IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

CASE STUDY: PREVALENCE OF STAPHYLOCOCCUS AUREUS IN FISHERY PRODUCTS

[1]Aarcha B R, [2]Dr. Noha L, [3]Parvathy J,[4]Abhinsha Z

[1]M Sc Microbiology, [2]Assistant Professor Microbiology, [3]M Sc Microbiology, [4] M Sc Microbiology

[1]Department of Microbiology,

[1]A J college of Science and Technology, Thonnakal, Trivandrum, India

Abstract: A case study accidentally found the incidence of Pathogenic *Staphylococcus aureus* in ready to eat / ready to cook fishery products. Coagulase positive *S aureus* was detected in fish pickles. Methicillin Resistant *S aureus* was detected in 24% of fishery products. Hence the isolation of potentially pathogenic *S aureus* isolates from fishery products indicates risk to consumers; screening of prevalence, pathogenicity potential and antibiotic resistance is essential to implement control measures. This is a serious public health risk and highlights the need to implement good hygienic practices. These findings emphasize the need to presence of *S aureus* strains and Staphylococcal enterotoxin production in foods.

***Index Terms - Staphylococcus aureus* (*S aureus*), Coagulase positive *Staphylococcus aureus* (CPS).**

I. INTRODUCTION

Staphylococcus aureus is a common pathogen associated with serious community and Hospital acquired diseases and has for long been considered as a major problem of Public Health (Pesavento et al., 2007). Seafood's are rich in protein and their breakdown into low molecular weight peptides and amino acids support the growth of S. aureus. The foods involved in outbreaks are canned, smoked and salted products, frozen fishery products, boiled fish paste and fish sausages which inhibit the growth of competing organisms (Bryan, 1980; Nakano et al., 2004; Sanjeev, Iyer, Rao, & James, 1986). Freshly caught seafood's are free from *S. aureus* and contamination takes place during handling (Bryan, 1980; Shewan, 1962). Staphylococcal food poisoning is one of the most prevalent causes of gastroenteritis worldwide, which is caused by the ingestion of food that contains preformed toxins (Jab-lonski and Bohach, 2001). Some strains of this organism can cause food-poisoning by production of enterotoxins (SEs) when growing in foods; SEs have been divided into different serological types initially SEA through SEE and later the existence of new types of SEs have also been reported (Monday and Bohach, 1999; Omoe et al., 2005; Chiang et al.,2006; Chiang et al., 2008). Biological tests and immunoassays may be used to detect SEs (Dolman and Wilson, 1940; Casman, 1965; Celano et al., 1999; Normanno et al.,2001). Molecular biology methods, such as Polymerase Chain Reaction, are able to detect the genes encoding for the SEs (Johnson et al., 1991; Beker et al., 1998; Mehrotra et al., 2000). Immunoassays are both very sensitive and specific and reverse passive latex agglutination is acknowledged to be a sensitive, rapid and simple method for the detection of SEs (Rose et al.,1989; Brett, 1998; Soriano et al., 2002). Detection of SEs is paramount to food safety and protection of the food supply. *S. aureus* isolates from intensive care units across the country and from blood culture isolates worldwide are increasingly resistant to a greater number of antimicrobial agents. Inevitably this has left fewer effective bactericidal antibiotics to treat these often life-threatening infections. As rapidly as new antibiotics are introduced, *staphylococci* have developed efficient mechanisms to neutralize them. Recent reports of *S. aureus* isolates with intermediate or complete resistance to vancomycin portend a chemotherapeutic era in which effective bactericidal antibiotics against this organism may no longer be readily available. Most of the nosocomial *S. aureus* infections are caused by methicillin-resistant *S. aureus* (MRSA) strains and have become a widely recognized cause of morbidity and mortality throughout the world (Ardic et al., 2006; Pesavento et al., 2007; Ho et al., 2008). In addition, MRSA strains resistant to quinolones or multiresistant to other antibiotics have been emerging, leaving a limited choice for their control (Mee-Marquet et al., 2004; Nejma et al., 2006; Pesavento et al., 2007). Hence this study emphasizes the prevalence of *S aureus* in fishery products and to characterize its antibiotic susceptibility.

Samples analyzed in the study

II. MICROBIOLOGICAL ANALYSIS OF FISHERY PRODUCTS

About 76 RTC/RTE Samples were collected from various supermarkets, hypermarkets in and around Kochi, Trissur, and Kollam. Microbiological procedures were carried out to detect the presence of *S aureus* and following results were obtained:

2.1 23.68% of the total samples was contaminated with Staphylococcus sp among which 2.68% were coagulase positive S.aureus. Moreover 35 % of the products shows antimicrobial count of above 5 Log (cfu/g) which exceeds standard APC limit of 5 log (cfu/g).

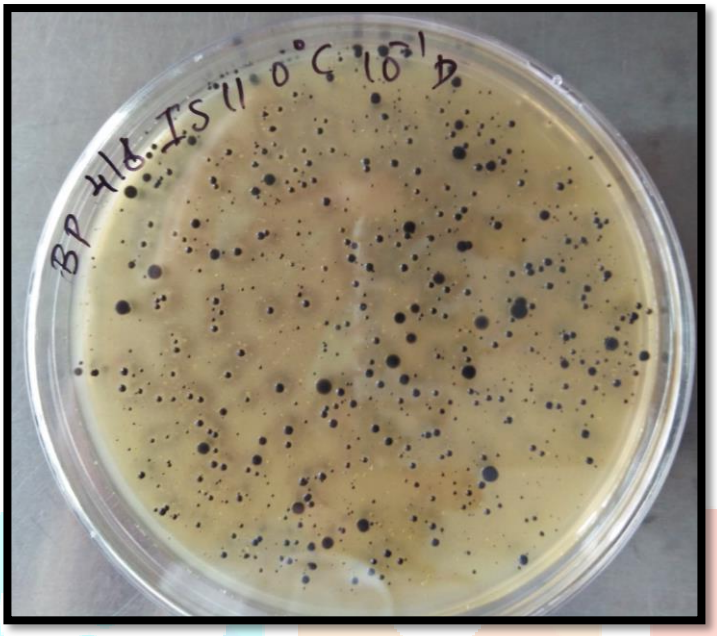
Microbial load of RTC/RTE fishery products from different markets of Kerala

Sl. no	Sample	No of samples	APC (log cfu/g) (Range)	Coagulase positive *staphylococci* (log cfu/g)
1	Dried fish products	13	3-6.18	ND
2	Fish Pickles	13	1-4.25	1-2.66
3	Prawn Pickles	11	1-3.9	ND
4	Fish cutlet	1	3.81	ND
5	Fish fillets	1	5.85	ND
6	Fish fingers	1	6.05	ND
7	Canned tuna products	10	0-3.39	ND
8	Canned sardine	1	2.30	ND
9	Dried prawn	5	3.8-5.25	ND
10	Prawn chutney powder	10	4.05-5.95	ND
11	Fried Prawn	1	5.69	ND
12	Prawn roast	1	4.47	ND
13	Shrimp cocktail	1	3.94	ND
14	Mussel Pickle	1	1.00	ND
15	Fish samosa	1	4.27	ND
16	Clam pickle	4	1-2.4	ND
17	Squid Pickle	1	1.77	ND
	Total	76		

Coagulase positive *S. aureus* were isolated in Baird Parker agar plates from RTC/RTE products. Total 76 samples were used for the present study. About 50 *S. aureus* isolates were obtained out of these 17 isolates obtained from two samples were coagulase positive. CPS were present in 2.63 % of the total samples. The isolated then subjected to biochemical confirmation.

Morphological characters of the isolates

Source	Morphology
Fish pickle 1	Small, round black colonies with opaque zone
Fish pickle 2	Small, round black colonies with opaque zone.



Characteristics colonies of *S. aureus* on Baird-Parker agar plate. Colonies appear as small, black, convex round colonies with clear zone.

2.2. Biochemical test was performed with 50 isolates out of which 17 were coagulase positive, catalase positive, Mannitol salt agar positive, and DNase test positive. In our study prevalence of coagulase positive *S. aureus* was detected only in fish pickles. The contamination could be the result of a combination of improper and unsanitary handling, improper storage and cross contamination (Huang, Weng, and Chiou, 2001; Ng and Tay, 1993; Synder and Poland, 1991; Tranter, 1990). Other products viz. dried fish products, frozen fish products, mussel pickle and value-added products did not show the presence of Coagulase Positive S. aureus. This low level of incidence could be attributed to the improvements in the handling and sanitary procedures and adaptation of good manufacturing practices (GMP) and hazard analysis and critical control points (HACCP) in the processing units.

2.3. Enterotoxigenic strains were not detected in any of the isolates.

2.4. Antibiotic sensitive test was performed with isolates and obtained that 24% of isolates were resistant to methicillin, 6 % showed multidrug resistance to methicillin, ampicillin, oxacillin, and erythromycin, while 80-100 % were sensitive to ampicillin, trimethoprim, tetracycline, erythromycin, clindamycin, gentamycin, chloramphenicol, cephalothin, and oxacillin. However, the prevalence of MRSA in our study (24 %) which was higher than that (0.6%) of the study by Wang et al. (2014). Contamination of food with antimicrobial-resistant bacteria is a threat to public health.

2.5. Test to detect the presence of virulence factors was performed on blood agar. β-hemolysis was exhibited by 52.9 % and 47.1 % exhibits δ-hemolysis.

III. INFERENCE

S. aureus is an indicator of hygiene and sanitary conditions hence the presence of this organism indicates the unhygienic condition during processing, storage etc. (Synder & Poland, 1991). In conclusion, these findings highlight the high potential risk for consumers in the absence of strict hygienic and preventative measures to avoid the presence of *S. aureus* isolates and SEs production in foods, emphasising the need for improved hygiene practices during food processing and also during the distribution and consumption of the final food products. It is important to identify the origin of food-related MRSA and to evaluate the potential pathogenicity of these MRSA isolates. However, the findings of our study are even more serious in terms of public health because RTE foods are consumed without further cooking, which would eliminate or reduce the microbial load. Consequently, the incidence of *S. aureus* and MRSA in RTE foods, along with the spread of antibiotic resistant strains, represents a potential health hazard to humans.

REFERENCES

[1] Ardic, N., Sareyyupoglu, B., Ozyurt, M., Haznedaroglu, T. and Ilga, U., 2006. Investigation ofaminoglycoside modifying enzyme genes in methicillin-resistant *staphylococci*. Microbiological research, 161(1), pp.49-54.

[2] Ayulo, A.M.R., Machado, R.A. and Scussel, V.M., 1994. Enterotoxigenic Escherichia coli and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. International Journal of Food Microbiology, 24(1-2), pp.171-178.

[3] Becker, K., Roth, R. and Peters, G., 1998. Rapid and specific detection of toxigenic Staphylococcu*S aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. Journal of Clinical Microbiology, 36(9), pp.2548-2553.

[4] Brett, M.M., 1998. Kits for the detection of some bacterial food poisoning toxins: problems, pitfalls and benefits. In Symposium series (Society for Applied Microbiology) (Vol. 27, pp.110S-118S).

[5] Bryan, F.L., 1980. Foodborne diseases in the United States associated with meat and poultry. Journal of Food Protection, 43(2), pp.140-150.

[6] Casman, E.P., 1965. Staphylococcal enterotoxin. Annals of the New York Academy of Sciences, 128(1), pp.124-131.

[7] Celano, G.V., Normanno, G., Sebastio, P. and Nirta, F., 1999. Colture cellulari per evidenziare l'enterotossina stafilococcica tipo A. Industrie alimentari, 38(384), pp.938-940.

[8] Chiang, Y.C., Chang, L.T., Lin, C.W., Yang, C.Y. and Tsen, H.Y., 2006. PCR primers for the detection of staphylococcal enterotoxins K, L, and M and survey of staphylococcal enterotoxin types in *Staphylococcus aureus* isolates from food poisoning cases in Taiwan. Journal of food protection, 69(5), pp.1072-1079.

[9] Chiang, Y.C., Liao, W.W., Fan, C.M., Pai, W.Y., Chiou, C.S. and Tsen, H.Y., 2008. PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. International journal of food microbiology, 121(1), pp.66-73.

[10] Dolman, C.E. and Wilson, R.J., 1940. The kitten test for staphylococcus enterotoxin. Canadian Public Health Journal, 31(2), pp.68-71.

[11] Evenson, M.L., Hinds, M.W., Bernstein, R.S. and Bergdoll, M.S., 1988. Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. International journal of food microbiology, 7(4), pp.311-316.

[12] Ho, P.L., Chuang, S.K., Choi, Y.F., Lee, R.A., Lit, A.C., Ng, T.K., Que, T.L., Shek, K.C., Tong, H.K., Cindy, W.S. and Tung, W.K., 2008. Community-associated methicillin-resistant and methicillin-sensitive Staphylococcu*S aureus*: skin and soft tissue infections in Hong Kong. Diagnostic microbiology and infectious disease, 61(3), pp.245-250.

[13] Jablonski, L.M. and Bohach, G.A., 2001. *Staphylococcus aureus* in: Food Microbi-ology: Fundementals and Frontiers. 2" Edn.(Doyle, MP, Beuchat, LR and Montville, TJ Eds) pp 411- 434

[14] Johnson, W.M., Tyler, S.D., Ewan, E.P., Ashton, F.E., Pollard, D.R. and Rozee, K.R., 1991. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. Journal of Clinical Microbiology, 29(3), pp.426-430.

[15]Mehrotra, M., Wang, G. and Johnson, W.M., 2000. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. Journal of clinical microbiology, 38(3), pp.1032-1035.

[16] Monday, S.R. and Bohach, G.A., 1999. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. Journal of clinical microbiology, 37(10), pp.3411-3414.

[17] Nakano, S., Kobayashi, T., Funabiki, K., Matsumura, A., Nagao, Y. and Yamada, T., 2004. PCR detection of Bacillus and Staphylococcus in various foods. Journal of food protection, 67(6), pp.1271-1277.

[18] Nejma, M.B., Mastouri, M., Frih, S., Sakly, N., Salem, Y.B. and Nour, M., 2006. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated in Tunisia. Diagnostic Microbiology and infectious disease, 55(1), pp.21-26.

[19] Normanno, G., Celano, G., Dambrosio, A., Lassandro, L. and Buonavoglia, C., 2001. Enterotoxins of *Staphylococcus aureus* induce a cytopathic effect in cell lines. The new microbiologica, 24(4), pp.341-346.

[20] Omoe, K., Hu, D.L., Takahashi-Omoe, H., Nakane, A. and Shinagawa, K., 2005. Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. FEMS microbiology letters, 246(2), pp.191-198.

[21] Pesavento, G., Ducci, B., Comodo, N. and Nostro, A.L., 2007. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA). Food control, 18(3), pp.196-200.

[22] Rodma, P., Satjapala, T. and Suwanvitaya, P., 1991. Study of *Staphylococcus aureus* in frozen food product. Ahan. Rose, S.A., Bankes, P. and Stringer, M.F., 1989. Detection of staphylococcal enterotoxins in dairy products by the reversed passive latex agglutination (SET-RPLA) kit. International journal of food microbiology, 8(1), pp.65-72.

[23] Sanjeev, S., Mahadeva Iyer, K., Rao, C.C.P. and Arul James, M., 1986. Occurrence of enterotoxigenic *Staphylococci* in frozen fishery products. Shewan, J.M., 1962. The bacteriology of fresh and spoiling fish and some related chemical changes. Recent advances in food science, 1, pp.167-193.

[24] Sokari, T., 1991. Distribution of enterotoxigenic *Staphylococcus aureus* in ready-to-eat foods in eastern Nigeria. International journal of food microbiology, 12(2-3), pp.275-279.

[25] Soriano, J.M., Font, G., Rico, H., Molto, J.C. and Manes, J., 2002. Incidence of enterotoxigenic *staphylococci* and their toxins in foods. Journal of food protection, 65(5), pp.857-860

[26] Van Der Mee-Marquet, N., Domelier, A.S., Girard, N., Quentin, R. and Bloodstream Infection Study Group of the Relais d'Hygiene du Centre, 2004. Epidemiology and typing of *Staphylococcus aureus* strains isolated from bloodstream infections. Journal of clinical microbiology, 42(12), pp.5650-5657.

[27] Wang, X., Li, G., Xia, X., Yang, B., Xi, M. and Meng, J., 2014. Antimicrobial susceptibility and molecular typing of methicillin-resistant *Staphylococcus aureus* in retail foods in Shaanxi, China. Foodborne pathogens and disease, 11(4), pp.281-286.