices, hygiene standards, and environmental exposures. These findings will contribute valuable baseline data to support future surveillance programs and the implementation of targeted antimicrobial stewardship strategies in Malaysian livestock farming.

# MATERIALS AND METHODS

## **Ethical Approval**

Informed written and signed consent was obtained from animal owners or their representatives. This research was reviewed and approved on 27th October 2022 by the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (Reference: UMK/FPV/ACUE/PG/003/2022)

# Study Design and Sampling

This study was conducted as a surveillance investigation to detect multidrug resistance in *Staphylococcus* 

aureus and coagulase-negative staphylococci (CoNS) in Kelantan, Terengganu, and Pahang. The data collection period spanned from November 2022 to April 2023. Sampling was carried out between November 2022 and April 2023. A total of 396 nasopharyngeal swabs were collected from 290 goats and 106 cattle owned by small-holder farmers and university farms.

Nasal swabs were collected using sterile swabs inserted approximately 2 cm into each nostril, rotated against the nasal wall, and repeated for the opposite nostril using the same swab (Chai *et al.*, 2022). The swabs were then immersed in a modified transport medium containing Baird Parker Agar Base supplemented with 6.5% sodium chloride (Baird-Parker, 1962) to preserve bacterial viability during transport to the laboratory.

#### **Bacterial Strains Isolation**

In the laboratory, tubes containing the modified transport medium and swabs were supplemented with 5 mL of modified enrichment medium (Baird Parkerbased broth without agar, supplemented with 6.5% sodium chloride). The samples were incubated at 37 °C for 24 hours to enrich bacterial populations. Following enrichment, the samples were cultured on Baird Parker Agar (BPA) (Becton Dickinson, USA) and incubated at 37 °C for 24 hours. Presumptive *Staphylococcus* colonies with characteristic black pigmentation on BPA were subcultured onto Mannitol Salt Agar (MSA) (Oxoid, UK) to obtain pure isolates (Baird-Parker, 1962; Tallent *et al.*, 2001).

# Molecular Identification of Staphylococci Isolates

Presumptive staphylococcal colonies from MSA were confirmed via PCR assays targeting the species-specific *nucA* gene for *S. aureus* and *mecA* gene for methicillin resistance. DNA extraction was performed using the boiling method, where bacterial cells were suspended in sterile distilled water, heated at 95 °C for 10 minutes, and centrifuged to obtain DNA from the supernatant. The primers for *nucA* (278-bp amplicon) and *mecA* (533-bp amplicon) were used as previously described (Aklilu & Ying, 2020; Zarizal *et al.*, 2018). The PCR conditions included initial denaturation at 95°C for 3 minutes, followed by 30 cycles of 95 °C for 15 seconds, 55 °C for 15 seconds, and 72 °C for 20 seconds.

# **Antimicrobial Susceptibility Test**

Antimicrobial susceptibility testing was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2022). A bacterial suspension prepared from MSA colonies was inoculated onto Mueller Hinton Agar (MHA). Antibiotic discs included cefoxitin (30 µg), ceftaroline (30 µg), clindamycin (2 µg), erythromycin (15 µg), azithromycin (15 µg), gentamicin (10 µg), tetracycline (30 µg), minocycline (30 µg), doxycycline (30 µg), ciprofloxacin (5 µg),

norfloxacin (10  $\mu g)$ , rifampicin (5  $\mu g)$ , linezolid (30  $\mu g)$ , chloramphenicol (30  $\mu g)$ , and fusidic acid (10  $\mu g)$  (Oxoid, UK). Results were interpreted after 18 hours of incubation at 35 °C by measuring the zones of inhibition around the discs.

# **Data Analysis**

Antimicrobial susceptibility profiles were summarized as percentages of resistant, intermediate, and susceptible isolates for each antibiotic. Multidrug resistance (MDR) was defined as resistance to three or more antibiotic classes.

#### **RESULTS**

### Phenotypic and Genotypic Identification of Isolates

A total of 396 nasal swab samples, comprising 290 goats and 106 cattle, were collected from livestock in Kelantan, Terengganu, and Pahang. Among these, 55 isolates (13.9%) were identified as *Staphylococcus aureus*, while the remaining 341 isolates (86.1%) were classified as coagulase-negative staphylococci (CoNS). Genotypic confirmation was conducted via PCR targeting the *nucA* gene for *S. aureus* and *mecA* for methicillin resistance.

Methicillin resistance was detected in 32 isolates (8.08%), with one MRSA isolate identified in goats (0.25%) and 31 MRCoNS isolates distributed among 8 cattle and 23 goats. Notably, the single MRSA isolate harbored the *mecA* gene but demonstrated susceptibility

to cefoxitin, indicating a 'stealth' phenotype, which may lead to false-negative detection using standard phenotypic methods. The breakdown of isolates is presented in Table 1.

# **Antimicrobial Susceptibility Profiles**

The antimicrobial susceptibility profiles of 55 *S. aureus* isolates revealed high susceptibility (>80%) to most tested antibiotics, including clindamycin, azithromycin, tetracyclines, fluoroquinolones, and linezolid (Table 2). Cefoxitin resistance was observed in 55.6% of *S. aureus* isolates; however, none of these carried the *mecA* gene. The MRSA isolate (harboring *mecA*) remained susceptible to cefoxitin and all other antibiotics tested.

Among the 341 CoNS isolates, 58.1% showed resistance to cefoxitin and 47.2% to fusidic acid. Other antibiotics, such as clindamycin and erythromycin, exhibited moderate resistance rates, 10.6% and 14.1%, respectively (Table 3). Ultimately, 14 MRCoNS isolates were classified as multidrug-resistant (MDR), displaying resistance to 3 to 7 antibiotic classes (Table 4). These MDR MRCoNS isolates were predominantly obtained from goats and exhibited consistent resistance to cefoxitin and fusidic acid.

### Comparison of Resistance Patterns by Livestock Type

Goats exhibited a higher prevalence of staphylococcal colonization and methicillin resistance compared to cattle. Among the 290 goat samples, 26.76% yielded

Table 1. Distribution of Staphylococci nasal colonization according to the animal types

Sample	Staphylococcus aureus colonisation		Coagulase-negative Staphylococci (CoNS) colonization		Total
•	MRSA	Non-MRSA	MRConS	Non-MRCons	
Cattle	0 (0%)	21 (5.30%)	8 (2.02%)	77 (19.44%)	106
Goats	1 (0.25%)	33 (8.33%)	23 (5.81%)	233 (58.83%)	290
Total	1	54	31	310	396

 $Note: MRSA=Methicillin-resistant \ \textit{Staphylococcus aureus}, MRCoNS=Methicillin-resistant \ coagulase-negative \ staphylococci.$ 

Table 2. Antimicrobial drugs used and susceptibility percentages of 55 Staphylococcus aureus isolates

Cl ( ('11' ('	A (:T-:	D: 1	Staphylococcus aureus		
Class of antibiotics	Antibiotics	Disc used	Resistant	Intermediate	Susceptible
Cephems	Cefoxitin (30 µg)	FOX30	31 (55.6%)	N/A	24 (44.4%)
	Ceftaroline (30 µg)	CPT30	0	0	55 (100%)
Lincosamides	Clindamycin (2 µg)	DA2	3 (5.6%)	4 (7.4%)	48 (87%)
Macrolides	Erythromycin (15 μg)	E15	1 (1.9%)	8 (14.8%)	46 (83.3%)
	Azithromycin (15 μg)	AZM15	1(1.9%)	0	54 (98.1%)
Aminoglycosides	Gentamicin (10 µg)	CN10	4 (7.4%)	0	51 (92.6%)
Tetracyclines	Tetracycline (30 μg)	TE30	0	1 (1.9%)	54 (98.1%)
	Minocycline (30 μg)	MH30	1 (1.9%)	0	54 (98.1%)
	Doxycycline (30 µg)	D030	1(1.9%)	0	54 (98.1%)
Fluoroquinolones	Ciprofloxacin (5 µg)	CIP5	3 (5.6%)	0	52 (94.4%)
-	Norfloxacin (10 μg)	NOR10	1 (1.9%)	0	54 (98.1%)
Ansamycins	Rifampicin (5 μg)	RD5	1 (1.9%)	1 (1.9%)	53 (96.2%)
Oxazolidinones	Linezolid (30 µg)	LZD30	2 (3.8%)	N/A	53 (96.2%)
Phenicols	Chloramphenicol (30 µg)	C30	2 (3.7%)	1 (1.9%)	52 (94.4%)
*Fusidane	*Fusidic acid (10 µg)	FD10	5 (9.3%)	N/A	50 (90.7%)

Note: All antimicrobial susceptibility breakpoint interpretations according to CLSI 2020, except \*Fusidic acid breakpoint guideline used EUCAST 2022. N/A = Not applicable (no intermediate category for these antibiotics). Percentages were calculated from the total of 55 *S. aureus* isolates.

S. aureus isolates, with 5.81% classified as MRCoNS. In contrast, only 5.3% of cattle samples contained S. aureus isolates, with 2.02% were MRCoNS-positive. These findings suggest that goats may serve as a more significant reservoir for multidrug-resistant staphylococci in the studied region.

# Prevalence and Characteristics of MDR MRCoNS

MDR MRCoNS isolates were characterized by resistance to various antibiotic classes, including cefoxitin, clindamycin, macrolides (erythromycin, azithromycin), phenicols (chloramphenicol), and fusidic

Table 3. Antimicrobial drugs used and susceptibility percentages of 341 coagulase-negative staphylococci isolates

Class of antibiotics	Antibiotics	Disc used	Coagulase negative Staphylococci		
Class of antibiotics		Disc used -	Resistant	Intermediate	Susceptible
Cephems	Cefoxitin (30 μg)	FOX30	198 (41.9%)	N/A	143 (58.1%)
	Ceftaroline (30 µg)	CPT30	3 (0.9%)	3 (0.9%)	335 (98.2%)
Lincosamides	Clindamycin (2 µg)	DA2	36 (10.6%)	55 (16.1%)	250 (73.3%)
Macrolides	Erythromycin (15 μg)	E15	48 (14.1%)	33 (9.7%)	260 (76.2%)
	Azithromycin (15 μg)	AZM15	17 (5%)	5 (1.5%)	319 (93.5%)
Aminoglycosides	Gentamicin (10 μg)	CN10	17 (5%)	4 (1.2%)	320 (93.8%)
Tetracyclines	Tetracycline (30 μg)	TE30	33 (9.7%)	16 (4.7%)	292 (85.6%)
	Minocycline (30 μg)	MH30	3 (0.8)	6 (1.6%)	332 (97.6%)
	Doxycycline (30 μg)	D030	11 (3.2%)	5 (1.5%)	325 (95.3%)
Fluoroquinolones	Ciprofloxacin (5 µg)	CIP5	9 (2.6%)	3 (0.9%)	329 (96.5%)
-	Norfloxacin (10 µg)	NOR10	5 (1.5%)	3 (0.8%)	333 (97.7%)
Ansamycins	Rifampicin (5 µg)	RD5	14 (4.1%)	8 (2.3%)	319 (93.5%)
Oxazolidinones	Linezolid (30 µg)	LZD30	17 (5%)	N/A	324 (95%)
Phenicols	Chloramphenicol (30 µg)	C30	11 (3.2%)	2 (0.6%)	328 (96.2%)
Fusidane	*Fusidic acid (10 μg)	FD10	164 (47.2%)	N/A	177 (52.8%)

Note: All antimicrobial susceptibility breakpoint interpretations according to CLSI 2020, except \*Fusidic acid breakpoint guideline used EUCAST 2022. N/A = Not applicable.

Table 4. Resistance phenotype of multidrug-resistant MRCoNS isolates

Methicillin-resistant coagulase-negative isolates	Antibiotic resistance	Number of antibiotic classes (Classes)
MRCoNS KL2L74	Cefoxitin, Clindamycin, Erythromycin, Azithromycin, Chloramphenicol, Fusidic Acid	5 (Cephems, Lincosamides, Macrolides, Phenicols, Fusidane)
MRCoNS KL2L77	Cefoxitin, Clindamycin, Erythromycin, Azithromycin, Gentamicin, Ciprofloxacin, Norfloxacin, Chloramphenicol, Fusidic Acid	6 (Cephems, Lincosamides , Macrolides, Fluoroquinolones, Phenicols, Fusidane)
MRCoNS B12K9	Cefoxitin, Ceftaroline, Tetracycline, Fusidic Acid	3 (Cephems, Tetracyclines, Fusidane)
MRCoNS B12K17	Cefoxitin, Clindamycin, Erythromycin, Azithromycin, Gentamicin, Tetracycline, Doxycycline, Linezolid, Fusidic Acid	7 (Cephems, Lincosamides, Macrolides, Aminoglycosides, Tetracyclines, Oxazolidinones, Fusidane)
MRCoNS BK1L6	Cefoxitin, Tetracycline, Fusidic Acid	3 (Cephems, Tetracyclines, Fusidane)
MRCoNS BK2L15	Cefoxitin, Erythromycin, Azithromycin, Fusidic Acid	3 (Cephems, Macrolides, Fusidane)
MRCoNS BK2L26	Cefoxitin, Erythromycin, Azithromycin, Rifampicin, Fusidic Acid	4 (Cephems, Macrolides, Ansamycins, Fusidane)
MRCoNS BK2L33	Cefoxitin, Erythromycin, Azithromycin, Fusidic Acid	3 (Cephems, Macrolides, Fusidane)
MRCoNS BK3K10	Cefoxitin, Erythromycin, Azithromycin, Fusidic Acid	3 (Cephems, Macrolides, Fusidane)
MRCoNS BK3K11	Cefoxitin, Clindamycin, Erythromycin, Azithromycin, Tetracycline, Chloramphenicol, Fusidic Acid	6 (Cephems, Lincosamides, Macrolides, Tetracyclines, Phenicols, Fusidane)
MRCoNS BK3K12	Cefoxitin, Clindamycin, Erythromycin, Azithromycin, Tetracycline, Chloramphenicol, Fusidic Acid	6 (Cephems, Lincosamides, Macrolides, Tetracyclines, Phenicols, Fusidane)
MRCoNS BK3K23	Cefoxitin, Clindamycin, Erythromycin, Azithromycin, Chloramphenicol	4 (Cephems, Lincosamides, Macrolides, Phenicols)
MRCoNS JK1K9	Cefoxitin, Erythromycin, Azithromycin, Chloramphenicol, Fusidic Acid	4 (Cephems, Macrolides, Phenicols, Fusidane)
MRCoNS JK1K25	Cefoxitin, Clindamycin, Erythromycin, Azithromycin, Tetracycline, Fusidic Acid	5 (Cephems, Lincosamides, Macrolides, Tetracyclines, Fusidane)

acid. Notably, isolate B12K17 exhibited the broadest resistance, spanning seven antibiotic classes. The resistance phenotypes of MDR MRCoNS isolates are detailed in Table 4.

#### **DISCUSSION**

This study provides critical insight into the prevalence and antimicrobial resistance profiles of Staphylococcus aureus and coagulase-negative staphylococci (CoNS) in livestock from the East Coast region of Peninsular Malaysia. Methicillin resistance was detected in 8.08% of isolates, including one MRSA and 31 MRCoNS. The low prevalence of MRSA (0.25%) aligns with findings from Malaysia and Southeast Asia, where livestock-associated MRSA rates are generally lower than other regions (Chai et al., 2020; Aklilu & Ying, 2020). However, the identification of MRCoNS as the predominant methicillin-resistant staphylococci underscores their significance as reservoirs of antimicrobial resistance genes. Goats exhibited higher colonization and methicillin resistance rates compared to cattle, with MRCoNS detected in 5.81% of the former's samples versus 2.02% of the latter's. This difference may reflect variations in farming practices, hygiene standards, and environmental exposures, as goats are often raised in semi-intensive systems where they are more exposed to environmental contaminants and antibiotic residues (Kalai et al., 2021; Terreni et al., 2021).

The detection of a single 'stealth' MRSA isolate adds an interesting dimension to the findings. This isolate harbored the mecA gene but remained phenotypically susceptible to cefoxitin. Stealth MRSA is of concern because it can evade detection by conventional phenotypic methods, leading to underreporting of methicillin resistance (Liang et al. 2022; Guo et al., 2020). The presence of cefoxitinsusceptible MRSA isolates, often referred to as 'stealth MRSA, highlights the potential for false-negative results in conventional phenotypic detection methods, reinforcing the need for molecular-based confirmation. The identification of this isolate emphasizes the importance of incorporating molecular methods such as PCR in routine diagnostics to ensure accurate detection of resistance. Although stealth MRSA is uncommon, its potential public health implications warrant further investigation, particularly in resource-limited settings, where access to molecular diagnostics may be limited (Silva et al., 2022).

The detection of multidrug-resistant (MDR) MRCoNS is a significant concern. These isolates exhibited resistance to multiple antibiotic classes, including beta-lactams, macrolides, tetracyclines, and fusidic acid. Fusidic acid resistance, observed in 47.2% of CoNS isolates, is particularly alarming because this antibiotic is frequently used to treat staphylococcal infections in humans (Boloki *et al.*, 2021). The high rates of resistance observed in this study are consistent with the findings from other regions, where CoNS have emerged as important reservoirs of resistance genes due to their ability to acquire and transfer mobile genetic elements (Silva *et al.*, 2022). The presence of

MDR MRCoNS in livestock also raises concerns about their potential dissemination to humans through direct contact or contaminated animal products, further emphasizing the need for stringent biosecurity measures and prudent antibiotic use in farming practices (Khairullah *et al.*, 2023).

In comparison to Thailand, the MRSA prevalence in livestock there ranges from 2% to 30%, with higher rates reported in intensively farmed pigs compared to cattle and goats (Tanomsridachchai et al., 2021). Similarly, studies in Indonesia have documented MRSA prevalence rates of approximately 15.8% in goat farms, where resistant strains have been frequently isolated from milk and hand swabs of farmers (Khairullah et al., 2023). These higher prevalence rates may reflect differences in farming practices, regulatory frameworks, and antibiotic usage patterns. Malaysia's relatively stricter regulations on antibiotic use in livestock, including the prohibition of certain antibiotics as growth promoters, may explain the lower prevalence observed in this study (Jabatan Perkhidmatan Veterinar Malaysia, 2023). However, it is important to note that regional differences in hygiene standards, feed quality, and the intensity of farming practices also play a critical role in shaping resistance patterns (Terreni et al., 2021).

This study utilized a modified transport and enrichment method for staphylococcal isolation. The transport medium, based on Baird Parker Agar Base supplemented with 6.5% sodium chloride, was designed to maintain bacterial viability during transport suppressing non-halotolerant organisms. After enrichment in a selective broth with the same composition, samples were plated onto Baird Parker Agar (BPA) to isolate characteristic black colonies with halos indicative of staphylococcal lipase activity (Baird-Parker, 1962), followed by subculturing onto Mannitol Salt Agar (MSA) for differentiation based on mannitol fermentation. Findings from this study suggest that this two-step workflow may enhance the recovery of staphylococcal isolates and reduce contamination. The high bacterial counts obtained from this process were compatible with downstream molecular analyses, demonstrating its applicability in antimicrobial resistance surveillance. Future studies should explore the reproducibility of this method across different sample types and laboratory settings, particularly in resource-limited environments. While this approach shows promise, the study's limited geographical scope and sample size suggest the need for future research incorporating larger, more diverse samples and longitudinal surveillance to understand AMR trends in Malaysian livestock better.

The findings of this study underscore the critical need for enhanced surveillance, education on prudent antibiotic use, and improved biosecurity measures in livestock farming to mitigate the risks of antimicrobial resistance. The detection of MDR MRCoNS highlights the potential for zoonotic transmission and the growing threat these bacteria pose to public health. A One Health approach, which recognizes the interconnectedness of human, animal, and environmental health, is essential to addressing the complex challenges posed by

antimicrobial resistance. Collaborative efforts among veterinarians, public health officials, policymakers, and researchers are necessary to develop sustainable interventions that protect public health while ensuring the sustainability of livestock production systems (Velazquez-Meza *et al.*, 2022).

#### **CONCLUSION**

This study determined the prevalence of MRSA (0.25%) and MRCoNS (7.8%) in East Coast Malaysian livestock, with goats showing higher colonization rates than cattle. The antimicrobial resistance assessment revealed 14 multidrug-resistant MRCoNS isolates exhibiting resistance to 3-7 antibiotic classes, with notable fusidic acid resistance (47.2%) raising zoonotic transmission concerns. Environmental exposures, management practices, and antibiotic use patterns were identified as key factors influencing resistance development, particularly in goat farming systems. These findings provide essential baseline data for implementing targeted antimicrobial stewardship strategies and underscore the urgent need for enhanced surveillance programs across broader geographical regions. The detection of MDR MRCoNS highlights the critical importance of adopting a One Health approach to prevent zoonotic transmission and ensure sustainable livestock production in Malaysia.

# **CONFLICT OF INTEREST**

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

#### **ACKNOWLEDGEMENT**

This research was funded by the FRGS grant (FRGS/1/2020/WAB04/UNISZA/03/2) from MOHE Malaysia (Dr. Zarizal Suhaili, Universiti Sultan Zainal Abidin Terengganu). We thank the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, for their support. We are grateful to Universiti Malaysia Kelantan (Agro TechnoPark, Kampus Jeli), Universiti Sultan Zainal Abidin (Kampus Besut), and East Coast Malaysia smallholder farmers for providing cattle and goats. We also acknowledge individuals and institutions that contributed to this study.

### REFERENCES

- Aklilu, E., & Ying, C. H. (2020). First *mecC* and *mecA* positive livestock-associated methicillin resistant *Staphylococcus aureus* (*mecC* MRSA/LA-MRSA) from dairy cattle in Malaysia. Microorganisms, 8(2). https://doi.org/10.3390/microorganisms8020147
- Baird-Parker, A. C. (1962). An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. Journal of Applied Bacteriology, 25(1), 12–19. https://doi.org/10.1111/j.1365-2672.1962.tb01113.x
- Boloki, H. A., Al-Musaileem, W. F., AlFouzan, W., Verghese, T., & Udo, E. E. (2021). Fusidic acid resistance determinants in methicillin-resistant *Staphylococcus aureus* isolated in

- Kuwait hospitals. Medical Principles and Practice, 30(6), 542–549. https://doi.org/10.1159/000518408
- Chai, M. H., Faiq, T. A. M., Ariffin, S. M. Z., Suhaili, Z., Sukiman, M. Z., & Ghazali, M. F. (2020). Prevalence of methicillin-resistant *Staphylococcus aureus* in raw goat milk from selected farms in Terengganu, Malaysia. Tropical Animal Science Journal, 43(1), 64–69. https://doi.org/10.5398/tasj.2020.43.1.64
- Chai, M., Sukiman, M. Z., Kamarun Baharin, A. H., Ramlan, I., Lai, L. Z., Liew, Y., Malayandy, P., Mohamad, N. M., Choong, S., Ariffin, S. M. Z., & Ghazali, M. F. (2022). Methicillin-resistant *Staphylococcus aureus* from Peninsular Malaysian animal handlers: molecular profile, antimicrobial resistance, immune evasion cluster and genotypic categorization. Antibiotics, 11(1), 103. https://doi.org/10.3390/antibiotics11010103
- Clinical and Laboratory Standard Institute (CLSI). (2020). Performance Standards for Anti-Microbial Susceptibility Testing (30th ed., M100). https://clsi.org/media/3481/m100ed30\_sample.pdf
- EUCAST. (2022). Clinical breakpoints breakpoints and guidance. Version 12.0. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\_files/Breakpoint\_tables/v\_12.0\_Breakpoint\_Tables.pdf
- Guo, Y., Song, G., Sun, M., Wang, J., & Wang, Y. (2020). Prevalence and therapies of antibiotic-resistance in *Staphylococcus* aureus. Frontiers in Cellular and Infection Microbiology, 10, 107. https://doi.org/10.3389/fcimb.2020.00107
- Jabatan Perkhidmatan Veterinar Malaysia. (2023). Perangkaan Ternakan 2022/2023. Jabatan Perkhidmatan Veterinar Malaysia. https://www.dvs.gov.my
- Kalai, S., Roychoudhury, P., Dutta, T. K., Subudhi, P. K., Chakraborty, S., Barman, N. N., & Sen, A. (2021). Multidrug-resistant staphylococci isolated from pigs with exudative epidermitis in the northeastern region of India. Letters in Applied Microbiology, 72(5), 535–541. https://doi.org/10.1111/lam.13448
- Khairullah, A. R., Kurniawan, S. C., Silaen, O. S. M., Effendi, M. H., Sudjarwo, S. A., Ramandinianto, S. C., Gololodo, M. A., Widodo, A., Riwu, K. H. P., Kurniawati, D. A., & Rehman, S. (2023). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolation and *mecA* gene detection from milk and farmer hand swab in Tulungagung, Indonesia. Tropical Animal Science Journal, 46(2), 231-238. https://doi.org/10.5398/tasj.2023.46.2.231
- Liang, B., Xiong, Z., Liang, Z., Zhang, C., Cai, H., Long, Y., Gao, F., Wang, J., Deng, Q., Zhong, H., Xie, Y., Huang, L., Gong, S., & Zhou, Z. (2022). Genomic basis of occurrence of cryptic resistance among oxacillin- and cefoxitin-susceptible mecA-positive Staphylococcus aureus. Microbiology spectrum, 10(3), e0029122. https://doi.org/10.1128/spectrum.00291-22
- Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. A. (2023). Antimicrobial resistance: a growing serious threat for global public health. Healthcare, 11(13), 1946. https://doi. org/10.3390/healthcare11131946
- Silva, V., Caniça, M., Ferreira, E., Vieira-Pinto, M., Saraiva, C., Pereira, J. E., Capelo, J. L., Igrejas, G., & Poeta, P. (2022). Multidrug-resistant methicillin-resistant coagulase-negative Staphylococci in healthy poultry slaughtered for human consumption. Antibiotics, 11(3), 365. https://doi. org/10.3390/antibiotics11030365
- Tallent, S., Hait, J., Bennett, R. W., & Lancette, G. A. (2001). Staphylococcus aureus. In FDA Bacteriological Analytical Manual (Chapter 12). U.S. Food and Drug Administration. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-12-staphylococcus-aureus
- Tanomsridachchai, W., Changkaew, K., Changkwanyeun, R., Prapasawat, W., Intarapuk, A., Fukushima, Y., Yamasamit,

- N., Flav Kapalamula, T., Nakajima, C., Suthienkul, O., & Suzuki, Y. (2021). Antimicrobial resistance and molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from slaughtered pigs and pork in the central region of Thailand. Antibiotics, 10(2), 206. https://doi.org/10.3390/antibiotics10020206
- Terreni, M., Taccani, M., & Pregnolato, M. (2021). New antibiotics for multidrug-resistant bacterial strains: Latest research developments and future perspectives. Molecules, 26(9), 2690. https://doi.org/10.3390/molecules26092671
- Velazquez-Meza, M. E., Galarde-López, M., Carrillo-Quiróz, B., & Alpuche-Aranda, C. M. (2022). Antimicrobial resistance: One Health approach. Veterinary World, 15(3), 743–749. https://doi.org/10.14202/vetworld.2022.743-749
- Zarizal, S., Yeo, C. C., Faizal, G. M., Chew, C. H., Zakaria, Z. A., Al-Obaidi, M. M. J., Amin, N. S., & Nasir, M. D. M. (2018). Nasal colonisation, antimicrobial susceptibility, and genotypic pattern of *Staphylococcus aureus* among agricultural biotechnology students in Besut, Terengganu, east coast of Malaysia. Tropical Medicine and International Health, 23(8), 905–913. https://doi.org/10.1111/tmi.13090