ISOLATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM PORK MEAT IN KOTA BHARU, KELANTAN

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(D18A0013)

A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI MALAYSIA KELANTAN IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE

FEBRUARY 2023

UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

This is to certify that we have read this research paper entitled "**Isolation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Pork Meat in Kota Bharu, Kelantan"** by Lak Ong'A A/P Udom, and in our opinion, it is satisfactory in term of scope, quality and presentation as partial fulfillment of the requirements for the course DVT55204 - Research Project.

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ACKNOWLEDGEMENT

Special thanks to those who have given their support, guidance, advice, and aid for the completion of this project paper:

Dr. Intan Noor Aina Kamaruzaman

Dr. Mohammad Sabri bin Abdul Rahman

Puan Norsyamimi Farhana binti Mat Kamir

Family

DVM 5 Class of 2018/2023

Thank you

DEDICATIONS

I dedicate my dissertation work to my family and many friends. Special gratitude to my loving parents, Udom and La'O, whose encouragement and push for tenacity ring in my ears. My brother, Uson Udom, has never left my side and is very special.

I also dedicate this dissertation to many lecturers and seniors who have supported me throughout the process. I will always appreciate everything they have done, especially Dr. Intan Noor Aina and Dr. Mohammad Sabri bin Abdul Rahman for helping me develop my skills as a veterinary student.

I also dedicate this spadework to Bacteriology and Mycology lab assistant, Puan Norsyamimi Farhana binti Mat Kamir, for helping me throughout the project.

I also dedicate this work and give special thanks to my wonderful classmates, Lim Chee Chee, Abdul Muhaimin bin Simunir and Jeremy Tan for being there for me throughout the project. All of them have been my best cheerleaders.

Table of contents

Contents

List of tables

List of Figures

List of appendices

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT55204 - Research Project.

Foodborne disease, often known as food poisoning, refers to any ailment induced by ingesting contaminated food containing hazardous bacteria, such as *Staphylococcus aureus* from pork meat. The condition could have been worse if the bacteria was antimicrobial-resistant, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), which is regularly found in pigs. This study aims to evaluate whether MRSA is present in pork meat in Kota Bharu, Kelantan. Thus, 30 samples of fresh pork meat were gathered from the Kota Bharu market, bacteria were isolated and identified, and an antibiotic sensitivity test (AST) using the Kirby-Bauer disk method was conducted. The obtained data were analyzed by manual data analysis. The results showed that 21 of 30 (70%) pork meat samples from the market were colonized with *S. aureus*, and 60% of them tested positive for MRSA. The outcome shows that MRSA in pork meat can be spread to humans through meat handling and potentially cause food poisoning.

Keywords: Foodborne disease, antimicrobial-resistant, *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), pork meat

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT55204 - Projek Penyelidikan.

Penyakit bawaan makanan, selalunya dikenali sebagai keracunan makanan, merujuk kepada sebarang penyakit yang disebabkan oleh pengambilan makanan tercemar yang mengandungi bakteria berbahaya, seperti *Staphylococcus aureus* daripada daging babi. Keadaan ini boleh menjadi lebih teruk jika bakteria itu tahan antimikrob, seperti Methicillin-Resistant *Staphylococcus aureus* (MRSA), yang kerap ditemui dalam babi. Kajian ini bertujuan untuk menilai sama ada MRSA terdapat dalam daging babi di Kota Bharu, Kelantan. Oleh itu, tiga puluh sampel daging babi segar telah dikumpulkan dari pasar Kota Bharu, dan bakteria telah diisolasikan dan dikenalpasti, sertaujian sensitiviti antibiotik (AST) dijalakan menggunakan kaedah disk Kirby-Bauer telah dijalankan. Data yang diperolehi dianalisis secara manual data analisis. Hasil keputusan menunjukkan 21 daripada 30 (70%) sampel daging babi dari pasaran positif *S. aureus*, dan 60% daripadanya diuji positif untuk MRSA. Hasil keputusan menunjukkan bahawa MRSA yang di dalam daging babi berupaya untuk disebarkan kepada manusia melalui pengendalian daging babi serta berpotensi untuk menyebabkan keracunan makanan.

Keywords: Penyakit bawaan makanan, bakteria tahan antimirkrob, *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), daging babi

1.0. INTRODUCTION

Foodborne disease, often known as food poisoning, refers to any sickness induced by consuming contaminated, pathogen-infected food. From the farm to the table, foodborne infections present a variety of obstacles. The food production process, from growing to harvesting to transporting and cooking in filthy settings without adequate temperature and environmental controls, creates an avenue for infection in humans and animals. The financial costs of foodborne illness can be catastrophic for individuals, food companies, and the reputation of a nation (Adley & Ryan, 2016).

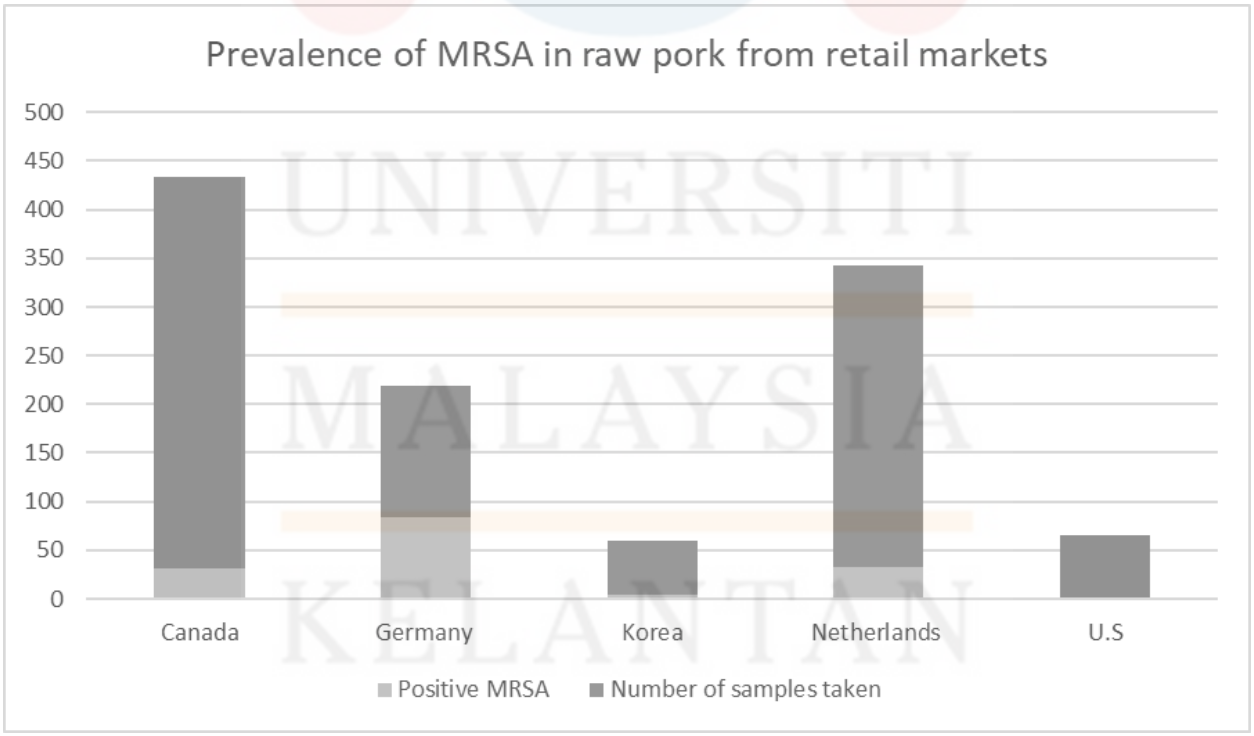
Foodborne diseases may be acute or chronic, and their causes may be biological, chemical, or physical. Bacteria, viruses, and parasites are the principal biological hazards responsible for acute foodborne diseases. *Bacillus cereus*, *Campylobacter* spp, *Clostridium perfringens*; pathogenic *Escherichia coli*, *Salmonella* ssp, *Shigella* spp, *Staphylococcus aureus*, *Vibrio cholerae* and *Vibrio parahaemolyticus* are among the bacterial agents identified in food (Mortarjemi et al., 2013).

According to Delgado and others (2001), pork is the world's most widely consumed type of meat. Though pork is not most consumed meat in Malaysia, it was estimated that in 2021 the locals consumed 5.2 kg of pork per capita (Statista, 2021). Due to its widespread use in a range of products, foodborne diseases related to pork meat are regarded as serious, as pork carries a variety of pathogens in addition to *S. aureus*, including *Salmonella* spp., *Toxoplasma gondii*, *Campylobacter* spp., and *Listeria monocytogenes*, respectively (Baer and others, 2013).

S. aureus is one of the common contaminant bacteria found in the food system, which can result in low levels of enterotoxin generation upon ingestion. *S. aureus* contamination is therefore, frequently associated with handling meat products. Despite the fact that *S. aureus* is a very low-risk bacterium, the emergence of MRSA has become a public health concern, as MRSA is now considered endemic to hospitals and has recently been found in a range of pets and livestock, including pigs

Since MRSA is an ongoing topic of concern in the retail meat sector, a number of prevalence studies on raw pork meat have been conducted in several countries. The pork samples were collected from retail markets in Canada, Germany, Korea, the Netherlands, and the United States (Figure 1.1). However, no research on the frequency of MRSA in pork meat in Malaysia has been conducted.

Figure 1.1. Prevalence of MRSA in raw pork meat from retail markets (Bhunia, 2018)



1.1. Research Problem

Various studies on MRSA isolation and detection have been conducted in Malaysia, but the majority of them involved molecular detection from a nasal swab in live animals, primarily in domesticated animals such as dogs, cats, and cattle. However, no reports of the agent being detected in fresh pork meat in Kelantan, Malaysia.

1.2. Research questions

1.2.1. Does *S. aureus* present and localize in fresh pork meat in Kelantan market?

1.2.2. Does *S. aureus* present and localized in fresh pork meat in Kelantan resistant to Methicillin?

1.3. Research hypothesis

1.3.1. There is a high level of contamination of *S. aureus* in fresh pork meat in Kelantan market.

1.3.2. *S. aureus* isolated from pork meat in Kelantan market is resistant to Methicillin.

1.4. Objectives

1.4.1. To isolate and identify *S. aureus* from fresh pork meat in Kelantan market.

1.4.2. To determine the degree of Methicillin-resistance exhibited by *S. aureus* isolated from fresh pork meat in Kelantan.

2.0. LITERATURE REVIEW

2.1. Overview of foodborne disease caused by bacterial specifically in pork meat

According to Lee and Yoon (2021), tainted food is responsible for 600 million cases of foodborne illness and 420 thousand of deaths annually. The present global population is 7.8 billion, and 56 million people die annually. 7.69 percent of people develop foodborne infections annually, and foodborne illness accounts for 7.5% of annual mortality.

Salmonella spp., *Listeria monocytogenes, Campylobacter* spp., *Staphylococcus aureus,* and *Toxoplasma gondii* are among the main causes of foodborne illness and death in the United States annually (CDC 2011). It is well-documented that these bacteria are prevalent in pigs and pork products, making pork a possible contributor to foodborne illness.

2.2. Description of *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, facultatively anaerobic, *Staphylococcus*-genus, Staphylococcae-family bacterium. *S. aureus* is non-motile, non-sporulating, and catalase and coagulase tests were positive. On non-selective media; Nutrient agar, Blood agar, and selective media; Mannitol Salt Agar, they are typically grouped in clusters or clusters of grapes with an average diameter of 1 m. *S. aureus* typically inhabits the skin, upper respiratory system, lower urogenital tract, and digestive tracts of animals and humans (Quinn et al, 2001). According to Baer et al. (2013), *S. aureus* was also detected in around fifty percent of retail raw pork samples from the Netherlands, the United States, and Germany.

2.3. Description of Methicillin-Resistant *S. aureus* (MRSA)

Barber (1914) discovered that Staphylococci-produced toxin was responsible for staphylococcal food poisoning. Infections caused by *S. aureus* were initially treated with the β-lactam antibiotic penicillin, despite the fact that bacteria usually develop resistance by producing penicillinase (β-lactamase). Thus, the β-lactamase-resistant drug methicillin was developed to tackle penicillin resistance; however, certain bacteria established resistance to methicillin and are now referred to as Methicillin-Resistant *Staphylococcus aureus* (MRSA). In the United States, MRSA was first recognised in the late 1960s, when an outbreak occurs at Boston City Hospital. At that time, resistant staphylococci had firmly established themselves in the hospitals of Europe's leading cities (Weigelt, 2016).

S. aureus's ability to respond to selective pressures has contributed to the spread of antibiotic resistance, particularly methicillin-resistant strains in hospitals, communities, and livestock herds. Methicillin resistance is caused by acquiring the *mecA* gene, which codes for an alternative penicillin-binding protein. The altered surface protein has a low binding affinity for β-lactam antibiotics, hence diminishing their bactericidal effects (Lassok & Tenhagen, 2013).

2.4. Colonization of Methicillin-Resistant *S. aureus* (MRSA) in pork meat in general

Since the initial revelation of MRSA in the meat-producing pig population and a regionally high incidence rate of MRSA among pig farmers in The Netherlands in 2005, awareness of MRSA in livestock has increased. Numerous research has been conducted in several nations to determine the incidence of MRSA, its transmission dynamics within the pig primary production sector, and its public health implications (Lassok & Tenhagen, 2013).

According to Lassok and Tenhagen (2013), the transmission of MRSA in pork meat could occur either between herds due to animal trading and transportation or during the meat processing process itself.

3.0. MATERIALS AND METHOD

3.1. Sample collection and preparation

A total of 30 fresh pork meat samples (~20 g) were collected from different sellers in a wet market in Kota Bharu, Kelantan, within 3 weeks to obtain a different batch of pigs. Tissue samples were collected aseptically with a glove. The samples were kept in an individual seal zip-lock bag, stored, and frozen at a temperature of -80°C for bacterial isolation and identification.

3.2. Isolation, identification and antibiotic sensitivity of *S. aureus*

Swab samples were obtained from the collected samples by using sterile swab cottons and it was inoculated onto Mannitol Salt agar which is a selective medium for *S. aureus* since the presence of sodium chloride in MS results in partial or complete inhibition of bacterial organism other than staphylococci. The inoculated plates were incubated at 37˚C for 24 hours. After 24 hours, the colonies growth on the inoculated plates were observed and coagulase test was performed using EDTA-treated plasma to differentiate between *S. aureus* and *S. epidermis*. An antibiotic sensitivity test was performed on Mueller Hinton agar using Kirby Bauer method with 5 different antibiotic discs: oxacillin, ampicillin, amoxicillin, trimethoprim and clindamycin.

Various antibiotics from different class were used to detect the range of resistance of the microorganism. The principle of Kirby Bauer test is that the bacteria is swabbed on Mueller Hilton agar, and the antibiotic discs are dispensed on top. The antibiotic disperses from the disc into the agar in tapering concentration as it moves away from the disc. If the bacteria are killed or inhibited by the attention of the antibiotic, no growth will be observed in the area around the disc. This is called the zone of inhibition. The zone sizes are compared on a standardized chart to give a result of sensitivity, resistant, or intermediate (Table 3.2). The inoculated MHA plates were incubated

for 24 hours at 37˚C, and the zone of inhibitions exhibited by the colonies was observed the next day.

Table 3.1. CLSI guideline for AST inhibitory zone for S. aureus

Antibiotic agent	Disc content	Resistant	Intermediate	Susceptible
Oxacillin (OX1)	1 μg	≤ 21 mm	-	≥ 22 mm
Clindamycin (DA2)	2 μg	≤ 14 mm	15 – 20 mm	≥ 21 mm
Trimethoprim (W5)	5 μg	≤ 10 mm	11 – 15 mm	≥ 16 mm
Penicillin				
Ampicillin (AMP10)	10 μg	≤ 28 mm	-	≥ 29 mm
Amoxicillin (AML10)				

3.3. Data analysis

Data were recorded and statistically analysed manually by using manual record and tabulation by Microsoft Office on the identification of *S. aureus*.

4.0. RESULTS

4.1. Isolation and identification of *S. aureus*

Table 4.1. Colony morphology on MSA, Gram staining result and coagulase test result.

Seller ID	Sample (pork meat)	Colony morphology on MSA	Gram staining results	Coagulase test
Seller A	1	Yellow colonies, yellow zones	Gram-positive cocci	+
	2	Yellow colonies, yellow zones		+
		Pinkish colonies, red zones		-
	3	Pinkish colonies, red zones		-
	4	Pinkish colonies, red zones		-
	5	Yellow colonies, yellow zones		+
	6	Yellow colonies, yellow zones		+
	7	Yellow colonies, yellow zones		+
		Pinkish colonies, red zones		-
	8	Pinkish colonies, red zones		-
	9	Yellow colonies, yellow zones		+
	10	Pinkish colonies, red zones		-
Seller B	11	Pinkish colonies, red zones	Gram-positive cocci	-
	12	Yellow colonies, yellow zones		+
	13	Yellow colonies, yellow zones		+
	14	Yellow colonies, yellow zones		+
	15	Pinkish colonies, red zones		-
Seller C	16	Pinkish colonies, red zones	Purple cocci	-
	17	Yellow colonies, yellow zones		+
	18	Yellow colonies, yellow zones		+
	19	Pinkish colonies, red zones		-
	20	Yellow colonies, yellow zones		+
		Pinkish colonies, red zones		-
Seller D	21	Pinkish colonies, red zones	Gram-positive cocci	-
	22	Yellow colonies, yellow zones		+
		Pinkish colonies, red zones		-
	23	Yellow colonies, yellow zones		+
		Pinkish colonies, red zones		-
	24	Yellow colonies, yellow zones		+
	25	Yellow colonies, yellow zones		+
	26	Yellow colonies, yellow zones		+
	27	Yellow colonies, yellow zones		+
		Pinkish colonies, red zones		-
	28	Yellow colonies, yellow zones		+
	29	Yellow colonies, yellow zones		+
	30	Yellow colonies, yellow zones		+

Table 4.1 shows the colony morphology on MSA, Gram stain and coagulase test result. The results were based on the growth obtained from a single colony on the MSA. All samples from four different sellers were positive for bacterial growth.

Ten pork meat samples were taken on various dates from Seller A. Out of ten samples, only two samples show two types of colony growth on the agar, which are yellow colonies surrounded by yellow zone and pinkish colonies surrounded by red zones. In contrast, the rest of the samples show the same colony growth: only yellow colonies surrounded by a yellow zone. As for Gram stain, all the colonies appear to be Gram-positive cocci, in a formation of grapes bunches. After coagulase test was done, it appears that yellow colonies with yellow zone are coagulase positive while pinkish colonies with red zone are coagulase negative.

All five samples from Seller B show Gram positive cocci bacteria in bunches of grapes formation once observed under a microscope. Three out of five samples taken from Seller B show pure yellow colonies surrounded by yellow zone without a mixture of any other colony. Two of the samples show complete red zone surrounding pinkish colonies, which turned out to be negative coagulase. Yellow colonies growth with yellow zone is all coagulase positive.

For Seller C, yellow colonies with yellow zones were observed on three of the samples, while the other two samples show a mixture of yellow and pinkish colonies surrounded by yellow and red zones. All the colonies show grapes bunches formation in a Gram positive cocci shape under Gram stain. Three out of five samples were positive coagulase.

Lastly, from Seller D, six out of ten samples show single yellow colonies surrounded by yellow zones, Gram stain revealed Gram-positive cocci in the formation of grapes bunches and positive coagulase. Three out of ten samples offer a mixture of yellow and pinkish colonies with purple cocci shapes where the yellow colonies are positive coagulase while the pinkish colonies are negative coagulase. Single pinkish colonies, purple cocci, and negative coagulase can be seen in one of the samples.

Thus, in a conclusion, yellow colonies surrounded by yellow zone, which Gram stain shows Gram-positive cocci in the shape of grapes bunches and positive coagulase test are *S. aureus*, while those pinkish colonies surrounded by red zone, purple cocci, and negative coagulase test are *S. epidermis.* Based on that, 21 out of 30 fresh pork meat samples obtained from four different sellers in the Kota Bharu wet market are positive for *S. aureus*.

4.2. Antibiotic Sensitivity Test (AST)

As a result, 18 out of 21 *S. aureus* isolated are resistant to oxacillin, ampicillin , and amoxicillin. As for trimethoprim, 11 out of 21 isolates were susceptible, four intermediates, and three were resistant. Eight out of 21 *S. aureus* were susceptible to clindamycin, six were resistant, and Three were intermediate. Three out of 21 inoculated plates produced zero growth. In conclusion, 18 out of 30 pork samples were positive for MRSA (Figure 4.2).

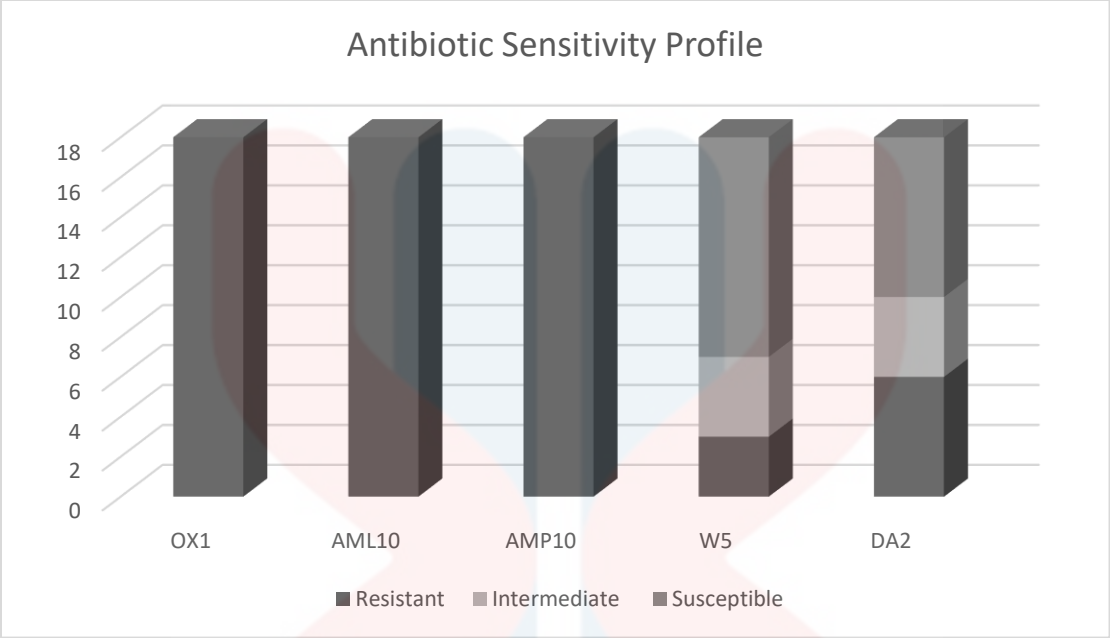


Figure 4.2. Antibiotic sensitivity results for 18 resistance samples (OX1: Oxacillin, AML10:Amoxicillin, AMP10:Ampicillin, W5:Trimethoprim, DA5:Clindamycin)

5.0. DISCUSSION

S. aureus causes around 241,000 episodes of foodborne disease in people each year in the United States (Scallan et al., 2011). According to Atanassova et al. (2001) *S. aureus* was found to contaminate 50% of raw pork samples in the retail market from The Netherlands, United States and Germany. From this study, we obtained that *S. aureus* can be isolated from 70% (21 out of 30) of the pork meat samples taken from the wet market which indicates high contamination, almost as seen in the previous studies.

In addition, *S. epidermidis* was found in 9 out of 30 raw pork meat samples. A study on the prevalence of staphylococci in the pork production chain was done by Chile by Velasco and others (2018) shows that *S. aureus* has higher prevalence in pork, but other species of Staphylococci bacteria such as *S. epidermidis, S. lentus, S. warneri, S. simulans, S. saprophyticus, S. hominis, S. sciuri, S. haemolyticus, S. chromogenes, S. hyicus, and S. caprae* can be found too, but in an insignificant number of percentages.

According to Widerström (2016), recent research has focused on the significance of *S. epidermidis* as a source of livestock-associated illnesses. However, there has been a lack of simple and reliable methods for species identification for a number of years, which has hindered the assessment and understanding of the epidemiology of *S. epidermidis* and the evaluation of bacteria of this species in clinical cultures. Moreover, the inherent characteristics of *S. epidermidis* infections contribute to the difficulty in making a correct microbial diagnosis and distinguishing between contamination, colonisation, and true infection. Instead, *S. epidermidis* is the most common cause of prosthetic joint infections, prosthetic valve endocarditis, and other biomedical

device-related infections. It is also the most common cause of catheter-related bloodstream infections and early-onset neonatal sepsis

Pork is significantly more frequently contaminated with MRSA than beef, chicken, or turkey. MRSA was only found in samples of pork in an Iowa evaluation of retail meat (Weese et al., 2010 & Hanson et al., 2011). Based on this study, we found that 60% (18 out of 30) of the pork meat obtained from the wet market is MRSA-positive. In comparison to other studies that have been carried out in the USA, Germany, and The Netherlands, MRSA contamination in raw pork, including ground pork and pork chops, is estimated to range between 3.1% and 10.7% (Van Loo et al., 2007; de Boer et al., 2001).

The MRSA contamination shown by this result indicates that pork meat in Kota Bharu retail market is way higher than in other countries. The reason for this might be due to the poor handling of retail products in the wet market, as the market is not in a very hygienic state. According to de Boer et all (2001), numerous researchers have discovered that the MRSA strains recovered from retail meat are not related with livestock but are frequently carried by humans, which is a strong indicator of poor product handling and human cross-contamination.

Panchal and others (2020) stated that penicillin binding proteins (PBP) are a class of proteins that metabolise the peptidoglycan component of bacterial cell walls. Since β-lactam antibiotics make covalent bonds when they come into contact with the active PBP site, they stop PBP from doing its work and block the synthesis of the wall. Methicillin-resistant strains of bacteria produce a penicillin-binding protein (PBP2a or PBP2′) with a low affinity for β-lactam

antibiotics. This protein is made by the *mecA* gene. A unique movable genetic element called the staphylococcal cassette chromosome *mec* (SCC mec) contains the *mecA* gene, which results in methicillin resistance (Ito et al., 2013). Antibiotics are no longer upheld on PBP2a. As a result, peptidoglycan synthesis is not blocked, and cellular lysis is not stopped, becoming MRSA resistant to particular antibiotics (Panchal and others, 2020).

The study of the molecular ecology of *S. aureus* has provided significant insight into the capacity of specific bacterial clones to demonstrate "inter-species animal jump or spillover". While some MRSA clonal complexes appear to be connected with particular animal hosts (such as MRSA-CC398 in pigs and MRSA-CC5 in poultry), others, such as CC1 and CC130, appear to have a broad host spectrum. Thus, the transmission of MRSA occurs when wild animals produce nasal and oral (saliva) secretions that may serve as significant transient or persistent vectors for MRSA transmission to humans and other animals (Abdullahi et. al., 2012). Hence, MRSA can be transmitted from human to animals or vice versa through human-livestock direct contact, indirect contact between human, livestock and pest, migratory birds and ingestion of infected carcasses that were not properly cooked (Figure 5.1)

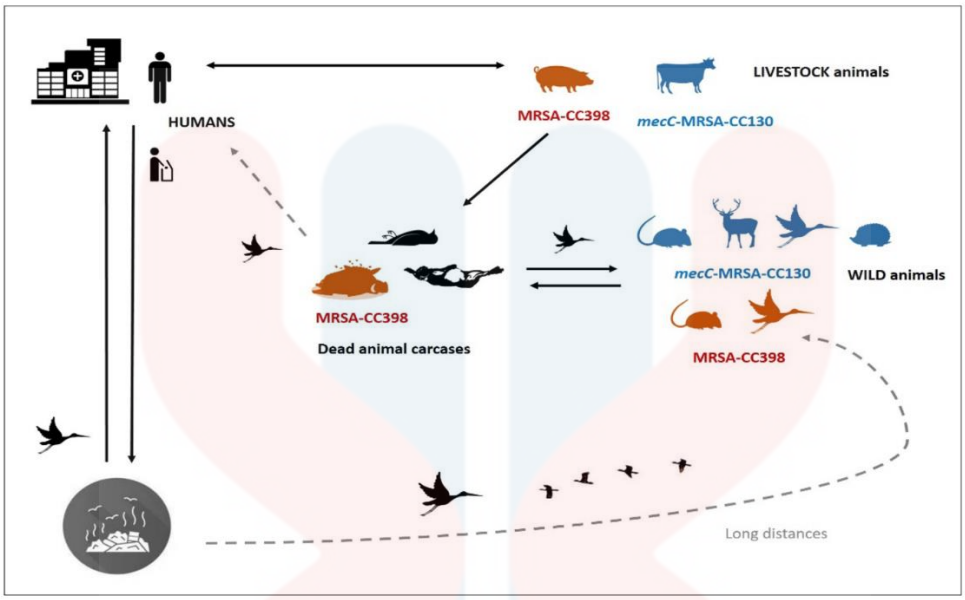


Figure 5.1. Transmission cycle of special MRSA between humans, animals (livestock and wild) and the environment (such as landfills and hospitals) (Abdullahi and others, 2021)

According to Chambers & Deleo (2009), midway through the 1940s, the proportion of hospital infections caused by *S. aureus* strains resistant to penicillin increased. Soon thereafter, penicillin-resistant strains began to cause community infections, and by the early 1950s, they had hit a growing public health problem. Hence, it is not a surprise that this study reveals that *S. aureus* does not only resistant to oxacillin (methicillin) but also penicillin beta lactam antibiotics such as amoxicillin and ampicillin.

Due to the limited number of effective antibiotics available, infections brought on by Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) constitute a serious and ongoing threat to human healthcare. These organisms are also continually changing in order to become resistant to additional drugs. As a result, there is a need for fresh, clinically effective antibiotics, and the Streptomyces bacteria, which is the source of vancomycin and other anti-

MRSA medications, is one such source. Streptomyces are filamentous gram-positive bacteria that are categorised under the phylum Actinobacteria. When it comes to its capacity to survive under unfavorable soil conditions, Streptomyces is recognised to be a highly resilient genus of bacteria. The 124 compounds generated by Streptomyces that exhibit moderate to powerful anti-MRSA action have been highlighted in the literature to date. According to a numerical analysis of these substances, polyketides (PKS) make up the largest group, followed by non-ribosomal peptides (NRPS), while other compounds have lower amounts of alkaloids, PKS/NRPS hybrids, and PKS/terpenoids. The majority of Streptomyces-derived conventional antibiotics come from the polyketide and NRPS biosynthesis pathways, and accordingly, these classes also make up a sizable portion of the compounds exhibiting anti-MRSA activity (Kemung et al.,2018). This provides hope for combating emerging MRSA.

6.0. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

In conclusion, *S. aureus* was successfully isolated and identified from raw pork meat from a wet retail market in Kota Bharu, Kelantan. More than one-half of the isolated *S. aureus* were resistant to methicillin, which indicates the presence of MRSA in the pork meat supplied from the wet market. This bacterium can cause foodborne diseases in humans, raising public health concerns among the consumer in the affected area, and require a proper cooking and handling of food to prevent emerging of foodborne illness and overcome the antimicrobial resistance altogether with superbug issue.

6.2. Recommendation and future work

Regarding the recommendation and future work, it might be enhanced by increasing the number of samples and the target market. Consequently, the aim and samples of raw pork meat can be expanded. Another improvement that may be made is isolating fresh samples. After obtaining the samples from the market that is being targeted, a process of isolation has to be immediately process to prevent possible contamination. Furthermore, more biochemical tests, such as catalase, should also be done to further confirm the presence of *S. aureus* in the samples rather than relying on coagulase test only. The use of molecular or serological approaches to further identify the bacteria, such as, serology for *S. aureus* is another recommendation for future study that should be considered. The findings of this study provide a preliminary basis for future screening of *Staphylococci* pathogens in different food kinds, as well as notifying health authorities and educating the public about food safety code, so that pathogens causing foodborne diseases can be prevented within the community.

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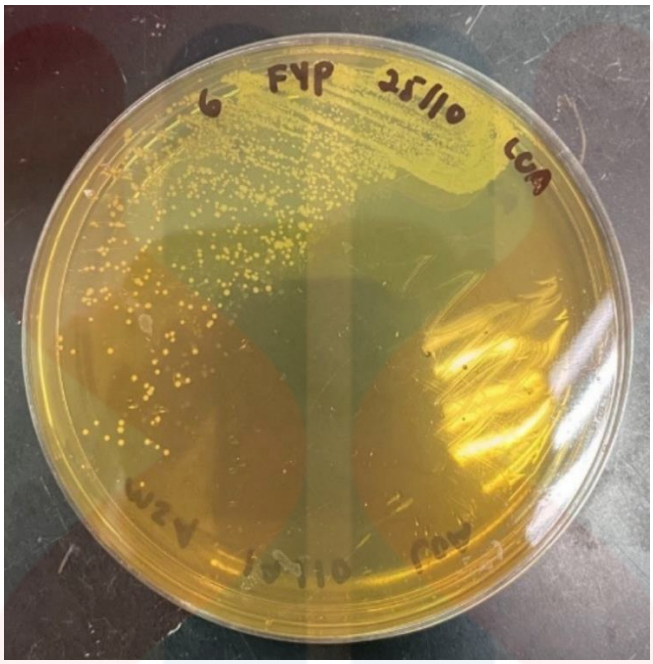
Appendix A

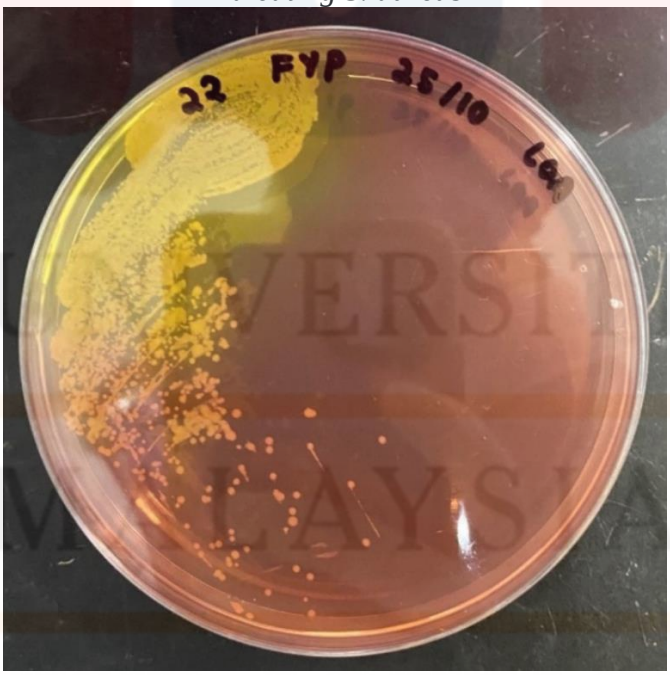
Appendix A1: Pork wet market in Kota Bharu, Kelantan

Appendix A2: Pork meat samples were kept in a sterile ziplock bag, stored, and frozen at a temperature of -80°C

Appendix B

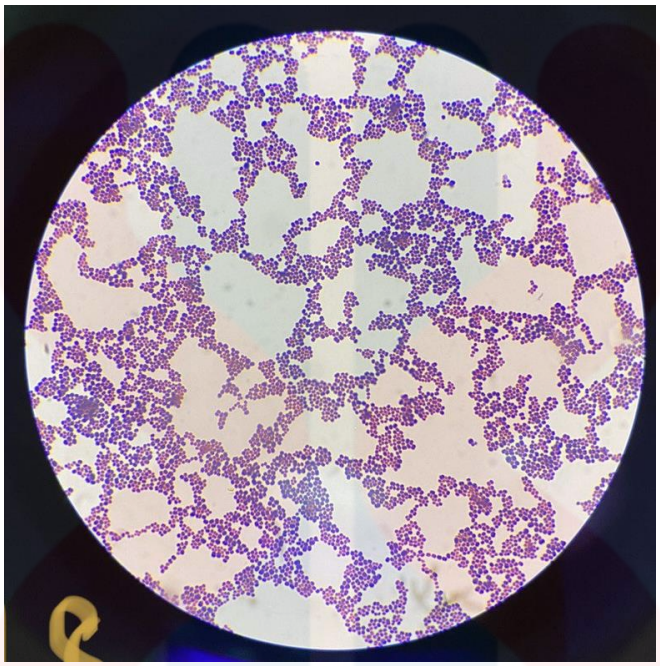


Appendix B1: Colonies morphology on MSA – yellow colonies surrounded by yellow zone indicating S. aureus

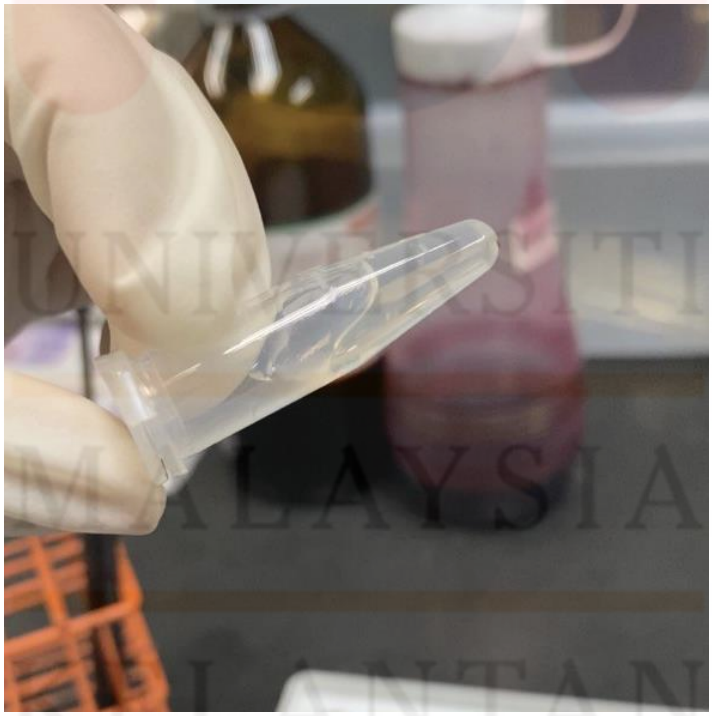


Appendix B2: Colonies morphology on MSA – yellow-pinkish colonies surrounded by red zone indicating S. epidermidis

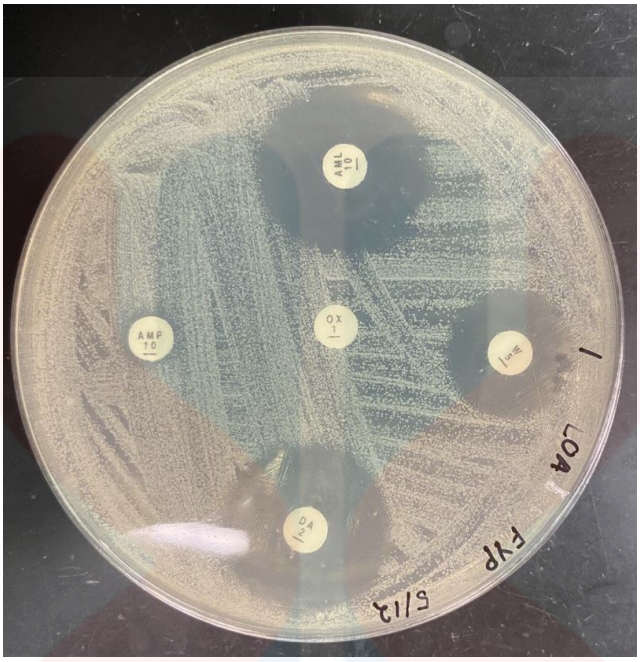
Appendix C



Appendix C1: Gram staining result: Gram-positive cocci bacteria in a formation of bunches of grapes



Appendix C2: Tube coagulation test: clumping of the mixture indicating coagulase-positive Staphylococcus



Appendix C3: AST result on MHA following Kirby Bauer disc method – showing zone of inhibition