

## Prevalence and Survival of *S. aureus* in Street Vended Indian Green Chutneys

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**Abstract:** Green chutneys, served with Ready-to-eat (RTE) foods sold in street vended shops in five major locations throughout the main city of Patiala, were analyzed for their microbiological qualities. The total viable counts of all the chutneys were around log 6 with loads of total staphylococcal counts, total faecal coliform counts and total faecal streptococcal counts. Qualitative analysis showed the presence of coagulase positive *S. aureus* in seventy-two samples (48%), but *Salmonella* was not detected in any of the samples. The survival characteristics of three isolated, enterotoxigenic strains of *S. aureus* belonging to group VII were determined in both green chutneys and coriander pastes at storage temperatures of 4, 8, 15, 21 and 28°C. In Coriander pastes, the strains of *S. aureus* was able to survive at refrigeration temperatures for one day, after which there was a gradual decrease in counts to about log 2 CFU g<sup>-1</sup> after four days in samples stored at 21 and 28°C. The strains of *S. aureus* showed reduced survival in green chutneys, with a higher inactivation rate at temperatures of 21 and 28°C where the counts decreased to about log 3 CFU g<sup>-1</sup> after a period of four days.

**Key words:** Green chutneys, Street foods, faecal coliforms, Coagulase positive *Staphylococcus*, survival, coriander pastes

### Introduction

Street-vended foods are ready-to-eat foods prepared and sold by vendors on streets and similar public places; street-vended-foods provide ready available, inexpensive, nutritional meals, while providing a source of income for the vendors, the types of street-vended food differs greatly between countries and cultures. Despite the advantages that street vended foods provide in terms of inexpensiveness and convenience, concerns for the safety and quality of such foods have been raised, because the vendors lack an adequate appreciation of basic food safety issues. The hygienic aspects of street vended foods are a major source of concern and epidemiological associations between street vended foods and illnesses have been reported (Mensah *et al.*, 2002 and WHO, 1996). In countries where street vending is prevalent, there is commonly a lack of information on incidence of food-borne diseases related to street-vended foods however, microbiological studies on such foods in American, Asian and African Countries have revealed high bacterial counts and a high incidence of food-borne pathogens in the food (Kapdia, 1984; Lary, 1995; Spika, 1987 and WHO, 1996).

In developing countries like, India, a large proportion of ready-to-eat food is sold on the streets a huge section of people from all age and income groups consume such ready-to-eat foods everyday, most of these foods are accompanied by a paste of coriander, mint and tamarind known as green chutney. Green chutneys are popular since it offers a refreshing taste to both ready to eat foods and meals; it is also believed to be nutritious. However, they undergo no processing and are consumed raw. There is a paucity of information concerning the incidence of food-borne diseases related to Indian street foods, especially green chutneys which is consumed raw, in addition, the behavior of predominant bacterial food-borne pathogens in these products are not known. The aim of the present study was to examine the microbiological quality of street vended green chutneys in Patiala city, India, the last part of the work includes studying the fate of predominant food borne isolates of *S. aureus* in both green chutneys and coriander preserves (used to make Green chutneys) at different storage temperatures, our study is the first to report the survival of food-borne bacterial pathogens in Indian traditional foods which are street-vended.

### Materials and Methods

**Sampling:** A total of 150 samples were collected from five major areas, which represented almost whole of the city and comprised of shops preparing ready-to-eat foods and served at least 100 customers (comprising of upper, middle and low-income groups) per day. All samples were collected from the vendors in pre-sterilized 250 ml bottles and maintained on icepacks. during transport. In the laboratory these samples were analyzed within two hours of procurement.

**Microbiological Analysis:** 25 g of the Chutney samples were added to 225 ml Butterfield's Phosphate Buffer (BPB), blended for about 15 minutes to give appropriate dilutions for aerobic plate and coliform counts. Aerobic plate count (37°C/24hrs.) was performed using tryptone soya agar; total coliform count was done using Violet Red Bile

agar (37°C/24 hrs). Presence of faecal coliforms was determined using Brilliant Green Lactose bile broth (44.5°C/48hrs.), followed by confirmation of gas positive tubes using Eosin Methylene blue agar. *S. aureus* was isolated on Baird Parker agar (37°C/24hrs.). The isolates were identified and confirmed by biochemical tests (Brackett and Splitsoesser, 1992 and FAO, 1979). Coagulase positive strains were checked for enterotoxin (A, B, C and D) using a reversed passive latex agglutination based kit (SET RPLA, Oxoid). Strains which were enterotoxin positive were coagulase typed (Ushioda *et al.*, 1981). For Salmonella, 10g portions of chutneys were pre-enriched with 100ml Universal Preenrichment Broth for 24 hrs at 37°C, 0.1ml of the preenriched culture was inoculated in semisolid Rappaport Vassilidis Agar and incubated at 42°C for 24 hrs, loopfull of the latter were then streaked on Xylose-Lysine Deoxycholate agar and incubated at 37°C for 24-48 hrs (USFDA, 2002). Presumptive colonies were sent to Central Research Centre, Kasuali, India for further confirmation.

### Studies on Growth Pattern of *S. aureus* in Coriander Pastes and Green Chutneys

**Preparation of Coriander Pastes and Green Chutneys:** For preparing coriander pastes, coriander, mint leaves and green chilies were washed with 70% ethanol, twice with sterile distilled water and dried; they were chopped with sterile knife under aseptic conditions and blended together in a household blender, sterilized by applications of soap water, 70% ethanol and sterile distilled water. Green chutneys were made by mixing the coriander paste with filter sterilized tamarind juice (25ml), sterile water (50ml) and table salt (0.5%). Coriander pastes and Green chutneys so prepared were stored in sterile glass bottles and inoculated within 1 hour from preparation.

**Culture Preparation:** Three *S. aureus* strains which produced enterotoxins A, B and C were used for survival studies; the strains were designated as C1, C2 and C3 and maintained in 75% glycerol at -20°C. Prior to use the cultures were propagated in tryptic soy broth at 37°C for 24 hrs, under static condition, harvested by centrifugation at 10,000x g for 10min. the supernatant was discarded, and the pellet was washed twice with sterile BPB. The cells were resuspended in BPB to provide an inoculum of about 8 log CFU ml<sup>-1</sup>; 100g of Coriander pastes and chutneys prepared as described below were inoculated with approximately and incubated at 4, 8, 15, 21 and 28°C.

**Enumeration of *S. aureus*:** After incubation for 0, 1, 2, 3 and 4 days approximately 25g portions was aseptically removed, blended in 225 ml of sterile BPB and appropriate dilutions plated on Baird-parker medium, which was incubated at 35°C for 24 hr. Typical colonies were counted and three representative colonies from each plate (triplicates) were then picked and transferred into culture tubes containing 200µL of brain heart infusion broth (BHI). These tubes were then incubated at 35°C for 24h. After incubation, 500µL of rabbit coagulase plasma was added to each tube and they were then incubated at 35°C for an additional four to six hours. Each tube was then examined for coagulation. Since control samples did not show any positive growth on BP plates, all coagulase positive tubes indicated a confirmed result for *S. aureus*.

### Results and Discussion

All samples of green chutneys showed high total counts of *staphylococcus* of about log 7.5 CFU g<sup>-1</sup>, a significant proportion (sixty four samples) of the total staphylococcal counts was accounted for by coagulase positive *S. aureus*. Three coagulase positive strains of *S. aureus* strains (C1, C2 and C3) produced enterotoxins A, B and C, all three strains belonged to group VII, a description of the strains is shown in (Table 2). The total viable counts, total faecal coliform and total faecal streptococcal counts were 6, 5 and log 5.5 CFU g<sup>-1</sup> respectively (Table 1), salmonella was not detected in any of the samples. Microbiological studies on street-vended foods (Mohapatra *et al.*, 2002) and green chutneys (Kakar and Udipi, 2000) in India, have revealed high bacterial counts and a high incidence of food-borne bacterial pathogens, the total *Staphylococcal* counts reported in case of green chutneys were > log 3 with 5.9% coagulase positive *S. aureus*. Green chutneys undergo no processing either before or after preparation and may provide substrates for growth or survival of bacterial pathogens; in street vended green chutneys, the raw materials viz: coriander and mint are not sourced and undergoes unhygienic handling during transport to street vendors the presence of *S. aureus* have been demonstrated in raw vegetables (Heldman, 1974 and Nguyen-the and Carlin, 1994) earlier and recently in Indian street vended raw vegetables (Kaur and Vishwanathan, 2001), including those which are used for preparing green chutneys. Additionally, the water and unsanitary conditions in which the foods are prepared and stored serve as possible routes for entry of faecal coliforms and other pathogens into these products. *S. aureus* is an important food borne pathogen and *Staphylococcal* food poisoning ranks among the food as one of the most prevalent causes of gastroenteritis worldwide (Jablonsky and Bohach, 1997) besides, *S. aureus* is known to be a hardy organism and is able to grow over a broad range of pH (4.98-9.3), temperature (7-43°C) and water activities.

The Green chutneys analyzed in our study showed very high levels of *Staphylococcus*, importantly, coagulase positive *S. aureus* indicating complete lack of hygiene and handling practices, although the presence of *S. aureus*

Table 1: Mean bacterial counts in 150 street vended green chutney samples

Area	TVC	TSC	TFCC	TFSC
1	4.60	7.5	4.80	5.21
2	5.22	8.1	5.84	5.5
3	4.23	7.4	5.40	3.74
4	6.11	7.0	5.40	4.88
5	6.89	7.52	5.22	5.54
Mean	5.41	7.5	5.33	4.97
SE ( $\pm$ )	0.488	0.17	0.16	0.33

Table 2: Characteristics of *S. aureus* isolates used in survival studies

Name	Origin	Coagulase type	Enterotoxin type
C1	Green Chutneys	VII	A
C2	Green Chutneys	VII	A& C
C3	Green Chutneys	VII	B

is not uncommon in street vended foods, a population of coagulase positive *S. aureus* amounting to  $10^6$  per gram of food can produce sufficient toxin necessary for initiating illness (Banwart, 1989). The prevalence of high numbers of *S. aureus* in street vended green chutneys especially coagulase positive strains prompted us to investigate the survival of such strains at different storage temperatures in green chutneys and coriander pastes, which are prepared before in many cases by vendors and mixed with tamarind juice to make chutneys. We used a high level of *S. aureus* contamination in the experimental coriander base and chutneys to facilitate enumeration of these organisms, also large numbers was expected to provide a model demonstrating the effects of temperature or other inherent factors on survival of any population size of *S. aureus*.

At refrigeration temperatures (4°C or 8°C) survival of *S. aureus* in coriander pastes (pH 5.3) was not affected significantly within a period of two days with a decrease of about 1 log after three days (Fig. 2), reductions in counts was however apparent at other temperatures, namely, 15, 21 and 28°C, these results are similar to those of Pao *et al.* (1988). In samples of chutneys, *S. aureus* levels declined throughout refrigeration temperatures (4 and 8°C) after one day, the decline was more at temperatures of 15, 21 and 28°C and was approximately 3 log for samples at the latter storage temperatures, after four days (Fig. 1). There was a significant reduction in counts at temperatures of 4, 8, 15, 21 and 28°C after four days, in the samples of chutneys, it is probable that tamarind juice present in green chutneys (pH 3.5) may have inactivated *S. aureus*- tamarind juices contain tartaric acid which possess strong bactericidal activity towards cultures of *S. aureus* (Ray and Majumdar, 1976). The pH of both chutneys and coriander pastes did not change from their initial values of 3.5 and 5.3 during the course of the experiments, since *S. aureus*, is able to multiply above pH values of 4.98 (HACCP- TQM, 1998), its inability to do so in coriander pastes may be attributed to a reduced availability of nutrients.

Coriander pastes are usually made in advance by many street-vendors (in household refrigerators whose temperatures vary from 4-10°C) this paste is used several times for making chutneys the following day, at times the unsold chutneys are also mixed with fresh lots and displayed for sale for periods varying usually between seven to ten hours. Toxin production in *S. aureus* is initiated only at a temperature of 10°C and at pH values of 5.1 or more (HACCP- TQM, 1998). The lower inactivation of *S. aureus* strains and survival in coriander pastes in refrigeration temperatures assumes importance particularly, with an initial contamination of sufficiently high

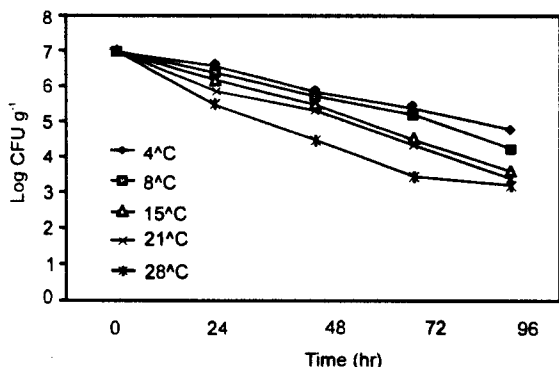


Fig. 1: Survival of *S. aureus* in Green chutneys at different storage temperatures

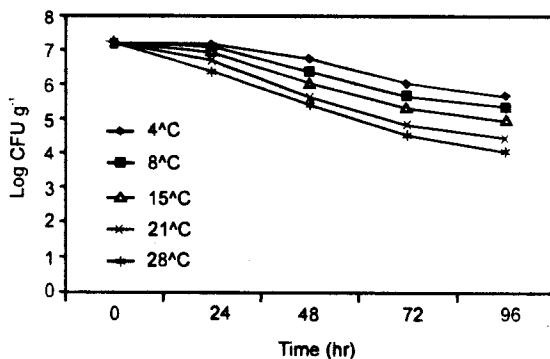


Fig. 2: Survival of *S. aureus* in Coriander pastes at different storage temperatures

numbers *S. aureus* would be able to produce enterotoxins once the coriander pastes are removed from refrigeration, and kept at ambient temperatures by the vendors, for making chutneys.

Our study clearly shows the prevalence of *S. aureus* in street vended green chutneys and its ability to survive in this product, especially in coriander pastes used for making such chutneys at least for a period of two days after which it is gradually inactivated. Given a lower level ( $< \log 3 \text{ CFU g}^{-1}$ ) of initial contamination and the fact that inoculum size of *S. aureus* may not affect survival rate, freshly prepared green chutneys may not pose significant risks to consumer health since levels of *S. aureus* population is likely to decline with time, not grow to population densities where it could produce toxin, also preparation and storage of coriander pastes in advance would be risky. The high levels of *S. aureus* and the ability of the latter to survive within short periods of time is definitely of concern in these street vended raw foods and Government intervention is mandatory in order to protect consumers and ensure safety standards of such foods.

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