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Drug resistance *Staphylococcus* and MRSA in slaughterhouse environment

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Abstract
Emergence of methicillin-resistant *S. aureus* (MRSA), associated with the *mecA* gene, has increased health concern in livestock-related settings including slaughterhouse environment. In this study, environmental samples (N = 346) from slaughterhouses were processed and 112 presumptive staphylococcal isolates obtained. Five of them were confirmed as *S. aureus* and one as MRSA through phenotypic and molecular tests. Fifty isolates of *Staphylococcus* spp. subjected to Kirby-Bauer disc diffusion assay where highest resistance was observed for erythromycin (58%), tetracycline (50%) and penicillin (48%), followed by cefoxitin (44%) while highest susceptibility was recorded to chloramphenicol (98%), gentamicin (96%), enrofloxacin (96%) and trimethoprim-sulfamethoxazole (96%). Despite the very low (0.28%) prevalence of MRSA, the detection of multidrug-resistant staphylococci within slaughterhouse environments highlights the need for continued monitoring and consistent sanitation practices to limit the spread of resistant strains.

Keywords: *mecA*, MRSA, multidrug-resistant, slaughterhouse, *Staphylococcus aureus*

Introduction
Worldwide, zoonotic diseases are at the forefront of emerging infectious threats, with over 60% of new infections having their origins in animals, and almost 75% of recently discovered pathogens being traced back to animal sources (WHO, 2020) [20]. With the growing demand for animal protein, coupled with rapid urban expansion and the intensification of livestock production, the likelihood of zoonotic spill over events has increased, especially in regions where veterinary oversight and disease surveillance remain limited (FAO, 2019) [10]. Slaughterhouses are one such place that occupies an essential position in the food production system; however, they also represent settings where zoonotic and antimicrobial-resistant organisms can accumulate when hygiene and operational standards fall short. Within slaughterhouses, contamination risks are inherent to routine activities such as slaughtering, evisceration and carcass dressing. These operations involve direct contact with biological materials like blood, hide, faecal matter, urine and internal organs, all of which can harbour and disseminate microorganisms (Espinosa *et al.*, 2020) [8]. Surfaces such as carcasses, slaughter floors, workers' hands, knives, cutting tools and water used for rinsing are well-established points where microbes can persist or spread (Macedo and Van Der Sand, 2005) [15]. Water plays a dual role serving both as a medium for cleaning and as a potential source of contamination, especially when untreated, reused or drawn from contaminated supplies (EPA, 2021) [9]. Effluents containing blood and organic matter frequently enter surrounding drains, agricultural lands, or natural water bodies, promoting environmental persistence of pathogens and antimicrobial-resistant bacteria (Adebowale *et al.*, 2019; Iroha *et al.*, 2016) [3, 13]. In contrast, poorly managed unorganized slaughterhouses or meat shops serve as a direct source of environmental contamination.
Among the various pathogens associated with slaughterhouse contamination, *Staphylococcus aureus* remains one of the most relevant and common contaminants. Further, the drug resistant and sensitive staphylococci are commonly colonizing the skin and mucous membranes of both animals and humans, enabling its transfer through direct handling, contaminated carcasses, equipment, aerosols and fomites.

Although once regarded as a hospital-associated organism, MRSA has expanded into community settings and animal production systems, including abattoirs and meat processing chains. Several reports describe MRSA colonization among slaughterhouse workers and its detection on carcasses and environmental surfaces, illustrating occupational exposure risks and possible entry into the food chain and the environment (Ivbule *et al.*, 2017) [14]. Slaughterhouse wastewater frequently contains residual antibiotics, resistant bacteria, and mobile genetic elements, thereby acting as a hotspot for the spread of AMR into nearby ecosystems (Van Boeckel *et al.*, 2015) [19]. Given the public health significance and the scarcity of detailed information from Indian slaughterhouses particularly with respect to the occurrence and antimicrobial susceptibility of *S. aureus* and MRSA, this study aimed to investigate their occurrence in environmental samples collected from both organized and unorganized slaughterhouse facilities. The antimicrobial resistance profiles of the isolates were also assessed to contribute evidence toward AMR dissemination.

Materials and Methods

Experimental design

Prior to commencing this research, approval was obtained from Biosafety Committee of the ICAR-National Institute of Veterinary Epidemiology and Disease Informatics. The study was carried out from December 2024 to June 2025 across two organized (Amberpet and Chengicherla) and four unorganized slaughterhouses located in Hyderabad, Telangana, India. A total of 346 samples were collected over four sampling visits conducted during this period.

Sample Collection

Environmental samples consisting of drinking water, wastewater, drainage water, surface swabs and swabs from floors, walls and equipment were collected from multiple sections of both organized (N = 280) and unorganized slaughterhouses (N = 66), including unloading pens, lairage areas, slaughter halls, vehicles, workers' hands, the effluent treatment plant and surrounding premises, depending on accessibility. Sterile swabs moistened with buffered peptone water (BPW) were used for sampling, with each swab or sponge rubbed over two separate 10 × 10 cm areas at each site before being placed into trypticase soy broth as the transport medium. A total of 272 swabs, 33 drainage water, 19 drinking water, 10 washing water, 6 effluent inlet and 6 effluent outlet samples were collected and processed. All the samples were collected under strict aseptic conditions, transported in insulated containers with appropriate cooling elements until further processing.

Isolation and identification of *Staphylococcus* spp.

ISO 6888-2 (2017) protocol was followed for isolation and identification of *Staphylococcus aureus*. For the isolation of *Staphylococcus* spp., samples were inoculated into trypticase soy broth supplemented with 10% sodium chloride and 1% sodium pyruvate (primary enrichment media), followed by incubation at 37 °C for 24 hours. After primary enrichment, sample from trypticase soy broth was streaked onto Baird-Parker agar and incubated at 37 °C for 24-48 hours. After overnight incubation, the characteristic colonies on BP agar were further confirmed by Gram's staining followed by biochemical tests such as, catalase test, slide coagulase test, Tube coagulase test and DNase test.

Molecular confirmation of *S. aureus*

Isolates identified as *Staphylococcus aureus* based on cultural, morphological, and biochemical characteristics were subjected to molecular confirmation. Fresh cultures were grown in BHI broth until the mid-to-late logarithmic phase, after which genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. PCR assays were performed to detect the *nuc* and *coa* gene. Primers details of the genes targeted in this study are presented in Table 1.

The reaction volume of 10 µL of the PCR mixture comprised of 5 µL of 2X PCR Master mix consisting of dNTPs, Taq DNA polymerase and $MgCl_2$, 0.5 µL of each forward and reverse primers, 3 µL of DNA template and nuclease free water (1 µL) was added to make a final volume of 10 µL. Amplification of genes were carried out in a thermal cycler (Bio-Rad, USA) under routine cycling conditions consisting of initial denaturation at 94 °C for 10 min followed by 30 cycles each of denaturation at 94 °C for 30 sec, annealing temperature (refer Table 1 for genes) and extension at 72 °C for 45 sec and the final extension at 72 °C for 10 min. Amplified PCR products were resolved by electrophoresis on 1.5% agarose gel and visualized using ethidium bromide staining.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of confirmed *Staphylococcus* spp. isolates (N = 50) was performed using the Kirby-Bauer disc diffusion method in accordance with CLSI guidelines using eight antibiotics (CLSI 2020) [7]. These eight antibiotics representing different antimicrobial classes, including cefoxitin (30 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), penicillin G (10 units), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (25 µg). Each isolate was inoculated into 5 mL of tryptone soya broth and incubated at 37 °C until the turbidity matched the 0.5 McFarland standard. The standardized inoculum was then evenly spread onto Mueller-Hinton agar plates using sterile cotton swabs. After drying or absorption of inoculum, antibiotic discs were placed. The inoculated plates were incubated aerobically in an inverted position at 37 °C for 18-24 hours. Following incubation, inhibition zone diameters were measured using a transparent ruler and susceptibility or resistance was interpreted according to CLSI performance standards.

Detection of methicillin resistance

Phenotypic detection of methicillin resistance was assessed using cefoxitin (30 µg) disk diffusion method. Resistant isolates were further confirmed by molecular test. The genomic DNA extracted from each *Staphylococcus* spp. isolate was subjected to genus specific PCR assay using specific primer pair targeting *mecA* and *mecC* gene.

Results

All the 346 samples when streaked onto Baird Parker agar, 112 number of samples showed presence of staphylococci by showing characteristic black colour colonies. All the isolated colonies after purification were subjected to Gram staining where Gram positive cocci showing cluster like grapes were further subjected to biochemical tests such as catalase test, slide coagulase test, tube coagulase test and DNase test. Finally, catalase positive isolates were

presumed to be *Staphylococcus* spp. isolates while coagulase and DNAse producing isolates were suspected of *S. aureus* isolates were targeted to *nuc* (Fig. 1) and *coa* gene (Fig. 2). Interesting to note that, water samples, vehicle swabs and hand swabs were found to be negative for staphylococci while unloading pen swabs, lairage swabs and slaughter hall swabs contained the staphylococci. The occurrence of *Staphylococcus aureus* in slaughterhouse environments was comparatively low, with an overall detection rate of 1.44% (5/346 samples). All *S. aureus* isolates originated from the organized slaughterhouses at Chengicherla and Amberpet. None of the samples collected from the unorganized slaughterhouse yielded *S. aureus*. Detection was restricted to specific environmental sampling points, suggesting localized contamination rather than widespread distribution within the slaughterhouses (Table 2).

Antimicrobial susceptibility testing revealed notable variability in resistance patterns. The highest resistance rates were observed for erythromycin (58%), tetracycline (50%) and penicillin (48%), indicating limited efficacy of these agents against environmental isolates. Moderate resistance to cefoxitin (44%) was detected, whereas the isolates exhibited high susceptibility to chloramphenicol (98%), gentamicin (96%), enrofloxacin (96%) and trimethoprim-sulfamethoxazole (96%), suggesting these antimicrobials remain broadly effective (Fig. 3).

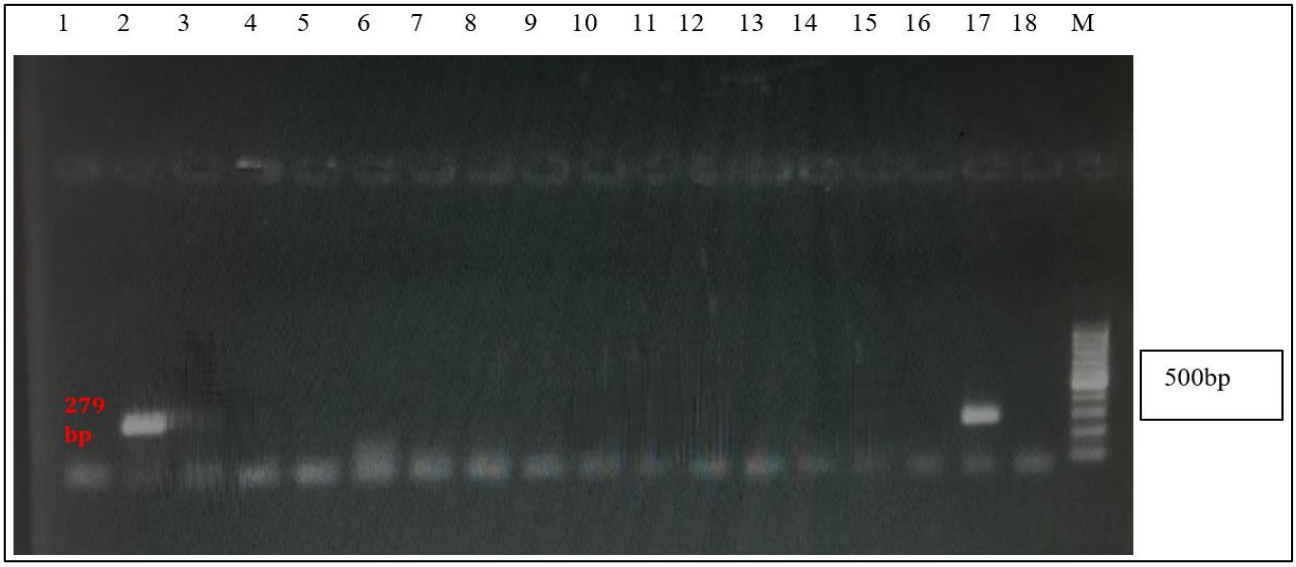
Of the 50 isolates screened for methicillin resistance, 22 isolates (44%) were phenotypically resistant to cefoxitin. Molecular detection identified the *mecA* gene in only three isolates (6%), while *mecC* was not detected. Importantly, only one *mecA* (Fig. 4) positive isolate corresponded to *S. aureus*, resulting in an overall MRSA prevalence of 0.28% (1/346 samples) while two isolates that harboured *mecA* gene were coagulase negative staphylococci. This MRSA isolate originated from the unloading pen floor of the Chengicherla slaughterhouse.

Table 1: list of primers and cyclic conditions

Sl. No.	Target Gene	Primer Sequence (5'-3')	Annealing Temp. (°C)	Amplicon Size (bp)	Reference
1	*nuc*	F: GCGATTGATGGTGATACGGT R: AGCCAAGCCTTGACGAACTAAAGC	52 °C	279	Brakstad *et al.* (1992) [6]
2	*coa*	F: ATAGAGATGCTGGTACAGG R: GCTTCCGATTGTTCGATGC	58 °C	603	Hookey *et al.* (1998) [12]
3	*mecA*	F: ACTGCTATCCACCCTCAAAC R: CTGGTGAAGTTGTAATCTG	51 °C	163	Mehrotra *et al.* (2000) [17]
4	*mecC*	F: ACTGCTATCCACCCTCAAAC R: CTGGTGAAGTTGTAATCTG	53.5 °C	138	Pajic *et al.* (2014) [18]

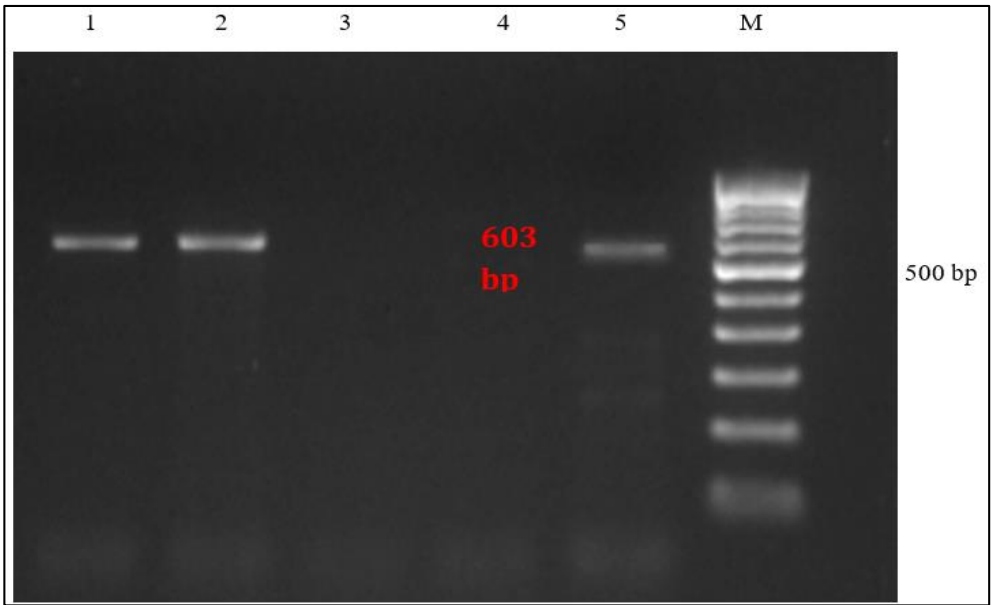
Table 2: Occurrence of *Staphylococcus* spp. in slaughterhouse environment

Sample Type	Chengicherla		Amberpet		Unorganized SH	
	No. of samples collected	Positive for staphylococci	No. of samples collected	Positive for staphylococci	No. of samples collected	Positive for staphylococci
Unloading pen swabs	24	9	24	9	12	8
Lairage swabs	24	10	24	9	12	8
Slaughter hall swabs	47	18	47	19	26	16
Water samples	36	0	26	0	12	0
Vehicle swab	8	3	8	1	0	0
Hand swab	6	1	6	1	4	0
Total	145	41	135	39	66	32



Lane M-100 bp ladder; Lane 18-Negative control; Lane 17-Positive control of *nuc* gene for *S. aureus*; Lane 2-Positive sample for *nuc* gene of *S. aureus*; Lane 3-16-Negative sample

Fig 1: PCR confirmation of *Staphylococcus aureus* isolates by amplification and detection of *nuc* gene



Lane M-100 bp ladder; Lane 5-Positive control for *coa* gene of *S. aureus*; Lane 4-Negative control; Lane 3-Negative sample; Lane 1 and 2-samples positive for *coa* gene of *S.aureus*

Fig 2: PCR confirmation of *Staphylococcus aureus* isolates by amplification and detection of *coa* gene

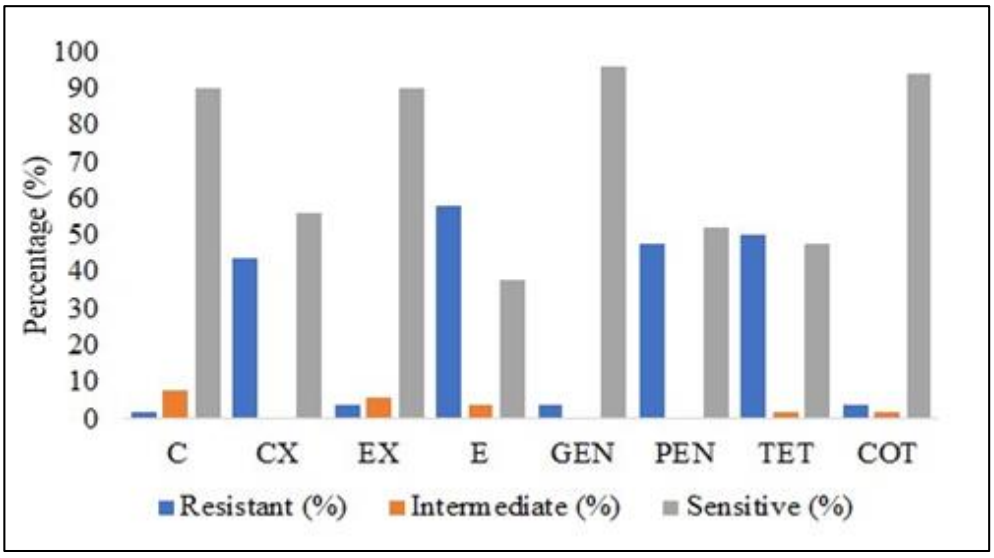
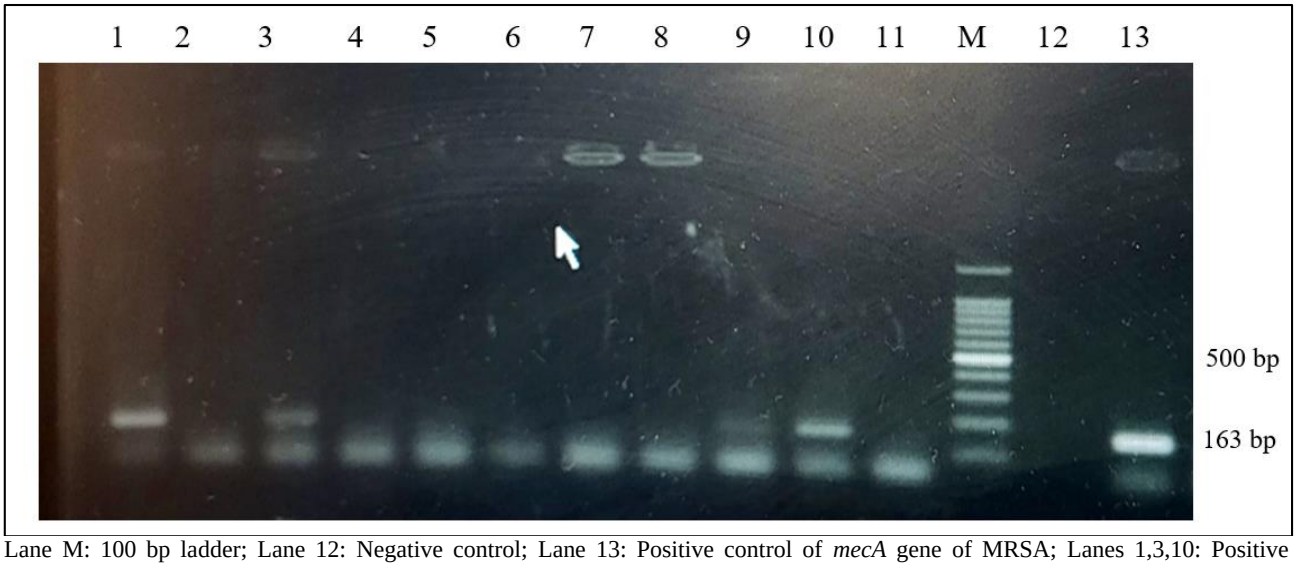


Fig 3: Antimicrobial resistance profile of *Staphylococcus* spp.



Lane M: 100 bp ladder; Lane 12: Negative control; Lane 13: Positive control of *mecA* gene of MRSA; Lanes 1,3,10: Positive samples of MRSA; Lanes 2,4-9,11: Negative samples of MRSA

Fig 4: PCR confirmation of MRSA by amplification of *mecA* gene

Discussion

The findings from this study offer an understanding of the occurrence of *Staphylococcus aureus* and its antimicrobial resistance patterns in the slaughterhouse environments of Chengicherla, Amberpet and an unorganized facility. Overall, *S. aureus* was detected in only 1.44% of the samples, a level noticeably lower than what has been described in several earlier investigations. For example, Awaad *et al.* (2024) [2] reported prevalence levels above 60% in similar environments and Abunna *et al.* (2016) [1] documented substantial contamination on carcasses, knives and floor surfaces. Though the present study demonstrated low level of occurrence of *S. aureus* but the other species of staphylococci were found to be dominating @ 42-44% at unloading pen, lairage and slaughter hall. As slaughterhouses differ widely in their structural quality, degree of mechanization, sanitation, routine and general operational workflows. These factors collectively shape the microbial load and persistence of pathogens in abattoir settings.

The comparatively low prevalence observed here could reflect better hygiene practices in the organized slaughterhouses, including more reliable drainage systems, scheduled routine washing and consistent surface disinfection. In contrast, studies reporting higher contamination often involved older or less organized plants where manual carcass handling, irregular cleaning and poor water management favour bacterial survival (Adebowale *et al.*, 2019; Iroha *et al.*, 2016) [3, 12]. Since *S. aureus* can withstand dry conditions and persist on surfaces such as steel, plastic and wood (Harris *et al.*, 2002) [11], even small differences in cleaning frequency may influence whether the organism is recovered during sampling.

An interesting observation was the absence of *S. aureus* in the unorganized slaughterhouse, despite such facilities generally having weaker sanitation infrastructure. Although this appears contradictory, similar findings have been noted in studies where lower detection was attributed to fewer sampling zones, smaller working areas, or reduced surface moisture conditions that may limit the recovery of staphylococci, which often thrive in moist environments (Espinosa *et al.*, 2020) [7]. The relatively smaller number of water and surface samples collected from the unorganized facility may also have contributed to non-detection in this setting.

The antimicrobial resistance patterns recorded in the present study follow trends commonly described in the literature, with high resistance to erythromycin, tetracycline and penicillin (Ahaduzzaman *et al.*, 2014; Awaad *et al.*, 2024) [4, 16]. These antibiotics are widely used in food animals, both for prophylactic purposes and metaphylaxis, which likely explains the sustained selective pressure reported by Marshall and Levy (2011) [16]. Conversely, the strong susceptibility to gentamicin, enrofloxacin, and chloramphenicol parallels findings from Ivbule *et al.* (2017) [14] in meat-processing environments, suggesting that these antimicrobials may be used less frequently in the local livestock sector. The MRSA prevalence in this study was particularly low (0.28%), which is substantially below the 5-20% range reported in several European and African studies (Awaad *et al.*, 2024; Abunna *et al.*, 2016) [2, 1]. Only one *mecA*-positive *S. aureus* isolate was identified, indicating that MRSA contamination in slaughterhouses within this region remains limited. Nevertheless, the fact that MRSA was detected at the unloading pen in Chengicherla, as contamination at this stage may originate from animal hides, transport vehicle surfaces or human contact routes frequently implicated in the spread of MRSA into abattoirs (Bhargava *et al.*, 2011) [5].

Taken together, the results highlight the importance of regular monitoring of slaughterhouse environments, both to minimize contamination of meat and to reduce occupational exposure to antimicrobial-resistant bacteria. Although the low prevalence of *S. aureus* and MRSA reflects reasonably effective sanitation within the examined facilities, the detection of resistance to commonly used antibiotics emphasizes the continuing need for responsible antimicrobial use in livestock production. Strengthening routine hygiene practices, ensuring adherence to cleaning protocols and conducting periodic microbial assessments would further support food safety and help in limit the dissemination of resistant strains.

Conclusion

Overall prevalence of *S. aureus* was low (1.44%) and MRSA was detected only rarely (0.28%) indicated good hygienic practices followed at these slaughterhouses. Even the antimicrobial resistance profiling of the present study isolates showed better sensitivity compared to isolates from other animal settings. However, the detection of a *mecA*-positive MRSA isolate, even at a very low level, reinforces the need for sustained monitoring and responsible antimicrobial use in food-animal systems. Collectively, the findings highlight the importance of continued investment in hygiene programs, proper water management and routine microbial surveillance within slaughterhouses. Strengthening biosecurity practices and integrating a One Health oriented monitoring approach will aid in early detection and control of zoonotic and antimicrobial-resistant hazards, ultimately contributing to safer meat production and reduced public health risks.

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