



Isolation and Identification of Bacterial Agents from Intramuscular Abscesses in Condemned Pig Carcasses

P. N. Arpitha¹, P. M. Priya¹ , V. Niranjana¹, P. Santhanalakshmi¹, B. K. Mani¹ and V. N. Vasudevan²

¹Dept. of Veterinary Microbiology, ²Dept. of Livestock Products Technology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University Mannuthy, Thrissur, Kerala (680 651), India



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Corresponding  priya@kvasu.ac.in

 0000-0001-7671-1923

ABSTRACT

The experiment was conducted during August, 2023 to September, 2024 at the College of Veterinary and Animal Sciences, Mannuthy, to isolate and identify bacterial agents from intramuscular abscesses in condemned pig carcasses detected during slaughter. Pus samples collected from twenty-five pigs were cultured on blood agar for bacterial isolation and the obtained isolates were identified using conventional biochemical tests along with the automated microbial identification system. Antibiotic susceptibility testing was performed and the multiple antibiotic resistance index was calculated to determine the resistance profile of each isolate. A total of twenty bacterial isolates were recovered, of which eighteen were positive on bacterial culture. Gram-positive bacteria were more prevalent than Gram-negative ones. The most frequently identified organisms were *Staphylococcus* spp. followed by *Escherichia coli* and *Streptococcus* spp. while less frequently isolated species included *Acinetobacter lwoffii*, *Providencia rettgeri*, *Globicatella sulfidifaciens*, *Granulicatella adiacens*, *Lactobacillus species* and an unidentified Gram-positive rod. Most isolates showed susceptibility to chloramphenicol, gentamicin, tetracycline, cefoperazone, levofloxacin, ofloxacin, amikacin, co-trimoxazole, ceftriaxone combined with sulbactam, amoxicillin combined with clavulanic acid and ciprofloxacin, whereas a high level of resistance was observed against penicillin G, clindamycin and methicillin. Among the twenty isolates, fifteen exhibited a multiple antibiotic resistance index greater than 0.2, indicating a multidrug-resistant profile. The emergence of multidrug-resistant bacteria highlights the growing concern of antimicrobial resistance and the findings of this study emphasise the significant role of bacterial pathogens in the formation of abscesses, ultimately contributing to carcass condemnation.

KEYWORDS: Abscess, bacteria, condemnation, pigs, *Staphylococcus*, *Escherichia coli*

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Pigs are widely recognised as one of the most efficient meat-producing animals worldwide owing to their high reproductive capacity, excellent maternal ability, rapid growth rate, efficient feed conversion, short gestation period, and high carcass yield. These attributes make pig farming an economically viable and sustainable option for meeting the increasing global demand for animal protein. According to the 20th Livestock Census of India (Anonymous, 2019), the pig population in the country is approximately 9.06 million, which is considerably lower than the global pig population of about 975.40 million reported (Anonymous, 2021). Despite this disparity, pig farming plays a significant role in supporting rural livelihoods in India. Pork production in the country is estimated at around 3.2 lakh tonnes annually, with nearly half a million people directly or indirectly involved in pig farming activities (Bhaduria et al., 2023). Furthermore, India possesses a rich genetic resource, with fourteen indigenous pig breeds identified and registered to date (Jeyakumar and King, 2024). Kerala is characterised by a predominantly meat-consuming population and exhibits a substantial demand for pork. The state records the annual slaughter of approximately 1.13 lakh pigs, contributing to nearly 8.1 thousand tonnes of pork production (Anonymous, 2020). However, meat inspection frequently reveals pathological conditions that compromise carcass quality and safety. One of the common findings during post-mortem inspection is the presence of purulent exudates or intramuscular abscesses disseminated along the carcass, primarily resulting from bacterial infections. Such abscesses adversely affect meat quality and often lead to partial or total carcass condemnation (Evans and Pratt, 1978), thereby reducing marketable pork and causing significant economic losses to farmers and the meat industry. Several studies have documented the prevalence and cause of carcass condemnation in pigs. Garcia-Diez and Coelho (2014) reported that out of 160,763 pigs slaughtered, 392 (0.24%) were condemned during post-mortem inspection. The major pathological conditions responsible for condemnation included osteomyelitis (38.5%), granulomatous lymphadenitis (22.7%), and pleurisy or pneumonia (21.2%). Muscular abscesses have been strongly associated with unhygienic practices during vaccination or antibiotic administration, injection at inappropriate anatomical sites, and handling by untrained personnel (King et al., 2010; George et al., 1995; Thomas-Bachli et al., 2014). These practices facilitate bacterial entry into muscle tissue, leading to localized or disseminated infections. The distribution of abscesses in various anatomical regions of pigs indicates susceptibility to bacterial infections originating either from endogenous normal flora or from environmental pathogens (Jones, 1980). Several

bacterial agents have been implicated in abscess formation, with *Trueperella pyogenes* being one of the most frequently reported pathogens (Yassin et al., 2011). Other commonly isolated organisms include *Staphylococcus aureus* (Ho et al., 2012) and *Escherichia coli* (Abubakar et al., 2017), along with *Klebsiella*, *Pseudomonas*, *Micrococcus*, and *Streptococcus* species (Popova and Kanchev, 2013; El-Dakhly et al., 2008; Takizawa et al., 2018; Olatubi et al., 2021). Ante-mortem inspection poses considerable challenges in identifying abscesses, particularly in clinically normal animals, as most lesions remain subclinical until slaughter. Consequently, post-mortem inspection outcomes play a crucial role in safeguarding public health and minimising economic losses by identifying unfit carcasses. Therefore, systematic isolation and identification of bacterial agents associated with abscesses in pig carcasses are essential. Such studies can aid in understanding disease epidemiology, formulating effective control strategies, improving on-farm management practices, and ultimately reducing carcass condemnation and financial losses within the swine industry.

2. MATERIALS AND METHODS

2.1. Sample collection

The present study was conducted on twenty-five crossbred pig carcasses exhibiting intramuscular abscesses (with or without concurrent generalized abscesses in other regions of the carcass or offal) detected during slaughter and post-mortem inspection at the Meat Technology Unit, KVASU, Mannuthy. Information on the age, sex and breed of the animals was recorded. The location of abscesses varied among the carcasses. A total of twenty-eight pus samples were aseptically collected in sterile vials and transported under refrigerated conditions to the Department of Veterinary Microbiology for routine microbiological analysis aimed at determining the bacterial causative agents.

2.2. Isolation and identification of the bacterial agents

The samples were inoculated onto 5–10% BA and incubated at 37°C for 24–48 hours. For selective isolation, subculturing was performed on Mannitol Salt Agar (MSA), Eosin Methylene blue agar (EMB), MacConkey agar (MCA), Xylose Lysine Deoxycholate agar (XLD), Brilliant Green agar (BGA), and serum agar. Presumptive identification of the isolates was carried out based on colony morphology, Gram's staining, and a series of biochemical tests, including IMViC (Indole, Methyl red, Voges Proskauer and Citrate), urease, oxidative/fermentative (O/F) test, nitrate reduction, catalase, and oxidase test (Barrow and Feltham, 1993; Quinn et al., 1994). Final confirmation of the bacterial species was performed using the VITEK 2 system.

2.3. Antibiotic sensitivity test (ABST)

Antibiotic susceptibility test was performed in accordance

with the guidelines of the Clinical and Laboratory Standards Institute (Anonymous, 2020). Fourteen antibiotics were employed to determine the antibiogram of each isolate, including tetracycline (TE), cefoperazone (CPZ), amoxycylav (AMC), levofloxacin (LE), penicillin G (P), methicillin (MET), ceftriaxone/sulbactam (CIS), amikacin (AK), ciprofloxacin (CIP), clindamycin (CD), ofloxacin (OF), chloramphenicol (C), gentamicin (GEN) and cotrimoxazole (COT). Muller Hinton Agar (MHA) was the medium used for the culture. Finally, the multiple antibiotic resistance index (MAR) was calculated as the ratio of several antibiotics to which an organism was resistant to the total number of antibiotics to which the organism was exposed (Ayandele et al., 2020).

3. RESULTS AND DISCUSSION

Out of 28 pus samples examined, 18 tested positive for bacterial culture, yielding a total of 20 bacterial isolates. Among them, 70% of the isolates belonged to Gram-positive bacteria, and the remaining were Gram-negative organisms. The most frequently identified bacteria were *Staphylococcus* spp. (25%) followed by *Escherichia coli* (20%) and *Streptococcus* spp. (15%). According to Liljegren et al. (2003), *Staphylococcus aureus* (*S. aureus*) and *Arcanobacterium pyogenes* (*A. pyogenes*) induce extensive abscesses in slaughtered pigs.

A total of five *Staphylococcus* isolates were identified, of which four were Coagulase-Negative Staphylococci (CoNS): *Staphylococcus lugdunensis* (n=1), *Staphylococcus hominis* subsp. *hominis* (n=1) and *Staphylococcus warneri* (n=2). The remaining isolate was a Coagulase-Positive Staphylococci (CoPS), identified as *S. aureus*. *Staphylococcus* spp. appeared as Gram-positive cocci arranged in grape-like clusters on Gram stain. The isolates tested positive for catalase, MR, VP and nitrate reduction test, whereas negative for oxidase, indole, citrate utilization and urease (except *S. aureus* positive for urease). All the isolates were fermentative on the OF test. Final species confirmation was performed using the VITEK 2 system.

Four Gram-negative short rods identified as *E. coli*, were produced pink colonies on MCA and displayed a characteristic greenish metallic sheen on EMB agar. On BGA and XLD agar, the isolates formed a slightly variant type of yellow coloured colony. They exhibited +++- for IMViC reaction and were fermentative in the OF test. All the isolates were confirmed by the VITEK 2 system.

Three *Streptococcus* isolates appeared as Gram-positive cocci arranged in chains or pairs on Gram stain. On BA, they produced beta-haemolytic colonies (Figure 1). Based on biochemical profiling and confirmation with the VITEK 2 system, the isolates were identified as *Streptococcus uberis*, *Streptococcus pseudoporcinus* and *Streptococcus porcinus*.

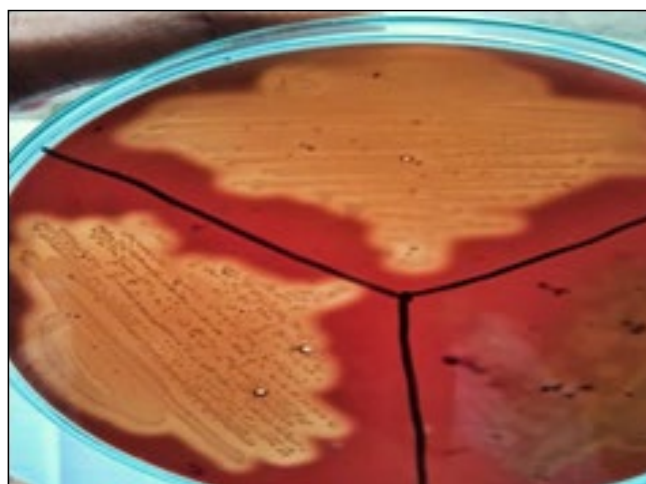


Figure 1: Beta haemolytic colony of *Streptococcus* spp.

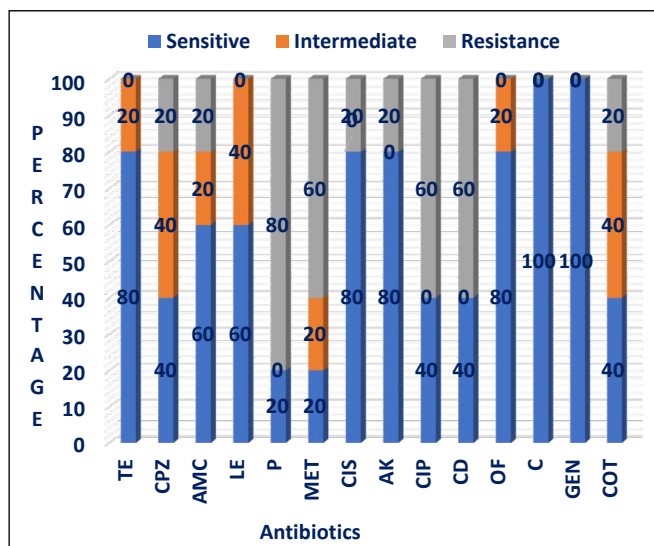
Other bacterial species identified and confirmed using the VITEK 2 system included *Acinetobacter lwoffii* (n=1), *Providencia rettgeri* (n=1), *Globicatella sulfidifaciens* (n=1), *Granulicatella adiacens* (n=1), and *Lactobacillus* spp. (n=2) and one unidentified Gram-positive rod (n=1). Similarly, Ling et al. (2001) and Muder et al. (2006) identified Gram-negative bacteria, including *Enterobacteriaceae* and *Pseudomonas aeruginosa*, as well as *S. aureus* in their studies using the VITEK 2 system.

A majority of the isolates were susceptible to chloramphenicol, followed by gentamicin, tetracycline, cefoperazone, levofloxacin, ofloxacin, amikacin, cotrimoxazole, ceftriaxone/sulbactam, amoxycylav, and ciprofloxacin. In contrast, marked resistance was recorded against penicillin G, clindamycin and methicillin. Among the 20 isolates, 15 isolates had a MAR index more than 0.2, as depicted in Table 1, which indicates the multidrug-resistant nature. In the present study, *S. aureus* was resistant to penicillin-G, methicillin, ceftriaxone/sulbactam, amikacin, and ciprofloxacin (Figure 2). A study conducted by Ho et al. (2012) revealed that *S. aureus* was resistant to fluoroquinolones, tetracyclines, macrolides/lincosamides, and chloramphenicol.

A study by Said et al. (2021) found that *E. coli* was the most frequently isolated bacterium, followed by *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., and *Acinetobacter* spp. The isolates were sensitive to amikacin, imipenem, fosfomycin, nitrofurantoin, and streptomycin but resistant to cefuroxime, ceftazidime, tetracycline, ofloxacin, ampicillin, cefepime, piperacillin-tazobactam, cefotaxime, ciprofloxacin, and levofloxacin. In the current study, *E. coli* isolates exhibited resistance to penicillin-G, methicillin, clindamycin, amikacin, ofloxacin, ciprofloxacin, and amoxicillin/clavulanate, while showing sensitivity to levofloxacin, chloramphenicol, ceftriaxone/sulbactam, gentamicin, and

Table 1: The multiple antibiotic resistance index of the organism is shown below for different antibiotics tested

Organism	Sample number	Multiple antibiotic resistance index
		Significant bacterial isolates
<i>Staphylococcus</i> spp.	AR2	0.35
<i>Staphylococcus</i> spp.	AR4-b	0.42
<i>Staphylococcus</i> spp.	AR15	0.07
<i>Staphylococcus</i> spp.	AR18-a	0.14
<i>Staphylococcus</i> spp.	AR24	0.35
<i>Escherichia coli</i>	AR5	0.42
<i>Escherichia coli</i>	AR6	0.42
<i>Escherichia coli</i>	AR13	0.42
<i>Escherichia coli</i>	AR18-b	0.42
<i>Streptococcus</i> spp.	AR12	0.42
<i>Streptococcus</i> spp.	AR25	0.21
<i>Streptococcus</i> spp.	AR27	0.35
<i>Acinetobacter lwoffii</i>	AR26	0.35
Less significant bacterial isolates		
<i>Providencia rettgeri</i>	AR23	0.42
<i>Gemella bergeri</i>	AR28	0.35
<i>Globicatella sulfidifaciens</i>	AR7	0.28
Unidentified Gram-positive rod	AR4-a	0.21
<i>Granulicatella adiacens</i>	AR10	0.14
<i>Lactobacillus</i> spp.	AR16	0.14
<i>Lactobacillus</i> spp.	AR17	0.07

Figure 2: Antimicrobial sensitivity pattern of *Staphylococcus* spp.

tetracycline. These findings align with the study by Shama et al. (2018), which reported that *E. coli* demonstrated high resistance to penicillin and cefotaxime while showing the least resistance to cefoperazone/sulbactam and gentamicin.

Streptococcus exhibited sensitivity to tetracycline, which contradicts the findings of Zhang et al. (2019), who reported that *S. suis* was resistant to tetracycline.

The location of the abscesses is indicative of the introduction of bacteria through wounds and invasive procedures. Generalisation of abscesses from a primary location might warrant total condemnation of the carcass. The current study revealed that the bacterial agents had a significant role in causing abscesses and led to carcass condemnation.

4. CONCLUSION

Staphylococcus spp. were the most common isolates from condemned carcasses, followed by *E. coli* and *Streptococcus* spp. Among 20 isolates, 15 showed a MAR index greater than 0.2, indicating multidrug resistance. The findings emphasized the role of bacterial pathogens in abscess formation leading to carcass condemnation. As ante-mortem detection remained difficult, antibody-based screening and preselection of finishing pigs were suggested to improve inspection efficiency and reduce post-mortem risks.

5. FURTHER RESEARCH

Further studies are recommended to investigate the molecular characteristics and virulence factors of the isolated bacterial species with particular emphasis on their role in abscess formation in pigs. Long-term surveillance across multiple slaughterhouses would help to determine the prevalence and distribution of multidrug-resistant bacteria associated with carcass condemnation. Additionally, exploring rapid diagnostic tools for early detection of abscess-causing pathogens in live pigs could improve ante-mortem screening and reduce economic losses.

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