Molecular Characterization of Food Borne Staphylococcus Aureus and Designing of Novel Anti Biofilm

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ABSTRACT

Staphylococcus aureus is a Gram-positive cocci involved in food spoilage. It is one of opportunistic pathogens causes serious surgical wound infections, lifethreatening blood stream and bone infections, or pneumonia, osteomyelitis, meningitis and sepsis. It is frequently found in skin causing serious infections such as pimples, boils, and abscesses. According to world health organization (WHO), consumption of any food or water contaminated with microbes or its toxic nature leading to foodborne illnesses posing to be a major healthcare problem throughout the world. The contagious nature of these diseases makes itimperative for early diagnosis of the microbes to preventthe spread of epidemics. Conventional standard diagnostic methods have limitations such as difficulty in sample preparation, time consuming, and poor sensitivity. The inappropriate use of antimicrobial drugs increases the prevalence of biofilm forming; drug-resistant methicillin-resistance Staphylococcus aureus (MRSA) is one of great challenging factors in the control of S. aureus in the food processing environments and disease conditions. Hence, robust field deployable diagnostic modalities arein urgent need to identify, and efficient technology to combat the problems of food-spoilage and infection caused by S. aureus. This thesis aims to screen various food-samples from different areas of Mysuru to understand the prevalence of food-borne pathogens, developing robust identification method, nanotechnology approach to control its infection, and developing novel food wrap to stop food-spoilage caused by S. aureus suitable for developing countries. To realize these objectives, we screened different food samples and isolated 500 food-borne pathogens. Among them, methicillin-resistant S. aureus (MRSA) showed highest prevalence in the region of Mysuru. The MALDIBiotyper evolved as robust and sensitive method by identifying MRSA in 2 min compared to conventional methods. Also, PCR-SSCP evolved as novel sensitive nucleic acid based method to identify food-borne pathogens by discriminating at species level. Both MALDI-Biotyper and PCR-SSCP recognized for point-of-care and unsophisticated laboratories applications respectively, from the present study.

Keywords *– Food Packaging, Staphylococcus Aureus*

INTRODUCTION

Food is a primary source for living organism obtained directly from plants; even though animals used food sources are obtained by plants or plant derived products. The important essential nutrients in food contain carbohydrates, proteins, fats, minerals and vitamins. These elements ingested by organism and assimilated by cells to generate energy to stimulate continuous growth and to maintain life (https://www.britannica.com/topic/food). Food causes mild illnesses to life threatening outbreaks which are triggered by number of agents consumed along with food. Food safety is a complex issue that has an impact on all segment of society, from general public to government and food industries which causes considerable social and economic burdens. According to the World Health Organization (WHO), food may contaminate at any stage during food production to consumption (farm to fork) which is result from number of environmental factors such as air soil, pollution of water, or food handlers. According to WHO, both developing and developed countries these are all considered as major public health problem (WHO, 2018).

Microbial spoilage of food, caused by pathogenic microorganisms, including bacteria (Bacillus cereus, Campylobacter jejuni, Escherichia coli O157:H7, Listeria monocytogenes, Staphylococcus aureus, S. epidermidis, S. haemolyticus, Salmonella typhimurium, Vibrio cholerae and V. parahaemolyticus) and fungi (Alternaria alternata, Bipolaris spp. Curvularia lunata, Candida albicans, Aspergillus niger, A. flavus and Fusarium oxysporium) reduces the quality of foods and inturn elevate the risk of food-borne illness, presents a major global concern (Gram et al., 2016; Quintavalla and Vicini, 2019 Bohme et al., 2018). Approximately 50 food-borne pathogens have been reported (Velusamy et al., 2010) in that Staphylococcus aureus, Listeria monocytogenes, Shigella and E. coli strains (nonO157 STEC), and Vibrio sp. are leading causes of food-borne diseases. Food-borne pathogens are increasingly being studied due to their ability to change and adapt to different environment and surviving conditions. There is a wide variety of microorganisms that are able to produce toxins into food and cause diseases mainly by B. cereus, Clostridium botulinum, Clostridium perfringens, E. coli O157:H7, S. aureus, and Vibrio cholera (Fusco et al., 2019). Assurance of food safety in food processing company includes both control and an assurance activity for finished product is a pre-requisite. Control activities are involved in prevention or reduction of a bio-hazard and in preventive measures cleaning, sanitation, temperature control, hygiene of the workers etc., programs are prerequisite at production environment to avoid food microbial contamination exacerbate the human out breaks (Luning et al., 2018).

OBJECTIVE OF THE STUDY

1. To study the molecular characterization of food borne staphylococcus aureus.

2. To study the designing of novel anti biofilm material for food packaging.

FOOD CONTAMINATION

Many food-borne pathogens in nature cause wide varieties of major health issues through food chain and lead to life threatening diseases. So, improving microbiological food safety is very important in food industries due to concern of consumers. Food may contaminate through number of ways including water, air, soil, food handling, hospitals, also during growing food

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crop, harvesting, processing and many food chain stages exacerbate the microbial population involved in food spoilage. A wide range of illness triggered by biological agents consumed along with contaminated food leads to emergence of public health problems throughout the globe (WHO, 2017; CDC, 2011; Srey et al., 2018). There are many physical, chemical and biological agents involved in many public health issues and diseases. Of which, the biological agents proved its significant role by causing majority of food-borne diseases. The diseases causing biological agents associated with raw material, food transporting, storing, processing, serving, consuming, and many other unknown routes are responsible for diseases.

Even some microbes associated with food and leads to changes in food properties and cause food spoilage by deteriorating food organoleptic quality by releasing toxins (Cortese et al., 2016; Khan et al., 2017). In many countries, the income of low poverty level, people are preparing inexpensive meal prepared at streets and other places for public consumption (Cortese et al., 2016). Without basic infrastructures like water, refrigeration and compromised sanitary quality becoming a public health risk to consumers every day (WHO, 2016; FDA, 2016). To avoid this problem, need an international call to boost the food safety measures at the stages of food chain and global rudimentary guidelines to all food related sectors to avoid diseases burden (FAO, 2019).

Food-borne pathogens

In Europe, 60% of food-borne outbreaks and diseases are still a major concern and 22% take place in collectivities. Therefore, catering services play a very important role in health and especially supplied food to target population made up of vulnerable users like child care, hospitals, schools and nursing homes (Albrecht and Nagy-Nero, 2009; EFSA, 2015). The many bacterial microorganisms present in skin and gut of human body as normal flora, which are risk-free and helpful in many important functions of the body. Even though many microorganisms pathogenic, gastrointestinal tract is one of the major routes for pathogens to enter the complex human body and defending to cause many food-borne diseases through contaminated water or undercooked food. Such organisms majorly include B. cereus, B. subtilis, C. perfringens, C. jejuni, E. coliO157:H7, L. monocytogenes, Salmonella typhimurium, Staphylococcus aureus, S. epidermidis, S. hemolyticus, S. xylosus, Vibrio cholerae and Yersinia pestis (Priyanka et al., 2016). Among these, S. aureus is notorious and frequent pathogens associated with human disease outbreaks (CDC, 2016; Lira et al., 2016). S. xylosus is coagulase-negative staphylococci belongs to skin microflora of mammals and habitually isolated from meat, milk, cheese and sausages (Kloos and Schleifer, 2016; Talon et al., 2018).

Antibiotic resistance

Even though in 2015 antibiotics were discovered, they are revolutionized during 20thcentury in the field of medicine. In nature, bacterial antibiotic resistance is well phenomenon due to misuse of antibiotics and leads to serious public health problem, also it increase many fold by human neglect and misuse. Among bacterial group, gastrointestinal tract containing Enterococci are Gram-positive cocci and certain species of Enterococcus related to human infections, namely, E. faecalis (90%), E. faecium (5-15%), and E. gallinarum (causing global disease burden. CNS reported for virulence genes (example: mecA) for antibiotics and implicated in food poisoning to cause immediate threat to the public health (Veras et al., 2018; Bhumbra et al., 2016; Chajęcka et al., 2015; Osman et al., 2016)

GREEN CHEMISTRY APPROACH FOR BIOMEDICAL APPLICATIONS NATURE

"Green chemistry" approach is a versatile and innovative method to design and develop efficient goods to minimize health hazard substances to minimum and developing tunable nanomaterials to replace unhealthy nano based materials to prove useful in this respect (Rejeski and Lekas, 2018). The research efforts need to find out exposure and toxicity of these nanomaterials to measure its efficacy inturn by preventing targeted risk. Current green approach involves use of starting material such as natural sources, nonhazardous solvents and less energy efficient technology in nanomaterial preparation. Numerous natural substances serve as natural reducing agents such as plant extracts, biopolymers, vitamins, proteins, peptides (e.g., glutathione), and sugars (e.g., glucose, fructose) for the safe synthesis of nanoparticles (Virkutyte and Varma, 2016). To-date, plant extracts are the main studied category and promising natural reducing agent in nanotechnology. One of the milestones in nanotechnology is by synthesizing metal nanoparticles using plant extracts as reducing agents. Also biopolymers such as cellulose, carbohydrates, chitosan, and dextran which are derived from plants, exoskeleton of crustaceans, and sugar cane respectively used as reducing and capping agents for noble elements in nanoparticles synthesis (Shamim et al., 2017; Lu and Ozcan, 2015). Also to improve biocompatibility nontoxic reducing agents, plants, algae, bacteria and fungi sources are used continuously in the biomedical applications such as gene and drug delivery using noble metal elements includes Au, Ag, Pt, Pb; semiconductors CdSe, CdS, ZnS, TiO2, PbS, InP, Si; magnetic compounds Fe3O4, Co, CoFe2O4, FePt, CoPt, and its combinations with drug carriers are in the biomedical trial (Thanh and Green, 2016).

FOOD SPOILAGE AND PACKAGE

Food contaminated by a number of pathogenic bacteria and fungi, inturn reduces the quality and shelf-life of the food and increases the food-borne illness representing major global outbreaks (Krepker et al., 2017). Many food industries facing microbial spoilage during food packaging leading to decrease the quality of the product which leads consumers to reject any brand having less quality. Hence food packaging industries need an effective technology to inactivate or reduce/eliminate food-borne microbial agents on food-contact surfaces. In this regard antimicrobial technology plays a promising role in food industries by increasing shelf-life of food and retaining the nutritional and sensory quality of food. Generally in food packaging industries, antimicrobials such as organic, inorganic and biological active substances are used. Especially, the active substance derived from plants tagged having nontoxic, efficient, and biocidal agents are preferred due to health and ecological concern (Wen et al., 2016). EOs are kind of naturally gifted with powerful beneficial agent to human and as antimicrobial against diverse food-borne pathogens such as B. cereus, B. subtilis, E. coli, L. monocytogenes, and S. aureus etc. Without approval they can be used as food ingredients in food industries and they are categorized as Generally Recognized as Safe (GRSA) by U.S. Food and Drug Administration (FDA). However, there is some limitation to use EOs in food as preservatives due to their poor solubility in water and unique flavor which may alter the original sensory quality of the food (Wen et al., 2016). Currently many antimicrobials are in food packaging industries and agent's silver and copper as oxidizing ions provide limited efficacy against food pathogens upon direct contact (Krepker et al., 2017).

Loop-mediated isothermal AMPlification (LAMP)

The available traditional methods for diagnosis based on isolation and biochemical characterization. But, due to time consuming makes it unsuitable for many a field for diagnosis and many a times number of diseases are overlooked. Many serological tests are carried out such as complement fixation test (CFT), indirect haemagglutination (IHA) tests, lateral flow assay and enzyme-linked immunosorbent assay (ELISA) (Arun et al., 2015; Kumar et al., 2015). Various, nucleic acid amplification based molecular diagnostic methods developed over a long time includes PCR (Rekha et al., 2015), PCR-RFLP (Foddai et al., 2005), PCRDGGE analysis, (McAuliffe et al., 2003) RT-PCR (Becker et al., 2018), and immunomagnetic capture-PCR (Sanna et al., 2017) require thermocycler and makes applications of PCR based techniques to become difficult due to cost involved.

For the first time Notomi et al. (2000) developed an easy, sensitive, accurate, fast and cost effective LAMP test base on strand displacement activity by specialized enzyme called Bst polymerase from Bacillus stearothermophilus (Lu et al., 2016) stable at ambient temperature. In LAMP method, six set of primers have been used for the amplification of target DNA. This involves each two inner and outer primers and two loop primers. The amplification is carried out under isothermal condition with enzyme optical activity at 50 to 65 oC, this makes LAMP independent of thermo cycler and decreased reaction time with higher enzymatic activity (Nagamine et al., 2017). The amplified reaction can be interpreted visually by changing color of the initial reaction solution by fluorescent intercalating dye SYBR Green I (Rekha et al., 2015) or hydroxy naphthol blue (HNB) (Wang et al., 2017), Calcein and EvaGreen (Wachiralurpan et al., 2017). This simplicity and sensitive of LAMP used for the detection of many pathogens such as E. coli (Stratakos et al., 2017), B. cereus (Zhu et al., 2017), S. typhimurium (Liu et al., 2017), Clostridium perfringens (Hong, 2017), S. flexneri (Yan et al., 2017), Potato virus Y (PVY) (Rekha et al., 2015), resistant

MICROBIAL PATHOGENS AND FOOD SPOILAGE

Natural active agents in food packaging

Packaging is one of the most important processes to maintain the quality of food products for storage, transportation, and end use. Oxygen, light, water vapor, bacteria and other contaminants can affect the product without protective packaging after processing. It prevents the quality deterioration and facilitates distribution and marketing. Suitable packaging can slow down the deterioration rate and hence extend the shelf life of food. Now-a-days people are more conscious about the environment and they move towards environmental friendly packaging systems (Ahvenainen, 2017). The basic characteristics of food packaging materials such as antimicrobial properties, barrier properties, strength, and stability to heat and cold are being focused through innovative advancements through specific researches in their respective area. Different kinds of polymers like polyvinyl chloride, polyethylene, nylon and starch are used to produce packaging materials. The strong antimicrobial characteristic enhances the packaging materials by increasing shelf life and keeps it safe for human consumption (Jalal et al., 2017).

Phenolic compounds in plants play an important role in protection of plants against pathogens and predators. Cinnamaldehyde is a principal component of cinnamon essential oil comprising approximately 60–75% of the total oil. Cinnamaldehyde is one of the molecules of interest for development as a food antimicrobial agent due to its demonstrated activity against both Gram-positive and Gram-negative bacteria, including organisms that are of concern for food safety. Cinnamaldehyde has been reported to inhibit the growth of C. botulinum, S. aureus, E. coli O157:H7 and S. enterica serovar typhimurium. Cinnamaldehyde derived from cinnamon essential oil strongly inhibits C. perfringens and Bacteroid esfragilis and moderately inhibits Bifidobacter iumlongum and Lactobacillus acidophilus isolated from human faeces. It was also reported that cinnamaldehyde inhibits fungal cell wall synthesis enzymes. Cinnamaldehyde is registered as a flavoring agent by the Food and Drug Administration (FDA) and is allowed to be added to food (Makwana et al., 2019).

Screening and isolation of Staphylococcus aureus from food samples

In the present study, 500 food samples were collected from different regions of Mysuru area, Karnataka, India and screened for food-borne Staphylococci. Food samples collected from Devaraj Urs road and Bamboo bazaar areas are highly contaminated as compared to others. The main source of contamination is possibly the population crowd of the area arriving from different environments. The food-borne pathogens isolated from food include Staphylococcal spp. (160±0.01), Escherichia coli (92±0.03), Bacillus cereus (108±0.01), Salmonella typhimurium (85±0.11), Shigella flexneri (29±0.04), Vibrio cholera (20±0.04) and V. parahaemolyticus (8±0.02) on selective Baired Parker Agar (BPA) media, Eosin methylene blue (EMB), Bacillus Cereus Agar (BCA), Xylose Lysine Deoxycholate (XLD), Deoxycholate Citrate Agar (DCA) and Thiosulfate-Citrate-Bile Salts (TCBS) respectively. Among 160 Staphylococcal spp., 152 isolates were confirmed as S. aureus (95±0.02%), 6 as S. epidermidis (3.75±0.01%) and 2 confirmed as S. haemolyticus (1.25±0.03%). This shows, the occurrence of food contamination is high in processed, vegetarian, non-vegetarian foods, bakery, and dairy products was observed. The total 500 samples were screened and isolated 502 pathogens on selective agar. The experiments were repeated thrice for concordant confirmation, and significant results (P

Antimicrobial susceptibility to pure cinnamaldehyde

The minimum inhibitory concentration (MIC) of cinnamaldehyde was determined against bacterial strains by disc diffusion method according to Ghanwate et al. (2016) with slight modifications. The overnight cultures were centrifuged at 6000 rpm for 10 min, resuspended in phosphate-buffered saline (PBS, pH 7.3, 10 mM) and the absorbance was adjusted exactly to O.D. of 0.1 at 600 nm. About 15-20 mL of molten nutrient agar was poured into each of the sterile plates, dried and 100 µL of bacterial cultures were added and smeared with the aid of L-shaped loop. Sterile discs were placed on media and were loaded with different concentrations of cinnamaldehyde, streptomycin, and bacitracin (10 mg/mL concentration) as standard antibiotics and ethanol as a negative control. Then the plates were incubated for 24 h at 37 °C and zone of inhibition (mm) were measured

FOOD WRAP EXPERIMENT

Antimicrobial verification test with food samples

The antimicrobial effectiveness of Cin-C-LDPE films against MRSA090 was tested with chicken, mutton, cheese, and grape foods according to the protocol of Ahmed et al. (2017) with slight modification. The 1 g of sterilized grapes and minced chicken, mutton and cheese samples were kept in two different Petri dishes. Then, a 1 mL aliquot of 1×107 CFU/mL inocula of MRSA090 was added independently into each Petri dish, spread over the food samples by sterile spreaders, and kept in a laminar hood for 30 min for bacterial adherence to food samples. Afterwards, about 0.1 g of contaminated food samples with MRSA090 was wrapped individually with LDPE and Cin-C-LDPE film (5 × 5 cm), heat sealed and stored in Petri dishes at 4 °C. Wrapped samples were collected and tested for the existence of MRSA090 by counting at different time intervals of days (0 to 10 day) during the storage period. Food samples wrapped with bare LDPE films considered as the control sample.

CONCLUSION

Large numbers of people are affected by food-borne pathogens and suffer diseases due to contamination of food by number of pathogens. Literature suggests that, about 30-50% of the population has been carriers of Staphylococcus aureus at one time in their lives and about 20% of them are long-term carriers. S. aureus can express a broad range of virulence factors including surface proteins that are covalently attached to peptidoglycan layer known as cell wall anchored (CWA) proteins. These surface proteins are crucial to the success of the organism as commensal bacterium and also as a pathogen. S. aureus is one of the most important food-borne pathogens with clinical relevance in community-acquired infections. It can cause numerous symptoms from minor skin infections to life threatening diseases such as nausea, stomach cramps, diarrhea, abscesses, pneumonia, meningitis, endocarditis and septicemia. In present scenario, there is an increasing push off in public for food related issues particularly food safety and quality. So, there should be a quick and simple analysis for the detection of food borne pathogens for proper prevention and cure of diseases caused. With rise in public insight into food safety, detection of food-borne pathogen in food became prime concern, especially in lesser developed part of the globe. Over the time, there has been considerable improvement in the detection methodology, in order to make test more rapid, sensitive and specific. Nanoparticles are being incorporated into products and materials due to their significant capability that enhances and improvises particles properties, characteristics and used indifferent fields like medicine, biology, chemistry, catalysis, photonics, electronics, and bio-labeling. It is believed that both traditional and modern knowledge will enable better understanding the basis of active components for the suppression of diseases with lesser side effects.

REFERENCES

[1]. Abdallah, M., Khelissa, O., Ibrahim, A., Benoliel, C., Heliot, L., Dhulster, P. and Chihib, N. E. (2015). Impact of growth temperature and surface type on the resistance of Pseudomonas aeruginosa and Staphylococcus aureus biofilms to disinfectants. International Journal of Food Microbiology 214: pp. 38-47.

[2]. Ba, X., Harrison, E. M., Edwards, G. F., Holden, M. T., Larsen, A. R., Petersen, A., Skov, R. L., Peacock, S. J., Parkhill, J., Paterson, G. K. and Holmes, M. A. (2017). Novel mutations in penicillin-binding protein genes in clinical Staphylococcus aureus isolates that are methicillin resistant on susceptibility testing, but lack the mecA gene. Journal of Antimicrobial Chemotherapy 69(3): pp. 594-597.

[3]. Calo, J. R., Crandall, P. G., O'Bryan, C. A. and Ricke, S. C. (2015). Essential oils as antimicrobials in food systems–A review. Food Control 54: pp. 111-119.

[4]. da Silva Luz, I., Neto, N. J. G., Tavares, A. G., Magnani, M. and de Souza, E. L. (2016). Exposure of Listeria monocytogenes to sublethal amounts of Origanum vulgare L. essential oil or carvacrol in a food-based medium does not induce direct or cross protection. Food Research International 48(2): pp. 667-672.

[5]. EFSA (European Food Safety Authority). (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal 13(3991): pp. 1e162.

[6]. Ferreira, J. V. N., Capello, T. M., Siqueira, L. J., Lago, J. H. G. and Caseli, L. (2016). Mechanism of action of thymol on cell membranes investigated through lipid Langmuir monolayers at the air–water interface and molecular simulation. Langmuir 32(13): pp. 3234-3241.

[7]. Gallas-Lindemann, C., Sotiriadou, I., Mahmoodi, M. R. and Karanis, P. (2018). Detection of Toxoplasma gondii oocysts in different water resources by Loop Mediated Isothermal Amplification (LAMP). Acta Tropica 125(2): pp. 231-236.

[8]. Hahn, M. A., Keng, P. C. and Krauss, T. D. (2018). Flow cytometric analysis to detect pathogens in bacterial cell mixtures using semiconductor quantum dots. Analytical Chemistry 80(3): pp. 864-872.

[9]. Ilinskaya, A. N. and Dobrovolskaia, M. A. (2019). Nanoparticles and the blood coagulation system. Part II: safety concerns. Nanomedicine 8(6): pp. 969-981.

[10]. Jadhav, S., Gulati, V., Fox, E. M., Karpe, A., Beale, D. J., Sevior, D., Bhave, M. and Palombo, E. A. (2015). Rapid identification and source-tracking of Listeria monocytogenes using MALDI-TOF mass spectrometry. International Journal of Food Microbiology 202: pp. 1-9.

[11]. Jevon, M., Guo, C., Ma, B., Mordan, N., Nair, S. P., Harris, M., Henderson, B., Bentley, G. and Meghji, S. (1999). Mechanisms of Internalization of Staphylococcus aureus by Cultured Human Osteoblasts. Infection and Immunity 67(5): pp. 2677-2681.

[12]. Manukumar, H. M. (2017) http://hdl.handle.net/10603/256859, Department of Biotechnology, University of Mysore.