



PS5-03

# PREVALENCE OF MRSA IN GOAT AND SHEEP MILK IN TERENGGANU, MALAYSIA

(Proteomic analysis of goat milk in experimentally induced MRSA)

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# Background

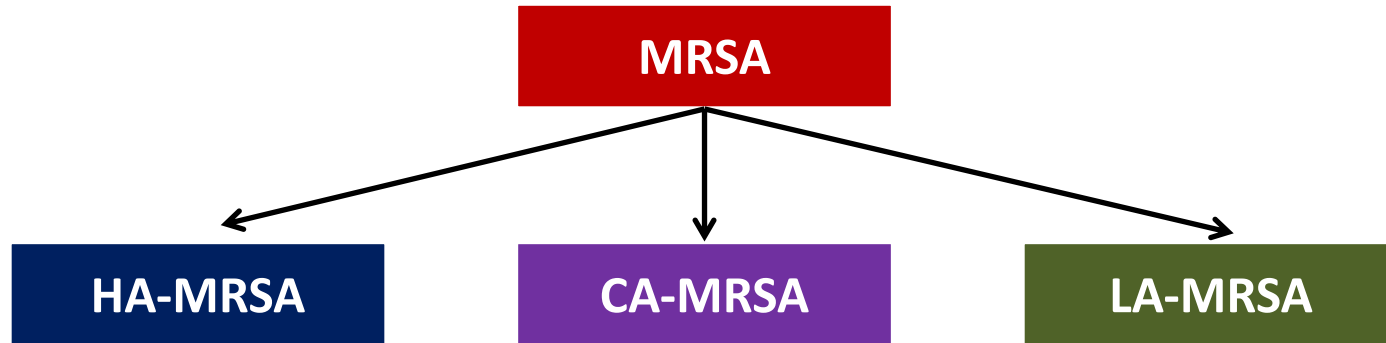
- AMR has been an emerging worldwide concern including in Malaysia.
- It is expected that the deaths attributable to AMR by 2050 will be up 10 million deaths per year. Besides, world GDP is also expected to decrease for 2 to 3% and costing the world \$100 trillion (*WHO, 2017*)
- The 71st session of the UN General Assembly identified AMR as a dominant global health concern, placing it high on the agenda of national policy makers, international organisations, and financial institutions in developed and developing countries alike (*United Nation, 2016; Maldives et al., 2017*)

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# Introduction

- *S. aureus* has been identified as one of the major risk pathogens associated with the development of AMR (Voss *et al.*, 2005; Harkins *et al.*, 2017). MRSA are found in human and animals. It is emerging zoonotic and can overcome species barrier (Voss *et al.*, 2005; Cuny *et al.*, 2008).

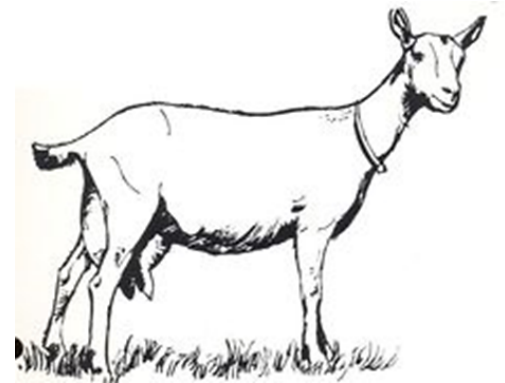


# Introduction

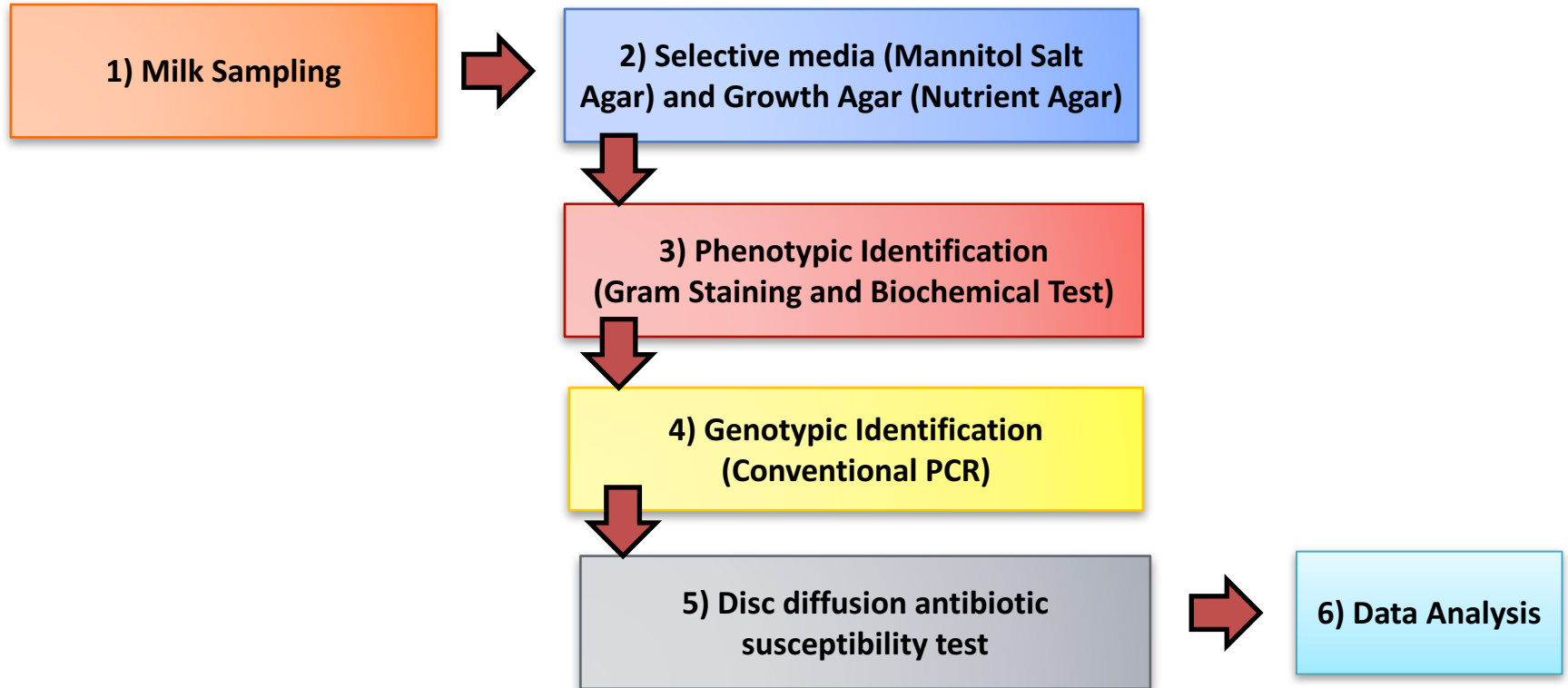
- The first report on the emergence of LA-MRSA in ruminants was from an outbreak of mastitis in cattle in Belgium (*De Vriese et al., 1972*).
- The prevalence of MRSA in bovine milk and its zoonotic transmission between farmers and ruminants have been reported by several studies carried out in Belgium, Germany, Czech Republic, Turkey and Iran (*Stastkova et al. 2009; Feßler et al. 2010; Vanderhaeghen et al., 2010a, b; Spohr et al. 2011; Aras et al., 2012 and Mirzaei et al., 2012*).
- Currently, there is no study conducted on the prevalence of MRSA in goat milk especially in the east coast region of peninsular Malaysia.

# Objectives

1. To investigate the **prevalence** rate of *S. aureus* and MRSA in goat and sheep milk from farms in Terengganu.
2. To determine the **antimicrobial resistance** profile of *S. aureus* isolates obtained in milk.



# Overview of methodology



# Milk sampling

- A total of 600 udder milks samples (R and L) were taken from 300 small ruminants (279 goats and 21 sheep) from 36 selected farms in 7 districts of Terengganu.
- Milk samples were collected using proper aseptic techniques , stored in icebox and send to Microbiology Laboratory, Campus Besut, UniSZA for analysis.





# Bacteria culture and isolation

- The milk samples were first smeared on Mannitol Salt Agar and incubated for 24 hours at 37 °C.
- The morphology of the growth colonies of bacteria was observed and suspected colonies were collected and cultured on Nutrient Agar supplemented with 3% Sodium Chloride further incubated at 37 °C for 24 hours.

# Phenotypic identification

| <b>Test</b>    | <b>Expected Results</b>    |
|----------------|----------------------------|
| Gram Stain     | Gram Positive, Cocci Shape |
| Oxidase Test   | Negative                   |
| Hemolysis Test | Beta-hemolysis             |
| Catalase Test  | Positive                   |
| Coagulase Test | Positive                   |

*(Sperber & Tatini, 1975; Saiful et al., 2006)*

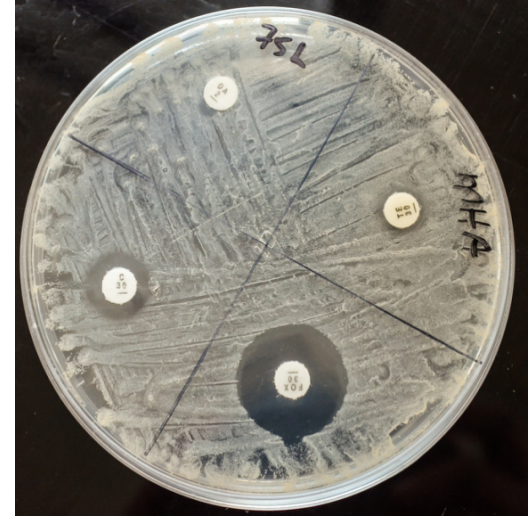
# Genotypic identification

- The identity of the bacteria isolates were further confirmed using the conventional PCR.
- Specific designed primers were used to detect the presence of *nuc* gene of *S. aureus* (278bp).
- *MecA* gene primers (533bp) were used to investigate the prevalence of MRSA among the *S. aureus* isolates.

| Primer              | Sequence                               |
|---------------------|--|
| Nuc gene (Forward)  | 5¢-GCG ATT GAT GGT GAT ACG GTT-3¢      |
| Nuc gene (Reverse)  | 5¢-AGC CAA GCC TTG ACG AAC TAA AGC-3¢. |
| MecA gene (Forward) | 5¢-AAA ATC GAT GGT AAA GGT TGG C-3¢    |
| MecA gene (Reverse) | 5¢-AGT TCT GCA GTA CCG GAT TTG C-3¢.   |

# Disc diffusion antibiotic susceptibility test

- The susceptibility of the *S. aureus* and MRSA isolates towards different antibiotics were tested.
- The bacteria isolates were cultured on Muller Hinton Agar and antibiotic discs (Oxoid) were placed on the agars.
- The agars were incubated for 24 hours at 27 °C. The diameter of inhibition zone surrounding the antibiotic discs were measured and recorded. *Disc diffusion breakpoints are based on the Clinical And Laboratory Standard (CLSI) guidelines.*
- The data collected were recorded and analyzed using Microsoft Excel.



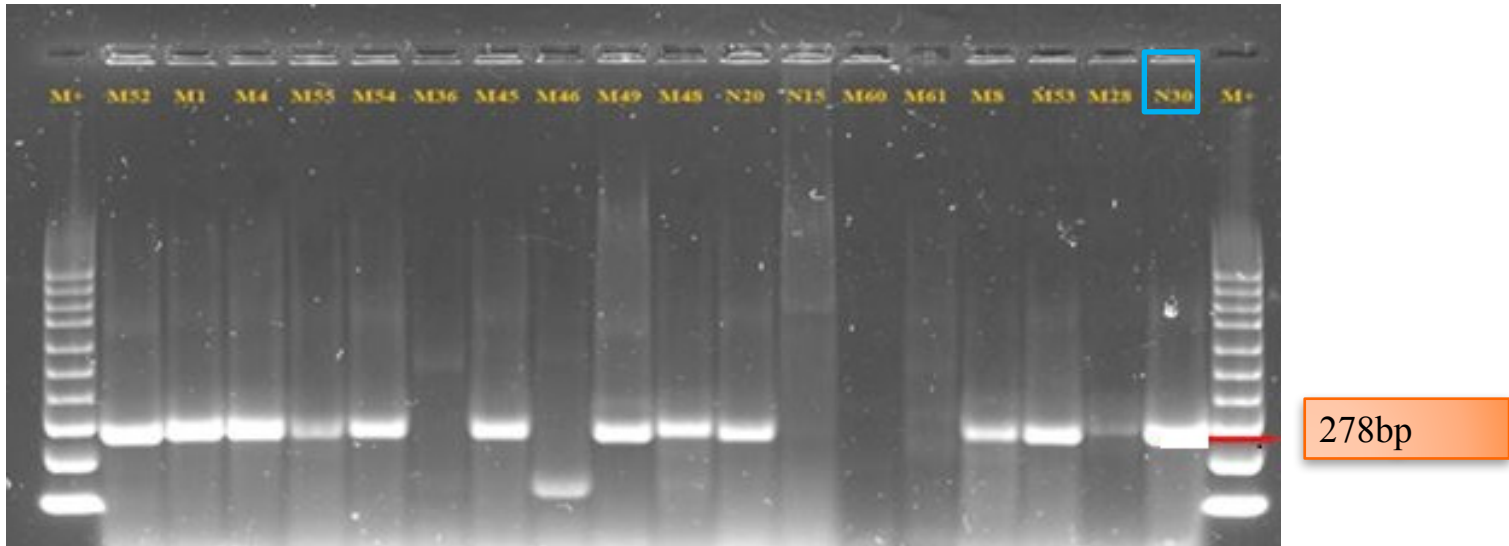
# Disc diffusion antibiotic susceptibility test

List of antibiotic used:

- 1) Oxacillin, 1  $\mu\text{g}$
- 2) Cefoxitin, 30  $\mu\text{g}$
- 3) Tetracycline, 30  $\mu\text{g}$
- 4) Chloramphenicol, 30  $\mu\text{g}$
- 5) Amoxicillin, 10  $\mu\text{g}$
- 6) Vancomycin, 30  $\mu\text{g}$
- 7) Penicillin, 10  $\mu\text{g}$
- 8) Cefotaxime, 30  $\mu\text{g}$
- 9) Doxycycline, 30  $\mu\text{g}$
- 10) Amikacin, 30  $\mu\text{g}$
- 11) Norfloxacin, 10  $\mu\text{g}$
- 12) Clindamycin, 2  $\mu\text{g}$
- 13) Kanamycin, 30  $\mu\text{g}$
- 14) Cephalothin, 30  $\mu\text{g}$

# RESULTS AND DISCUSSION

# Prevalence of *S. aureus* (nuc gene)

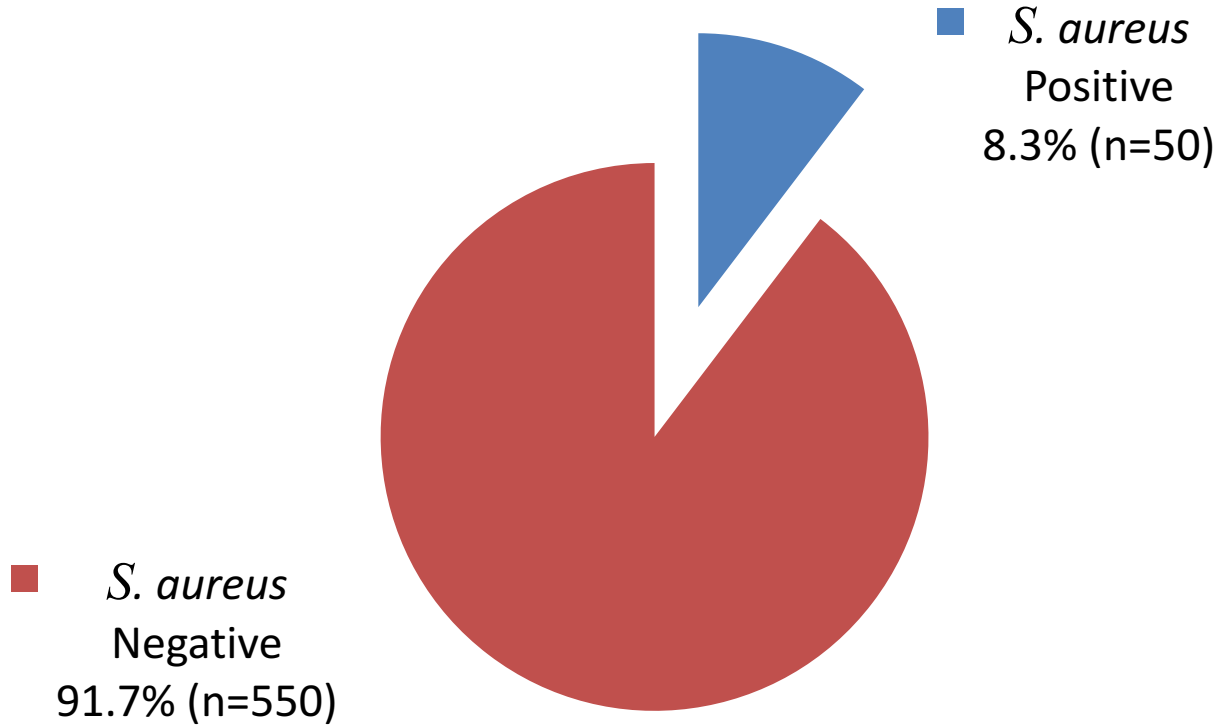


M+ : DNA Ladder (100bp)

M52 – M28: Bacteria isolates from milk samples (*S. aureus* Positive PCR band at 278bp)

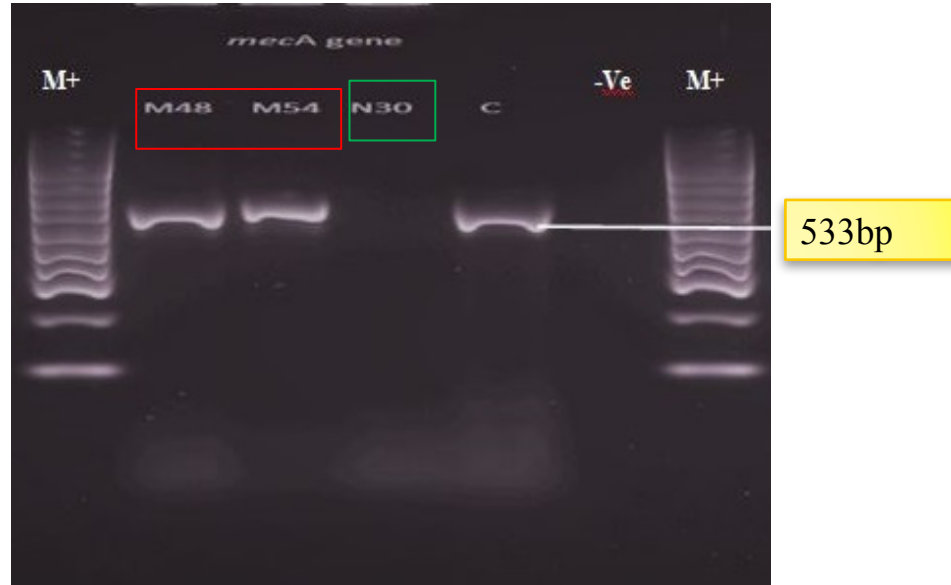
N30 : Positive Control (278 bp)

# The prevalence of *S. aureus* in SR milk samples in Terengganu (n=600)





# Prevalence of MRSA (*mec A* gene)



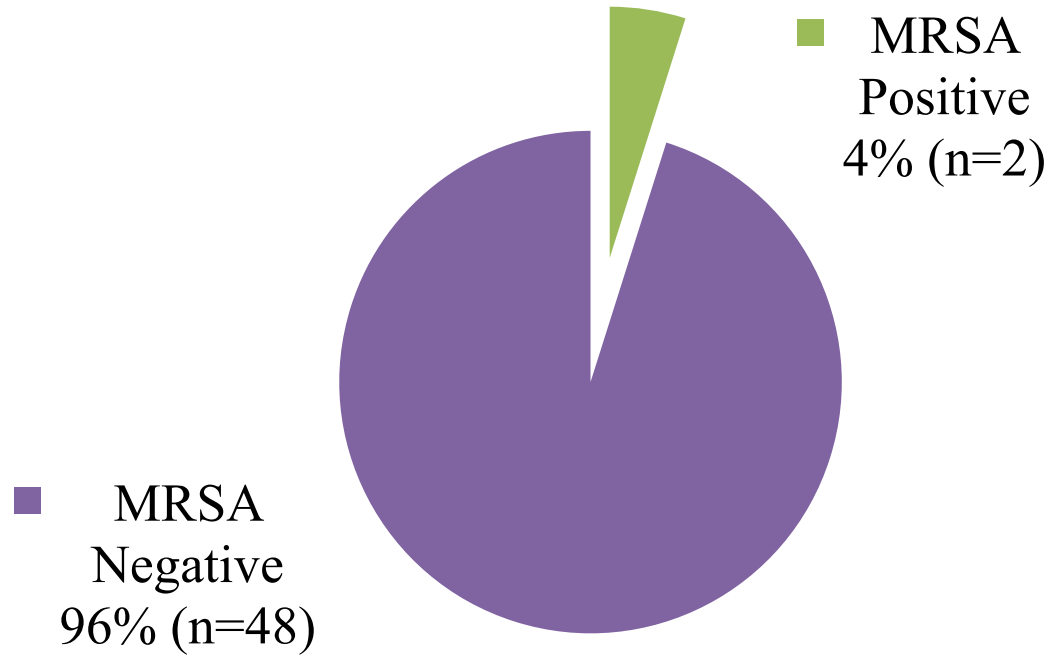
M+ : DNA ladder (100bp)

M48- N30: *S. aureus* isolates from milk samples (MRSA Positive PCR band at 533bp)

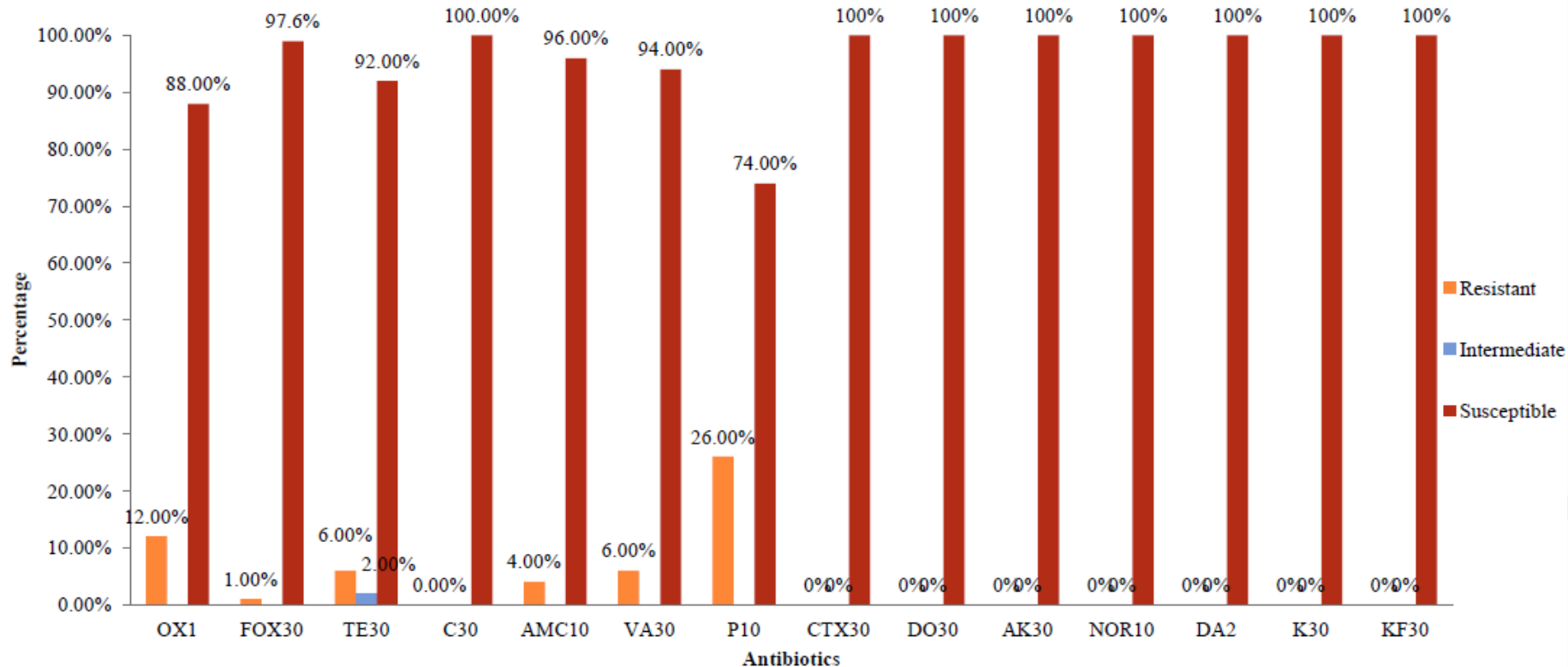
C : Positive Control

-Ve : Negative Control

# Prevalence of MRSA : *mec A* gene (n=50)



### The Antibiotic Susceptibility of *S. aureus* Isolates



n =50

#### Antibiotics used in this study:

OX1 =Oxacillin, 1 µg; FOX30 = Cefoxitin, 30 µg; TE30 = Tetracycline, 30 µg; C30 = Choramphenicol, 30 µg; AMC10 = Amoxicillin, 10 µg, VA30 =Vacomycin, 30 µg; P10 = Penicillin, 10 units, CTX30= Cefotaxime, 30 µg; DO30 = Doxycycline, 30 µg; AK30= Amikacin, 30 µg; NOR10 = Norfloxacin, 10 µg; DA2= Clindamycin, 2 µg, K30= Kanamycin, 30 µg, , KF 30= Cephalothin, 30 µg

# Disc diffusion antibiotic susceptibility test

| Antibiotics    | M48 | M54 |
|----------------|-----|-----|
| Oxacillin      | R   | R   |
| Cefoxitin      | S   | S   |
| Tetracycline   | S   | R   |
| Choramphenicol | S   | S   |
| Amoxicillin    | S   | S   |
| Vancomycin     | R   | S   |
| Kanamycin      | S   | S   |
| Cephalotin     | S   | S   |
| Penicillin     | R   | R   |
| Cefotaxime     | S   | S   |
| Doxycycline    | S   | S   |
| Amikacin       | S   | S   |
| Norfloxacin    | S   | S   |
| Clindamycin    | S   | S   |

# Disc diffusion antibiotic susceptibility test

- The *S. aureus* isolates show different degree of resistances towards various antibiotics. *S. aureus* isolates were found to have higher tendency to be resistance toward Oxacillin (12.0%) and Penicillin (26.0%).
- Staphylococci have two mechanisms for resistance to beta-lactam antibiotics. One is the production of beta-lactamases, enzymes that hydrolytically destroy beta-lactams. The other is the expression of penicillin-binding protein 2a (PBP 2a), which is not susceptible to inhibition by beta-lactam antibiotics (*Fuda et al., 2005*).
- 3 of the *S. aureus* are resistant Tetracycline. This result is support by a study conducted by *Rubin et al (2011)* where the *S. aureus* isolates collected from various animals shown resistance towards Tetracycline.

# Disc diffusion antibiotic susceptibility test

- 3 (6%) of the *S. aureus* isolates shown resistance towards Vancomycin.
- A study carried out by *Bhattacharyya et al (2016)* revealed the presence of 7 *S. aureus* isolates originated from bovine and caprine milk that shown resistance towards Vancomycin.
- Another study carried out *Adegoke and Okoh (2014)* also reported the discovery of *S. aureus* isolates from pigs that are resistant to Vancomycin.

# Conclusion

- 2 MRSA isolates have been found the goat milk samples in Terengganu, suggesting the emergence of LA-MRSA in goats.
- Antibiotic susceptibility test also revealed that *S. aureus* and MRSA isolates has shown resistant toward multiple antibiotics.
- More studies must be done to further investigate the epidemiology of LA-MRSA and other form of AMR in various samples and livestock animals.

# Ongoing works

- Gene sequencing of the MRSA isolates obtained.
- Induction of MRSA into the mammary gland of the targeted goats and collection of milk samples.
- Proteomic analysis of the milk samples collected from the induction of MRSA process.



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**Thank you**  
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