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Isolation and Detection of Antibiotic-Resistant *Staphylococcus aureus* from Street-Vended Chicken Intestines in a Philippine Urban Setting

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ABSTRACT

Foodborne pathogens, particularly *Staphylococcus aureus*, is commonly associated with the street food, causing a wide array of diseases such as staphylococcal food poisoning (SFP), which leads to diarrhea, nausea, and more. This study aimed to detect and isolate putative antibiotic-resistant *S. aureus* isolates in *isaw* and tomato-based condiment randomly collected from the food stalls around Barangay Ampayon, Butuan City, Philippines. The putative isolates were selectively grown and morphologically characterized using Tryptic Soy Broth and Baird-Parker Agar. Further characterization was conducted through biochemical testing using assays such as Gram staining, Indole test, Catalase test, Methyl-red test, Triple Sugar Iron Test, and Coagulase test. The study revealed that 19 isolates of the presumptive *S. aureus* were detected, with 15 isolates expressing the characteristics of typical *S. aureus* through biochemical tests. All 15, or 100%, of the suspected *S.aureus* isolates expressed resistance to Oxacillin. However, on the other hand, 100% of these isolates were also susceptible to Ciprofloxacin and Cotrimoxazole, with only 14 isolates, or 93%, showing susceptibility to Gentamicin and Tetracycline. One isolate was characterized as multidrug resistant (MDR) due to its resistance to Oxacillin, Gentamicin, and Tetracycline. The presence of MDR *S. aureus* in this study implies significant health risks to both the vendors and consumers of *isaw*, highlighting the importance of proper food preparation and food safety regulations in the Philippines, especially towards commonly sold consumables such as chicken products and sauces.

Keywords: *foodborne pathogens, food stalls, multidrug resistance, condiment, isaw*

1 Introduction

Street food, delicacies prepared and sold by vendors on the streets, has gained significant popularity worldwide due to its affordability, convenience, and diverse flavors (Ceyhun Sezgin and Şanlıer 2016). However, the preparation and consumption of street food carries potential health risks, as these vendors, the ingredients, and even the hygiene practices and food handling techniques they utilize can act as carriers of foodborne pathogens.

One of the most common street foods in the Philippines is *isaw*, or grilled chicken intestines (Harkins 2017). It is revered for its unique taste among locals and tourists alike, especially with the addition of tomato sauce, which plays a crucial role in enhancing the flavors of street food dishes. Behind this success and popularity, however, lies an increased presence of foodborne pathogens, specifically *Staphylococcus aureus*, a Gram-positive, coccus-shaped pathogen (Moloi et al. 2021).

Being frequently found in undercooked poultry, meat, and eggs, *S. aureus* initiates infection

using its ability to produce enterotoxins that can cause Staphylococcal foodborne disease (SFD), resulting in symptoms such as vomiting, nausea, stomach cramps, and diarrhea (Hennekinne et al. 2012). Recorded cases of *S. aureus* infections in the Philippines have also been known to cause necrotizing pneumonia and toxic shock syndrome in extreme cases, which is exacerbated by the challenge of detecting the microbe and its toxins due to its lack of odor in street foods (Valle et al. 2016).

Moreover, the emergence of antibiotic resistance and multidrug resistance (MDR) in *S. aureus* poses a significant public health concern due to the extensive use and misuse of antibiotics in human medicine and agriculture (Gatadi et al. 2019). One such variant that has been labeled as the one of the leading causes of both nosocomial and community infections worldwide is the Methicillin-Resistant *Staphylococcus aureus* (MRSA), which has grown to ignore the effects of the staph disease-treating Methicillin since the 1960's and has cloned, disseminated, and acquired greater resistance against other treatments extensively to the point of being able to cause local outbreaks if left undetected and unresolved (Masim et al. 2021). The nigh-impossible challenge of reversing antimicrobial resistance back towards susceptibility, the decreasing reliability of modern drugs, and the time-gated race against the constantly evolving MDR bacteria leads to the burden of detecting and documenting the presence of S. aureus, especially in countries that have a known scarcity of information, like the Philippines (Medina & Pieper 2016).

Despite numerous studies on *S. aureus* contamination in street food around the world, there is a lack of information on its presence and antibiotic resistance profile in isaw and tomato sauce sold in Barangay Ampayon, Butuan City, Philippines — an area with high student consumption due to its proximity to Caraga State University. Addressing this gap is crucial to understanding local food safety risks and antibiotic resistance patterns. With street food consumption being a popular trend among many Filipinos, *S. aureus* and its unique resistance patterns, as well as its tendency to be found in local delicacies, make this a prime target for study.

This study aims to detect antibiotic-resistant *S. aureus* from isaw and tomato-based condiment samples obtained from food stalls around Barangay Ampayon, Butuan City, by isolating, characterizing the isolates through their morphology and biochemical reactions, and determining the antibiotic susceptibility profiles of the presumptive *S. aureus* isolates.

The findings of this study will raise public awareness of the potential risks associated with consuming street food, such as *isaw*, and provide valuable data for local health officials, policymakers, and food vendors. The opportunity to provide ideas for appropriate interventions and regulations may also be garnered from the results of the study, reducing the prevalence of foodborne diseases in the study area while ensuring the microbiological safety of street food, alongside the promotion of safer street food practices and enhancing public health measures against the threat posed by *S. aureus* and its MRSA and MDR variants for the Philippines and the world.

2 Methodology

Study Area

The study was conducted along the roadside around Caraga State University in Barangay Ampayon, Philippines (Figure 1), specifically at locations where crowded street food stalls are commonly found.

Collection of Samples

The collection of samples was conducted in accordance with the street food collection protocol of Mamun et al. (2013), with some modifications. Fourteen samples of *isaw* and tomato-based condiment were collected in triplicate from seven street food stalls based on crowd density and prevalence of the street food mentioned. The samples were collected using pre-sterilized plastic containers and stored in a sealed ice box to maintain a low temperature before being immediately transported to the Caraga State University Microbiology laboratory for analysis. A permission letter was sent to the barangay captain of the municipality prior to sample collection.

Isolation of Staphylococcus aureus

The collected samples of *isaw* and sauce were homogenized with a 1:10 ratio, following the protocol of Lakhanpal et al. (2019). Specifically, 25 g of *isaw* and 25 mL of condiments were separately mixed with 225 mL of buffered peptone water. Then, it was transferred to the stomacher to homogenize the samples. The homogenized samples were then

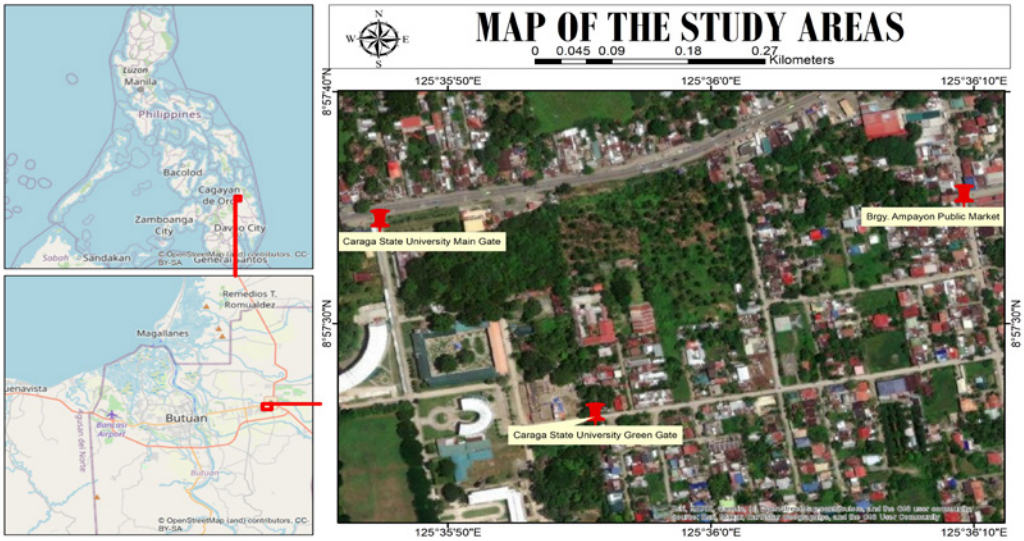

Figure 1. Location of the sampling area. The top left panel displays a map of the Philippines, highlighting the location of Butuan City (red line). The bottom left panel shows Barangay Ampayon (red line) on the Butuan City Map. The right panel shows the map of the sampling areas (three sites) in Barangay Ampayon.

subjected to enrichment to support the growth of *Staphylococcus aureus* using Tryptone Soy Broth (TSB), as it is the most commonly used culture medium (González-Machado et al. 2024). Four replicates of sterile 9 mL TSB were prepared for each isaw and tomato-based condiment sample, and were then inoculated with 1 mL of homogenized sample into the test tubes. The TSB test tubes were then incubated for 24 h at 37°C.

The enriched isolates were inoculated into selective media plates using the freshly prepared Baird-Parker medium with Egg Yolk Tellurite and incubated at 37°C for 48 h (FDA 1998). The isolates were subjected to subculturing for purification and then further characterization through biochemical assays.

Characterization of the Staphylococcus aureus isolates

The putative *Staphylococcus aureus* isolates were characterized by the following biochemical tests: indole test (MacWilliams 2012), methyl red test (McDevitt 2009), coagulase test (Aryal 2022), triple sugar iron test (Lehman 2005), and catalase test (Reiner 2010).

For the indole test, the putative *S. aureus* isolates from the samples were inoculated into sterile Tryptone Soy Broth tubes and incubated at 37°C for 24 h. After incubation, five drops of Kovács reagent were added to the test tube, and color changes in the medium were observed. The expected result of S. aureus isolates is negative (MacWilliams 2012). The methyl red test involved inoculating the assumed *S. aureus* isolates onto freshly prepared Methyl Red and Voges-Proskauer (MR-VP) broth and incubating them at 37°C for 48 h. Five drops of methyl red reagent are then directly dropped into the MR-VP broth test tubes, with a positive result expected for *S. aureus* (McDevitt 2009). The slide or drop technique was employed in the Catalase test, with 3% hydrogen peroxide as the primary agent of this test. A small amount of *S. aureus* was carefully deposited onto the slide. Then, the slide containing the bacterium was treated with a drop of hydrogen peroxide. Bubbles were expected as a result, indicating a catalase-positive result (Reiner 2010).

During the coagulase test, presumptive *S. aureus* isolates were inoculated into sterile TSB for broth culture, then freeze-dried rabbit plasma was diluted with a ratio of 1:10 in physiological saline. The diluted plasma was then pipetted into sterile test tubes, each containing 0.5mL. Afterwards, the broth culture of *S. aureus* isolates was pipetted into the diluted plasma test tubes at 0.1mL. After mixing, the test tubes were incubated at 37°C. Examination for clotting was checked every six hours, but not more than 24 hours of incubation. *S. aureus* isolates were expected to be coagulase-positive (Aryal 2022). The TSI test was conducted using the stab-and-streak method, wherein *S. aureus* isolates were inoculated by vertically inserting a sterile needle

into the agar deep to near the base of the tube, followed by streaking of the agar slant. Afterward, TSI slants were incubated for 18 to 24 h at 37°C. In this test, *S. aureus* isolates were expected to be negative (Lehman 2005).

Antimicrobial susceptibility assay

All the putative *S. aureus* isolates that exhibited the expected biochemical test results were subjected to antibiotic susceptibility screening following the Kirby-Bauer Disk Diffusion Susceptibility test protocol by Hudzicki (2009). This CLSI-recommended test utilizes antibiotic discs with a fixed volume, aimed at identifying the resistance of *S. aureus* isolates to these agents (Yang et al. 2019). The *S. aureus* isolates were tested for antibiotic susceptibility to Oxacillin (penicillin), ciprofloxacin (fluoroquinolones), tetracycline (tetracyclines), gentamicin (aminoglycosides), and cotrimoxazole (Table 1). Commercially available antibiotic disks were used with antibiotic concentrations prescribed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The plate disk diffusion technique was determined to be the primary method of initiating this assay, as there is a greater opportunity to isolate and detect MRSA due to the presence of oxacillin discs in the test, which is known to be stable in the disc diffusion technique and is structurally and pharmacologically related to Methicillin (Drew et al., 1972).

3 Results and Discussion

Isolation of Staphylococcus aureus from isaw and tomato-based condiment

Nineteen suspected *S. aureus* were detected from the samples—ten from the isaw and nine from the tomato sauce. The presence of *S. aureus* in the samples can be attributed to several factors. A study by Wang et al. (2012) attributes the appearance of the bacterium from the production process, be it storage of chicken carcasses with damaged or lacking packaging with other food products, or the sheer demand for the popular street food and sauce causing a lack of attention in checking for cross-contamination and observing proper storage and freezing times compared to withdrawal and transportation times. Chicken intestines, alongside human skin, have also been determined to be a frequent source of *S. aureus* isolates, indicating that human error, particularly concerning local street vendors exposed to crowds and open air, can also be the cause of contamination by simply being unhygienic or improperly cleaning or preparing the *isaw* (Kitai et al. 2005).

Similar to the study by El-Hadedy and El-Nour (2012), the halo formation around the *S. aureus* isolates (Figure 2) indicates lipase activity. The grey-black color of the shiny colonies is formed due to the reduction of potassium tellurite from the egg yolk tellurite emulsion (Silva et al. 2000). Morphological characteristics of the *S. aureus* isolated from the *isaw* and tomato sauce samples are expressed as Gram-positive cocci arranged in clusters similar to the *S. aureus* isolates in the study of Sina et al. (2011).

Characterization of the suspected Staphylococcus aureus isolates

After confirming the morphology of the cell and colony of presumptive *S. aureus* isolates, biochemical tests, including the Indole test, Methyl Red test, Coagulase test, Triple Sugar Iron test, and Catalase test, were used for characterization.

Out of the nineteen presumptive *S. aureus* isolates, fifteen (79%) were positive for the methyl red test, coagulase test, and catalase test, then negative for the indole test and TSI test, which passed the guidelines for characterizing typical *S. aureus* (Bergey 1994, Aryal 2022).

All suspected *S. aureus* colonies exhibited the presence of yellow rings during the Indole test,

Table 1. Antimicrobial agents used for antibiotic resistance screening of *Staphylococcus aureus* and their zone diameter breakpoints (EUCAST, 2024).

Antimicrobial Agent	Disk Content	Zone Diameter Breakpoints to the nearest whole (mm)	
		Susceptible	Resistant
Oxacillin (Penicillin)	1 µg	≥ 13	<10
Ciprofloxacin (Fluoroquinolones)	5 µg	≥ 50	< 17
Tetracyline (Tetracyclines)	30 µg	≥ 22	< 22
Gentamicin (Aminoglycosides)	30 µg	≥ 18	< 18
Cotrimoxazole	25 µg	≥ 17	< 14

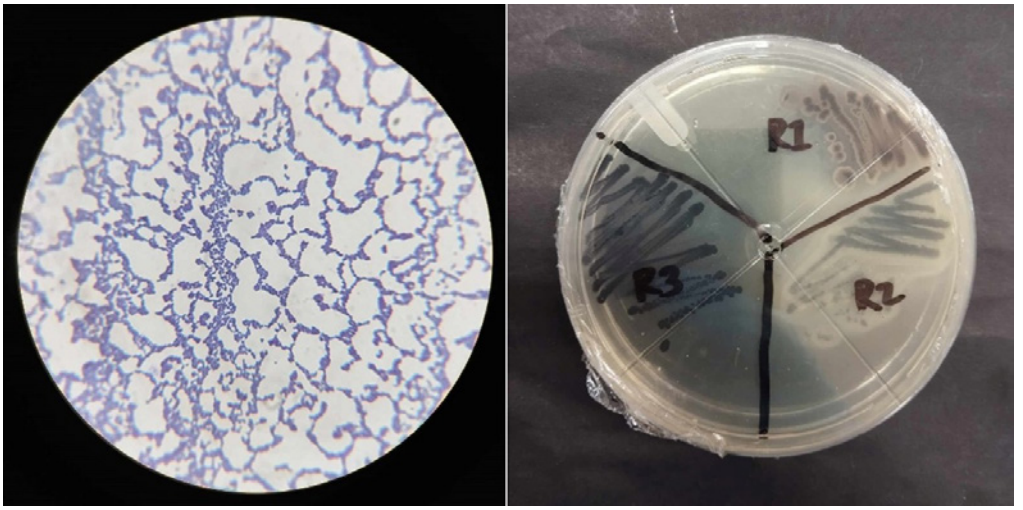

Figure 2. The left photo shows a suspected *Staphylococcus aureus* isolate gram stain under a microscope oil immersion objective lens (1000x). The cells are purple, cocci-shaped, and packed like grapes. The right photo shows colonies from the sample grown on Baird Parker media plates incubated at 37°C for 48 hours. *S. aureus* is black and shiny with a halo.

Table 2. Biochemical characterization of suspected *S. aureus* isolates from the tomato sauce and isaw samples in food stalls. Positive reaction (+), adverse reaction (-), acidic reaction (A).

***S. aureus* isolates**	**Colony Morphology**				**Gram Reaction**	**Cell Morphology**	
	Color	**Forms**	**Texture**	**Elevation**		**Shape**	**Arrangement**
Sauce							
KR1	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
KR2	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
KR3	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
KR4	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
KR5	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
KR6	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
KR7	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
KR8	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
KR9	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
Isaw							
IR1	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR2	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR3	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR4	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR5	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR6	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR7	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR8	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR9	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered
IR10	Black	Circular	Smooth	Convex	Positive	Cocci	Clustered

indicating a negative result of the biochemical test due to a lack of tryptophanase activity, indicating an inability to break down tryptophan to pyruvate and ammonia, a result typical of *S. aureus* (Hartline 2023). Meanwhile, in the Methyl Red test, all 19 suspected *S. aureus* isolates from the samples expressed a red ring similar to the positive control, indicating that the isolates can ferment one or more organic acids formed during glucose fermentation.

In the coagulase test, all suspected *S. aureus* isolates from both *isaw* and sauce samples exhibited a positive result, forming clumps of the diluted rabbit plasma, in line with results from other studies (Rakotovao-Ravahatra et al. 2019). This test serves as a benchmark for differentiating and characterizing *S. aureus* from other bacteria due to the species' ability to cause blood clots using the enzyme coagulase, which converts fibrinogen to fibrin, as it resembles prothrombin (Lucia et al. 2017).

All presumptive *S. aureus* isolates yielded an acid-positive reaction in the TSI test, characterized by yellow coloration of both the slant and agar deep, indicating glucose and lactose fermentation (Isnan et al. 2017). In the research of Hasan et al. (2016), vancomycin-resistant *S. aureus* and methicillin-resistant *S. aureus* strains isolated from burn wounds expressed similar results.

On the other hand, 15 (79%) out of 19 isolates were catalase-positive, indicating that the isolates were able to produce the enzyme catalase, a characteristic of *S. aureus*, as observed in the bovine-based study by Pumipuntu et al. (2017). Catalase production is a virulence factor of S. aureus, as the presence of this enzyme enables the bacteria to resist both intra- and extracellular killing via hydrogen peroxide (Mandell 1975, Kanafani and Martin 1985). However, four (21%) out of 19 isolates were catalase-negative, which could indicate the presence of a rare strain of the bacteria, such as in the case study by Yilmaz et al. (2005), which reported a catalase-negative methicillin-resistant *S. aureus* (MRSA) isolated from a patient who suffered from sepsis syndrome, which made detection methods complicated due to the nonconformance of the results.

Antibiotic susceptibility test

An antibiotic susceptibility test was conducted to determine the sensitivity of the 15 *S. aureus* isolates to Oxacillin (penicillin), gentamicin (aminoglycosides), ciprofloxacin (fluoroquinolones), cotrimoxazole, and tetracycline (tetracyclines).

The antibiotic resistance pattern of the presumptive *S. aureus* isolates shows that 15 (100%) of the isolates were susceptible to ciprofloxacin and cotrimoxazole, indicating that these drugs were effective against the presumptive S. aureus isolates. Gentamicin and tetracycline susceptibility was also observed in fourteen (93%) of the isolates, contrary to studies that have showcased the complete tetracycline resistance of modern *S. aureus* isolates (Grossman 2016).

On the other hand, all of the presumptive *S. aureus* isolates were resistant to Oxacillin, which belongs to the same antibiotic class as Methicillin. They thus could be labeled as MRSA (Eromo et al. 2016). This resistance can be attributed to the acquisition of staphylococcal cassette chromosome mec (SCCmec), which contains genes encoding proteins that render the bacterium resistant to most β-lactam antibiotics such as Methicillin and Oxacillin (Lee et al. 2018). Even worrying is that this high resistance usually comes at the cost of reduced virulence. However, it is largely unknown how most MRSA clones have achieved the goal of simultaneously expressing sufficiently high methicillin resistance and aggressive virulence characteristics, using different approaches in convergent evolution, which urges hasty intervention from global health organizations (Otto 2013).

The presence of one presumptive *S. aureus* isolate that is resistant to three different classes of antibiotics, namely Oxacillin, gentamicin, and tetracycline, can be characterized as being a multidrug-resistant (MDR) strain (Magiorakos et al. 2012). This result aligns with the study by Eromo et al. (2016), which also found MDR *S. aureus* in ready-to-eat street foods, as it was resistant to ampicillin, cloxacillin, ceftriaxone, and other tested antibiotics. The MDR phenotype in *S. aureus* has become increasingly prevalent over the past few decades, with the *ori*C environment being identified as the so-called storehouse of drug resistance genes, capable of safeguarding even foreign genes that would typically cause cell destruction if overexpressed (Hiramatsu et al. 2014).

Possible causes for the prevalence of these strains within isaw and its accompanying tomato sauce involves the presence of enzymes like catalase, which aids its survival by protecting it from the chicken and human hosts' immune system;

Table 3. Biochemical characterization of suspected *Staphylococcus aureus* isolates from the sauce and isaw samples in food stalls. Positive reaction (+), negative reaction (-), acidic reaction (A).

Isolate No.	Indole Test	Methyl Red Test	Coagulase Test	Triple Sugar Iron Test		Catalase Test
				Slant	Butt	
KR1	-	+	+	A	A	+
KR2	-	+	+	A	A	+
KR3	-	+	+	A	A	+
KR4	-	+	+	A	A	-
KR5	-	+	+	A	A	-
KR6	-	+	+	A	A	+
KR7	-	+	+	A	A	+
KR8	-	+	+	A	A	+
KR9	-	+	+	A	A	+
IR1	-	+	+	A	A	+
IR2	-	+	+	A	A	+
IR3	-	+	+	A	A	-
IR4	-	+	+	A	A	-
IR5	-	+	+	A	A	+
IR6	-	+	+	A	A	+
IR7	-	+	+	A	A	+
IR8	-	+	+	A	A	+
IR9	-	+	+	A	A	+
IR10	-	+	+	A	A	+

Table 4. Antibiotic susceptibility test results of S. aureus isolated from the isaw and sauce in street food stalls in Barangay Ampayon, Butuan City, Philippines. Susceptible (S) or resistant (R) oxacillin (OXA), gentamicin (GEN), ciprofloxacin (CIP), cotrimoxazole (COT), and tetracycline (TET) are displayed.

Type of Antibiotic	No. resistant	No. sensitive	Sensitive (%)	Resistant (%)
Oxacillin	15	0	00	100
Gentamicin	1	14	93.33	6.67
Ciprofloxacin	0	15	100	00
Cotrimoxazole	0	15	100	00
Tetracycline	1	14	93.33	6.67

and genes like the *Agr* gene, which helps *S. aureus* form biofilms and adhesion factors to remain in the food stalls and the products they serve (Siddique et al. 2024). The crowd density in these stalls, alongside the difficulty in upholding proper hygiene measures, also unwillingly aids the acquisition of MDR genes, as contact with the unseen *S. aureus* in *isaw* and tomato sauce by different people with varied microbiomes can lead to horizontal gene transfer, allowing the pathogen to become even more resistant and virulent (Evans et al. 2020).

4 Conclusion

The presence of multiple MRSA isolates and one MDR *S. aureus* strain in locally served *isaw* and tomato-based condiments highlights a significant risk, not only to the health of consumers and vendors in the community, but also to the current level of food safety in the country and its localities. Not only this, but the threat of a potential *S. aureus* outbreak in Butuan City has now become a possibility, especially considering the resilience and virulence factors of the pathogen in question. These findings prompt immediate action from both the local government unit and the health sector, including

seminars by vendors and relevant safety personnel, training, health education, and regular inspections of served street food. These measures are key factors in preventing the spread of the pathogen and instilling knowledge among the people regarding the issue.

With this in mind, this reasearch provides recommendations for further study such as: (1) utilizing methods like 16S rRNA or Whole Genome Sequencing to confirm the identity of the presumptive *S. aureus* isolates as well as the specific genes they utilize to confer multidrug resistance; (2) identify other foodborne pathogens in street food samples aside from *S. aureus*; (3) conduct microbiological analysis of other street food samples and food stalls that were sold in Barangay Ampayon, Butuan City and; (4) utilize other antibiotics for antimicrobial susceptibility testing for further analysis of antibiotic-resistant *S. aureus,* especially concerning MRSAs. To establish a link between street food contamination and vendors, an epidemiological study should be conducted.

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7 Author Contribution

V.D.B. Gaston conducted the study, collected data, performed the analysis, and contributed to the original draft. J.V. Rosen conceptualized the study design, supervised the project, and edited the manuscript. V.D.B. Gaston wrote the manuscript draft for the journal. All authors reviewed and approved the final version of the article.

8 Statement of Conflict of Interest

There is no conflict of interest among authors.

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