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Original Research Article

# Microbiological hazard identification in selective food products and their association with food safety practices in Hyderabad, India

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ABSTRACT

**Introduction:** Microbiological risk assessment (MRA) is an emerging tool for evaluating the safety of food and water supplies. In this study, identification of microbial hazards in selected samples and their association with food safety practices were seen.

**Methodology:** Data were analyzed using Analysis of Variance (ANOVA) through the General Linear Models (GLM) procedure of the statistical analysis system software (SPSS version-11.5, 2003). The least significant differences were used to test means at $p<0.05$. Odds ratio (OR) values with 95% CI were computed to obtain the risk of the presence of the foodborne pathogen in a particular food.

**Results:** Analysis of 600 food samples indicated that *Salmonella enterica* (50%) was high in raw chicken samples followed by carrot salad (41%). The other emerging foodborne pathogens like *Methicillin-resistant staphylococcus aureus* (MRSA), *Yersinia enterocolitica*, and *E.coli O157: H7* were not detected in any of the food samples. Pathogens like *S.aureus* (73.5%) in khoa, *E.coli* (45%) and *fecal coliforms* (62.7%) in paneer were detected. A total of *S. aureus* (n=143) cultures were analyzed for enterotoxin and coagulase enzyme. Nine cultures (6.3%) showed a positive result for enterotoxin production. For the risk assessment of *S. aureus* contamination in foods, coagulase test and toxin production of isolates have to be evaluated. A significant association was found between the type of storage and log concentration of *S. aureus* in khoa, whereas, with water for washing hands, the status of nails, and cleaning cloth were contributing to foodborne pathogens in other products.

**Conclusion:** There is a need to provide food safety training to food handlers to improve food safety.



## 1. Introduction

Microbiological risk assessment is one of the best tools in food safety management. Microbiological Hazard identification is one of the preliminary steps in finding risk assessment in foods. The global burden of foodborne disease caused by the 31 hazards in 2010 was 33 million Disability Adjusted Life Years (DALYs); children under five years old bore 40% of this burden. In 2010, thirty-one foodborne hazards caused 600 million foodborne illnesses and 420,000 deaths. Foodborne diseases, are most frequently caused due to diarrhoeal agents such as Norovirus and Campylobacter spp. Foodborne diarrheal disease agents like non-typhoidal *Salmonella enterica*, caused 230,000 deaths. Other significant causes of FBD

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deaths were *Salmonella typhi, Taenia solium*, hepatitis A virus, and aflatoxin.[1]

The majority of foodborne illnesses in India caused due to vegetarian foods.[2] In other countries, the incidence of foodborne diseases was more due to non-vegetarian kinds of stuff such as beef, pork meat, frozen steaks, poultry, and other meat items.[3] Among the foods implicated in India, milk, and milk products were predominantly involved in the foodborne disease outbreak.[4]*E.coli* 0157:H7 was detected in one sample, each of milk, paneer, and ice cream, according to a recent study in India.[5] The economic impact of foodborne diseases on an annual basis in Canada, the United States, and New Zealand ranges from 1.1 billion to 7 billion.[6,7] The economic cost of a foodborne disease outbreak in Hyderabad, India, for 60 persons was Rs 91901.[8]

According to reports of the Center for Control Disease and Prevention, 61% of foodborne illnesses were attributed to improper food handling and is the leading cause of outbreaks in the foodservice industry.[9] WHO encourages governments, industry, and consumer organizations to disseminate important food hygiene messages such as keep clean, separate raw and cooked, cook thoroughly, keep food at a safe temperature and use safe water and raw materials.[10] A better understanding of the distribution, epidemiology, and threat posed by emerging and uncharacterized pathogens is needed because they are poorly controlled, spread globally, rapidly through the food chain. In this context, to see the association of food pathogens and food safety practices followed in Hyderabad, India, this study was done. For safe food handling practices among food handlers**,** the development of key messages will be done based on the results obtained during the study.

## 2. Materials and Methods

### *2.1. Study area*

The study carried out in Hyderabad, which is the capital of Telangana, India. The twin cities of Hyderabad and Secunderabad come under the ambit of a single municipal unit, the Greater Hyderabad municipal corporation. The random sampling procedure was adopted to select five zones out of it. The type and the number of procurement points (wholesale or retail shops) were those opted by the majority of the people. Based on the prevalence of food pathogens in food products, the exact sample size calculated. The sample required for the study obtained using probability proportion to size method.

### *2.2. Questionnaire*

A standard proforma to collect information on food products from procurement points prepared. The proforma pretested and collected the information on demographic data, product information, and personal hygiene of the shop keeper. Date of purchase, procurement point, region, district, Mandal, area/locality, shop type, and owner name included in the information of demographic data. The product information such as the name of the food product, date of manufacture, date of expiry, lot/batch no (quality symbol), amount of product purchased, type of storage, type of container used for storage, storage temperature, duration of storage, mode of transportation, source of supply, type of milk used for preparation was also collected. The information on personal hygiene of the shop keeper like wearing gloves, frequent cleaning of hands, cleanliness of clothing, and status of nails were also collected. Verbal consent was taken from the volunteer street vendors participated in this study. They were informed about objectives and nature of the study. Confidentiality was kept by using code number rather the name of street vendor. Closed ended and open ended questions have been included to obtain all type of probable answers. In quantitative method questions were asked by interview mode at the vending site. In qualitative method, a non-participant observation method was used to keep a note on personal hygiene and food safety practices of street vendors. These personal hygiene questions were observed and recorded with the consent of vendors.

### *2.3. Sample collection and processing*

Based on the prevalence of foodborne pathogens in the pilot study, Rasmalai (milk based dessert), Kulfi-icecream, Paneer, raw chicken, carrot salad, and Khoa (desiccated milk product) were selected. As per the study design, a total of 600 samples collected from randomly selected procurement points of Hyderabad. Twenty-five Grams of each piece was weighed and transferred to 225 ml of sterile buffered peptone water. The diluents of buffered peptone water then inoculated on to the respective media.

### *2.4. Identification and enumeration*

Identification and enumeration of foodborne pathogens such as *Escherichia coli0157:H7 Listeria monocytogenes, Yersinia enterocolitica, Methicillin-resistant Staphylococcus aureus* (MRSA) and *Salmonella* spp. performed as described by standard methods of US-FDA bacteriological analytical manual. Detailed information on enrichment media used for isolation, selective media and biochemical tests for food pathogens given in Table 1.

Coagulase test is done by taking 0.05ml of an overnight broth culture of *Staphylococci* or 2-3 pure colonies picked from the agar plate on a clean glass tube and then by adding 0.5ml of rehydrated coagulase plasma (from rabbit) procured from Himedia Laboratories Pvt.ltd. Both the solutions were mixed well and incubated at 37°C in the incubator for 4h. Agglutination or clumping of cocci within 4h was considered a positive result.

**Table 1:** Biochemical tests used to detect selective food pathogens

| Foodborne pathogens | Enrichment media for isolation | Specific media & cultural characteristics | Morphological characteristics | Biochemical confirmation |
|---|---|---|---|---|
| *E. coli* | PEM - BPW | MacConkey Agar – Pink to red | Gram-negative rod-shaped; Motile | * Glucose fermentation: Positive<br>* Lactose fermentation with gas production: Positive<br>* Oxidase: Negative<br>* Citrate utilization: Negative |
| *Salmonella* spp. | Selenite broth | XLD Agar – Red colony with a black center;<br>SS Agar – Colorless colonies with black cent<br>er | Gram-negative rod-shaped; Motile | *H2S (TSI & LIA): Positive<br>* Urease: Negative (No color change)<br>* Lysine decaroboxylase broth: Positive (Purple color)<br>* KCN broth: Negative (No growth)<br>* Indole test: Negative (Yellow color at the surface)<br>* Polyvalent flagellar test: Positive (Agglutination)<br>* Polyvalent somatic test: Positive (Agglutination)<br>* Phenol red sucrose broth: Negative<br>* Simmons citrate: Variable |
| *Shigella* spp. | PEM - BPW | SS Agar – Colorless colonies | Gram-negative rod-shaped; non-motile | * H2S: Negative<br>* Urease, Glucose (gas), Lysine decarboxylase, Sucrose, Adonitol, Inositol, Lactose (2 days), KCN, Malonate, Citrate, Oxidase & Salicin: Negative<br>* Methyl red: Positive |
| *S. aureus* | PEM - BPW | BPA – Grey black shiny colonies<br>MS Agar – Yellow-colored conies surrounded by yellow zones | Gram-positive Spherical shaped; Non-motile | * Catalase activity: Positive<br>* Coagulase production: Positive<br>* Anaerobic utilization of glucose: Positive<br>* Mannitol: Positive |
| *B. cereus* | PEM - BPW | BCA – Peacock blue color conies surrounded by good egg yolk precipitation of the same color | Gram-positive rod-shaped in short to long chains; Motility +/- | * Phenol red glucose broth: Anaerobic fermentation Red to Yellow<br>* Catalase: Positive<br>* Reduction of nitrate: Positive<br>* Tyrosine decomposed: Positive<br>* Lysozyme resistant: Positive<br>* Egg yolk reaction: Positive<br>* VP reaction: Positive<br>* Acid from mannitol: Negative |
| *Listeria monocytogenes* | Listeria enrichment broth | Listeria identification Agar (Palcam) – Grey-green with clack center and a black halo | Gram-positive rod-shaped; Tumbling motility | * Catalase & Hemolysis: Positive<br>* Production of acid with Rhamnose: Positive<br>* Production of acid with Xylose: Negative<br>* CAMP test – S. aureus: Positive |
| *Yersinia enterocolitica* | Peptone sorbitol bile broth | Yersinia Selective Agar – Dark red colonies resembling bull's eye which are surrounded by a transparent border | Gram-negative rod shaped; Motile | * LAIA slant: Alkaline slant & acid butt; no gas & no H2S reaction<br>* Urease: Positive<br>* MR VP test: Agglutination (clumping) of bacteria along walls and bottom of the tube with clear supernatant fluid |
| Fecal coliforms (Indicator organism) | PEM - BPW | MFC Agar base – Aniline blue color colonies | Gram-negative rod shaped; motile | * Lactose fermentation with gas and acid production: Positive |
| *E.coli* 0157H7 | E.coli 0157H7 Enrichment broth | E.coli 0157H7 selective Agar Base | Gram-negative rod shaped; motile | Sorbitol Negative and Latex agglutination test<br>β-Glucuronidase negative |
| MRSA | PEM - BPW | MRSA selective Agar Base | Gram-positive | Strains of S. aureus having a zone of inhibition of ≤21 mm to cefoxitin disc (30 $\mu$g) |

**Table 2:** Incidence of foodborne pathogens (percentage of contamination)in food products sold in various localities of Hyderabad

| **Food products** | **Foodborne pathogens** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (n=600) | *S.aureus* | *Salmonella spp.* | *F.coliforms* | *E.coli* | *Listeria* spp | *Yersinia* Spp | MRSA | *Campylobacter* spp | *E.coli* 0157:H7 |
| Rasmalai | 32 (31.7%) | 6 (5.9%) | 21 (20.8%) | 10 (9.9%) | ND | ND | ND | ND | ND |
| Khoa | 75 (73.5%) | 10 (9.8%) | 48 (47.1%) | 32 (31.4%) | ND | 6 (5.8%) | ND | ND | ND |
| Paneer | 29 (28.4%) | 16 (15.7%) | 64 (62.7%) | 46 (45.1%) | ND | 1 (1%) | ND | ND | ND |
| Raw Chicken | 35(35%) | 50 (50%) | 35(35%) | 41(41%) | ND | ND | ND | ND | ND |
| Raw Carrot Salad | 27 (27%) | 41 (41%) | 15 15%) | 21(21%) | ND | ND | ND | ND | ND |
| Kulfi | 22 (22.0%) | 12 12%) | 13 13.0%) | 6 (6.0%) | ND | 1 (1%) | ND | ND | ND |

### *2.5. Preparation of bacterial culture for enterotoxin production*

Pure *S. aureus* culture was pre-enriched in Brain Heart Infusion (BHI) broth. Centrifugation of the bacterial culture done for 5 min at a minimum of 3500g/$10^0$C. Sterile filtration of the supernatant done and 100 $\mu$l of the filtrate per well used in the enzyme immunoassay.

### *2.6. Enterotoxin detection*

For the detection of enterotoxin assay, both coagulase-negative and positive *S. aureus* strains selected. RIDASCREEN SET total sandwich enzyme immunoassay kit, Manufactured in Germany, was used for the combined detection of *Staphylococcus* enterotoxins (SET) A, B, C, D and E from bacterial cultures. All reagents required for the enzyme immunoassay were there in the test kit.

### *2.7. Antibacterial resistance of food pathogens to commonly used antibiotics*

Agar disk diffusion technique used to check antibacterial resistance against commonly used antibiotics. In this experiment, antibacterial resistance of food pathogens tested along with bacterial pathogens isolated from diarrhoeal stool samples of under-five children. Mueller Hinton agar supplied by Himedia used in this experiment. Himedia antibiotic discs including Gentamycin (G), Norfloxacin (NX), Amikacin (AK), Amoxycillin (AM), Furazolidone (FR), Co-Tromoxazole (COT), Ampicillin (A), Ceftriaxone (CTR) and Cefotaxime (CTX) used in this experiment. Agar plates inoculated with a standard inoculum of a respective food pathogen. The antibiotic discs kept on the agar aseptically. Then the plates are incubated at 37°C for 24h.

### *2.8. Survival of food pathogen at different temperature and storage time*

The overnight culture of *S. aureus* diluted to get the initial inoculum level ($10^2$ CFU), and 1 ml of this known bacterial load transferred to the control tube and the milk product. Milk products tested for the presence of any contaminating bacteria before investigation. The inoculated milk product kept at 4, 12, and 37°C. During each sampling day, 1 ml of the milk product was aseptically drawn and dispensed in 9 ml peptone water. For each test sample, serial dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$) carried out for microbial assays in 9 ml peptone water and plating was done in duplicate using the spread plate technique onto selective agar. The plates were incubated aerobically at 37 °C for 24 h. After incubation, typical colonies of the presumptive pathogens counted, and the results interpreted as CFU/ ml.

### *2.9. Molecular characterization of food pathogens*

Reagent based method was used to extract genomic DNA. The samples electrophoresed on 1% Agarose gel. DNA yield determined from the concentration of DNA in the eluate, measured by absorbance at 260nm. DNA samples sent to Eurofins, Bangalore, for species-level identification.

### *2.10. Statistical analysis*

All microbial counts were converted to the base -10 logarithm of the number of colonies forming units per ml of milk samples (log CFU/ml) and from the means and their standard deviations. Data were analyzed using Analysis of Variance (ANOVA) through the General Linear Models (GLM) procedure of the statistical analysis system software (SPSS version-11.5, 2003). An association between food safety practices and food products contributing to foodborne pathogens found using chi-square analysis. Odds ratio values with 95% CI computed to obtain the risk of the

presence of the foodborne pathogen in a particular food. Least significant differences used to test means at $p<0.05$.

## 3. Results

Analysis of 600 food products indicated *Salmonella enterica* (50%) was high in raw chicken samples followed by carrot salad (41%). *Salmonella* also found in paneer, ras malai, khoa, and kulfi. In any of the food products, the other emerging foodborne pathogens like *Listeria* spp. *Methicillin-resistant staphylococcus aureus* (MRSA), *Yersinia enterocolitica*, and *E. coli O157: H7* were not detected. Pathogens like *S.aureus* (73.5%) in khoa, *E.coli* (45%) and *fecal coliforms* (62.7%) in paneer were detected. Khoa (52%) samples found to contain *S. aureus* above $10^6$ CFU/g, which is likely to produce heat-stable enterotoxin. However, the milk product like paneer is usually further cooked, and the pathogen may not survive in the final product. The presence of *Salmonella* Spp. in milk products is a cause of concern from the consumer point of view. The percentage contamination of foodborne pathogens was high in the summer season than in winter except for foodborne pathogen *S. aureus*.

The counts of *Salmonella* and *S.aureus* are not conforming to FSSAI (Food Safety Standards Authority of India)-Microbiological standards of foods. A significant association was found between the type of storage and log concentration of *S.aureus* in khoa, whereas, with water for washing hands, the status of nails, cleaning cloth were contributing to foodborne pathogens in other products. The information collected on milk products indicated that 70% of milk products kept in refrigerated condition, and 30% kept at ambient temperature. The type of milk used for the preparation of milk products indicated that 47.7% of milk products prepared from buffalo milk, and 4.2% prepared from cow milk. The information was not available for 27.2% of packaged milk products. The information on personal hygiene of shop keeper indicated that 92% of the shopkeepers were not wearing gloves while serving milk products. Only 31% of shop keepers wore clean clothes, and 33% of shop keepers cut their nails.

Statistical analysis was done to see the association between food safety practice and milk products contributing to foodborne pathogens. A significant association was found between the type of storage and log concentration of *S. aureus* in khoa, whereas, with water for washing hands, the status of nails, cleaning cloth were contributing to foodborne pathogens in other milk products.

Risk estimate of foodborne pathogens with food safety practices associated with milk products indicated that the odds ratio of *S. aureus* in ras malai was five times higher if they use bore well water for washing hands, the 95% confidence interval being 1.86-16.7. The odds ratio of fecal coliforms in panner was 9times more for bore well water usage instead of municipal water, the 95% confidence interval being 1.77-50.4. Similarly, the odds ratio of *Salmonella* in khoa was 17 times higher if the nail of the vendor is uncut, the 95% confidence interval being 1.52-195.8.

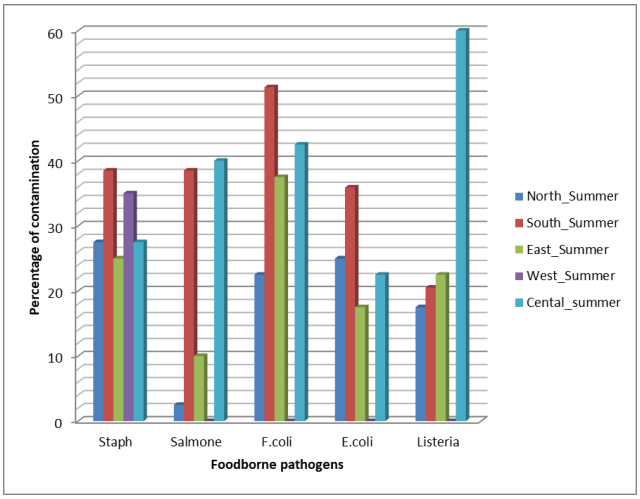


**Fig. 1:** Percentage of foodborne pathogen contamination in food samples during the summer season

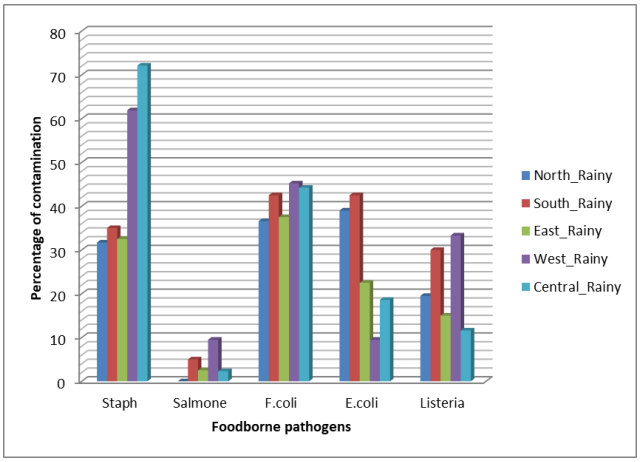


**Fig. 2:** Percentage of foodborne pathogen contamination in food samples during the rainy season

Study on persistence and survival of *S. aureus* at different temperatures (4, 10, and 37$^o$C) for different length of time (0-12 days) indicated that the *S. aureus* population varied with temperature and showed the highest population and viability at 37$^o$C. On the $12^{th}$ day, differences in the population observed at a lower and higher temperature. A total of *S. aureus* (n=143) cultures analyzed for enterotoxin and coagulase enzyme. Nine cultures (6.3%) showed a positive result for enterotoxin production (ELIZA). And 106 (74.1%) showed a positive result for coagulase enzyme production.

Nine commonly prescribed antibiotics were used against 113 isolates to check the antibiotic sensitivity. All the

**Table 3:** Association between food safety practices and food products contributing to foodborne pathogens

| Variable | Milk Product | Foodborne pathogen | Categories | | P-Value |
|---|---|---|---|---|---|
| **Type of storage** | | | **Refrigerated** | **Ambient** | |
| | Khoa | *E.coli* | 42.1 | 85.7 | 0.05* |
| | Paneer | *S.aureus* | 59.6 | 88 | 0.001** |
| | Paneer | *E.coli* | 21.2 | 42 | 0.05* |
| | Kulfi | *F.coliforms* | 14.3 | 26.9 | 0.01** |
| **Water for washing hands** | | | **Borewell water** | **Municipal water** | |
| | Rasmalai | *S.aureus* | 47.2 | 19.6 | 0.05* |
| | khoa | *F.coliforms* | 57.1 | 31 | 0.01** |
| | khoa | *E.coli* | 53.1 | 11.9 | 0.01** |
| | Paneer | *F.coliforms* | 69.3 | 31.3 | 0.01** |
| **Status of nails** | | | **Cut** | **Uncut** | |
| | Rasmalai | *S.aureus* | 13.6 | 29.1 | 0.05* |
| | Khoa | *S.aureus* | 8.1 | 40 | 0.01** |
| | Kulfi | *S.aureus* | 14.3 | 38.4 | 0.01** |
| | Kulfi | *F.coliforms* | 10.7 | 24.7 | 0.05* |

* P<0.05 indicates statistical significance at 95%CI
**P<0.01 indicates statistical significance at 99%CI

**Table 4:** Risk estimate of foodborne pathogens with food safety practices associated with food products

| Variable | Milk product | Foodborne pathogen | OR (CI) | P-Value |
|---|---|---|---|---|
| Water for washing hands (Ref- Municipal water, Risk- Borewell water) | Rasmalai | *S.aureus* | 5.58 (1.86-16.7) | 0.01* |
| | Khoa | *F.coliforms* | 3.20 (1027-8.06) | 0.01* |
| | Paneer | *F.coliforms* | 9.44 (1.77-50.4) | 0.05** |
| | Khoa | *S.enterica* | 0.022 (0.001-0.5) | 0.05* |
| Status of nails (Ref-cut, Risk-uncut) | Khoa | *S. enterica* | 17.2 (1.52-195.8) | 0.05* |

* P<0.05 indicates statistical significance at 95%CI
**P<0.01 indicates statistical significance at 99%CI

**Table 5:** Screening of coagulase and enterotoxin in Staphylococcal enterotoxin producing *S.aureus* fromfood samples

| Type of foods | No. of isolates | Coagulase + | Coagulase - | Enterotoxin |
|---|---|---|---|---|
| Dhal | 7 | 5 | 2 | 2 |
| Hand Washings | 7 | 6 | 1 | 0 |
| Khoa | 36 | 27 | 9 | 0 |
| Kulfi | 5 | 4 | 1 | 0 |
| Non-veg | 12 | 5 | 7 | 2 |
| Paneer | 7 | 6 | 1 | 0 |
| Pineapple FJ | 2 | 2 | 0 | 0 |
| Rasmalai | 22 | 20 | 2 | 1 |
| Rice | 27 | 17 | 10 | 2 |
| Sapota FJ | 2 | 2 | 0 | 0 |
| Veg curry | 16 | 12 | 4 | 2 |
| Total | 143 | 106 (74.1%) | 37 (25.9%) | 9 (6.3%)* |

**Table 6:** Antimicrobial resistance of bacterial isolates from food samples for commonly used antibiotics

| Antibiotics Antibiotics | Bacterial pathogens resistant n(%) to antibiotics isolated from food (N=113) |
|---|---|
| Gentamycin | 0(0) |
| Norfloxacon | 9(7.9) |
| Amicacin | 1(0.8) |
| Amoxycillin | 4(3.5) |
| Furazolidone | 41(36.2) |
| CoTrimoxazole | 31(27.4) |
| Ampicillin | 27(23.8) |
| Ceftrioxone | 8(7) |
| Cefotaxime | 10(8.8) |

Bacterial pathogens: *Staphylococcus, E.coli, Salmonella*

isolates were resistant to the antibiotics except Gentamycin. Bacteria isolated were resistant to Furazonidole (36%), followed by Cotrimoxazole, Ampicillin, Cefotaxime, Ceftriaxone, Norfloxacin, and Amoxicillin. Antibiotic sensitivity of bacterial isolates also compared with the same isolates from clinical samples. The percentage of antibiotic resistance was more in clinical isolates compared to bacterial isolates isolated from food. Bacterial isolates of clinical samples showed more resistance towards antibiotic Cotrimoxazole (68%), followed by Amoxicillin (66%).

The antibacterial activity of nisin tested against the *S. aureus* and *Salmonella* spp, which was isolated from milk products. The antibacterial activity of nisin against *S. aureus* and *Salmonella* spp indicated that the minimum inhibitory concentration observed at 2.5 mg/ml. The effect of ozone on *S. aureus* in milk products was seen only in Rasmalai (50% reduction). Based on the results obtained during the study, following key messages developed for the food handlers such as, use hand gloves and mask while cooking and serving food, keep cooked foods and cut vegetables covered, store mik products in refrigerated condition, and trim long nails.

## 4. Discussion

In India, food safety is gaining a lot of importance to control disease burden in the population. FSSAI (Food Safety Standards Authority of India) has recently released guidelines for recall as an activity to remove food products from the market at any stage of the food supply chain, including that possessed by a consumer, which may pose a threat to the public health or food that violate the Act, or the rules or regulations made thereunder.[11]

To ensure food safety in the supply chain, ISO22000 established which is also known as Food Safety Management Systems (FSMS). FSMS developed as an international solution for improving food safety. It incorporates a critical control point and hazards analysis system in a more improved form. FSMS endorses the conformity of services and products for international trading by assuring reliability, food safety, and quality.[12]

This study mainly done to see the prevalence of emerging food pathogens and to estimate the risk of food pathogens in food products. The present study showed the presence of food pathogens in food products. A similar kind of study conducted reported that 29% of the ras malai and rasgulla food samples were contaminated with *E. coli*.[13] The percentage of contamination of *S. aureus* in khoa samples was high (74%) compared to other milk products. In another study, carried out at Madhya Pradesh, India reported that out of 50 khoa samples, 45 samples were contaminated with *S. aureus*.[14]

Khoa (52%) samples found to contain *S.aureus* above $10^6$ CFU/g, which is likely to produce heat-stable enterotoxin. However, the milk product like paneer is usually further cooked, and the pathogen may not survive in the final product. Very few *S.aureus* isolates (6.3%) were able to produce enterotoxin. Coagulase diversity among *S.aureus* isolates indicated that coagulase-positive cultures were more (74%) than the coagulase-negative (26%) cultures. Both coagulase-positive and negative cultures were able to produce enterotoxins.

In our study, among 143 (31.7%) cultures of *Staphylococci,* 106 (74.1%) showed coagulase enzyme production, and 37 (25.9%) isolates were coagulase-negative. Only nine cultures (6.3%) showed a positive result for enterotoxin production. It is known that $>10^6$ CFU/g of *S. aureus* is likely to produce an enterotoxin, however, in the present study, 17% of food samples have crossed the limit, but very less number of them were able to produce enterotoxin. A survey conducted by Cunha et al on detection of enterotoxin genes in coagulase-negative Staphylococci isolated from foods indicated that 22.7% were positive for CNS. Among them, four isolates were positive for enterotoxin genes.[15]

Our study showed a risk estimate of *S. aureus* 5 times higher if a vendor use bore well water for washing hands and for paneer nine times more for borewell water usage instead of municipal water. Similar kind of study on the bacteriological quality of water at the point of use and hand hygiene of primary food preparers showed that 48% and

20% of hand rinse samples were contaminated by fecal coliforms and *E. coli* respectively.[16]

In the present study, *S. aureus* population varied with temperature and showed a high population and viability at 37$^{o}$C. A study on the influence of holding temperature on the growth and survival of *Salmonella* spp. and *Staphylococcus aureus* and the production of staphylococcal enterotoxin in egg products indicated that Staphylococcal enterotoxin A and B are detected only in the egg products held at 37 or 22 degrees C. After holding at 37 degrees C for 36h, scrambled egg inoculated with *S. aureus* contains the highest levels of SEA and SEB.[17] In India, there are many studies to show the prevalence of Staphylococcus in food samples, but studies on its enterotoxin production are scanty.

Our study showed a significant association between the type of storage and log concentration of *S.aureus* in Panner. A similar kind of survey on Food Safety in Domestic Refrigerators – A Mixed Methods Study to Identify Key Messages for Promoting Safe Storage Practices among Households shows that *Salmonella spp. (44.4%), E. coli (27.7%), fecal coliforms (11.1%), and S. aureus (5.5%)* detected in leftover refrigerated foods.[18] The contamination by *Staphylococcus aureus in food products* may be due to its carriage in nasal passages of food handlers or infected workers.[19]

Our study showed seasonal variation among bacterial contamination in food products was more in summer compared to the rainy season. The survey of Seasonal influence, enteropathogenic microbial load and diarrhoeal enigma in the Gangetic Delta, India: Present scenario and health implications showed that total bacterial count proliferates at higher temperature whereas turbidity enhances their survival providing the substratum for the bacterial pool.[20]

## 5. Conclusion

Emerging foodborne pathogens like *Methicillin-resistant staphylococcus aureus* (MRSA), *Yersinia enterocolitica, Listeria monocytogenes*, and *E.coli O157:* H7 not detected in any of the food samples. Further molecular characterization of classical and novel genes encoding different Staphylococcal enterotoxins is necessary to find out different types of enterotoxins in Indian conditions. The significant association found between the type of storage and log concentration of *S.aureus* in khoa whereas, with water for washing hands, the status of nails, cleaning cloth contributing to foodborne pathogens in other products indicated that there is a need to provide food safety training to food handlers to improve food safety.

## 6. Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

## 7. Availability of data and materials

All the data generated or analyzed of the current study are available with the corresponding author.

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## 9. Authors Contributions

Naveen Kumar R: Conceptualization, Investigation, Methodology, Uday Kumar P: Project administration, Bhaskar V: Formal Analyis, data curation, Sudershan RV: Software, validation, Polasa K: Writing Original Draft, Hemalatha R: Resources, Supervision, Sudip Ghosh: Writing, review and editing

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