



Review Article

PREVALENCE AND CHARACTERIZATION OF *Staphylococcus aureus* ISOLATED FROM RETAIL VEGETABLES

FULARA V., SINGH J.K., VERMA S., TYAGI A. AND CHATTERJI A.*

Department of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, 201206, Chaudhary Charan Singh University, Meerut, 250001, Uttar Pradesh, India

*Corresponding Author: Email - arjun.chatterji@iamr.ac.in

Received: April 16, 2020; Revised: May 06, 2020; Accepted: May 07, 2020; Published: May 30, 2020

Abstract- Foodborne diseases are a major global health concern. There are many bacteria which cause toxicity and create major health issues. It can even destroy one's immune system and result in death. Out of all these bacteria, "*Staphylococcus aureus*" is one of the most prevalent bacterium which causes major foodborne diseases in vegetables and food from plant origins. *S. aureus* forms complex communities with unwanted bacteria in multispecies biofilms. To enhance the safety of the food products we need to work on improving hygiene conditions, especially in the food sectors. Particularly we need the removal of bacterial biofilms which adhere to food. Symptoms of *Staphylococcus aureus* food diseases (SFD) include nausea, vomiting, and abdominal cramps with or without diarrhoea. Preventive measures include safe food handling and processing practice, maintaining cold chain, adequate cleaning and disinfection of equipment and prevention of cross-contamination in home and in the kitchen. This study aims to evaluate the presence and contamination rate of *Staphylococcus aureus* in fresh food and vegetables in the markets of our country, along with providing a brief overview of SFD, contributing factors and the threat that it imposes on the consumers. The genes encoding for toxins will be identified by Polymerase Chain Reaction (PCR), however there is a limitation in using this technique as it does not show the expression of genes that encode for new toxins.

Keywords- PCR, SFD, Bacterium

Citation: Fulara V., et al., (2020) Prevalence and Characterization of *Staphylococcus aureus* Isolated from Retail Vegetables. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 12, Issue 5, pp.-1828-1831.

Copyright: Copyright©2020 Fulara V., et al., This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Academic Editor / Reviewer: Takemi Otsuki, Dr Mukundraj G. Rathod, Kumar D Shiva, Nishant Bhanu, Savita Kate

Introduction

Staphylococcus aureus is a pathogenic bacterium that causes major foodborne illnesses and several other diseases with symptoms like nausea, vomiting, diarrhea and abdominal pain.[1]. It has been shown that these bacteria is the most prevalent one that can cause intoxication and gastroenteritis worldwide [2].

There are various virulence factors which are linked with the pathogenesis of *S. aureus* strains [3]. Some of them are hemolysins, hyaluronidase, lipases, leukocidin, exfoliatin and thermo nuclease [4], which cause invasion in tissues of the host cell. The major virulence factors are heat-stable enterotoxins (SEs) that create food-poisoning syndrome or foodborne related symptoms in the human body. The toxin 'Toxic shock syndrome (TSST-1)' which is found in *S. aureus* impairs the immune system of a host leading to a pathetic condition.

Staphylococcal enterotoxins (SEs) are mainly associated with food poisoning outbreaks. It is shown that about 95% of staphylococcal food poisoning effects are caused by the classical SE (SEA~SEE) [5]. Human beings are mostly dependent on vegetable consumption for better survival, they are essential to the human diet. The presence of *S. aureus* in retail food has been reported previously in China [6] and in some retail vegetables, especially in tomato, cucumber, lettuce and caraway which are mostly consumed raw. The genetic background of *S. aureus* isolates is determined according to their antibiotic susceptibility profiles, enterotoxin genes, spa types and MLST types in China. The harvesting processes and requirements contribute highly to the contamination of fresh products. For example, in Pakistan, a quarter of fresh products are irrigated with waste water. The lack of thorough cooking in fresh cuisine can result in foodborne diseases when a pathogen contaminates it. To avoid foodborne illness while consuming fresh vegetables and salad it's important to be aware regarding the hygiene of consumed food [7]. This is due to the production of one or more toxins by the bacteria during growth at permissive temperatures [8]. However, the incubation period of SFD depends on the amount of toxin ingested [9].

Very small doses of SEs can cause SFD. Another hazard is the tendency of these bacteria to form complex communities with unwanted bacteria in multispecies biofilms. Biofilms are richly packed communities of microbes which adhere to the surface of some objects and surround themselves with secreted polymers also known as the slime layer.

Diverse types of food serve as an optimum growth medium for *S. aureus*. Foods that are frequently implicated in SFD are meat products, poultry and egg products, milk and related products, bakery products like creamy pastries and cakes, sandwich fillings and salads [10]. Foods associated with SFD vary from country to country, particularly due to varying consumption and food habits. If food is prepared centrally and circulated widely, SFD outbreaks can have grave consequences impacting thousands of people. For example, over 13,000 cases of SFD occurred in Japan in 2000 as a result of contamination of milk at a dairy-food production plant [11].

Review of Literature

Staphylococcal aureus Enterotoxins

S. aureus produces a wide array of toxins. Staphylococcal enterotoxins (SEs) are a family comprising nine major serological types of thermostable enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, and SEJ) which are a part of the class of pyrogenic toxin superantigens [12]. These cause super antigenic activity like immune suppression and nonspecific T-cell proliferation [13]. SEs are highly stable, heat-resistant and unyielding to environmental conditions such as freezing and drying [14]. They do not lose their functionality on exposure to the enzymes and low pH in the gastrointestinal tract [15]. The thermostable characteristic of *S. aureus* imposes a significant threat in food industries [16]. It was determined that the counts of *S. aureus* were not negatively impacted by heat.

Environmental surfaces which came in contact with pre-packaged foods that were contaminated with *S. aureus* during slicing and packaging, harboured *S. aureus*. *S. aureus* was also frequently found on food handler's gloves [17]. Pathogenic microbes adhering to the surface of the gloves worn by retail food employees may serve as a source of cross-contamination if not changed frequently. The practice of wearing gloves without proper hand washing can contaminate both the interior and exterior of the gloves. Hand washing is often neglected when gloves are used, which may promote rapid microbial growth on the hands as gloves provide a warm, moist environment for bacterial growth [18]. Hand-washing is an easy method of preventing many microbial contaminations [19].

Antimicrobial resistance mediated by Mobile Genetic Elements (MGEs)

Substances which kill or restrict the proliferation of microorganisms are called antibiotics. These are one of the greatest boons to medicine and mankind which are used to treat a wide array of infectious diseases caused by bacteria in animals and human beings [20]. Abuse of existing antibiotics contributes to the spread of antibiotic resistance and poses a predicament for the treatment of several bacterial infections, including therapy for individuals with food poisoning. From a demonstration study conducted on 1,739 *Staphylococcus* isolates from a hospital in Guangzhou, China, from 2001 to 2010, antimicrobial resistance of tested drugs (exclusively for teicoplanin and vancomycin) was generally observed among the isolates examined, with high resistance rates for β -lactamases (94.0% and 73.7% for penicillin and oxacillin, respectively) and resistance percentages for cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, trimethoprim-sulfamethoxazole, and tetracycline ranging from 83.9% to 19.4% [21]. Consequentially, antibiotic resistance in microorganisms continues its baleful effect in global public health, with methicillin-resistant staphylococci (MRS) strains representing one important group, commonly considered as "super bugs" [22]. Since their first discovery in 1961, MRS (including MRSA and MRCNS) have become among the most prevalent pathogens causing nosocomial infections throughout the world [23]. With the first report of an MRSA-mediated gastrointestinal illness outbreak [24], MRS strains have been considered a major contributor to both health-care-associated and foodborne illnesses. MRS had been identified from contaminated food samples, such as milk, pork, chicken, veal, beef, turkey, and lamb meat [25] as well as in food production animals, such as cattle, chickens, pigs, and cows and are closely connected with the newly discovered MRSA designated as livestock-associated MRSA (LA-MRSA) [26]. Carriage of MRS strains in a wide variety of food and food production livestock may not be limited to only food hazard but also poses a significant occupational risk for the industrial staff, such as unaffected individuals, handlers and asymptomatic carriers. MRS strains are neither susceptible to most β -lactam antibiotics nor multiple other drugs due to the *mecA* and other resistance genes carried by an MGE designated as staphylococcal cassette chromosome *mec* (SCC*mec*). Additionally, the role of integrons as a mobile genetic mechanism in horizontal transfer of antibiotic resistance has also been well established [27].

Mobility and evolution of MGEs in staphylococci

MGE is a commonly found gene with an antibiotic resistance gene (*mecA*) and site-specific recombinase genes (*ccrA* and *ccrB*). SCC*mec* has been divided into 11 types. There are a variety of subtypes and variants which show an important role in antibiotic resistance, molecular epidemiology, and development of staphylococci. Through recognition of recombination sites (*attB*, *attSCC*, *attI1*, *attC*, secondary sites, etc.) and via this site-specific recombination event, MGEs are competent of capturing foreign genes. The mobility of MGEs is stated as being associated with mobile DNA elements (transposons or plasmids) and antibiotic resistance genes in addition to having a small collection size and substantial heterogeneity in recombination sites [28]. Southern hybridization analysis performed by Alam M revealed that 58 staphylococci isolates were found to harbor one copy of class 1 integron on the chromosomal instead of plasmid DNA compared with their frequent location on plasmids for facilitation of conjugative-mediated transfer [29]. As natural capture systems and assembly platforms, MGEs in *Staphylococcus* (SCC*mec* or integrons system) allow bacteria to incorporate foreign genes and convert them to functional proteins by ensuring the correct

expression. Despite affinity for self-transposition, integron systems are commonly associated with the transposons and conjugative plasmids serving as vehicles for the intra- and interspecies transmission of genetic material as well as gene cassettes capable of mobilizing to other integrons or to secondary sites in the bacterial genome [30]. This event has been regarded as a key mechanism in the dissemination and spread of resistance genes responsible for the swift spread of resistance genes and the rapid evolution of resistance to a wide range of unrelated antibiotics among diverse bacteria [31]. Any ORF existing in the environmental "gene pool" is conceivably capable of being structured into the bacterial genome through the recombination platforms, and MGEs consequently have the potentially limitless capacity to exchange and amass functional genes, which enables rapid adaptation to selective pressure and may ultimately bestow additional fitness and advantage to the bacterial host. Additionally, a horde of MGEs (such as conjugative plasmids, transposons, insertion sequences, and even entire chromosome) and the captured genes comprise the vast reservoirs of integrons and lead to the longstanding concept of a single massive "gene pool" that is available and temporally shared among bacteria. The common observation of MGEs in microorganisms from the general environment and its enormous sequence diversity detected from such microbes, as well as various products unrelated to antibiotic resistance, strongly suggests that MGEs are ancient genomic structural elements and have played a general role in evolution and adaptation for a considerable period of time [32]. As a genomic island (G island) and MGE demarcated by a pair of DRs and inverted repeats, SCC*mec* has a set of site-specific recombinase genes (*ccrA* and *ccrB*) required for its movement and is inserted at the 3' end of *orfX* and located adjacent to the replication origin [33]. In the chromosome of staphylococci, SCC*mec* may have evolved from a primordial mobile element SCC, into which the *mec* complex was inserted. However, the function of the putative SCC*mec* may not be limited as the conveyor of antimicrobial resistance (mediated by *mec* complex) alone, and this MGE may serve as a carriage for the exchange of beneficial genes for improving the chances of survival of staphylococci in various environments. In addition, SCC*mec* is a general genetic information exchange system of staphylococci with *ccrA* and *ccrB* involved in the recombination events (integration and excision), which plays a significant role in the evolution of *Staphylococcus*. MGEs serve as the reservoir for various genes and possess the function of interspecies genetic exchange. It is interesting to speculate whether multiple MGEs carried by staphylococci would speed up the rate of gene exchange or genome evolution, although these hypotheses require further investigation. From previous surveys, the influence of carriage of multiple MGEs on antimicrobial resistance had been investigated in MRSA. The presence of multiple MGEs was found to be strongly correlated with antimicrobial resistance, including erythromycin, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole, which further limits the therapeutic options for deep-seated *Staphylococcus* infection and diseases. For treatment of complicated *Staphylococcus* infections, gentamicin is commonly prescribed by many clinicians in combination with vancomycin due to enhanced efficacy based on synergistic antibacterial activity [34]. For penicillin-allergic patients, erythromycin has been frequently used. As the first choice for suspected CA-MRSA cutaneous infections, trimethoprim-sulfamethoxazole has also been commonly used in combination with rifampin for MRSA in carriers despite the high recurrence (up to 50%) and frequent emergent resistance of this organism.

Prevention

SFD is preventable. Consumers need to be aware of potential food contamination in home and during cooking in kitchen. Cooking food thoroughly is important, but preventing contamination and cross-contamination and maintaining critical points are the most effective ways to prevent SFD [35]. Since research findings and outbreak investigations have suggested that SFD is largely due to faulty food handling practices, knowledge and skills in food industry workers are warranted. Nevertheless, public health intervention should be designed to prevent *S. aureus* from pre- and post-harvesting in food processing facilities. Public awareness regarding safe vegetables and fruits handling would help to prevent cross-contamination as well as potential colonization of handlers from contaminated food products.

Other public health interventions such as personalized and tailored food safety education program targeting diverse socio demographic people could be a keystone in preventing the SFD outbreak [36].

Conclusion

As all human beings are highly dependent on food ingestion for basic living, for making our lives healthy we require vitamins and nutrients which we can receive from fresh vegetables, fruits and food. Therefore, quality of food should become our major concern. A number of illnesses are reported from food borne diseases. Food borne microorganisms and bacteria create complex interactions with the ecosystem and many other environmental factors are associated with food safety [6]. In countries, particularly those having very low rain fall, irrigation of salads and vegetables is done with waste water and this may lead to several foodborne diseases. For example, in Quetta region, due to insufficiency of water, farmers grow leafy green vegetables and salads in untreated waste water which is full of microbial contaminants and such practices make the food unhealthy and create food borne illness. *S. aureus* can be eradicated by exposure to heat and by competition with other flora in pasteurized and fermented foods respectively. SEs produced by *S. aureus* still possesses the capacity of causing SFD because of their thermostability [14]. This fact should be considered in risk evaluation and devising appropriate public health interventions. Prevention of *S. aureus* contamination from farm to fork is crucial. Current findings of high prevalence of *S. aureus* including MRSA in raw retail vegetables pose a potential hazard to consumers, both as classic SFD and as a potential source of colonization in food handlers.

Application of review: The lack of a sufficient quantity of quality literature impedes general awareness leading to improper food storage and subsequent proliferation, growth, metabolism and contamination by *Staphylococcus aureus* [37]. This review paper is an effort to promote awareness about the hygienic conditions for safe food storage and handling. Additionally, knowledge of the route of entry and the neurotoxic pathogenesis of *Staphylococcus aureus* can help greatly to diagnose current and future outbreaks [38].

Review Category: Microbial Biotechnology

Abbreviations:

SFD: *Staphylococcus aureus* Food Diseases
 PCR: Polymerase Chain Reaction
 SE: Staphylococcal Enterotoxin
 TSST-1: Toxic Shock Syndrome Toxin 1
 MLST: Multilocus Sequence Typing
 MGE: Mobile Genetic Element
 MRS: Methicillin-Resistant *Staphylococci*
 MRSA: Methicillin-Resistant *Staphylococcus aureus*
 LA-MRSA: Livestock-Associated MRSA
 (SCCmec): Staphylococcal Cassette Chromosome mec
 DNA: Deoxyribonucleic acid
 ORF: Open Reading Frame
 G Island: Genome Island

Acknowledgement / Funding: Authors are thankful to Department of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, 201206, Chaudhary Charan Singh University, Meerut, 250001, Uttar Pradesh, India

****Research Guide or Chairperson of research: Dr Arjun Chatterji**

University: Chaudhary Charan Singh University, Meerut, 250001, India
 Research project name or number: Review study

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained

from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, 201206

Cultivar / Variety / Breed name: Nil

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

References

- [1] Chiang Y.C., Liao W.W., Fan C.M., Pai W.Y., Chiou C.S. and Tsen H.Y. (2008) *Int J Food Microbiol.*, 121(1), 66-73.
- [2] Stewart G.C. (2005) *Emerg Infect Dis.* ISBN, 1-9044550-00-X.
- [3] Kérouanton A., Hennekinne J.A., Letertre C., Petit L., Chesneau O., Brisabois A. and De Buyser M.L. (2007) *Int J Food Microbiol.*, 115(3), 369-375.
- [4] Sandel M.K. and McKillip J.L. (2004) *Food Control*, 15(1), 5-10.
- [5] Altarazi Y.H., Albetar M.A. and Alaboudi A.R. (2009) *Food Res. Int.*, 42, 374-379.
- [6] Yang X., Zhang J., Yu S., Wu Q., Guo W., Huang J. and Cai S. (2016) *Front. Microbiol.*, 7, 816
- [7] Eisenberg M.S., Gaarslev K., Brown W., Horwitz M. and Hill D. (1975) *The Lancet*, 2(7935), 595-599.
- [8] le Loir Y., Baron F. and Gautier M. (2003) *Genetics and Molecular Research*, 2(1), 63-76.
- [9] Murray R.J. (2005) *Internal Medicine Journal*, 35, supplement 2, S106-S119.
- [10] Tamarapu S., McKillip J.L. and Drake M. (2001) *Journal of Food Protection*, 64(5), 664-668.
- [11] Asao T., Kumeda Y., Kawai T., et al., (2003) *Epidemiology and Infection*, 130(1), 33-40.
- [12] Argud M.A., Mendoza M.C. and Rodicio M.R. (2010) *Toxins*, 2(7), 1751-1773.
- [13] le Loir Y., Baron F. and Gautier M. (2003) *Genetics and Molecular Research*, 2(1), 63-76.
- [14] Hennekinne J.A., deBuyser M.L. and Dragacci S. (2012) *FEMS Microbiology Reviews*, 36, 815-836.
- [15] Balasubramanian D., Harper L., Shopsis B., Torres V.J. (2017) *Pathogens and Disease*, 75, 1.
- [16] Balaban N. and Rasooly A. (2000) *International Journal of Food Microbiology*, 61(1), 1- 10.
- [17] Syne S.M., Ramsubhag A. and Adesiyun A.A. (2013) *Infection Ecology and Epidemiology*, 3.
- [18] Ayçiçek H., Aydoğan H., Küçükkaraaslan A., Baysallar M., Ba ustao lu A.C. (2004) *Food Control*, 15, 253-259.
- [19] Lues J.F.R. and van Tonder I. (2007) *Food Control*, 18(4), 326- 332.
- [20] Martyn D.K., Ian M., Gill V.H. (2008) *Clin Infect Dis.*, 47, 392-400.
- [21] Deng Y., Liu J., Peters B.M. (2015) *Microb Drug Resist.*, 21, 102-104.
- [22] Xu Z., Li L., Shi L. (2011) *Molecular Biology Reports*, 38, 5261-5279.
- [23] Xu Z., Li L., Alam M.J. (2008) *Curr Microbiol.*, 57, 264-268.
- [24] Hall R.M., Stokes H.W. (1993) *Genetica*, 90, 115-132.
- [25] Andreoletti O., Budka H., Buncic S. (2008) *EFSA J.*, 765, 2-87.
- [26] Khanna T., Friendship R., Dewey C. (2008) *Vet Microbiol.*, 128, 298-303.
- [27] Stokes H.W., Hall R.M. (1989) *Mol Microbiol.*, 3, 1669-1683.
- [28] Ito T., Katayama Y., Asada K. (2001) *Antimicrob Agents Chemother.*, 45, 1323-1336.
- [29] Kuroda M., Nagasaki S., Ito R. and Ohta T. (2007) *FEMS Microbiology letters*, 273(1), 28-34.

- [30] Rowe-Magnus D.A., Mazel D. (2001) *Curr Opin Microbiol.*, 4, 565-569.
- [31] Correia M., Boavida F., Grosso F. (2003) *Antimicrob Agents Chemother.*, 47, 2838-2843.
- [32] Christianson K.K., Tweten R.K., Iandolo J.J. (1985) *Appl Environ Microbiol.*, 50, 696-697.
- [33] Teodoro C.R., Mattos C.S., Cavalcante F.S. (2012) *Microbiol Immunol.*, 56, 647-650.
- [34] Mulazimoglu L., Drenning S.D., Muder R.R. (1996) *Antimicrob Agents Chemother.*, 40, 1534-1535.
- [35] Ziakas et al., (2015) *Critical Care Medicine*, 43(2), 382-393.
- [36] Whittington et al., (2017) *Critical Care Medicine*, 45(8), 1304-1310.
- [37] Narciso et al., (2019) *Food Microbiology*, 82, 107-110.
- [38] Drwiega et al., (2019) *Infectious Diseases in Clinical Practice*, 27(5), 268-272.