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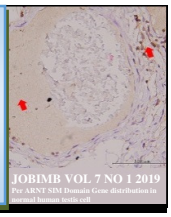

Multiple Drug Resistance Among *Staphylococcus aureus* Strains Isolated from Cutting Boards of Commercial Food Premises: A Threat to Food and Public Health Safety

Shu'aibu Isa[1,4], Nor Ainy Mahyudin[1,3]*, Son Radu[2], Nor Khaizura Mahmud @Ab Rashid[2] and Shakira Mohd Sahil[1]

[1]Department of Food Service and Management, Faculty of Food Science and Technology, Universiti Putra Malaysia, Malaysia.
[2]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Malaysia.
[3]Halal Products Research Institute, Universiti Putra Malaysia, Malaysia.
[4]Department of Microbiology, Gombe State University, Gombe, Gombe State Nigeria.

*Corresponding author:
Assoc. Prof. Dr. Nor Ainy Mahyudin
Department of Food Service and Management,
Faculty of Food Science and Technology,
Universiti Putra Malaysia
43400 UPM Serdang
Selangor, Malaysia.
Tel: +603- 97698517
Email: norainy@upm.edu.my

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ABSTRACT

Multidrug resistance among bacterial pathogens is an issue of global concern, especially in commercial food premises. This study aimed to isolate and identify the multidrug-resistant *S. aureus* from cutting boards of 45 restaurants in Sri Serdang, Selangor, Malaysia. Samples were obtained from the surfaces of 55 plastic cutting boards by swabbing and analyzed using 3M™ Petri film™ Staph Express Count Plate and Disk and further identified using Gram staining, coagulase and catalase tests and growth on mannitol salt agar and Baird Parker agar. Agar-disk diffusion technique was employed for antibiotic resistance against 11 antibiotics, and the results were analyzed based on the Clinical and Laboratory Standards Institute criteria. Out of 55 samples , 43.6% (n=24) were found to be positive for *S. aureus*. Of the 24 *S. aureus*, 45.8% (n=11) were multidrug-resistant. Resistance to the members of penicillins was 100%, to nalidixic acid (79.2%), to ciprofloxacin (66.7%), to cephalothin (33.3%) and ceftazidime (20.8%). There was no resistance to gentamicin, streptomycin, ceftriaxone, cefotaxime, and sulphafurazole. The emergence of multidrug-resistant *S. aureus* on cutting boards is an indication of poor personal and sanitary hygiene and could pose a danger of outbreak due to staphylococcal food poisoning among consumers.

INTRODUCTION

There is a reduced alternative treatment to diseases caused by microorganisms that have already become antibiotic-resistant. This antibiotic resistance is widespread throughout the world by a wide range of microorganisms; it has an increased incidence that poses a threat to the health of both man and animals [1]. Direct and indirect consequences are associated with the infections caused by antibiotic-resistant microbes; the direct ones being lasting and severe illnesses, increase in mortality rate, prolonged hospital stay, unsure protection for patients facing hospital procedures and operations, and the increase in expenses. Resistance to antibiotics affects all health areas with involvement in many sectors and a strong effect on the entire society.

However, the indirect effects of antibiotic resistance are those beyond health-related risks. They are those that have several consequences on the development of the public, such as draining the world economy associated with economic loss as a result of low productivity because of the sickness of humans and animals as well as high treatment cost [1]. Therefore, supports including monetary and technical such as developing new methods of diagnosis, vaccines, antibiotics and other interventions as long-lasting investments for ensuring effective healthcare systems are needed to combat antibiotic resistance.

The poor cleaning and sanitization of food contact surfaces such as cutting boards, cross-contamination and food leave traces of pathogenic microorganisms that may consequently

cause infections so difficult to treat [2-3]. In Malaysia and other developing countries, non-standard disinfectants are mostly employed in the cleaning food contact surfaces, and this may eventually lead to the persistence of microbes especially if the chemical compositions of the cleaning agents do not reach the global standard.

S. aureus is a Gram-positive coccus that is implicated in food poisoning as a result of the ingestion of preformed enterotoxins secreted. It's the third most prevalent bacterial pathogen involved in food poisoning after *Salmonella* and *Vibrio* in the previous decades [4]and second after Salmonella [5]. Its pathogenicity is attributed to the possession of various virulent factors that help them attach and establish themselves in human and animal hosts to cause infection [6-7]. Antibiotic-resistant bacteria, particularly the multidrug-resistant ones, are able to cause serious infections with effects including long-lasting hosting hospitalization, failure of treatments and eventual death [8]. This has made infections due to multidrug *S. aureus* difficult to treat and the organism difficult to deal with in addition to its capability of outsmarting the host immune system [9]. Considering the severity of illnesses as a result of infections due to *S. aureus*, its wider prevalence, difficulty of treatment of infections associated with it and the risk of economic loss, this study aimed to detect the presence and multidrug resistance in *S. aureus* from cutting boards of some selected restaurants of Sri Serdang, Selangor, Malaysia.

MATERIALS AND METHODS

Sampling Technique and Identification of Isolates

A total of 55 samples from cutting boards where raw meats are processed in 45 restaurants (some restaurants having more than one cutting boards) were collected. Sample collection was done after use and cleaning of the cutting boards prior to another use. The samples were then collected by standard swabbing technique using standard templates of an area size of 10cm^2. Targeted areas of the cutting boards were swabbed horizontally, vertically and diagonally using sterile swabs removed from coded test tubes containing 5ml sterile phosphate-buffered saline (PBS pH 7.4±0.1) (Waltham, Massachusetts, USA). They were transported to the laboratory for analysis.

The samples were analyzed according to AOAC [10]. They were vortexed for 10 seconds, and a 1 mL aliquot of the sample was dispensed onto the centre of Petri film Staph Express plates (3M^{TM} Microbiology, St. Paul, USA). Plates were finally incubated in stacks of up to 20 with the clear side up at 35^{o}C ± 1^{o}C for 24hr ± 2hr. Red-violet colonies observed on the Petri film following incubation indicated the presence of *S. aureus*. However, Petri film plates presenting other colonies than the red-violet indicated the masking of the *S. aureus* and were thus treated further with 3M Petri film Staph Express Disk which applied on top of the colonies re-incubated. Colonies considered for counting in this regard, range from 15-150 [10-11]. Further identification was conducted using conventional methods such as Gram-staining, catalase and coagulase tests and cultural characteristics on selective media; Baird Parker Agar (BPA) (Oxoid, England) and Mannitol Salt Agar (MSA) (Merck, Germany).

Antibiotic Resistance and Multidrug Resistance Definition

Susceptibility testing to 11 antibiotics was carried out on the identified *S. aureus* isolates using agar-disk diffusion technique on Mueller-Hinton agar in accordance with the CLSI criteria (CLSI, 2017) and the resistance was examined and recorded. The antibiotics tested against the isolates were ciprofloxacin (CIP: 5µ g), amoxycillin (AML: 10 µg), gentamicin (CN: 10 µg), penicillin G (P: 10µg), ceftriaxone (CRO: 30µg), Sulphafurazole (SF: 300µg), streptomycin (S: 25µg), ceftazidime (CAZ: 30µg), cephalothin (KF: 30µg), nalidixic acid (NA: 30µg) and cefotaxime (CTX: 30µg). The antibiotics were chosen based on their modes of action, the spectrum of activity and molecular structures. The diameters for the zones of inhibition were recorded as resistant, intermediate or susceptible. Multidrug-resistant isolates were then examined and defined as that *S. aureus* isolates resistant to antibiotics belonging to three or more classes of antibiotics [13-15].

RESULTS

Sampling and Identification of Isolates

Out of the 55 swab samples from cutting boards, 24 (43.6%) were contaminated with *S. aureus* based on the analysis using the technique of Petri film Staph Express plates, Gram staining, mannitol fermentation on MSA, growth on BPA, catalase and coagulase tests. The remaining (31 samples; 56.4%) were negative for *S. aureus,* although they might be positive for other bacterial pathogens.

Antibiotic Resistance, Resistance Pattern, Multiple Antibiotic Resistance (MAR) Index and Multidrug-Resistant *S. aureus* Isolates

The antibiotic resistance of *S. aureus* isolated from cutting boards of the restaurant is displayed in **Fig. 1**. All the 24 (100%) isolates were resistant to amoxycillin and penicillin G and were susceptible to gentamicin, ceftriaxone, sulphafurazole, streptomycin and cefotaxime. However, 19 (79.2%) of them were resistant to nalidixic acid, 16 (66.7%) to ciprofloxacin, 8 (33.3%) to cephalothin and 5 (20.8%) to ceftazidime. Intermediate resistance was exhibited to ceftazidime by 15 (62.5%), to cephalothin by 11 (45.8%) isolates, to ciprofloxacin by8 (33.3%) isolates and to nalidixic acid by5 (20.8%) isolates. susceptibility was also observed to cephalothin by 5(20.8%) isolates and to ceftazidime by 4(16.7%) isolates.

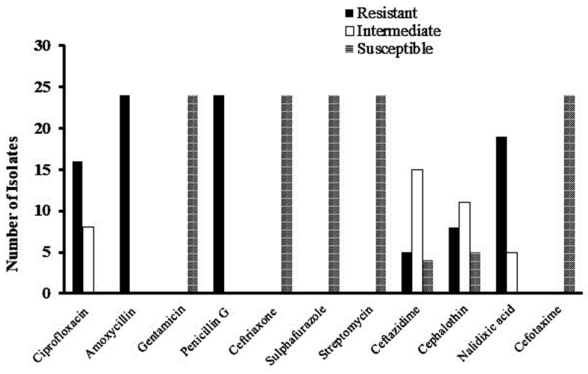

Fig 1. Antimicrobial resistance of *S. aureus* (n=24) isolated from cutting boards' surfaces of restaurants to 11 commonly used antibiotics.

It should be noted that the percentage positive of the strains was from the mean of four replications. The resistance, susceptibility and intermediate resistance were determined on the basis of CLSI, (2017) guideline. The antibiotic resistance patterns, multiple antibiotic resistance (MAR) index and multidrug resistance profile of the isolates are displayed in **Table 1**. Nine patterns of antibiotics resistance were observed

overall with CIP AML P NA observed in isolates SA002, SA008, SA009, SA012, SA013, SA015, SA021 and SA022. Other patterns include resistance to CIP AML P KF NA by SA001, SA003, SA016 and SA019; resistance to AML and P only by SA004, SA018 and SA020; resistance to AML P KF NA to SA005 and SA023; resistance to AML P CAZ NA by SA006 and SA007; resistance to CIP AML P CAZ NA by SA010 and SA017; resistance to CIP AML P CAZ KF NA by SA011 only; resistance to CIP AML P by SA014 and lastly resistance to AML P KF by SA024.

Table 1. Antibiotic resistance profile and multiple antibiotic resistance indices of individual *S. aureus* isolated from cutting boards.

Isolate*	Antibiotic resistance pattern**	No. of antibiotics***	MAR Index****
SA001[a]	CIP AML P KF NA	5(3)	0.45
SA002[b]	CIP AML P NA	4(2)	0.36
SA003[a]	CIP AML P KF NA	5(3)	0.45
SA004	AML P	2(1)	0.18
SA005[a]	AML P KF NA	4(3)	0.36
SA006[a]	AML P CAZ NA	4(3)	0.36
SA007[a]	AML P CAZ NA	4(3)	0.36
SA008[b]	CIP AML P NA	4(2)	0.36
SA009[b]	CIP AML P NA	4(2)	0.36
SA010[a]	CIP AML P CAZ NA	5(3)	0.45
SA011[a]	CIP AML P CAZ KFNA	6(3)	0.55
SA012[b]	CIP AML P NA	4(2)	0.36
SA013[b]	CIP AML P NA	4(2)	0.36
SA014[b]	CIP AML P	3(2)	0.27
SA015[b]	CIP AML P NA	4(2)	0.36
SA016[a]	CIP AML P KF NA	5(3)	0.45
SA017[a]	CIP AML P CAZ NA	5(3)	0.45
SA018	AML P	2(1)	0.18
SA019[a]	CIP AML P KF NA	5(3)	0.45
SA020	AML P	2(1)	0.18
SA021[b]	CIP AML P NA	4(2)	0.36
SA022[a]	AML P KF NA	4(3)	0.36
SA023[b]	CIP AML P NA	4(2)	0.36
SA024[b]	AML P KF	3(2)	0.27

*a=Multidrug resistant (MDR) isolates (resistant to 3 or more classes of antibiotics)
*b=non-multidrug-resistant but resistant to two classes of antibiotics
**AML=Amoxycillin,P=Penicillin G,CRO=Ceftriaxone, CTX=Cefotaxime, CIP=Ciprofloxacin, CN=Gentamicin, SF=Sulphafurazole, S=Streptomycin, CAZ=Ceftazidime, KF=Cephalothin, NA=Nalidixic Acid,.
***The number of antibiotics to which each isolate was resistant. The number in the parenthesis indicates the total number of the classes the antibiotics belong.
****Multiple antibiotic resistance index.

The range of MAR index was 0.18 to 0.55. Most of the *S. aureus* isolates (n=12) were resistant to four antibiotics (MAR=0.36) followed by those resistant to five antibiotics (13 isolates; MAR=0.45), to two antibiotics (3 isolates; MAR=0.18) to three antibiotics (2 isolates; MAR=0.27) and finally to six antibiotics (1 isolate; MAR=0.55). In addition, 11 (45.8%) of the isolates were multidrug-resistant (MDR) and include SA001, SA003, SA005, SA006, SA007, SA010, SA011, SA018, SA019, SA019 and SA022. The remaining 13 (54.2%) were non-MDR 10 (41.7%) resistant to two different antibiotic classes as SA002, SA008, SA009, SA012, SA013, SA014, SA015, SA021, SA023 and SA024 and three (12.5%) resistant to only one antibiotic class as SA004, SA018 and SA020.

DISCUSSION

Food contact surfaces, including cutting boards, have been continuously labelled as reservoirs of cross-contamination in the kitchens of commercial food premises. In addition, cutting boards give a wide surface area for contamination by pathogenic bacteria. In this study, detection of *S. aureus* from visibly clean cutting boards is an indication of contamination as well as the inefficacy of cleaning agents used. When cutting boards are contaminated with bacterial pathogens, the bacteria tend to survive and multiply and become disseminated to other food contact surfaces in an amount sufficient enough to induce hazards [16]. This is especially if they are able to aggregate and form biofilms. Various studies have reported the detection of pathogenic bacteria such as *S. aureus*, *L. monocytogenes*, *B. cereus,* and *Salmonella* spp. from food contact surfaces [16-17]. Epidemiological surveys have indicated that several foodborne outbreaks resulted from cross-contamination from poor hygienic practices involving food handlers' hands and food contact surfaces [18-20]. Detection of *S. aureus* by the methods employed has also been demonstrated by several studies [10-11, 21].

Antimicrobial resistance of *S. aureus* has been for a long period of time and this may be attributed to the ability of the organism to neutralize the antibiotic ring and evade the protein synthesis and nucleic acid inhibitors [22]. In this study, resistance of the 100% of the *S. aureus* isolates was reported against the members of the penicillins family (amoxycillin and penicillin G) used and this is common as 83.7% of *S. aureus* isolates from retailed foods in China were resistant to penicillin [15] and 100% of *S. aureus* isolated from fish market and fish handlers in Brazil were resistant to ampicillin. Resistance to ciprofloxacin, a fluoroquinolone as well as to nalidixic acid, a member of quinolones was observed in this study. This was similar to the study obtained in the US by Waters et al. [23] where *S. aureus* was isolated from meat and poultry.

There was no resistance to the Aminoglycosides members (gentamicin and streptomycin) reported. This study is supported by others conducted around the world including that of Tan et al. [24] where all the *S. aureus* isolated from the hands of food handlers at primary schools in Hulu Langat district, Selangor Malaysia were susceptible to gentamicin. Similarly, all *S. aureus* isolates from a dairy plant in Brazil were susceptible to Gentamicin [25]. In this study, *S. aureus* resisting sulphafurazole was not observed. Multidrug resistance, considered as complete or intermediate resistance to three classes of antimicrobials or more [14-15, 26], is widespread in the strains of *S. aureus*.

The MAR indices study indicates that 87.5% (n=21) have MAR index above 0.25. It is determined as the ratio of a number of antibiotics the organism resisted to the total number of antibiotics it was exposed. This MAR index is a vital tool for the assessment of health risk that helps to identify if an isolate is from a region of low or high use of antibiotics [27]. MAR index values greater than 0.2 were considered important for risk assessment of contamination. Further study is needed on the ability of these isolates to form biofilms and produce enterotoxins responsible for causing food poisoning outbreaks. However, the samples size involved herein is not sufficient to represent the incidence in the study area, and as such, high throughput data is required to be analyzed to qualify the prevalence of *S. aureus* from the cutting boards of restaurants.

CONCLUSION

S. aureus from cutting boards exhibited varying antibiotic resistance. High resistance (100%) was observed against amoxycillin and penicillin G, and lowest resistant was observed against ceftazidime (16.7%). In addition, 100% susceptibility was observed to gentamicin, ceftriaxone, sulphafurazole, streptomycin and cefotaxime. MAR index ranged from 0.18 to 0.55 (resistance to 2-6 different antibiotics). Majority of the *S. aureus* isolated were resistant to four different antibiotics (MAR=0.36), but CIP AML P NA pattern was the commonest.

In addition, 45.8% were multidrug resistant, 41.7% non-MDR but resistant to two classes of antibiotics and 12.5% were non-MDR resisting one antibiotic class only. In Malaysia, the use of antibiotics is being controlled. Therefore, there is a need for the improvement in the cleaning and washing of cutting boards, preferably the use of standard sanitizers as well as hot water to totally eliminate the adherent *S. aureus* so as to avoid the occurrence of staphylococcal food poisoning outbreaks.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
BPA	Baird Parker Agar
MSA	Mannitol Salt Agar
CLSI	Clinical and Laboratory Standard Institute
AML	Amoxycillin
CN	Gentamicin
P	Penicillin G
CRO	Ceftriaxone
CIP	Ciprofloxacin
S	Streptomycin
SF	Sulphafurazole
CAZ	Ceftazidime
KF	Cephalothin
NA	Nalidixic Acid
CTX	Cefotaxime
MAR	Multiple Antibiotic Resistance
MDR	Multidrug Resistance

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