Detection, antibiogram and molecular characterization of MRSA and MSSA isolated from swine

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Abstract: The emergence of bacteria with antimicrobial resistance traits such as methicillin-resistant Staphylococcus aureus (MRSA) in food-producing animals is a significant public health concern. The aim of this study is to determine the prevalence rate, antibiotic susceptibility and the molecular characteristics of Staphylococcus aureus (S. aureus) in swine from selected swine farms in Peninsular Malaysia. The oral and nasal swabs of 200 live swine were collected. Screening and isolation of S. aureus isolates were carried out using phenotypic identification techniques. The identity of the bacteria isolates was confirmed by detection of nuc gene (S. aureus) and mecA gene (MRSA). Antibiogram of S. aureus against 19 antibiotics was developed using Kirby-Bauer test. Molecular detections of antimicrobial resistance and virulence genes as well spa typing of S. aureus was carried out. 25% of swine were carrying S. aureus with 9% being MRSA carriers. S. aureus and MRSA have shown high resistance against clindamycin, tetracycline, chloramphenicol, oxacillin, penicillin, erythromycin, cefoxitin and amoxicillin/clavulanate. Antimicrobial resistance and virulence genes, including tetK, tetL, tetM, ermA, ermB, ermC, msrA and scn genes were detected. 93% of S. aureus were multiple-drug resistant (MDR), suggesting the emergence of MDR S. aureus in swine in Malaysia.

Keywords: Antibiotic resistance, methicillin-resistant Staphylococcus aureus, methicillin-susceptible Staphylococcus aureus, swine

1. Introduction

Staphylococcus aureus (S. aureus) is a commensal pathogen that was often found in the respiratory tract and skin surface of living hosts, causing severe disease in humans as well as economic impact on farm industry [23]. By nature, S. aureus is susceptible to every antibiotic that has been developed by mankind. However, this pathogen also demonstrated the ability to develop antibiotic resistance mechanisms to aid its survival against antibiotics [23]. Shortly after the usage of penicillin commercially, S. aureus strains that are resistant to penicillin were widely spread worldwide [20]. Later, superbug bacteria called methicillin-resistant S. aureus (MRSA) that are resistant to multiple common antibiotics emerged from medical settings, causing difficulties in treating persistent S. aureus infections. Today, MRSA has spread
beyond hospitals to become a significant “community-associated” and “livestock-associated” health burden to both public and animal health [23]. For instance, livestock-associated MRSA (LA-MRSA) ST398 and ST9 that are now commonly found in farm workers and food animals [7]. Studies have revealed that unregulated usage of antimicrobial agents in farms that practice intensive systems such as those in the swine farming industry may facilitate the transmission of LA-MRSA. Notably, an increase of LA-MRSA infections in human and LA-MRSA outbreaks in hospitals were also observed [19]. Therefore, the presence of S. aureus with multidrug resistant traits in food animals such as swine have become a significant public health concern and need to be monitored regularly. Aside from antimicrobial resistance, S. aureus in animals were also reported to carry various virulence genes that encode virulence factors that allow these bacteria to adhere to the surface, invade or avoid the human immune system as well causing diseases to humans [6, 23]. Thus, screening of virulence genes is also vital to determine the pathogenicity of S. aureus from swine.

In Malaysia, the pork production was relatively stable with the production rate in 2018 recorded over 200 thousand metric tons with self-sufficiency as high as 91% generating more than RM 3.9 billion annually [13]. However, there is less information regarding the latest antibiotic resistant pattern and molecular profile of the S. aureus isolated from swine, particularly those from major pork production states in Peninsular Malaysia. The extent of public health impact of MRSA and multidrug-resistance S. aureus (MDRSA) carried by swine and their associated products is still not well-known. Hence, this study serves to provide some insight on the current status of occurrence rate, antibiogram and molecular profile of S. aureus in swine from Peninsular Malaysia.

2. Materials and methods

2.1. Sample collection
A total of 400 swab samples (200 nasal and 200 oral swabs) from 200 finisher swine were collected randomly from swine farms that practiced intensive farming in the states of Selangor, Perak and Johor, Malaysia. Prior to sampling, the history of the swine was obtained and physical examinations were performed. Nasal swabs were collected by inserting the swab into nostrils (2 cm from the opening) and rotated against the nostril wall for a few seconds. Meanwhile, oral swabs were taken by inserting the swab in the back of the oral cavity of the swine and swabbing the soft palate vigorously for at least 5 seconds. The oral swabs were kept into a transport medium in falcon tubes and kept in ice box to maintain at 4 °C before transporting back to laboratory for further analysis. The study and method of sampling were approved by the Universiti Sultan Zainal Abidin Animal and Plant Research Ethnic Committee (UAPREC). Protocol code: UAPREC/04/18/006.

2.2. Bacterial isolation and identification
The swab samples were streaked on mannitol salt agar (MSA) plates (Oxoid, UK) and incubated at 37°C for 24 hrs to promote the growth of S. aureus. Presumptive S. aureus colonies (yellow colonies with yellow surrounding zones) were subcultured onto nutrient agar (NA) plates (Oxoid, UK) supplemented with 6.5% of sodium chloride and incubated at 37 °C for 24 hrs. The isolates then underwent routine microbiological tests, including Gram-staining, catalase, oxidase and coagulase test to filter out Gram negative staphylococci. The bacterial genomic DNA of the isolates was extracted using heat lysis methods [44]. The identity of the presumptive S. aureus isolates was confirmed via detection of nuc genes at size 278 base pair (bp) using the nuc primers mentioned in Table 1 [42]. The presence of MRSA among the isolated S. aureus was investigated by detecting the presence of mecA (533 bp), and mecC (138 bp) genes as mentioned in Table 1 [42-43]. Bacterial isolates with the presence of both nuc and mecA or mecC genes are categorized as MRSA while those with only nuc genes were considered as methicillin-susceptible S. aureus (MSSA).
2.3. Antibiotic susceptibility testing
The antibiotic susceptibility profile of the isolated *S. aureus* was determined using Kirby-Bauer disk diffusion test as recommended by Clinical and Laboratory Standards Institute (CLSI). A total of 19 antibiotics representing 11 different classes of antibiotics were involved in the antibiotic susceptibility testing. These antibiotics included amikacin (30 µg), amoxicillin/clavulanate (10 µg), cefotaxime (30 µg), cefoxitin (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (30 µg), clindamycin (2 µg), doxycycline (30 µg), erythromycin (15 µg), gentamicin (30 µg), kanamycin (30 µg), linezolid (30 µg), norfloxacin (10 µg), oxacillin (1 µg), penicillin (10 units), quinupristin-dalfopristin (15 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg). The diameter of inhibition zones of each isolates were measured and compared to the antimicrobial susceptibility breakpoints as listed in CLSI 2018 guidelines [9]. Isolates that show resistances against 3 or more classes of antibiotics were classified as multidrug-resistant *S. aureus* [31]. The relationships between antibiotic exposure and overall antibiotic resistance in *S. aureus* isolates were assessed using multiple antimicrobial resistance index (MARI). The MARI was calculated as the proportion of antibiotics tested to which the isolate was phenotypically resistant [22].

2.4. Molecular characterization of *S. aureus*
*S. aureus* isolates were further screened using PCR to detect the presence of various antimicrobial resistance genes, including the tetracycline resistant (tetK, tetL, tetM and tetO), erythromycin resistant (ermA, ermB, ermC and msrA) and vancomycin resistant (vanA) genes as listed in Table 1. The prevalence of several virulence determinants, including *tst*, *lukPV*, immune evasion cluster (IEC) gene cluster (*scn, chp, sak, sea* and *sep*) and exfoliative toxin genes (*eta* and *etb*) among the *S. aureus* isolates were also been investigated via PCR using the primers listed in Table 1. In regard to IEC genes, *S. aureus* isolates were classified into 8 different IEC types (A to H) after PCR detection of IEC genes with the presence *scn* gene is mandatory for the consideration of IEC types.

2.5. Spa typing of MRSA and MDRSA
The X region of the *spa* gene of each *S. aureus* isolates was amplified using PCR with the primers *spa* 1095F (5’-AGACGATCCTTCGGTGAGC-3’) and *spa* 1517R (5’-GCTTTTGCAATGTCATTTACTG-3’) [21]. The PCR amplification of *spa* started at initial denaturation at 95 °C for 5 min followed by 30 cycles of 94°C for 30 secs, 59°C for 1 minute and 72°C for 1 minute, with the final extension at 72°C for 10 min. The PCR products of the amplified X-region of *spa* genes (200 to 600 bp) were sent to Bio Basic Asia Pacific Pte Ltd (Singapore) for cleanup and DNA sequencing. The DNA sequences obtained were analysed using spatyper (http://spatyper.fortinbras.us/) to obtain the *spa* repeats and *spa* type. Novel *spa* repeats and sequences were uploaded to Ridom Spa Server (https://spaserver.ridom.de/) for the new *spa* repeats and *spa* type assignment.

2.6. Statistical analysis
The occurrence rate of *S. aureus*, antimicrobial resistance and virulence genes were verified and presented in percentages (%). Categorical data were analysed and compared using Chi-square test (Minitab 19, 2019) with 95% confidence interval (p< 0.05) was set to indicate the significant difference. Prevalence of antibiotic resistance was calculated as the proportion of isolates tested that had an inhibition zone that below the respective antibiotic breakpoint. A phylogeny tree illustrating the relationship of the isolates was generated via UPGMA method using Mega X version 10.1.8. software.

3. Results
The detection of *nuc* genes using PCR have resulted in 50 swine (25%; 50/200) were confirmed carrying *S. aureus* (Figure 1). 4 of the swine were carrying *S. aureus* in both nasal and oral while the other 46 swine have one of their swab samples taken to be positive for the presence of *S. aureus*, yielding a total of 54 *S. aureus* isolates. A total of 20 MRSA (Figure 2) were able to be isolated from 18 different swine,
with the overall MRSA occurrence rate in swine being 9% (18/200). No significant differences were observed between the occurrence rates of MSSA or MRSA in both nasal and oral swab samples.

The antibiogram of *S. aureus* and MRSA isolates were summarized in Table 2 and Table 3. *S. aureus* isolates from swine were highly resistance against clindamycin (96.3%), tetracycline (88.9%), chloramphenicol (81.5%), oxacillin (81.5%), penicillin (74.1%), erythromycin (63.0%), cefoxitin (59.3) and amoxicillin/clavulanate (55.6). In another hand, all of the MRSA isolates were 100% resistant towards clindamycin, tetracycline, chloramphenicol, penicillin and erythromycin. Linezolid is the only antibiotic that is 100% effective against *S. aureus* and MRSA. Out of the 54 *S. aureus* isolates, 50 (92.6%) isolates were resistant towards three different classes of antibiotics and have been categorized as multidrug resistant *S. aureus*. MARI (Table 4) assessments have shown that 52 out 54 (96.3%) isolates have MAR index value of 0.2 and above. 50 isolates were categorized as multidrug-resistant *S. aureus* as they have shown resistance to at least one antimicrobial agent from three different classes of antibiotics.

Molecular characterization of *S. aureus* isolated from swine revealed the presence of multiple antimicrobial resistance genes, including mecA (37%; 20/54), tetK (48.1%; 26/54), tetL (22.2%; 24/54), tetM (44.4%; 24/54), ermA (11.1%; 6/54), ermb (3.7%; 2/54) and ermC (18/54; 33.3). MecC, tetO, msrA and vanA genes were not detected among the isolated *S. aureus*. For virulence genes, only (14.8%; 8/54) *scn* genes were detected. Eight (14.8%; 8/54) of the *S. aureus* isolates were typable by IEC type H while the rest of the isolates were considered as no type (Table 5). In regards to *spa* typing, PCR products were detected among 48 out of 54 *S. aureus* isolates. No PCR products were detected in 6 of the isolates (4 MSSA and 2 MRSA) and thus considered to be non-typable (NT).

Analysis of the DNA sequences of amplified PCR products revealed the presence of four known *spa* types (t189, t315, t548 and t4171), followed by t315 (12/48; 25%), t189 (5/48; 10.4%), t548 (4/48; 8.3%) and t19675 (25/48; 52%). The most prominent *spa* type in MRSA was t4171 (25/48; 52%), followed by t315 (12/48; 25%), t189 (5/48; 10.4%), t548 (4/48; 8.3%) and t19675 (2/48; 4.2%). The most prominent *spa* type in MRSA was t4171 (25/48; 52%), followed by t315 (12/48; 25%), t189 (5/48; 10.4%), t548 (4/48; 8.3%) and t19675 (2/48; 4.2%). The most prominent *spa* type in MRSA was t4171 (25/48; 52%), followed by t315 (12/48; 25%), t189 (5/48; 10.4%), t548 (4/48; 8.3%) and t19675 (2/48; 4.2%). The phylogenetic relationship of the *S. aureus* isolates using the *spa* type sequences was shown in Figure 2.

**Table 1.** List of primers for the detection of AMR and virulence genes used in this study.

| No. | Primer | Primer Sequence (5’-3’) | Product Size (bp) | Annealing Temperature (˚C) | Reference |
|-----|--------|--------------------------|-------------------|---------------------------|-----------|
| 1.  | *Nuc*  | F-GCCATGTGCGGATAGATCCG | 278               | 56˚C                      | [42]      |
| 2.  | *mecA* | R-AGGCAACGCTGCTGAAGATGAGC | 533               |                           |           |
| 3.  | *mecC* | R-AGTTTCTCGAGTACCCGAGGGG | 138               | 59˚C                      | [43]      |
| 4.  | *tetK* | R-TGCAGAATGGACACGAGTA    | 169               | 55˚C                      | [35]      |
| 5.  | *tetM* | R-CGGTAAGAGTTCGCTACACAC | 406               |                           |           |
| 6.  | *tetL* | R-GATCCAGCTCCTACCTCCTT  | 267               |                           |           |
| 7.  | *tetO* | R-CAAGTCGCGCCGCTCAC   | 515               |                           |           |
| 8.  | *msrA* | R-GGCCAACATGAGGTTGTTAAAAGG | 940               | 50˚C                      | [30]      |
| 9.  | *ermA* | R-AGTTTCTATGTAGAAGGGTCCTT | 421               | 55˚C                      | [42]      |
| 10. | *ermB* | R-GGTCAGGCGCCGTAATATTATC | 421               | 55˚C                      | [42]      |
| 11. | *ermC* | R-GTTTTACGAGCTGATGGTTGG | 572               | 52˚C                      |           |
| 12. | *vanA* | R-ATGAACTGCTAAACCCTATTTT  | 1032              | 62˚C                      | [41]      |
| 13. | *sca*  | R-CCAGAAGGTGCTGACCATCG | 258               | 50˚C                      | [48]      |
| 14. | *sak*  | R-AGCCGATCAGGCCAGTTAT | 223               |                           |           |
15. **sea**
   
   | Forward Primer | Reverse Primer | Annealing Temperature | Reference |
   |----------------|----------------|-----------------------|-----------|
   | 5'-AGATCATTCGTGGTATAACG-3' | 5'-TGCAAATGAGTGGCTCCTCAA-3' | 50°C | [12] |

16. **sep**
   
   | Forward Primer | Reverse Primer | Annealing Temperature | Reference |
   |----------------|----------------|-----------------------|-----------|
   | 5'-TGCAAATGAGTGGCTCCTCAA-3' | 5'-TGCAAATGAGTGGCTCCTCAA-3' | 50°C | [12] |

17. **chp**
   
   | Forward Primer | Reverse Primer | Annealing Temperature | Reference |
   |----------------|----------------|-----------------------|-----------|
   | 5'-AGATCATTCGTGGTATAACG-3' | 5'-TGCAAATGAGTGGCTCCTCAA-3' | 50°C | [12] |

18. **lak-PV**
   
   | Forward Primer | Reverse Primer | Annealing Temperature | Reference |
   |----------------|----------------|-----------------------|-----------|
   | 5'-AGATCATTCGTGGTATAACG-3' | 5'-TGCAAATGAGTGGCTCCTCAA-3' | 50°C | [12] |

19. **tst**
   
   | Forward Primer | Reverse Primer | Annealing Temperature | Reference |
   |----------------|----------------|-----------------------|-----------|
   | 5'-AGATCATTCGTGGTATAACG-3' | 5'-TGCAAATGAGTGGCTCCTCAA-3' | 50°C | [12] |

20. **eta**
   
   | Forward Primer | Reverse Primer | Annealing Temperature | Reference |
   |----------------|----------------|-----------------------|-----------|
   | 5'-AGATCATTCGTGGTATAACG-3' | 5'-TGCAAATGAGTGGCTCCTCAA-3' | 50°C | [12] |

21. **etb**
   
   | Forward Primer | Reverse Primer | Annealing Temperature | Reference |
   |----------------|----------------|-----------------------|-----------|
   | 5'-AGATCATTCGTGGTATAACG-3' | 5'-TGCAAATGAGTGGCTCCTCAA-3' | 50°C | [12] |

**Figure 1.** Agarose gel electrophoresis image of the *nuc* genes (278 bp). Lane M+ was the 100 bp DNA marker; Lane C+ was the positive control (ATCC 700699); Lanes 1 to 17 were the representative *S. aureus* isolates.

**Figure 2.** Agarose gel electrophoresis image of the *mecA* genes (533 bp). Lane M+ was the 100 bp DNA marker; Lane C+ was the positive control (ATCC 700699); Lanes P-1 to P-13 were the representative *mecA* gene positive *S. aureus* isolates.
Figure 3. The evolutionary history using spa DNA sequences of the isolates was inferred using the UPGMA method. The optimal tree with the sum of branch length = 0.23812277 is shown.

Table 2. Antibiogram of *S. aureus* from swine (n=54).

| Antimicrobials                | Disk Potency | Number of isolates (%) |
|------------------------------|--------------|------------------------|
|                              | Resistant    | Intermediate | Sensitive |
| Clindamycin                  | 2 µg         | 52 (96.3) | 2 (3.7) | 0 (0) |
| Tetracycline                 | 30 µg        | 48 (88.9) | 4 (7.4) | 2 (3.7) |
| Chloramphenicol              | 30 µg        | 44 (81.5) | 0 (0)  | 10 (18.5) |
| Oxacillin                    | 1 µg         | 44 (81.5) | 0 (0)  | 10 (18.5) |
| Penicillin                   | 10 units     | 40 (74.1) | 0 (0)  | 14 (25.9) |
| Erythromycin                 | 15 µg        | 34 (63.0) | 0 (0)  | 20 (37.0) |
| Cefoxitin                    | 30 µg        | 32 (59.3) | 0 (0)  | 22 (40.7) |
| Amoxicillin/clavulanate      | 10 µg        | 30 (55.6) | 0 (0)  | 24 (44.4) |
| Quinupristin-Dalfopristin    | 15 µg        | 20 (37.0) | 12 (22.2)| 22 (40.7)|
| Doxycycline                  | 30 µg        | 18 (33.3) | 0 (0)  | 36 (66.7) |
| Trimethoprim-Sulfamethoxazole| 25 µg        | 18 (33.3) | 0 (0)  | 36 (66.7) |
| Ciprofloxacin                | 30 µg        | 16 (29.6) | 6 (11.1)| 32 (59.3) |
| Cefotaxime                   | 30 µg        | 14 (25.9) | 10 (18.5)| 28 (51.9)|
| Norfloxacin                  | 10 µg        | 12 (22.2) | 4 (7.4) | 38 (70.4) |
| Kanamycin                    | 30 µg        | 8 (14.8)  | 18 (33.3)| 28 (51.9)|
| Gentamicin                   | 30 µg        | 6 (11.1)  | 2 (3.7) | 46 (85.2) |
| Cephalothin                  | 30 µg        | 4 (7.4)   | 0 (0)  | 50 (92.6) |
| Amikacin                     | 30 µg        | 2 (3.7)   | 0 (0)  | 52 (96.3) |
| Linezolid                    | 30 µg        | 0 (0)     | 0 (0)  | 54 (100)  |

Table 3. Antibiogram of MRSA from swine (n=20).

| Antimicrobials               | Disk Potency | Number of isolates (%) |
|------------------------------|--------------|------------------------|
|                              | Resistant    | Intermediate | Sensitive |
| Chloramphenicol              | 30 µg        | 20 (100) | 0 (0)  | 0 (0) |
| Clindamycin                  | 2 µg         | 20 (100) | 0 (0)  | 0 (0) |
| Tetracycline                 | 30 µg        | 20 (100) | 0 (0)  | 0 (0) |
| Penicillin                   | 10 units     | 20 (100) | 0 (0)  | 0 (0) |
| Erythromycin                 | 15 µg        | 20 (100) | 0 (0)  | 0 (0) |
| Cefoxitin                    | 30 µg        | 18 (90)  | 0 (0)  | 2 (10) |
| Antibiotic                        | MARI | Number of isolates | Total (%) |
|----------------------------------|------|--------------------|-----------|
| Oxacillin                        | 1 µg | 16 (80)            | 4 (20)    |
| Amoxicillin/clavulanate          | 10 µg| 16 (80)            | 4 (20)    |
| Doxycycline                      | 30 µg| 14 (70)            | 6 (30)    |
| Cefotaxime                       | 30 µg| 12 (60)            | 4 (20)    |
| Quinupristin-Dalfopristin        | 15 µg| 10 (50)            | 4 (20)    |
| Ciprofloxacin                    | 30 µg| 8 (40)             | 8 (40)    |
| Norfloxacin                      | 10 µg| 8 (40)             | 10 (50)   |
| Trimethoprim- Sulfamethoxazole   | 25 µg| 8 (40)             | 12 (60)   |
| Kanamycin                        | 30 µg| 6 (30)             | 8 (40)    |
| Amikacin                         | 30 µg| 4 (20)             | 16 (80)   |
| Gentamicin                       | 30 µg| 2 (10)             | 16 (80)   |
| Cephalothin                      | 30 µg| 2 (10)             | 18 (90)   |
| Linezolid                        | 30 µg| 0 (0)              | 10 (100)  |

### Table 4. MARI assessment of *S. aureus* isolated from swine (n=54).

| Number of antibiotic | MARI | Number of isolates | Total (%) |
|----------------------|------|--------------------|-----------|
| 0                    | 0    | 0                  | 0         |
| 1                    | 0.05 | 0                  | 0         |
| 2                    | 0.11 | 2                  | 3.7       |
| 3                    | 0.16 | 0                  | 0         |
| 4                    | 0.21 | 6                  | 11.1      |
| 5                    | 0.26 | 6                  | 11.1      |
| 6                    | 0.32 | 8                  | 14.8      |
| 7                    | 0.37 | 6                  | 11.1      |
| 8                    | 0.42 | 4                  | 7.4       |
| 9                    | 0.47 | 0                  | 0         |
| ≥ 10                 | 0.52 | 22                 | 40.7      |

### Table 5. Summary of IEC type of MSSA and MRSA isolates.

| IEC Type | IEC genes composition | MSSA (n=34) | MRSA (n=20) | Total number of isolates (%) |
|----------|-----------------------|-------------|-------------|------------------------------|
| A        | scn, chp, sak, sea    | 0           | 0           | 0 (0)                        |
| B        | scn, chp, sak         | 0           | 0           | 0 (0)                        |
| C        | scn, chp              | 0           | 2           | 2 (3.7)                      |
| D        | scn, sak, sea         | 0           | 0           | 0 (0)                        |
| E        | scn, sak              | 0           | 4           | 4 (7.4)                      |
| F        | scn, chp, sak, sep    | 0           | 0           | 0 (0)                        |
| G        | scn, sak, sep         | 0           | 0           | 0 (0)                        |
| H        | Scn                   | 6           | 2           | 8 (14.8)                     |
| Non-typable | Absence of scn gene, but other IEC genes are present. | 0 | 0 | 0 (0) |
| No Type  |                       | 28          | 10          | 38 (70.4)                    |

### 4. Discussion

In recent years, the emergence of MRSA, particularly livestock-associated strains (LA-MRSA) in food-producing animals has gained great attention in the context of food safety and public health concerns, mainly due to the zoonotic capability of these bacteria. In the present study, the occurrence rate of *S. aureus* isolated from the selected swine farms was 25.0% (50/200 swine). This finding is higher than *S. aureus* prevalence rates in swab samples collected from live swine in China (9.5% to 23.4%), Thailand (1.4%) and Senegal (12.3%) as reported in previous studies [16, 19, 37, 50]. However, some other studies recorded a much higher *S. aureus* prevalence rate. A study in the USA reported a high prevalence rate of 76% in nasal swab samples collected from growing swine [45]. Another study by Linhares et al (2015) demonstrates that 91.1% of the sampled swine in Minnesota were harboring *S. aureus* after
analyzing the swabs collected from various body sites, including nares, tonsils, axilla, vagina and rectum [28]. Further screening of mecA and mecC genes revealed that the overall occurrence rate of MRSA in swine in the present study was 9% (18/200). This finding is higher than the 0% (USA), 0% (Punjab), 0.68% (Thailand) and 3.3% (China) of MRSA prevalence rate in live swine by studies conducted elsewhere [19, 28, 37, 49]. Nonetheless, other similar studies from Italy (46.1%) Portuguese (96%) and Neatherland (83% to 99%) reported a higher MRSA prevalence rate in swab samples collected from pigs [14, 29, 39].

Swine farming has been regarded as an industry with very high antimicrobial usage both for prophylactics and therapeutic purposes [24]. Thus, a continuous surveillance on the usage of antimicrobials in swine production and the antibiotic susceptibility of these bacteria are deemed necessary. In this study, the antibiotic susceptibility test has revealed that the isolated MSSA and MRSA were high resistance rate towards multiple antibiotics from different antimicrobial classes, including beta-lactams (penicillin, oxacillin, cefoxitin, and amoxicillin/clavulanate), tetracyclines (tetracyclines), macrolides (erythromycin), phenicols (chloramphenicol) and lincosamides (clindamycin). Similar finding was also reported by Conceição et al., (2017) where majority of MRSA isolated from swine in Portuguese swine farms showed resistant against tetracycline (100%), clindamycin (97%), erythromycin (96%) and chloramphenicol (84%) [10]. The isolated S. aureus in this study were fully susceptible towards linezolid, suggesting that linezolid antibiotics can be used to treat MRSA or persistence S. aureus infections in swine. However, further clinical study needs to be carried out to examine the effectiveness of antibiotics in living hosts colonized or infected with the S. aureus.

The high antibiotic resistance rate displayed by S. aureus isolates against beta-lactams, tetracyclines and macrolides is not surprising as penicillin, tetracycline and erythromycins have been reported to be the three most common antibiotics used by swine farmers around the globe [24]. As the development of antimicrobial resistance is strongly associated with the repeated therapeutic or indiscriminate use of antibiotics, it is possible that the isolated S. aureus were consistently exposed to antibiotics [5]. Indeed, 52 of the S. aureus were found to have MARI of 0.2 and above, indicating that the isolates may be frequently exposed to antibiotics or came from a surrounding with constant antibiotic usage [40]. Nevertheless, a high percentage of multidrug-resistant S. aureus (50/54; 92.6%), including both MRSA and MDRRSA were detected among the isolated S. aureus. Similar result was also reported by Guo et al (2018) where 97% of the S. aureus isolated from both live and slaughter swine were MDRSA [19]. Another study by Zhou et al. (2020) also reported that 66.7% of S. aureus isolates from swine and swine farm environments were multidrug-resistant, with high rates of resistance against penicillin, tetracycline, clindamycin, and clarithromycin [50]. The presence of such a high number of MDRSA is a concern to public health and welfare of farm workers as these bacteria may be transmitted to humans, spill over infections to other animals or contaminate the environments during meat processing, packaging or delivery process.

In order to determine the possible mechanism for antibiotic resistance among the S. aureus isolates, molecular detection of various antimicrobial resistance genes were carried out. In this study, antimicrobial resistance genes that responsible for methicillin or oxacillin resistance (mecA gene), tetracycline-resistance (tetK, tetL and tetM) and erythromycin resistance (ermA, ermB and ermC) have been detected. Briefly, the mecA gene encodes the low affinity penicillin binding protein 2a (PBP2a) that confers resistance to otherwise inhibitory concentrations of all β-lactam antibiotics [8]. The TetK and tetL genes are responsible for the tetracycline efflux pump system while the tetM gene is involved in ribosomal protection of bacteria against tetracyclines [25]. ErmA, ermB and ermC genes are responsible for the ribosomal binding site modification to protect the S. aureus against erythromycin antibiotics [17]. In Malaysia, the presence of tetracycline and erythromycin resistance genes in S. aureus from animals was not previously reported. However, other researches that study molecular characterization of S. aureus and MRSA in medical settings and community reported the presence of tetracycline and erythromycin resistance genes among the isolated S. aureus in Malaysia [15, 26, 36, 44]. In 2017, Zehra and co-author reported the presence of tetK (16.1%), tetL (19.4%), tetM (3.2%) and ermB (13.5%) among the isolated S. aureus from bovine and swine in Punjab [49]. Another study by
Zhou et al. (2020) reported the presence of tetK gene (46.4% to 99.5%), tetL gene (51.8% to 100%), tetM gene (1.6% to 67.9%), ermA gene (1.6% to 85.7%), ermB gene (8.1% to 85.7%) and ermC gene (0% to 9.1%) in S. aureus isolated from both healthy and diseased swine [50]. The presence of such a variety of antimicrobial resistance genes in S. aureus from swine indicated that these bacteria are well adapted to antibiotics that are commonly used in the swine farming industry. However, not all of the S. aureus isolates in this study that develop phenotypic resistance were carrying antimicrobial resistance genes. It is possible that these isolates may have developed phenotypic resistance through physiological changes or mechanisms, including biofilm formation, drug indifference and persistence [11]. Besides, the antibiotic resistance may be mediated by other genes that are not included in this study [4, 49]. Nonetheless, the existence of antimicrobial resistance genes among S. aureus should not be taken lightly as certain genes were carried on mobile genetic elements and can spread to other bacteria, capable of causing treatment failure in both human and veterinary medicine [49].

Aside from antimicrobial resistance genes, S. aureus often harbors different virulence genes that encode various virulence factors that enable infections and evade the host immune system. In the present study, only the scn gene was detected among 6 MSSA and 2 MRSA. Scn gene encoded staphylococcal complement inhibitor (SCIN), a 10 kDa protein that could inhibit human immune response by blocking opsonophagocytosis of S. aureus by human neutrophils and prevent neutrophil chemotaxis [1]. Scn along with other IEC genes were frequently present in S. aureus obtained in humans but generally absent in the animals [12]. The presence of scn genes among these four isolates suggested that the S. aureus may have originated from humans [12]. In regards to IEC types, as there is only the presence of one type of IEC gene (scn), only four S. aureus can be categorized as IEC type H while the rest of the S. aureus are categorized as non-type. This result is in contrast with the previous study that stated IEC type B was the predominant IEC type found in S. aureus [1]. These findings suggested that there are variations in IEC types among S. aureus according to the different geographical regions and source of origins. All S. aureus isolates, including MRSA described in this study, were negative for luk-PV, tst, eta and etb genes that code PVL and TSST. This finding is in contrast with a study conducted in Senegal that reported the presence of PVL, TSST and exfoliative toxins encoding genes in S. aureus isolated from food-producing animals [32]. The finding of low number of virulence genes suggested that S. aureus isolates from swine sampled in this study may be less pathogenic or virulence to humans in comparison to other strains.

To the authors knowledge, only a few studies that applied the usage of spa typing for molecular typing of selected S. aureus isolates that originated from animals such as swine (t4358), chicken (t2247), cats (t346) and dogs (t267 and t3590) [2-3, 33, 34]. Thus, there is a lack of data on the prevalence of spa types among different animal species in Malaysia. Spa typing is frequently used to study the relatedness and genetic diversity of the S. aureus isolates [18]. It is preferred as it is a cheaper, effective and rapid method with good discriminatory abilities for typing of S. aureus isolates [18]. In this study, spa typing analysis revealed that only five genotypes, which are t4171, t315, t189, t548 and novel spa type t19675 were isolated from the swab samples in swine, with t4171 being the dominant genotype in both MSSA and MRSA isolates. This finding is different from the results reported by Neela et al. (2009), where all the isolated MRSA from swine in the Selangor area were spa typed t4358 [34]. Interestingly, t189 and t548 were previously reported in MSSA isolated from selected patients and healthy undergraduate students in Malaysia [26]. Besides, t4171 was reported to be associated with S. aureus infections in Malaysia (http://spaserver.ridom.de/spa-t4171.shtml). Meanwhile, S. aureus t315 that was known as human-associated clonal was also reported to be present in cattle from Czech Republic [46]. These show that the majority of the spa types, with the exception of t19675 found in this study, are not host-specific and may indicate wide dissemination of these genotypes in humans and animals in Malaysia. Additional research such as collecting samples from farm workers with close contact with swine and running multilocus sequence typing (MLST) on the isolates may provide insight on the clonal complex and potential lineage of the isolates.
5. Conclusion
In conclusion, this study has demonstrated the presence and prevalence rate of MSSA (25%) and MRSA (9%) in swine from the peninsular of Malaysia. 92.6% of the isolated S. aureus from swine were considered to be MDRSA. The isolated S. aureus show different degrees of resistance against 18 different antibiotics, with the exception of linezolid. Furthermore, the S. aureus were carrying various antimicrobial resistance genes in swine. The isolates were found to be less pathogenic to humans as they contain a low number of tested virulence genes. The presence of only five different spa types may indicate that the S. aureus may have originated from the common lineages.

References
[1] Ahmadrajabi R, Khavidaki S L, Kalantar-Neyestanaki D and Fasihi Y 2017 J. Prev. Med. Hyg. 58 E308–E314
[2] Aklilu E, Zakaria Z, Hassan L and Cheng C H 2012 PLoS ONE 7
[3] Aklilu E, Zakaria Z, Hassan L and Cheng C H 2013 Vet. Microbiol. 164 352–358
[4] Ariffin M F T, Hasmadi N, Chai M H, Ghazali M F and Ariffin S M Z 2020 Malays. J. Microbiol. 16 104–110
[5] Ariffin S M Z, Hasmadi N, Syawari N M, Sukiman M Z, Ariffin M F T, Chai M H and Ghazali M F 2019 J. Anim. Heal. Prod. 7 32–37
[6] Bien J, Sokolova O and Bozko P 2011 J. Pathog. 2011 1–13
[7] Butaye P, Argudín M A and Smith T C 2016 Curr. Clin. Microbiol. Rep. 3 19–31
[8] Chai M H, Ariffin M F T, Ariffin S M Z, Suhaili Z, Sukiman M Z and Ghazali M F. 2020 Trop. Anim. Sci. J. 43 64–69
[9] CLSI 2018. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Clinical & Laboratory Standards Institute, Wayne, PA.
[10] Conceição T, De Lencastre H and Aires-de-Sousa M 2017 PLoS ONE 12
[11] Cuny C, Abdelbary M, Layer F, Werner G and Witte W 2015 Vet. Microbiol. 177 219–223.
[12] De Haas C J C, Veldkamp K E, Peschel A, Weerkamp F, Van Wamel W J B, Heezius E C J M, Poppelier M J J G, Van Kessel K P M and Van Strijp J A G 2004 J. Exp. Med. 199 687–695
[13] Department of Veterinary Malaysia 2018 Malaysia: Livestock Population 2017-2018 1–15. http://www.dvs.gov.my/dvs/resources/user_1/2019/BP/Perangkaan%20Terbaik%202018/PDF/Malaysia_Perangkaan_Ternakan.pdf. [accessed at 10 October 2020].
[14] Dierikx C M, Hengeveld P D, Veldman K T, de Haan A, van der Voorde S, Dop P Y, Bosch T and van Dijkereen E 2016 J. Antimicrob. Chemother. 71 2414–2418
[15] Elkammoshi A M, Ghasemzadeh-Moghadam H, Nordin S A, Taib N M, Subbiah S K, Neela V. and Hamat R A 2016 Jundishapur J. Microbiol. 9
[16] Fall C, Seck A, Richard V, Ndour M, Sembene M, Laurent F and Breurec S 2012 Foodborne Pathog. Dis. 9 962–965
[17] Ghanbari F, Ghajavand H, Havaei R, Jami M -S, Khademi F, Heydari L, Shahin M and Havaei A S 2016 Adv. Biomed. Res. 5 62
[18] Goudarzi M, Fazeli M, Goudarzi H, Azad M and Seyedjavadi S S 2016 Jundishapur J. Microbiol. 9
[19] Guo D, Liu Y, Han C, Chen Z and Ye X 2018 Infect. Drug Resist. 11 1299–1307
[20] Hagstrandsd Aldman M, Skovby A and Pahlman L I 2017 Infect. Dis. 49 454–460
[21] Harmsen D, Claas H, Witte W, Claas H, Turnwald D and Vogel U 2003 J. Clin. Microbiol. 41 5442–5448
[22] Jaja I F, Jaja C I, Chigor N V, Anyanwu M U, Madubuchi E K, Oguttu J W and Green E 2020 Biomed Res. Int. 2020
[23] Lakhundi S and Zhang K 2018. Clin. Microbiol. Rev. 31 1–103
[24] Lekagul A, Changcharoensathien V and Yeung S 2019 Vet. Anim. Sci. 7
[25] Lim K T, Hanifah Y A, Yusof M Y M and Thong K L 2012 Indian J. Med. Microbiol. 30 203–207
[26] Lim K T, Yeo C C, Suhaili Z and Thong K L 2012 Jpn. J. Infect. Dis. 65 502–509
[27] Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter M O, Gauduchon V, Vandenbosch F and Etienne J 1999 Clin. Infect. Dis. 29 1128–1132
[28] Linhares L L, Yang M, Sreevatsan S, Munoz-Zanzi C A, Torremorell M and Davies P R 2015 J. Vet. Diagn. Invest. 27 55–60
[29] Lopes E, Conceiçã T, Poirel L, de Lencastre H and Aires-De-Sousa M 2019 PLoS ONE 14
[30] Ma Y, Zhao Y, Tang J, Tang C, Chen J and Liu J 2018 CyTA J. Food 16 76–84
[31] Magiorakos A P, Srinivasan A, Carey R B, Carmeli Y, Falagas M E, Giske C G, Harbarth S, Hindler J F, Kahlmeter G, Olsson-Liljequist B, Paterson D L, Rice L B, Stelling J, Struelens M J, Tڅapotoulos A, Weber J T and Monnet D L 2012 Clin. Microbiol. Infect. 18 268–281
[32] Mama, O. M., Dieng, M., Hanne, B., Ruiz-Ripa, L., Diop, C. G. M. and Torres, C. 2019. Genetic characterisation of staphylococci of food-producing animals in Senegal. PVL detection among MSSA. BMC Vet. Res. 15: 1–6. https://doi.org/10.1186/s12917-019-2137-9
[33] Neela V, Ghaznavi-Rad E, Ghasemzadeh-Moghaddam H, Nor Shamshudin M, Van Belkum A and Karunanidhi A 2013 Iran. J. Vet. Res. 14 226–231
[34] Neela V, Zafrul A M, Mariana N S, Van Belkum A, Liew Y K and Rad E G 2009 J. Clin. Microbiol. 47 4138–4140
[35] Ng L, Martin I, Afša M and Mulvey M 2001 Mol. Cell. Probes 15 209-215
[36] Ong M H L, Ho W Y, Ng W W and Chew C H 2017 Jundishapur J. Microbiol. 10
[37] Patchanee P, Tadee P, Arjkumpa O, Love D, Chanachai K, Alter T, Hinjoy S and Tharavichitkul P 2014 J. Vet. Sci. 15 529–536
[38] Peacock S J, Moore C E, Justice A, Kcantzanou M, Story L, Mackie K, O’Neill G and Day N P J 2002 Infect. Immun. 70 4987–4996
[39] Pirolo M, Gioffrè A, Visaggio D, Gherardi M, Pavia G, Samele P, Ciambrone L, Di Natale R, Spatari G, Casalhuuovo F and Visca P 2019 BMC Microbiol. 19 1–12
[40] Riaz S, Faisal M and Hasnain S 2011 Afr. J. Biotechnol. 10 6325–6331
[41] Saha B, Singh A K, Ghosh A and Bal M 2008 J. Med. Microbiol. 57 72–79
[42] Saiful A J, Mastura M, Zarizal S, Mazurah M I, Shuhaimi M and Ali A M 2006 World J. Microbiol. Biotechnol. 22 1289–1294
[43] Stegger M, Liu C M, Larsen J, Soldanova K, Aziz M, Contente-Cuomo T, Petersen A, Vandezendriessen C, Jimenez J N, Mammina C, van Belkum A, Salmenlinna S, Laurent F, Skov R L, Larsen A R, Andersen P S and Price L B 2013 PLoS ONE 8
[44] Suhaili Z, Yeo C C, Ghazali M F, Chew C H, Zainul A Z, Mazen M J A-O, Nordin S A and Mohd Desa M N 2018 Trop. Med. Int. Health 23 905–913
[45] Sun J, Yang M, Sreevatsan S and Davies P R 2015 PLoS ONE 10 1–14
[46] Tegegne H A, Koláčková I and Karpíšková R 2017 Asian Pac. J. Trop. Med. 10 929–931
[47] Tokajian S, Haddad D, Andraos R, Hashwa F and Araj G 2011 ISRN Microbiol. 2011 1–9
[48] van Wamel W J B, Rooijakkers S H M., Ruyken M, van Kessel K P M. and van Strijp J A G 2006 J. Bacteriol. 188 1310–1315
[49] Zhou Y, Li X and Yan H 2020 Antibiotics 9 1–16

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