Staphylococcus aureus: A Common Contaminant of Coleslaw in Local Ras-Beirut Restaurants

Adam Fawaz, Ali Rteil,Malek Itani and Tarek Na'was*

Natural Sciences Department, Lebanese American University
Beirut - Lebanon

*Corresponding author's email: tnawas [AT] lau.edu.lb

ABSTRACT---- ***Staphylococcus aureus isa gram positive coccus thathas long been associated with many serious infections amongst which foodborne illnesses are common. The enteric pathogenicity of this bacterium is mediated mainly by its production of various enterotoxins. The role of S.aureus in causing numeroustypes of infections and its ability to rapidly become resistant to antibacterial agents have shadowed its associationwith food poisoning. This study, which aimed at detecting the prevalence of S.aureusin coleslaw, a salad commonly served in the restaurants in the Ras-Beirut area, revealed alarming results. Of the 37 samples tested, 32 (86.5%) were positive for the presence of S.aureus. Although the isolated strains were not tested for enterotoxin production, yet such a high prevalence is a clear indicator of the lack of application of proper sanitary protocols in the tested food outlets.***

1. INTRODUCTION

Staphylococcus species have long been associatedwith foodborne illness, specifically after 1884, when spherical organisms in cheese caused a large food-poisoning outbreak in the United States. Multiple outbreaks attributed to the consumption of staphylococcal-contaminated foods occurred in France in 1894, Michigan in 1907, and the Philippines in 1914. In 1930 Dack and his colleagues at the University of Chicago were able to demonstrate that the cause of food poisoning resulting from the consumption of contaminated sponge cake with cream filling was a toxin produced by an isolated S*taphylococcus* sp.[1].
S. aureus is a gram-positive, facultative,non-motile and capsulated coccus [2]. It has an optimum temperature for growth of 35°C, and an optimum pH between 7.0 and 7.5 [2]. Most strains of *S.aureus* are highly tolerant to the presence of salts and sugars and can grow over an Aw range of 0.83 to 0.99.

S. aureus is capable of producing a large number of extracellular enzymes, toxins, and other chemical components [2]. It has been shown that *S. aureus* is capable of producing at least 34 different extracellular proteins. Two enzymes that have been the most useful in the identification of *S. aureus* are coagulase, a soluble enzyme that coagulates plasma, and thermonuclease (TNase), which is a heat stable phosphodiesterase that can cleave either (DNA) or (RNA). Of the various metabolites produced by the *staphylococci*, the enterotoxins, pose the greatest risk to consumer health.

Staphylococcal enterotoxins are proteins produced by some strains of staphylococci [3], which, if allowed to grow in foods, may produce enough enterotoxin to cause illness when that contaminated food is consumed. These enterotoxins are produced primarily by *S. aureus*, although *Staphylococcus intermedius*, *Staphylococcushyicus*, and the coagulase-negative *Staphylococcus epidermidis* were reported to each having caused at least one food poisoning outbreak [4]. These incidents support testing staphylococci other than *S. aureus* for enterotoxigenicity if they were present in large numbers in a type of food suspected of causing a food-poisoning outbreak.

Foods that are frequently incriminated in staphylococcal food poisoning include meat and meat products, poultry and egg products, salads such as egg, tuna, chicken, potato, macaroni, and other mayonnaise rich salads including coleslaw[5]. Milk and dairy products are also contaminated frequently. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are also frequently involved in staphylococcal food poisoning. Many of these foods are contaminated during preparation in homes or food service establishments. Although the potential is there, it is only when incomplete fermentation (e.g., lactic acid failure) takes place that the development of staphylococcal enterotoxin occurs.

The aim of this study was to isolate and identify the presence of *S.aureus* in coleslaw samples obtained from different restaurants in the Ras-Beirut area of Beirut, the capital of Lebanon.

2. MATERIALS AND METHODS

Coleslaw was chosen as the salad to be tested because it is served with various dishes in the selected restaurants and because mayonnaise, a major component of coleslaw, is known to be an excellent medium in which bacteria can grow comfortably.The protocols used in this study are those recommended by the US food and drug administration [6-7].

Sample Collection:

Thirty seven coleslaw samples were collected from 37 different restaurants from the Ras Beirut area, including local and global franchises. The samples were bought on the spot, just as they are given to regular customers. The purchased food was dispensed in specific containers belonging to the restaurants, and transported quickly and within 15-20 min to the microbiology laboratory for processing. Opening and weighing food samples were done using an aseptic technique to avoid any external contamination. The samples were then numbered and the work was done using the assigned numbers to avoid bias during testing.

Sample Processing:

Portions of 25 g taken from the samples were placed in a blender containing 225 ml of trypticase soy broth (TSB) with 10% NaCl. They were homogenized for 2 mins at high speed. Then, usingan aseptic technic, 10 ml were pipetted into each of 3 sterile tubes for every food sample, and placed in an incubator at 37^{o}C for 18-24 hours. Only a very few organisms such as *S.aureus* can withstand such high salinity and therefore selection for *S.aureus*was favored. After incubation and proper mixing, a loop was used to withdraw a sample from each tube for subculture on a Mannitol Salt Agar (MSA) plate, thus three replicates for each sample were obtained. Organisms with a yellow halo surrounding their colony are suspected to be *S.aureus* as this selective differential medium has a high salinity of 7.5% NaCl which is tolerated by *S.aureus* and contains mannitol which is fermented by the organism (red to yellow change in color). Thesuspected organisms were gram stained and subcultured on Nutrient Agar (NA) plates and slants, which allowed for the growth of the characteristic golden colored typical colony. The slants were used to preserve the organisms for further testing.

Biochemical identification of isolates:

All the suspected isolates were gram stained and then definitivelybiochemically identified using the standard catalase, coagulase (slide and tube) and TNase tests as recommended [8-9].

3. RESULTS AND DISCUSSION

Of the 37 samples tested, 32 (86.5%) were positive for the presence of *S. aureus*, while only 5 of the samples tested were negative for the presence of the organism. Although more than one species of the genus *Staphylococcus* have been found to produce dangerous enterotoxins and were responsible for outbreaks in Europe and the US in the past decade [10], yet,*S. aureus* remains the most dangerous of the group.

The growth and proliferation of *Staphylococcus aureus* in foods presents a potential hazard to consumer health since many strains of *S. aureus* produce enterotoxins. The primary reasons for examining foods for *S. aureus* and/or their toxins are to confirm that this organism is the causative agent of a specific food-poisoning episode, determine whether a food or ingredient is a source of enterotoxigenic*staphylococci*, and demonstrate post processing contamination[3]. Post processing contamination is usually due to human contact with processed food or exposure of food to inadequately sanitized food processing surfaces. This represents a potential hazard because of the absence of competitive organisms that might otherwise restrict the growth of *S. aureus* and subsequent itsproduction of enterotoxins.

After culturing the sample/TSB homogenates on Mannitol Salt Agar, all, but 5 samples, showed growth of isolated colonies surrounded by a distinct yellow halo. A definitive negative for any single plate was established after leaving the plates to incubate at room temperature for an additional 24 hours. It is worth noting that the isolates that gave a negative slide coagulase result were also tested by the tube coagulase test to ensure that neither bound nor free coagulase is produced.

The isolation of*S. aureus*from processed foods usually indicates contamination from the skin, mouth, or nose of food handlers. This contamination may have been introduced directly into foods by process line workers with hand or arm lesions caused by *S. aureus* coming into contact with the food, or by their coughing and/or sneezing, which is a common symptom of respiratory tract infections. Contamination of processed foods may have also occurred when deposits of contaminated food collect on or are adjacent to the processing surfaces to which the food products are exposed. Large numbers of *S. aureus* encountered in processed food infer that sanitation, temperature control, or both were inadequate.

The result that 32 of the 37 tested samples were positive for *S. aureus* is alarming and a definitive proof of bacterial contamination of the specimens of coleslaw tested, as *S. aureus* is considered a general indicator of hygiene and general sanitation.The 86.5% contamination rate is an indicator of a generalized trend of a poor sanitation among food outlets,

which may translate into an increased incidence of food poisoning. The significance of the presence of *S.aureus* in food should be interpreted with caution. The presence of a large number of the organism in a specific type of food is not a sufficient cause to incriminatethat type of food as the vector of food poisoning as not all *S.aureus* strains produce enterotoxins [11]. The potential for staphylococcal intoxication cannot be asserted without testing the enterotoxigenicity of the *S.aureus* isolates and, more importantly, demonstrating the presence of staphylococcal enterotoxin in food. Neither the absence of *S.aureus* nor the presence of small numbers of the organism can provide complete assurance that the tested food is safe. Conditions inimical to the survival of *S.aureus* may result in a diminished population or death of viable microbial cells, while sufficient toxin remains to elicit symptoms of staphylococcal food poisoning.

As for the possible sources of contamination, human, animal, or environmental sources may be held responsible. *S.aureus* is a known inhabitant of the normal flora of human skin; As such, methods of transmission may include contact with naked skin while preparing food. Moreover, *S.aureus* is also a known inhabitant of the nasal cavity [12], because staphylococci adhere well to cells scraped from the anterior nares where surface glycoproteins and proteoglycans, present on the mucous membranes, contribute to the adhesion of bacteria. Thus a carrier employee, whether transient or permanent, may also be the contributor to contamination. There is a significant variation among individuals, with higher rates of staphylococcal binding being observed to cells from carriers than to non-carriers of the organism, to older babies than neonates in the first week of life, influenza A virus-infected volunteers than control uninfected individuals, and to moderately ill geriatric patients than to seriously ill elderly patients [13].

The environment of the working surfaces may also be responsible, since an unhygienic (dusty) surface may carry unwanted contaminants such as the organism in question. In addition, the potential for enterotoxin development is greater in foods exposed to temperatures that permit the growth of *S. aureus*.
A lack of understanding in many food service settings concerning the transmission and growth of pathogens can lead to potential foodborne outbreak situations.This gross error in hygiene should have been identified by the management, and appropriate information should have been relayed to employees.

Several possible explanations arise from this study. Considering that all the samples were collected from the same area, a possible explanation would be that the Ras-Beirut area houses a common reservoir for *S.aureus* that serves as a common source of contamination. This may be examined by studying the genetic relatedness of the bacteria in question (which is planned to be done). Considering the above, the particular source of contamination may be the cause of an increased frequency of *S.aureus* carriers who would then serve as vectors for consequent food contamination.
As a conclusion, one must state valid recommendations to ensure maintaining high standards of personal and food hygiene when storing, handling and pre-paring food. It is important to remind of the "four Cs" recommended by the Food Safety Agency: cleaning, cooking, chilling and avoiding cross-contamination(14).Care must be taken when handling food, this includes storing raw foods separately from other foods, preventing food items from touching or dripping onto other foods andasking food handlers to wear gloves and hair nets at all times in order to minimize exposure to possible S.*aureus*from carriers. Working surfaces, equipment and utensils must all be kept clean and void of dust and food remnants at all times andthe use of refrigeration and storage conditions should be standardized in order to minimize growth and spread of *S.aureus* as well as other common bacterial contaminants [14].Testing food handlers for nasal carriage of *S.aureus*is recommended, to prevent airborne contamination, as is stressingon the importance of hand-washing at all times, as this has proved to be the most effective single measure to minimize cross –contamination by *S. aureus* and other bacteria.

4. REFERENCES

1. Bergdoll, MS. Staphylococcal food poisoning. In: DO Cliver, DO, ed. Foodborne Diseases. San Diego: Academic Press, Inc., 1990, pp. 85–106.

2. Martin, SE and Myers, ER. *Staphylococcus aureus*. In: Hiu, YH, Gorham, JR, Murrell, KD, Cliver, DO, eds. Foodborne Disease Handbook, Diseases Caused by Bacteria. New York: Marcel Dekker, 1994, pp. 345–394.

3.Bergdoll, MS. The enterotoxins. In: Cohen, J, ed. The Staphylococci. New York: Wiley-Interscience, 1972.

4. Breckinridge,JC, Bergdoll, MS. Outbreak of foodborne gastroenteritis due to a coagulase negative enterotoxin producing staphylococcus. N Engl J Med 248:541–543, 1971.

5.Centers for Disease Control Surveillance Summaries 1993–1997, MMWR, U.S. DHHS, Atlanta, GA. Centers for Disease Control and Prevention, 2000, 49, #SS-1.

6. Andrews, WH, Hammack, TS. 1998. Food Sampling and Preparation of Sample Homogenate (Chapter 1) In: FDA Bacteriological Analytical Manual. Revision A. 8th ed., US Food and Drug Administration. Silver Spring, MD. USA.

7.International Commission on Microbiological Specifications for Foods. 1986. Microorganisms in Foods. 2. Sampling for Microbiological Analysis:Principles and Specific Applications, 2nd ed. University of Toronto Press, Toronto, Ontario, Canada.

8. Cowan, S T, and Steel, KJ. 1965. Identification of medical bacteria. University Press, Cambridge, MA.

9. Bartelt, M, 2000. Diagnostic bacteriology, a study guide. F. A. Davis Co., Philadelphia, PA. USA.

10. D.G. Hoover, DG, Tatini, SR, Maltais, JB. (1983) Characterization of staphylococci, Appl Environ Microbiol, 46 :649–660

11. M. Jett, M, Ionin, B, Das, R, Neill, R. (2001) Thestaphylococcal enterotoxins. Sussman, M, (Ed.), In: Molecular medical microbiology, Academic Press, San Diego, CA, USA (2001), pp. 1089–1116.

12. Aly, R., Shinefield, HR, Litz, C, and Maibach, HI. (1980). Role of teichoic acid in the binding of Staphylococcus aureus to nasal epithelial cells. J. Infect. Dis. 141:463–465.

13. Boelaert, J. R., Van Landuyt, HW, and Godard, CA.. (1993). Nasal mupirocin ointment decreases the incidence of S. aureus bacteremias in hemodialysis patients. Nephrol. Dial. Transplant. 8:235–239.

14. Food Standards Agency (FSA-UK). Preventing food poisoning. In: Food poisoning – Prevention. http://www.nhs.uk/Conditions/Food-poisoning/Pages/Prevention.aspx