Antibiotic Sensitivity Pattern of *Staphylococcus aureus* Isolated from Various Dry Foods

Golam Md. Sarwar[1], Maruf Abony[2], Suvamoy Datta[2*]

Abstract

Background: *Staphylococcus aureus* is a well-known pathogen responsible for a range of clinical and localized infections, including nosocomial methicillin-resistant *S. aureus* (MRSA) infections. The emergence of vancomycin-resistant *S. aureus* (VRSA) strains has further complicated treatment options. Globally, food-borne diseases (FBD), including those caused by *S. aureus*, pose significant public health concerns. This study investigates the prevalence and antibiotic resistance of *S. aureus* in ready-to-eat packaged foods in Dhaka, Bangladesh, to better understand food safety risks. Methods: Samples of ready-to-eat packaged foods were collected from superstores in Dhaka and transported to the laboratory under appropriate conditions. Bacterial isolation was performed using standard methods on Mannitol salt agar medium, followed by incubation at 37°C for 24 hours. Biochemical tests, including the Catalase Test, IMViC tests, and Coagulase Test, were conducted for bacterial identification. Antibiotic susceptibility testing was performed using the Kirby-Bauer method with a range of antibiotics including amoxicillin, gentamicin, penicillin, chloramphenicol, ciprofloxacin, ceftriaxone, cephradine, nalidixic acid, erythromycin, and azithromycin. Results: The study found that resistance among *S. aureus* strains varied significantly, from 0% for gentamicin to 100% for amoxicillin. Gentamicin was the most effective antibiotic, with 100% sensitivity, followed by chloramphenicol with 95% sensitivity. Of the samples, 2.5% showed resistance to six antibiotics, 12.5% were resistant to at least five antibiotics, and overall, 27.5% of the strains were multidrug-resistant. These results align with findings from similar studies conducted in China and Greece. Conclusion: The study highlights the significant presence of multidrug-resistant *S. aureus* in ready-to-eat packaged foods in Dhaka. Preventing staphylococcal food poisoning requires strict adherence to hygiene practices, proper food storage, and thorough cooking. Education of food handlers on Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP) is essential to reduce contamination risks. Further research on the impact of hygiene on the development of food-borne illnesses is recommended to enhance food safety measures.

Keywords: *Staphylococcus aureus*, food-borne, multidrug resistance, antibiotic susceptibility, packaged food.

Significance | This study underscores the critical need for vigilance in monitoring and controlling S. aureus in packaged foods to mitigate public health risks.

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Introduction

Staphylococcus aureus, a Gram-positive bacterium, is one of the most significant pathogens responsible for a wide range of clinical and localized infections. These infections vary from mild skin conditions to severe diseases such as pneumonia, endocarditis, and sepsis. The adaptability of *S. aureus* has contributed to its persistence as a public health threat, particularly with the emergence of antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA). MRSA infections, particularly in

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hospital settings, have become a global concern, with increasing instances of community-acquired MRSA infections reported as well (Tong et al., 2015; Hassoun et al., 2017, Md. Eaktear et al. 2020).

The first cases of VRSA were identified in 1996 in Europe, Asia, and the United States, signaling a critical challenge in the treatment of *S. aureus* infections (Cong et al., 2020; Shariati et al., 2020). The rise of VRSA strains has further complicated the therapeutic landscape, rendering many conventional antibiotics ineffective and necessitating the development of alternative treatment strategies (Tiwari & Sen, 2006).

Its role in clinical infections, *S. aureus* is also a prominent cause of food-borne diseases (FBDs). The World Health Organization (WHO) defines FBDs as diseases caused by the consumption of contaminated food or water, and they remain a major public health concern globally (Le Loir et al., 2003). In the United States alone, *S. aureus* is estimated to cause approximately 241,000 cases of FBD annually (Scallan et al., 2006). The Institute of Medicine has highlighted the potential for foods to serve as a source of emerging or reemerging infections, given the multiple stages at which food safety can be compromised during production, processing, and handling (Cohen, 2000). The presence of FBD pathogens in ready-to-eat foods, meat, and meat products not only poses a significant health risk to consumers but also results in substantial economic losses due to product recalls, healthcare costs, and lost productivity (Syne et al., 2013; Sofos, 2008).

In Bangladesh, the prevalence of *S. aureus* in packaged foods remains underexplored, particularly concerning the presence of antibiotic-resistant strains such as MRSA and VRSA. This lack of information poses a significant gap in understanding the risk of exposure to these pathogens through food consumption. The increasing global incidence of antibiotic-resistant *S. aureus* strains underscores the importance of monitoring their prevalence in food sources, especially in developing countries where food safety regulations may be less stringent.

This study aims to address this gap by investigating the occurrence of *S. aureus* in ready-to-eat packaged food samples collected from superstores in Dhaka, Bangladesh. The study further characterizes the isolated *S. aureus* strains to determine their resistance to commonly used antibiotics. The findings of this research will provide valuable insights into the potential health risks associated with the consumption of contaminated foods in Bangladesh and contribute to the broader understanding of food safety and public health. Additionally, the study emphasizes the need for stringent food safety practices, including proper handling, processing, and storage, to prevent the contamination of food products with *S. aureus*. By identifying the prevalence and antibiotic resistance patterns of *S. aureus* in packaged foods, this research will inform public health strategies aimed at reducing the burden of food-borne diseases and enhancing food safety standards in Bangladesh.

Materials and Methods

Collection of Samples

A total number of samples ready-to-eat packaged food samples were collected from various superstores located in Dhaka, Bangladesh. The selection of samples was based on their popularity and availability in the market, covering a diverse range of food types including dairy products, meat products, and bakery items. Each sample was aseptically collected to prevent external contamination, and all sample containers were appropriately labeled with unique identifiers indicating the type of food, date of collection, and the specific superstore from which they were obtained.

To preserve the physicochemical properties and prevent the growth of microorganisms during transportation

The samples were immediately placed in insulated coolers containing ice packs. The temperature within the coolers was continuously monitored to ensure it remained below 4°C. Upon arrival at the laboratory, the samples were either processed immediately or stored at 4°C and analyzed within 24 hours to maintain their integrity (Akhi et al., 2019).

Inoculation of Microbes

The isolation of Staphylococcus aureus, each food sample was prepared under sterile conditions to minimize external contamination. Approximately 25 grams of each sample was homogenized in 225 mL of buffered peptone water using a sterile stomacher bag. The homogenate was then serially diluted, and 0.1 mL of each dilution was inoculated onto Mannitol Salt Agar (MSA) plates. MSA is a selective medium specifically designed to isolate *S. aureus*, as it inhibits the growth of most other bacteria while allowing *S. aureus* to thrive (Akhi et al., 2019).

The inoculated plates were streaked using the standard streak plate method to obtain isolated colonies.

The plates were then incubated at 37°C for 24-48 hours under aerobic conditions. *S. aureus* colonies were identified by their characteristic golden-yellow coloration on the MSA plates, indicating mannitol fermentation. Colonies with these characteristics were further sub-cultured on nutrient agar plates to obtain pure cultures for subsequent analyses (Abebe et al., 2013).

Biochemical Studies of the Isolated Bacterial Strains

The Presumptive *S. aureus* isolates were subjected to a series of biochemical tests to confirm their identity. The tests conducted included:

Catalase Test: To differentiate *S. aureus* (catalase-positive) from other Gram-positive cocci. A drop of hydrogen peroxide was added to a small amount of bacterial culture, and the presence of bubbles indicated a positive catalase reaction (Addo et al., 2011).

IMViC Test (Indole, Methyl Red, Voges-Proskauer, Citrate Utilization Tests): This set of tests helped to further characterize the isolates. For example, the indole test was used to detect the production of indole from tryptophan, while the citrate utilization test determined the ability of the isolates to use citrate as a sole carbon source (Adesiji et al., 2011).

Coagulase Test: Since *S. aureus* produces coagulase, which can clot plasma, this test is a definitive marker for its identification. Both slide and tube coagulase tests were performed using rabbit plasma, with clot formation indicating a positive result (Ahmadi et al., 2009).

The results of these tests were carefully documented, and only those isolates that tested positive for the majority of the characteristic *S. aureus* markers were selected for further analysis.

Antibiotic Susceptibility Testing the confirmed *S. aureus* isolates were tested for their susceptibility to a panel of commonly used antibiotics using the Kirby-Bauer disk diffusion method. The antibiotics tested included Amoxicillin, Gentamycin, Penicillin, Chloramphenicol, Ciprofloxacin, Ceftriaxone, Cephradine, Nalidixic Acid, Erythromycin, and Azithromycin.

A standardized inoculum of each isolate, equivalent to 0.5 McFarland standard, was prepared in sterile saline and swabbed uniformly across the surface of Mueller-Hinton Agar plates. Antibiotic-impregnated disks were then placed on the surface of the agar using sterile forceps, and the plates were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zones around each disk was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008, 2012).

Results and Discussion

Prevalence of Staphylococcus aureus in Packaged Foods

The study aimed to determine the prevalence of Staphylococcus aureus in packaged foods collected from superstores in Dhaka, Bangladesh. A total of samples were analyzed, and *S. aureus* was isolated from of these samples. The high prevalence of *S. aureus* in ready-to-eat foods highlights the potential public health risk associated with the consumption of contaminated foods. The ability of *S. aureus* to cause food poisoning is well-documented, particularly due to its capacity to produce enterotoxins that can lead to gastrointestinal illnesses even in the absence of live bacteria in the food (Aydin, Sudagidan, & Muratoglu, 2011).

The presence of *S. aureus* in packaged foods suggests possible contamination during food processing, handling, or storage. Given that *S. aureus* is a common inhabitant of the skin and nasal passages of humans, food handlers are often the source of contamination. This emphasizes the importance of strict hygiene practices during food preparation and packaging to minimize the risk of contamination.

Antibiotic Resistance Patterns

One of the significant findings of this study is the antibiotic resistance pattern observed in the *S. aureus* isolates. The antibiotic susceptibility testing revealed varying levels of resistance among the isolates. Notably, 100% of the isolates were resistant to amoxicillin, making it the least effective antibiotic in this study. This high level of resistance is concerning as amoxicillin is commonly used to treat *S. aureus* infections, and its ineffectiveness suggests the presence of resistant strains in the food chain (Hennekinne, De Buyser, & Dragacci, 2012).

On the other hand, gentamycin exhibited 100% sensitivity, making it the most effective antibiotic against the *S. aureus* isolates. This result aligns with studies conducted in other regions, such as China and Greece, where gentamycin was also found to be highly effective against *S. aureus* isolated from food sources (Yang et al., 2016; Sergelidis et al., 2012). Chloramphenicol was the second most effective antibiotic, with a sensitivity rate of 95%. The high sensitivity to gentamycin and chloramphenicol indicates their potential as alternative treatments for *S. aureus* infections, particularly in cases where resistance to other antibiotics is prevalent.

Multidrug Resistance

The study also identified a concerning level of multidrug resistance (MDR) among the *S. aureus* isolates. Approximately 27.5% of the isolates were classified as MDR, meaning they were resistant to five or more of the antibiotics tested. The presence of MDR strains in the food supply poses a significant public health challenge, as these strains are more difficult to treat and can lead to more severe and prolonged illnesses (Beyene, 2016).

Multidrug resistance in *S. aureus* is often associated with the acquisition of resistance genes through horizontal gene transfer. The use of antibiotics in agriculture and food production, as well as the overuse and misuse of antibiotics in clinical settings, are major contributing factors to the emergence and spread of MDR strains. The detection of MDR *S. aureus* in ready-to-eat foods underscores the need for better antibiotic stewardship and the implementation of strategies to reduce the spread of resistant strains (Bryant, 2017).

Comparison with Global Studies

The findings of this study are consistent with global trends in the prevalence of antibiotic-resistant *S. aureus* in food products. For example, studies in China have reported similar levels of resistance to commonly used antibiotics, with high rates of MDR strains observed in ready-to-eat foods (Yang et al., 2016). Similarly, research in Greece has highlighted the presence of antibiotic-resistant *S. aureus* in various food products, raising concerns about the potential for foodborne transmission of resistant strains (Sergelidis et al., 2012).

These studies, along with the present research, demonstrate that antibiotic-resistant *S. aureus* is a global concern, and efforts to monitor and control its spread should be prioritized. The implementation of international standards and guidelines for antibiotic use in food production, as well as enhanced surveillance of antibiotic resistance in foodborne pathogens, are critical steps in

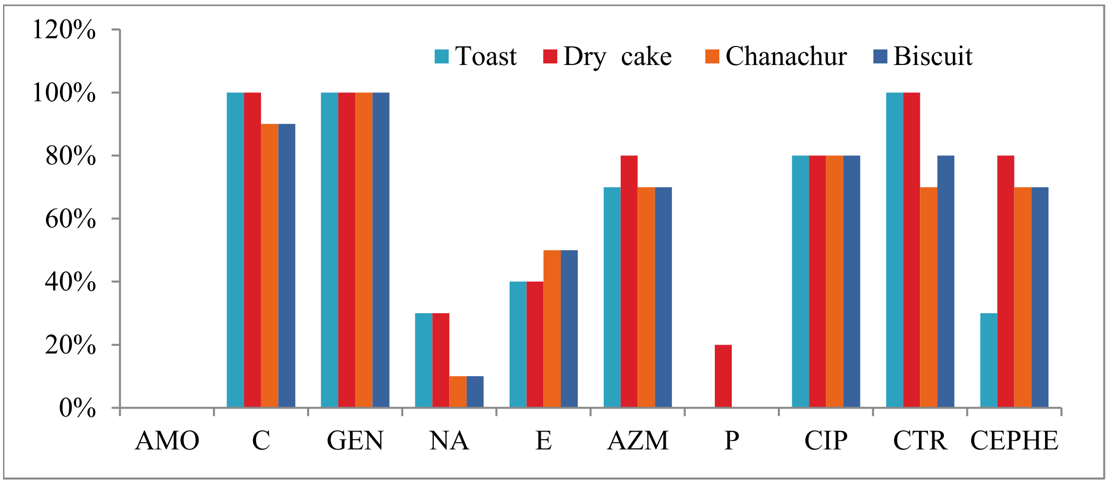

Figure 1. Comparative analysis of sensitivity (%) of *S. aureus* to antibiotics among age & sex groups

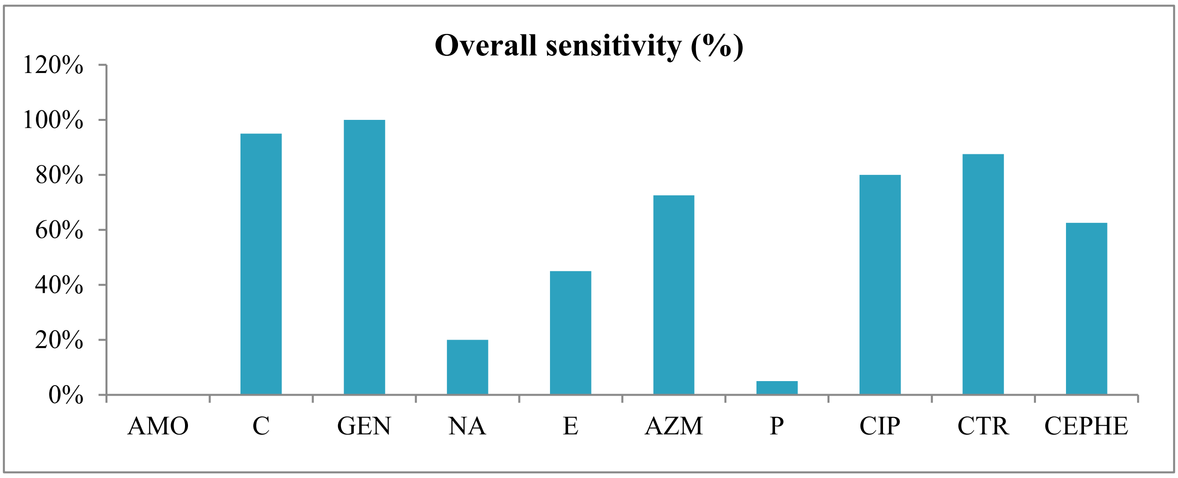

Figure 2. Overall Sensitivity (%) of *S. aureus* to each antibiotic in different age & sex groups

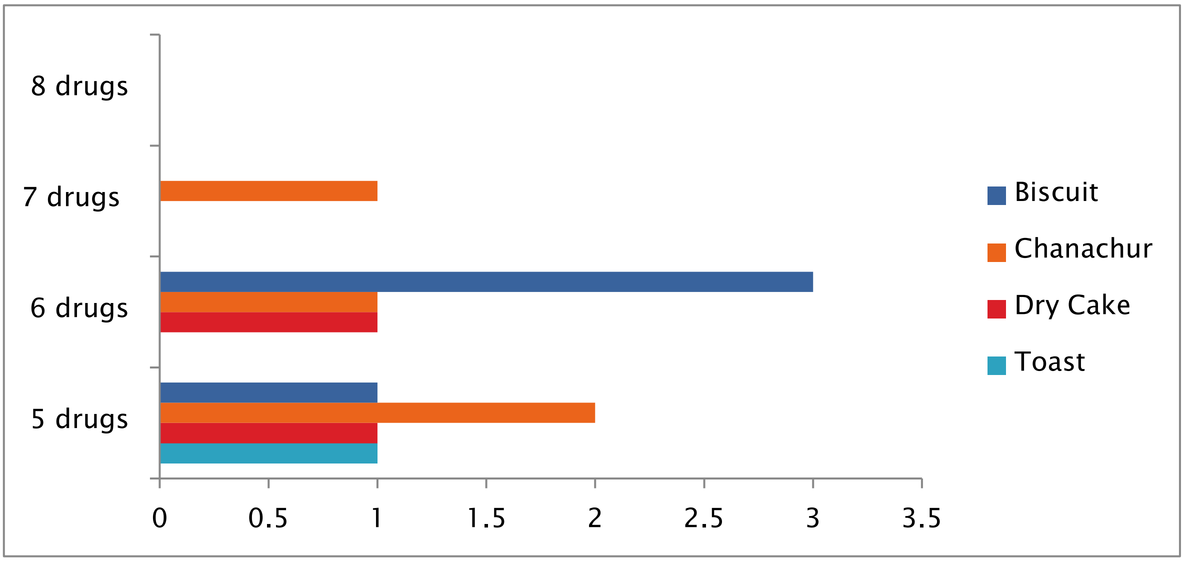

Figure 3. Multi-drug resistance (%) patterns of *S. aureus* in different age & sex groups

addressing this issue (Clinical and Laboratory Standards Institute [CLSI], 2008).

Implications for Public Health and Food Safety

The detection of antibiotic-resistant *S. aureus* in packaged foods has significant implications for public health and food safety. *S. aureus* is capable of causing a wide range of infections, from mild skin infections to life-threatening conditions such as septicemia and endocarditis. The presence of resistant strains in the food supply increases the risk of infections that are difficult to treat, particularly in vulnerable populations such as the elderly, young children, and immunocompromised individuals (Kadariya, Smith, & Thapaliya, 2014).

The health risks posed by antibiotic-resistant *S. aureus*, there are also economic implications. Foodborne illnesses can lead to significant economic losses due to healthcare costs, lost productivity, and food recalls. The presence of resistant strains further exacerbates these costs, as infections caused by MDR pathogens typically require more expensive and prolonged treatments (Clinical and Laboratory Standards Institute [CLSI], 2012).

To mitigate these risks, it is essential to implement strict food safety measures throughout the food production and supply chain. This includes adherence to microbiological guidelines such as Hazard Analysis and Critical Control Points (HACCP), Good Manufacturing Practices (GMPs), and Good Hygienic Practices (GHPs). Ensuring that food handlers are properly trained in hygiene practices and that food processing environments are regularly sanitized can help reduce the risk of contamination with *S. aureus* (Ekici, Bozkurt, & Isleyici, 2004).

Role of Cold Chain Management

The importance of maintaining an effective cold chain cannot be overstated in the prevention of *S. aureus* growth in food products. *S. aureus* is known to multiply rapidly at room temperature, and improper storage of food can lead to the production of enterotoxins, which are resistant to heat and can cause food poisoning even if the bacteria are subsequently killed by cooking or pasteurization (El-Gedawy, Ahmed, & Awadallah, 2014). Therefore, keeping food products at the correct temperatures throughout storage and transportation is crucial in preventing the growth of *S. aureus*.

Cold chain management should be coupled with regular monitoring and control of other critical factors such as raw ingredient quality, processing conditions, and equipment sanitation (Gooraninejad, Ghorbanpoor, & Salati, 2007). For instance, controlling the salt content in certain foods, as *S. aureus* can thrive in high-salt environments, is another important aspect of preventing contamination. Additionally, implementing stringent controls over raw ingredients, particularly those at high risk of contamination, can significantly reduce the chances of introducing *S. aureus* into the food supply (Hamid et al., 2017).

Future Directions and Recommendations

Given the findings of this study, there are several key areas where further research and action are needed. First, there is a need for more comprehensive surveillance of antibiotic-resistant *S. aureus* in the food supply, both in Bangladesh and globally. This should include regular monitoring of a wide range of food products, as well as the collection of data on the prevalence and types of resistance present.

Second, there should be a focus on developing and implementing more effective strategies for controlling the spread of antibiotic-resistant *S. aureus*. This includes not only improving hygiene practices in food production but also addressing the broader issue of antibiotic use in agriculture and food production. Reducing the use of antibiotics in these settings, and promoting alternatives such as probiotics or bacteriophage therapy, could help to limit the emergence and spread of resistant strains.

Finally, public awareness campaigns are needed to educate consumers and food handlers about the risks associated with antibiotic-resistant *S. aureus* and the importance of food safety practices. By raising awareness and promoting good hygiene practices, it is possible to reduce the incidence of foodborne illness and the spread of resistant bacteria.

Conclusion

The study highlights the significant presence of antibiotic-resistant Staphylococcus aureus in packaged foods in Dhaka, Bangladesh, emphasizing the associated public health risks. The detection of multidrug-resistant (MDR) strains underscores the growing challenge of managing foodborne pathogens that are increasingly difficult to treat. The findings call for urgent improvements in food safety practices, particularly in maintaining cold chain integrity and adhering to strict hygiene protocols during food processing and handling. Furthermore, the study advocates for enhanced surveillance of antibiotic resistance in foodborne pathogens and the implementation of better antibiotic stewardship practices in both clinical and agricultural settings. Public awareness campaigns on food safety and hygiene are also crucial in preventing contamination and reducing the incidence of foodborne illnesses. By addressing these areas, the risks posed by antibiotic-resistant *S. aureus* in the food supply can be mitigated effectively.

Author contributions

S.D. conceptualized and developed the methodology; M.A. prepared the original draft and collected; G.M.S. reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

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