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Presence of antimicrobial resistant *Staphylococcus aureus* in chicken meat and its potential public health implications

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ABSTRACT

Aim: Multi-drug resistant bacteria have become a global issue. Drug-resistant bacteria can be found in humans, animals, food and environmental sources. *Staphylococcus aureus* is one of many bacteria species known for its antimicrobial resistance. The current study is conducted to determine the antimicrobial resistance profiles of *S. aureus* isolated from raw chicken meat samples in Kota Bharu, Kelantan.
Methodology and results: Fifty raw and fresh chicken meat samples were purchased from 3 different wet markets in Kota Bharu, Kelantan and were transported to the laboratory aseptically. Routine isolation and identification of *S. aureus* was conducted and the isolates were confirmed by polymerase chain reaction (PCR) through the detection of a *S. aureus* specific gene, *nuc*A. Antimicrobial sensitivity tests were conducted according to Kirby-Bauer methods (Hudzicki, 2013). *Staphylococcus aureus* was isolated in 24% (12/50) of the samples. All the isolates were resistant towards at least two of the antimicrobials tested. Of these, 11 (91.67%), 10 (83.33%), 5 (41.67%), 3 (25%), 1 (8.33%) and 1 (8.33%) were resistant to ampicillin (AMP10), teicoplanin (TE30), amoxicillin (AML10), penicillin (P10), oxacillin (OX1) and mupirocin (MUP20) respectively. In addition to that, all the isolates were susceptible to streptomycin, vancomycin, teicoplanin and cefoxitin. However, all the isolates were negative for the methicillin resistance encoding gene, mecA while one of the isolates showed resistance towards oxacillin.
Conclusion, significance and impact of the study: The results from this study indicated that raw chicken intended for human consumption may be contaminated by antimicrobial-resistant strains of *S. aureus*. This may lead to the colonization or infection in humans. Nevertheless, further detailed investigation to determine the correlation between contamination of chicken meat and colonization of antimicrobial resistant *S. aureus* should be carried out. The relevance of the present study which showed contamination of fresh chicken meat with antimicrobial resistant *S. aureus* emphasizes the need to have stricter hygiene measures for retailers during the handling of the chicken meat to minimize or avoid possible health hazards for consumers.

Keywords: antimicrobial resistance, *Staphylococcus aureus*, chicken meat, public health

INTRODUCTION

Antimicrobial resistance (AMR) has become a global agenda and has been an issue of major concern for public health and in veterinary medicine. With the ever changing antimicrobial resistance profiles of formerly known resistant bacterial strains and with the emergence of new strains of known susceptible strains, antimicrobial-resistant bacteria continue to pose a serious threat. This problem is aggravated by the fact that different species of bacteria are becoming resistant towards multiple antimicrobial drugs. According to the recent reports from the World Health Organization (WHO), high occurrence of common illnesses caused by microbial-resistant bacteria have been observed around the world. The same reports warned that if this problem persists, antimicrobial-resistant bacteria will cause a cataclysmic event that endangers the world population (WHO, 2015). The irrational use of antimicrobials in food-producing animals has long been implicated as one of the contributing factors for the emergence and spread of antimicrobial resistant bacteria.

Staphylococci are widespread in nature although they are mainly found associated with the skin, skin glands and mucus membranes of warm-blooded animals and they also may be found in the air, water and soil (Freeman-Cook and Freeman-Cook, 2006). *Staphylococcus aureus* has been known to survive well both in the inside and the outside of its host cells. The ability of *S. aureus* to cause a variety of diseases in humans and animals may be related to its ability to produce a plethora of virulence factors (Archer, 1998; Dinges *et al.*, 2000; Novick *et al.*, 2001). These virulence factors include the production of exoproteins, such as secreted toxins, and factors that play diverse roles in pathogenesis but do not directly confer toxicity to host tissues, such as surface proteins (Dunman and Projan, 2002).

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Staphylococcus aureus is a frequent cause of human infections and is one of the most important nosocomial pathogens (Fenner *et al*., 2008). *Staphylococcus aureus* is perhaps the pathogen of great concern because of its intrinsic virulence, its ability to cause a diverse array of life threatening infections, and its capacity to adapt to different environmental conditions (Lowy, 1998; Waldvogel, 2000). While a variety of staphylococcal species are present on or in clinically normal individuals, staphylococci are also opportunistic pathogens, causing community-associated diseases in humans and animals worldwide (O'Mahony *et al*., 2005). Diseases caused by *Staphylococcus* sp. include suppurative disease, mastitis, arthritis, and urinary tract infections in animal species (Waldvogel, 1990). In humans, staphylococci cause a wide variety of clinical syndromes ranging from uncomplicated infections of the skin such as boils and carbuncles, to severe life threatening conditions like endocarditis and toxic shock syndrome (Murray, 2005; Todd, 2005). *Staphylococcus aureus* strains are also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones (Lee, 2003). Antimicrobial resistance is of great concern for various reasons such as prolonged therapy with antimicrobial agents, such as vancomycin or linezolid, which may then lead to the development of low-level resistance that compromises the therapy itself (Tenover *et al.*, 2004). Resistant bacteria may also spread and become broader infection-control problems, not only within healthcare institutions, but also in communities. Because of the considerable use of antimicrobial agents in human and veterinary medicine, as well as animal husbandry, antimicrobial resistance has developed into a prime illustration of how bacterial populations can readily adapt and react to selective pressure (Boerlin and Reid-Smith, 2008). Food-producing animals and contaminated animal products have also been often implicated as sources of antimicrobial-resistant bacteria. Several reports have indicated the occurrence and spread of multidrug resistant *S. aureus* from different sources (Lee, 2003; O'Mahony *et al*., 2005; Jaglic *et al*., 2010; WHO 2015). The current study was conducted to determine the antimicrobial resistance profiles of *S. aureus isolated* from raw chicken meat samples collected from fresh markets in Kota Bharu, Kelantan.

MATERIALS AND METHODS

Sampling and sample enrichment, isolation and identification of *S. aureus*

Fifty raw chicken meat samples were collected from three locations in Kota Bharu, Kelantan (Taman Bendehara, Pasar Siti Katijah and Kubang Kerian). A piece of fresh raw chicken meat measuring approximately 25 cm^2 was transferred into a sterile sampling bag containing saline solution (0.85% NaCl) and was transported to the laboratory in an ice box. The samples were soaked in 15 mL of saline solution (0.85%) at room temperature for 5 min and were shaken gently. Two millilitres of solution obtained from the soaked samples were pipetted into 10 mL Tryptone Soya Broth (TSB) and incubated at 37 °C for 48 h. The *S. aureus* colony which arise on TSB were then streaked onto blood agar with 7% horse blood and incubated aerobically at 37 °C for another 24 h. Routine bacteriological approach including observation of colonial morphology, Gram's staining and biochemical tests were conducted to identify *S. aureus* isolates. The isolates were also further confirmed by detection of *S. aureus*-specific gene (*nuc*A).

Antibiotic sensitivity test (Disc-diffusion method)

Antibiotic sensitivity tests were carried out by using disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2011) and Kirby-Bauer method. Briefly, three to four *S. aureus* colonies from an agar culture plate grown overnight were suspended directly into saline solution (0.85% NaCl) and vortexed to achieve a clear suspension of 0.5 McFarland turbidity (10^8 CFU/mL). Once the inoculum is ready, a sterile cotton swab was dipped into the suspension, removed and rotated several times on the wall of the tube to avoid excess inoculum. Then freshly prepared MHA plate was inoculated by streaking the cotton swab all over the agar's surface on the plate, with a rotation of approximately 60° each time to ensure even distribution of the suspension on the agar surface. MHA supplemented with 2% NaCl was used for oxacillin and methicillin susceptibility tests. The plates were left to dry for about 5 min after which the antibiotic discs were dispensed using the antibiotic disc dispenser (Oxoid, UK). The plates were incubated at 35 °C for 24 h and the zones of inhibition were analyzed and interpreted by Aura Image (Oxoid, UK) according to the standards of CLSI (2011). The antimicrobials tested were; ampicillin (AMP10), cefoxitin (FOX30), oxacillin (OX1), teicoplanin (TEC30), vancomycin (VA30), amoxicillin (AML10), streptomycin (S30), penicillin (P10) and mupirocin (MUP20). The results were interpreted following the CLSI (2011) guidelines.

Extraction of the genomic DNA

A single colony of *S. aureus* from blood agar was streaked onto Brain Heart Infusion Agar (BHIA) (Oxoid, UK) and incubated at 37 °C overnight. After incubation, two to three colonies were taken and emulsified into 1 mL of PBS buffer (pH 8.0) in a 1.5 mL sterile microcentrifuge tube. The extraction of genomic DNA was done using DNA Purification Kit (Promega, USA). The emulsified suspension was centrifuged for 10 min at 8000 rpm and the supernatant was discarded. The pellet was re-suspended in 180 µL enzymatic lysis buffer and incubated for 30 min at 37 °C. Twenty five microliter of proteinase K and 200 µL lysis buffer were added, vortexed and further incubated at 56 °C for 30 min. Two hundred microliters of 100% ethanol was added and mixed thoroughly by using a vortex until a homogenous mixture is seen. The total content of the tube was transferred into mini spin column

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and placed in 2 mL collection tube and centrifuged at 8000 rpm for 1 min. The flow through was discarded. The column was then placed in a new collection tube. Washing was conducted twice using a washing buffer. Finally, the DNA elution buffer was added and the solution was then centrifuged for 1 min at 8000 rpm to elute the DNA. The quality of the extracted DNA was assessed by using nanophotometer and the extracted DNA with the desirable quality was kept at −20 °C until further use.

Amplification of *S. aureus* specific gene (*nuc*A) and methicillin-resistance gene (*mec*A)

The PCR amplification of *nuc*A and *mec*A genes were conducted using the procedures described below. Specific primers for the amplification of the *nuc*A gene specific to *S. aureus* and *mec*A gene, for methicillin-resistant strains, were used. The *nuc*A primers were: 5'-GCGATTGATGGTGATACGGTT-3' and 5'-AGCCAAGCCTTGACGAACTAAAGC-3', while the *mec*A primers were: 5'-AAAATCGATGGTAAAGGTTGGC-3' and 5'-AGTTCTGCAGTACCGGATTTGC-3'. The PCR reactions were prepared in 50 µL volume, consisting 5 µL PCR buffer, 2 µL $MgCl_2$, 1 µL dNTPs, 2 µL of each primer and 0.5 µL of Taq polymerase. The amplifications were conducted using MyCycler™ thermal cycler (BioRad) programmed with the initial denaturation at 94 °C for 10 min, 30 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 45 sec and extension at 72 °C for 30 sec followed by a final extension at 72 °C for 10 min. The PCR products were then subjected to gel electrophoresis and the gels were analysed by using gel documentation system (GelDoc™, BioRad).

RESULTS AND DISCUSSION

Based on routine microbiological examination and PCR identification, 24% (12/50) of the samples were confirmed to be *S. aureus.* All the isolates were resistant towards at least two of the antimicrobials tested. Of these, 11 (91.67%), 10 (83.33%), 5 (41.67%), 3 (25%), 1 (8.33%) and 1 (8.33%) were resistant to AMP10, TE30, AML10, P10, OX1 and MUP20 respectively. However, all the isolates were susceptible to streptomycin, vancomycin, teicoplanin and cefoxitin. On the other hand, all the isolates were found to be negative for the gene (*mec*A) encoding for methicillin reistance in *S. aureus*. Although one isolate showed resistance to oxacillin by using disc diffusion, this isolate was confirmed to be negative for the methicillin resistance encoding gene, *mecA*. The absence of the *mec*A gene in this specific isolate can be attributed to several factors since there are non-*mec*-dependent mechanisms that contribute to methicillin resistance in staphylococci strains (Berger-Bachi, 1995; Berger-Bachi and Tschierske, 1998). Among these, overproduction of normal penicillin-binding proteins (PBPs) with an altered binding capacity or other unidentified factors were ascribed as potential contributors to the rise of methicillin resistance in *mec*A negative *S. aureus* strains (Chambers, 1997). Such features imply that it is possible that the *mec*A negative isolates might be among those MRSA strains showing border-line resistance towards oxacillin/methicillin (BORSAs). Nevertheless, the inherent deficiencies in sensitivity and specificity of disc diffusion method should also be taken in consideration while reporting methicillin resistance in *S. aureus*. As such, other more reliable methods of determining phenotypic resistance such as determination of minimum inhibitory concentration (MIC) should be considered.

The relevance and implications of detection of multidrug resistant *S. aureus* strains in the current study can be explained from different perspectives. Possible explanations for the detection of multidrug resistant *S. aureus* in the chicken meat can be due to contamination at any of the stages from slaughtering to marketing, the farm (source of live chicken), transport utilities (including vehicle and materials used as containers to transport the chicken meat), contamination by the meat handlers (at the slaughterhouse and the wet markets) and as a result of other direct and indirect contaminations from the environment. Considering the possibility of live chickens as sources for the resistant bacteria, the most rational explanation for this is the use of antimicrobials in poultry farms. Different groups of antibiotics have been widely and commonly used in livestock production as probiotics, growth promoters and also for therapeutic purposes. However, the use of antibiotics in animal production, including poultry industry, has been implicated as a catalyst for creating selective pressure that may lead to the emergence of resistant strains (Aarestrup *et al*., 2001). Heavy use of antibiotics in animal production has also been cited as one of the reasons for the emergence and the widespread of antibiotic-resistant foodborne pathogens (Shea, 2004). With the global expansion of intensive animal production systems, increased global human movement, wide-spread of animal diseases, limited options for treatment of infectious diseases of animals and limited reaches of the regulatory bodies, food-producing animals may become one of the major sources of antimicrobial resistant bacteria.

Considering the possibility of post-slaughter and during slaughtering contamination by extrinsic sources of antimicrobial resistant bacteria, carcasses can be contaminated by faecal microflora during these periods. The source of contamination can be due to unhygienic surroundings or manner of handling the meat during slaughtering or retail. Staphylococci are normal microflora found on human body surfaces and they are among the most ubiquitous bacteria in the environment. Moreover, staphylococci have the ability to form biofilm on inert materials used in the food-processing industry foodstuffs such as poultry meat to be easily contaminated with *S. aureus* strains upon exposure to these surfaces (Jaglic *et al*., 2010).

In conclusion, although the risk of human infection with multidrug-resistant *S. aureus* from contaminated meat is often considered minimal, its potential risk to the public health cannot be undermined. Rational use of antimicrobials in animal husbandry and regular surveillance for resistant organisms are recommended.

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Molecular typing and detailed epidemiological studies are necessary to establish the interrelatedness of resistant bacterial strains and to track the spread of these organisms. Meanwhile it is important to adhere to strict hygienic procedures to ensure that contamination of food stuff such as chicken meat with these bacteria is avoided or minimized.

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