

Ready-to-eat food sold in healthcare facilities could contribute to the increased disease burden of multidrug resistant *Staphylococcus aureus*

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Abstract

Food sold in healthcare facilities and environments are underestimated as sources of exposure of customers to potential pathogens. In the current study, we investigated the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in selected food items sold in two hospitals located in Benin City, Edo State, South-South Nigeria. A total of 100 food samples were obtained from food vendors and screened by pour plate method for the presence of heterotrophic bacterial and *Staphylococcus aureus*. Their antibiotic sensitivity patterns were determined by disc diffusion method and the presence of toxigenic and pathogenic genes by polymerase chain reaction and gel electrophoresis. Majority of the food samples investigated harbored *Staphylococcus aureus*, with varying levels of antibiotics resistance pattern. Only 5 of the isolates were observed to be methicillin resistant, with only one harboring the *mecA* gene. The results of this study are an indication that methicillin resistant *Staphylococcus aureus* could be a source of contaminant in ready-to-eat food sold in hospital facilities, and effort must be taken to prevent the occurrence of diseases arising from their presence.

Keywords: Methicillin resistant *Staphylococcus aureus*; Hospital; *mecA* gene; Ready-to-eat food; Public Health

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INTRODUCTION

Staphylococcus aureus is usually considered a notorious opportunistic pathogen in hospitalized patients, and its increased morbidity and mortality especially in recent times is believed to be due to antibiotics resistance (Tong *et al.*,

2015). This bacterium causes many types of human infections as well as diseases, most notably skin and soft tissue infections, and are often referred to as a widespread commensal and pathogenic bacterium (Feng *et al.*, 2008). Approximately 50 to 60% of individuals are

permanently or intermittently colonized with *Staphylococcus aureus*, thus, creating relatively high potential for infections (Liu *et al.* 2004).

Foodborne diseases are increasingly considered a major public health concern globally (Kadariya *et al.* 2014), and is particularly worrisome in developing countries where the majority of foodborne diseases are under reported (Odeyemi, 2016). For example, staphylococcal food poisoning outbreaks have been reported in Switzerland (Johler *et al.*, 2015), United States (CDCP, 2012), Italy (Ercoli *et al.*, 2017; Guidi *et al.*, 2018), Brazil (do Carmo *et al.*, 2003), Australia (Fletcher *et al.*, 2015), Japan (Suzuki *et al.*, 2017), Germany (Johler *et al.*, 2013), India (Basavegowda *et al.*, 2014) etc, while the only reported study on staphylococcal food poisoning outbreak in Africa was reported in Zimbabwe (Gumbo *et al.*, 2015). Interestingly, evidence exists on the practices that favour the proliferation of *Staphylococcus aureus* in retail ready-to-eat food (Wogu *et al.*, 2011; Farhadian, 2022).

On the other hand, methicillin is a class of antibiotics commonly used for treating of infections caused by *Staphylococcus aureus*. Although this class of antibiotics are proven to be effective in treating most of the infections caused by this bacterium, particularly the methicillin-sensitive strains of *Staphylococcus aureus* (MSSA), some species of *Staphylococcus aureus* have however developed resistance to methicillin and are no longer killed by this antibiotic (Omoruyi *et al.*, 2020a). *Staphylococcus aureus* are reported to cause life threatening infections such as staphylococcal food poisoning, mastitis, phlebitis, meningitis, as well as urinary tract infections (Asperger and Zangerl, 2003). The continuous emergence of methicillin-resistant as well as multi-drug resistant (MDR) strains of *S. aureus* in hospital foods further increases the mortality and morbidity rates of infections caused by this bacterium (Klein *et al.*, 2007). Unfortunately, research has majorly focused on healthcare associated MRSA, with limited data on the prevalence of MRSA in ready-to-eat foods, especially in developing countries. Multidrug resistant as well as methicillin resistant *Staphylococcus aureus* were previously thought to be associated with healthcare facilities alone (Neyra *et al.*, 2014). However, recent evidence revealed that MDR *S. aureus* and MRSA are prevalent in RTE retail food products globally (Yang *et al.* 2016, ChajECKa-WierZchowska *et al.*

2017). In Nigeria, MDR *S. aureus* and MRSA have been reported in RTE shellfish (Egege *et al.*, 2020), meats (Okoli *et al.*, 2018), fish and meat products (Bello *et al.*, 2013), raw milk and soft cheese (Omshaba *et al.*, 2018), amongst others. Despite increasing evidence on the prevalence of this organism in RTE retail foods there is however, paucity of data, on their presence in retail food sold in tertiary health care facilities. In the current study we investigated the prevalence of MDR *S. aureus* and MRSA in some selected food items sold in two hospitals in Benin City, their antibiotic susceptibility pattern and the distribution of pathogenic and virulent genes in them.

MATERIALS AND METHODS

Sample Collection

One hundred samples of ready-to-eat food sold in hospital environment were obtained from different food vendors in both Central hospital (40) and Stella Obasanjo hospital (60), both located in Benin City, Edo State, South-South Nigeria. Samples were collected over a ten-month period and included meat pie (20), egusi soup (10), white rice (20), spaghetti (15), doughnut (15), black soup (5) and white beans (15). Samples were obtained in sterile polyethylene bags and transported to Benson Idahosa University Laboratory on an ice-cold-pack for immediate analysis.

Microbiological Analysis

Twenty-five grams (25 g) of each food samples were weighed into a conical flask already containing 225 ml of sterilized peptone water. The food samples were thereafter macerated in the 225 ml of peptone water to form the stock. A ten-fold serial dilution was made from the stock solution up to the 6th dilution, and 1 ml of the third-dilution was adequately cultured on the appropriate medium by spread plate microbiological technique. All microbiological analysis were done within an hour of sample collection, in triplicates. All cultured plates were kept in the incubator for 24 hrs. at 37°C.

Total Heterotrophic Bacterial Count

One millimeter (1 ml) of each stock solution from a particular sample was collected using a sterile pipette and diluted into 9 ml of sterile peptone water done in a test tube. Further 10-fold dilutions

were made up to the sixth dilution, and 1 ml of the third dilution for the respective samples was cultured on Nutrient agar plates in triplicate. The plates were then incubated at 37°C for 24 hrs. The total numbers of colonies were counted from each plate using a colony counter (Labtech colony counter) and the average per sample was reported as mean \pm standard deviation.

Total *Staphylococcus aureus* Count

The mean *Staphylococcus aureus* count was determined using spread plate method by transferring 1 ml of the 3rd dilution into mannitol salt agar plates (in triplicate) as previously described (Omoruyi *et al.*, 2020b), following which the plates were kept in the incubator for 24 hrs at 37°C. Colonies characterized with yellow colour showing their ability to ferment mannitol were counted as *Staphylococcus aureus*. One anatomically discrete colony observed to be yellowish in colour as a result of mannitol fermentation, each from a plate was sub-cultured on nutrient agar plates. After streaking, the plates were then incubated at 37°C for 24 hrs. All pure isolates were stored on a slant at 4°C until further use.

Presumptive Identification of *Staphylococcus aureus*

Bacterial isolates with distinctive yellowish colony on Mannitol salt agar plates were sub-cultured, and confirmed by their morphological and biochemical characteristics (coagulase, citrate, catalase, oxidase and hemolysis).

Detection of MRSA and MSSA by Cultural Characteristics on Mannitol-Oxacillin Agar

Suspected *Staphylococcus aureus* isolates were assayed for their ability to grow on mannitol-oxacillin agar plates as previously described by Fooladi *et al.* (2015) and Omoruyi *et al.* (2020b). Briefly, freshly prepared mannitol-oxacillin agar was made by adding 0.4 g of oxacillin to already prepared mannitol salt agar (1L) before pouring. The medium containing the suspected *S. aureus* isolates was incubated for 24 hrs at 37°C. The presence of growth of the bacterium on the test medium showed methicillin-resistance while the absence of growth indicated methicillin sensitivity.

Antibiotic Sensitivity Test

Selected isolates (MRSA and MSSA) were screened for their sensitivity and/or resistance against some (10) commonly used antibiotics; septrin (30 μ g), gentamycin (30 μ g), pefloxacin (10 μ g), zinnacef (30 μ g), ampiclox (20 μ g), rocephin (25 μ g), amoxicillin (30 μ g), ciprofloxacin (10 μ g), streptomycin (30 μ g), and erythromycin (10 μ g) by disk diffusion method as described by Omoruyi and Ujubianja (2022). Briefly, isolates were cultured unto already prepared nutrient broth and incubated overnight. The turbidity of each culture was adjusted to match the opacity standard (BaSO₄ turbidity standard). The standard had a resulting broth culture of 10⁸cfu/mL. Freshly prepared Muller Hinton agar plates were seeded on standardized bacterial broth cultures by spread plate techniques. The inoculated culture plates were left to dry for approximately 15 min, and antibiotic discs were seeded on the agar plates, and incubated for 24 hrs at 37°C. The resultant zones of inhibition were interpreted based on the revised Clinical and Laboratory Standards Institute (CLSI) guideline for *Staphylococcus* spp. [EM100] (CLSI, 2020).

DNA Extraction

DNA from *Staphylococcus aureus* (MRSA AND MSSA) was extracted by a commercial DNA extraction kit (ZR Fungal/Bacterial DNA MiniPrep™, Zymo Research Corporation, USA), using the instruction provided by the manufacturers. The quantity and quality of DNA measure by UV spectrophotometer at an absorbance of 260 nm and 280 nm, using the formula, A_{260}/A_{280} . Only absorbance of 1.7 to 2.0, indicative of pure and quality DNA (Omoruyi and Ujubianja, 2022) was used in the current study. Pure DNA was kept at 4°C before use.

Polymerase Chain Reaction

Target genes using specific primers (forward and reverse) for each gene of interest (*mecA*, *entA* and *SCCmec1*) were amplified by polymerase chain reaction (PCR). The PCR reactions were carried out according to the method of Fooladi *et al.* (2015), while the targeted genes, primers used, their base pair and annealing temperatures are as presented by Omoruyi *et al.* (2020b).

Gel Electrophoresis

DNA fragments were separated as previously described (Omoruyi *et al.*, 2020b). Briefly, 1.5% agarose was prepared using 1XTAE buffer following which it was stained with ethidium bromide (3 μ l), before being poured into the gel containers. Ten microliters of PCR products containing the loading dye (New England Biolab, UK) were put inside each well, with DNA ladder (100-1000bp) placed in the appropriate well. All amplified DNA products were kept in the electrophoresis machine for 45mins at 100 Amp, following which the gels were viewed for bands of interest, with a UV transilluminator.

Statistical Analysis

The mean and standard deviation of heterotrophic bacterial and *Staphylococcus aureus* counts were calculated using the SPSS software (version 22).

RESULTS

The outcome of this study shows that heterotrophic bacteria and *Staphylococcus aureus* are present in selected food sold in Central Hospital and University of Benin Teaching Hospital, Benin City, Edo state, South-South Nigeria. The total heterotrophic bacterial counts obtained from Central Hospital was less ($107 \times 10^3 \pm 2.65$ CFU/g) in doughnut obtained from vendor 1 and highest ($321 \times 10^3 \pm 2.6$ CFU/g) in doughnut obtained from vendor 4 (Table 1). Interestingly, all 5-lots of white rice, spaghetti and white beans obtained from vendor 2 had mean heterotrophic bacterial counts of $300 \times 10^3 \pm 0.0$ CFU/g for all samples.

Total *Staphylococcus aureus* counts was lowest ($8.3 \times 10^3 \pm 10.9$ CFU/g) in white beans obtained from vendor 2 and highest in white rice obtained from vendor 3, with mean *Staphylococcus aureus* count of $300 \times 10^3 \pm 11.7$ CFU/g. The corresponding doughnut with the highest

heterotrophic bacterial counts of $321 \times 10^3 \pm 2.6$ CFU/g had total *Staphylococcus aureus* count of $100 \times 10^3 \pm 14.0$ CFU/g (Table 1).

Table 2 shows the total heterotrophic bacterial counts obtained from Stella Obasanjo Hospital, and ranged from $5.0 \pm 0.83 \times 10^3$ CFU/g in white rice from vendor 2 to $300.0 \pm 52.60 \times 10^3$ CFU/g in spaghetti from vendor 3, while total *Staphylococcus aureus* counts ranged from $0.00 \pm 0.00 \times 10^3$ CFU/g(ml) in doughnut from vendor 1, black soup, beans and white rice (all from vendor 2) and spaghetti from vendor 3 (Table 2), while beans from vendor 3 had the highest number of *S. aureus* count; $33.0 \pm 7.20 \times 10^3$ CFU/g.

Eight anatomically distinct *Staphylococcus aureus* isolates were isolated from all food samples obtained from Central Hospital. The majority of the isolates; all except 12.5% from white rice (Vendor 3) were resistant to ampiclox, while only few of the isolates were resistant to erythromycin (Table 3). All *Staphylococcus aureus* isolated from doughnut (Vendor 1) and white rice (Vendor 3) were resistant to septrin. Meanwhile, from Stella Obasanjo Hospital, all *Staphylococcus aureus* isolated from black soup and beans (Vendor 2) were resistant to septrin, all isolates from doughnut (Vendor 1), spaghetti (Vendor 3) and meat pie (Vendor 3) were completely resistant to erythromycin; meat pie (Vendor 1), spaghetti (Vendor 2) and egusi (Vendor 2) were also resistant to ampiclox; meat pie (Vendor 1), egusi (Vendor 3) and beans (Vendor 3) had 100% resistance to zinnacef. This was also the case for meat pie (Vendor 1) and beans (Vendor 3) in rocephin, and beans (Vendor 2) for ciprofloxacin (Table 4).

Five bacterial isolates (SOHV2WB004, SOHV2WB005, SOHV3SPOO2, CENV1M002 and CENV1M005) were phenotypically resistant to methicillin. Of the isolates, only one isolate (SOHV2WB005) obtained from white beans from Stella Obasanjo Hospital had the *mecA* gene (Plate 1).

Table 1: Total heterotrophic bacterial and *Staphylococcus aureus* counts of ready- to-eat food obtained from Central Hospital, Benin City, Edo State, Nigeria

Sample	N	THC ($\times 10^3$) Mean \pm SD	TSAC ($\times 10^3$) Mean \pm SD
V1Meat Pie	5	300.0 \pm 0.0	202.6 \pm 16.8
V1Doughnut	5	107.0 \pm 2.65	100.0 \pm 14.1
V2White Rice	5	300.0 \pm 8.0	101.0 \pm 14.0
V2Spaghetti	5	300.0 \pm 14.1	202.7 \pm 16.8
V2White Beans	5	300.0 \pm 0.0	8.3 \pm 0.9
V3White Rice	5	300.0 \pm 22.4	300.0 \pm 11.7
V4Doughnut	5	321.0 \pm 12.6	100.0 \pm 14.0
V4Meat Pie	5	247.0 \pm 90.0	170.0 \pm 90.6

Key: THC: Total Heterotrophic bacterial counts; TSAC: Total *Staphylococcus aureus* counts

Table 2: Total Heterotrophic bacterial and *Staphylococcus aureus* counts obtained from Stella Obasanjo Hospital, Benin City, Edo State, Nigeria

Sample	N	THC ($\times 10^3$) Mean \pm SD	TSAC ($\times 10^3$) Mean \pm SD
V1Meat Pie	5	59.0 \pm 4.00	10.0 \pm 2.86
V1Doughnut	5	10.0 \pm 5.17	0.0 \pm 0.00
V2Spaghetti	5	61.0 \pm 12.45	4.0 \pm 0.75
V2Egusi	5	300.0 \pm 0.00	3.0 \pm 0.15
V2Black Soup	5	16.0 \pm 3.94	0.0 \pm 0.00
V2White Rice	5	5.0 \pm 0.83	0.0 \pm 0.00
V2Beans	5	11.0 \pm 4.60	0.0 \pm 0.00
V3Spaghetti	5	300.0 \pm 52.60	0.0 \pm 0.00
V3Egusi	5	300.0 \pm 38.72	2.0 \pm 0.00
V3White Rice	5	108.0 \pm 11.60	4.0 \pm 1.00
V3Meat Pie	5	300.0 \pm 16.84	15.0 \pm 3.50
V3Beans	5	300.0 \pm 0.00	33.0 \pm 7.20

Key: THC: Total Heterotrophic Counts; TSAC: Total *Staphylococcus aureus* counts

Table 3: Antibiotics sensitivity pattern of *Staphylococcus aureus* isolated from ready-to-eat food obtained from Central Hospital, Benin City, Edo State, Nigeria

Sample	N	STX	E	PEF	CN	APX	Z	AMX	R	CPX	S
PERCENTAGE RESISTANCE											
V1Meat Pie	8	87.5	12.5	62.5	62.5	100.0	100.0	62.5	87.5	12.5	12.5
V1Doughnut	8	100	12.5	87.5	87.5	100.0	100.0	50.0	50.0	50.0	25.0
V2White Rice	8	87.5	25.0	87.5	87.5	100.0	87.5	62.5	87.5	50.0	50.0
V2Spaghetti	8	62.5	12.5	50.0	62.5	100.0	100.0	62.5	87.5	62.5	62.5
V2White Beans	8	87.5	50.0	62.5	50.0	100.0	87.5	62.5	62.5	87.5	87.5
V3White Rice	8	100.0	50.0	50.0	87.5	87.5	100.0	87.5	62.5	50.0	87.5
V4Doughnut	8	62.5	25.0	62.5	87.5	100.0	50.0	62.5	100.0	100.0	50.0
V4Meat Pie	8	100.0	50.0	87.5	50.0	100.0	87.5	87.5	87.5	62.5	62.5

Key: SXT: Septrin, E: Erythromycin, PEF: Pefloxacin, CN: Gentamin, APX: Ampiclox, Z: Zinnacef, AMX: Amoxacillin, R: Rocephin, CPX: Ciprofloxacin, S: Streptomycin

Table 4: Antibiotics sensitivity pattern of *Staphylococcus aureus* isolated from ready-to-eat food obtained from Stella Obasanjo Hospital, Benin City, Edo State, Nigeria

Sample	N	STX	E	PEF	CN	APX	Z	AMX	R	CPX	S
PERCENTAGE RESISTANCE											
V1Meat Pie	8	62.5	87.5	87.5	62.5	100.0	100.0	87.5	100	62.5	87.5
V1Doughnut	8	87.5	100.0	87.5	62.5	87.5	62.5	62.5	87.5	25.0	50.0
V2Spaghetti	8	87.5	87.5	87.5	50.0	100.0	50.0	87.5	87.5	62.5	62.5
V2Egusi	8	87.5	62.5	37.5	75.0	100.0	87.5	87.5	75.0	50.0	62.5
V2Black Soup	8	100.0	87.5	75.0	87.5	87.5	75.0	87.5	50.0	87.5	87.5
V2White Rice	8	62.5	87.5	87.5	62.5	62.5	50.0	62.5	62.5	62.5	87.5
V2Beans	8	100.0	87.5	75.0	50.0	87.5	75.0	87.5	87.5	100.0	62.5
V3Spaghetti	8	87.5	100.0	87.5	87.5	62.5	87.5	75.0	87.5	87.5	87.5
V3Egusi	8	75.0	87.5	87.5	62.5	75.0	100.0	87.5	87.5	62.5	87.5
V3White Rice	8	62.5	87.5	75.0	50.0	87.5	87.5	62.5	62.5	87.5	75.0
V3Meat Pie	8	75.0	100.0	87.5	87.5	62.5	62.5	75.0	62.5	87.5	87.5
V3Beans	8	87.5	87.5	87.5	62.5	87.5	100.0	87.5	100.0	87.5	87.5

Key: SXT: Septrin, E: Erythromycin, PEF: Pefloxacin, CN: Gentamin, APX: Ampiclox, Z: Zinnacef, AMX: Amoxacillin, R: Rocephin, CPX: Ciprofloxacin, S: Streptomycin

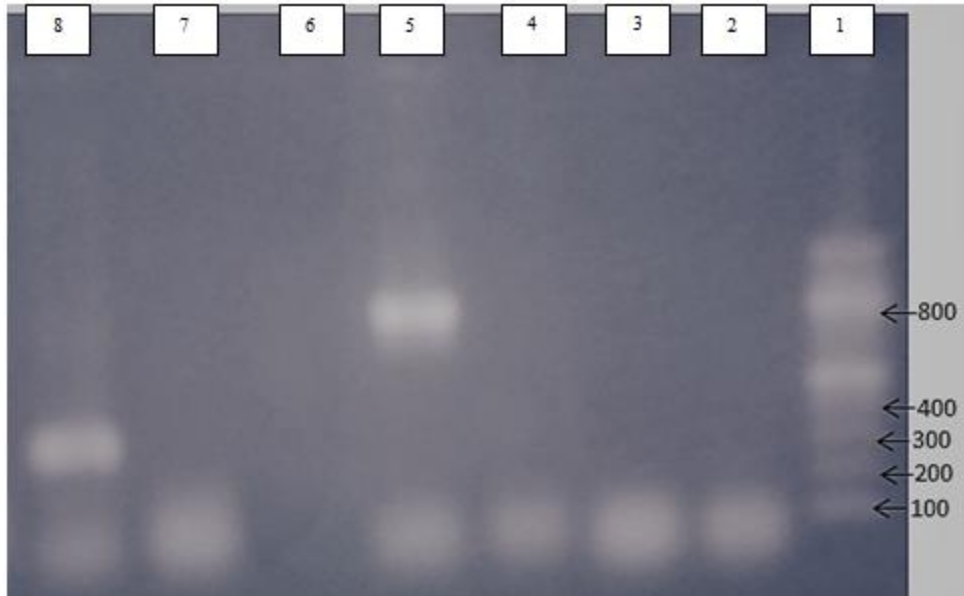


Plate 1: Plate showing the presence of *mecA* gene (857 bp) in white beans obtained from Central Hospital, Benin City, Edo State, Nigeria. 1: Molecular weight marker (100bp); 2: Isolate code SOHV2WB004; 3: Isolate code SOHV3SPOO2; 4: Isolate code CENV1M002; 5: Isolate code SOHV2WB005; 6: Isolate code CENV1M005; Negative control: *Escherichia coli*; 8: Isolate code SOHD3009

DISCUSSION

Food-borne diseases/infections are becoming endemic globally both in developed and developing nations and a threat to food security. For example, in China, there were 371 reported cases of food-borne diseases between 2008 and 2010, involving 20,062 people and 41 deaths (Yang *et al.*, 2016), 2003 – 2008 had a total of 1,930 foodborne disease outbreaks (Wu *et al.*, 2017), and 5,770 outbreaks between 1992 and 2001 (Liu *et al.*, 2004). In the United States, there are estimated 48 million people who get sick from the consumption of contaminated food alone, out of which, 128,000 get hospitalized with 3,000 deaths (CDC, 2016). In Nigeria, an estimated 200,000 persons die of food poison annually from the ingestion of contaminated food alone (Onyeneho and Hedberg, 2013; Ezirigwe, 2018). While paucity of data exists on the incidence of foodborne diseases in most developing countries, those caused by *S. aureus* are particularly lacking. Nkanga and Uraih (1981) had previously reported that information on the prevalence of staphylococcal food poisoning outbreaks in Nigeria was particularly lacking, owing to lack of statistical data. Unfortunately, the

situation has not significantly improved to-date. Although foodborne disease outbreaks have been documented in Nigeria, report on the causative organisms are particularly lacking in most cases. For example, Ojeniyi and Montefiore (1986) reported food poisoning outbreak in Ibadan, Nigeria, due to a new phage type of *Salmonella* Typhimurium, claiming 20 lives. Food poisoning in Kano state, Nigeria, arising from the consumption of yam flour was reported in 2009, without any causative agent (Adeleke, 2009). In a similar development, Adedoyin *et al.* (2008) attributed food poisoning in five families in Ilorin, Kwara State Nigeria, arising from the consumption of yam flour to the preservative used during processing, and not due to the presence of any pathogenic microorganism. In Nigeria, the knowledge on the cause of foodborne diseases is poor (Osagbemi *et al.*, 2010), with the majority of those patronizing street food more interested in availability and low cost (Osagbemi *et al.*, 2010). The unavailability of data on foodborne disease outbreak is linked to poor data collection, absence of appropriate surveillance systems, and willpower on the part of relevant agencies etc (FMH, 2021).

The results on the presence of heterotrophic bacteria and *Staphylococcus aureus* in ready-to-eat food sold in hospital environment reported in the current study is an indication that selected foods sold in hospitals in Benin City, Edo State, Nigeria, could be sources of exposure to *Staphylococcus aureus*. This is in agreement with previous studies where *S. aureus* was isolated from spoiled food samples (Alwan and Talak, 2015), desserts (Shimamura *et al.*, 2006), raw meat (Pesavento *et al.*, 2015) and reported to be associated with food poisoning in Shenzhen, China (Yang *et al.*, 2016a), and an outbreak in Hangzhou, China (Yang *et al.*, 2016b), further suggesting that the results obtained from this study should be given serious attention, to avoid potential outbreak of staphylococcal food poisoning. The presence of *Staphylococcus aureus* in ready-to-eat food can be attributed to unsafe food practices during preparation, processing and handling of food (Osagbemi *et al.*, 2010). For example, Ifeadike *et al.* (2012) reported fingernails of food handlers in Federal Capital Territory, Abuja, Nigeria to be contaminated with *S. aureus*, including the coagulase negative ones. Cooking and drinking water sources can also be a source of contamination of ready-to-eat food by *Staphylococcus aureus* including the methicillin resistant strains of *Staphylococcus aureus* (Santos *et al.*, 2020), as well as cross-contamination in the vicinity of food preparation and processing (Kadariya *et al.*, 2014).

Staphylococcus aureus are reported to be prevalent in ready-to-eat street foods sold in major cities and states in Nigeria; Agboe *et al.* (2016) reported 55.6% prevalence rate of *S. aureus* (N = 36) in street vended food sold in Calabar metropolis, while Ani *et al.* (2011), also reported a similar outcome in street vended foods sold in Aba, Abia State, Nigeria, with African salad (Abacha) being the most contaminated with *S. aureus*.

In a similar study, Dehkordi *et al.* (2018) reported 9.69% (n = 485) *S. aureus* prevalence in ready-to-eat hospital foods sold in Iran. Interestingly, 7.62% of the isolates were reported as methicillin-resistant strains of *S. aureus*, and harbored the highest prevalence of resistance against penicillin (100%), centerline (100%), tetracycline (100%), erythromycin (89.18%) etc. In a more recent study, Afshari *et al.* (2022) reported a prevalence rate of 24.37% (87/357) and 22.98% (20/87) for *Staphylococcus aureus* and methicillin

resistant *Staphylococcus aureus* respectively from food samples sold in selected hospitals around Mashhad, Iran, with chicken, meat, salad and barbecues being the most contaminated. Afshari *et al.* (2022) further reported resistance of the MRSA isolates to penicillin, erythromycin, clindamycin, azithromycin, tetracycline and gentamycin.

In this current study, all MRSA strains were completely resistant to septrin, erythromycin, pefloxacin, ampiclox, rocephin and streptomycin. Only one MSSA was isolated with the isolate showing resistance to only erythromycin. The presence of *S. aureus* and MRSA in ready-to-eat food is worrisome owing to the potential public health effects. Rodriguez-Lazaro *et al.* (2017) investigated the presence and characteristics of *S. aureus* and MRSA in foods of animal origin impounded from travellers on trips from 45 non-EU countries between 2012 and 2015 in 2 airports (Spain and Austria) as well as from foods in open markets close to EU land borders. Of the 868 food samples investigated, 15.7% were positive for *S. aureus*, while 3% were confirmed as MRSA. Antibiotics resistance is a major public health challenge and is accelerated by the overuse and misuse of antibiotics, particularly in developing countries where self-medication as well as indiscriminate use of antibiotics in farm animals remain a serious public health challenge (Omoruyi *et al.*, 2020b; Omoruyi and Ajayi, 2021).

Contrary to what was reported in the current study, all MRSA strains reported by Rodriguez-Lazaro *et al.* (2017) were *mecA* positive. In a similar study, Elhassan *et al.* (2015) reported 12 out of 123 MRSA isolates (9.8%) to be *mecA* negative, suggesting the possibility of methicillin resistant *Staphylococcus aureus* to be *mecA* negative, implying the use of alternative mechanisms for β -lactam resistance that may compete with *mecA* gene. Williams *et al.* (2020) also reported 43.2% of *mecA* negative *S. aureus* isolates to be phenotypically resistant to methicillin, while 27 (n = 234) representing 11.5% of *S. aureus* isolates found to be *mecA* positive were phenotypically susceptible to methicillin. Williams *et al.* (2020) attributed 20 out of the 27 results obtained as "unexplained discordance". However, the reasons for the results obtained in the current study remain unknown. The *mecA* gene is reported to confer resistance to methicillin and other beta lactam antibiotics. *MecA* gene is reported as part of a 21- to 60-kb staphylococcal chromosome cassette *mec* (SCC*mec*); a mobile

genetic element that may also contain genetic structures, which encodes resistance to non- β -lactam antibiotics (Wielders *et al.*, 2002). These organisms are an important cause of nosocomial infections worldwide, and their presence in ready-to-eat food sold in hospital environment is an indication that such unsuspecting ready-to-eat food could be a source of human exposure to infections, and efforts must be taken to ensure that food sold in hospital environments are free of pathogenic microorganisms.

CONCLUSION

In conclusion, the outcome of this study shows that ready-to-eat food sold in hospital environments are potential sources of human exposure to pathogenic and multi drug resistant bacteria, particularly methicillin resistant strains of *Staphylococcus aureus* possessing the *mecA* gene. Necessary awareness must be created amongst food vendors and patrons of ready-to-eat hospital foods to reduce the incidence of infection that could arise from the consumption of these food items.

Author Contribution

OIM designed the study. OIM and IOS both participated in the research and writing of the manuscript. Both authors approved the final version of the manuscript.

Conflict of Interest:

The authors declare that there are no potential conflicts of interest

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