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ASSESSMENT OF ADULTERATION AND MICROBIAL SAFETY IN RAW MILK: A CROSS -SECTIONAL STUDY IN THANJAVUR

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Abstract: Milk being vital food serves as an essential source of nutrients, crucial for the growth of children and the health of adults. However in recent times milk has been subjected to adulteration with various harmful substances that are primarily introduced to enhance the quantity and extend its shelf life often for financial gain. The present study was aimed evaluate the possible adulteration of milk (without added sugar) sold by street vendors and tea shops in and around Thanjavur distirct, Tamil Nadu, India. Approximately 45 milk samples (20 from tea shops and 25 from street vendors) were randomly collected and tested for common adulterants such as starch, sugar, formalin, hydrogen peroxide, pulverized soap, chromate or dichromate, annatto, boric acid, sodium chloride, water, urea, detergents, neutralizers, salt, and skim milk powder. The analysis revealed that milk samples from street vendors were predominantly adulterated with water (76%) and sugar (28%), while those from tea shops contained sugar (20%), starch (25%), sodium chloride (30%), skim milk powder (10%), and urea (10%). The results indicated that a significant number of milk samples were found to be adulterated with commonly used substances raising both economic and public health concerns. Therefore it is essential to raise awareness among consumers and local milk vendors about unethical practices in the milk supply chain that could pose serious health risks.

Keywords: Milk, Adulteration, Hygiene,Public health, Thanjavur, Cross-sectional analysis

INTRODUCTION

Milk is one of the most complete and widely consumed sources of nutrition across all age groups globally (Muehlhoff et al., 2013). Advances in cold chain logistics, pasteurization and processing technologies have significantly expanded milk's reach into rural and semi-urban markets, steadily driving up demand, particularly in a country like India which continues to lead the world in total milk production (NDDB, 2023; DAHD, 2024). Milk is susceptible to a wide range of chemical, physical and biological contaminants, and various traditional as well as modern biotechnology-based detection techniques have been developed to identify and quantify these adulterants (Darwesh et al., 2025; Azad & Ahmed, 2016). However, the persistent gap between farmer-level supply and rapidly growing consumer

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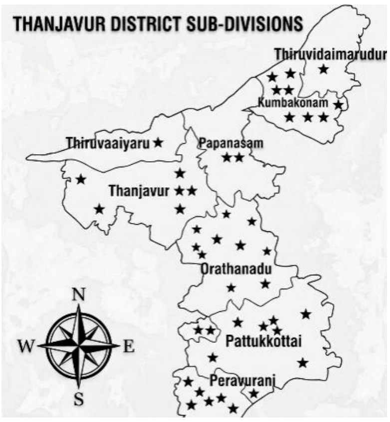
demand, combined with the temptation of higher profit margins at every stage of the supply chain, has made adulteration an increasingly common practice (Singh et al., 2020; Spink & Moyer, 2011). Among all food commodities, milk ranks as one of the most frequently adulterated products reported in global food fraud databases (European Commission, 2023; Moore et al., 2012). Adulteration is no longer confined to a single point — it occurs across the entire supply chain from the farmer and middleman to the processor and retail vendor (Singuluri, 2014; Azad & Ahmed, 2016). Surveys conducted by food regulatory bodies have repeatedly found that a significant proportion of milk samples collected across Indian states fail to meet established safety and quality standards (FSSAI, 2022; Singh et al., 2020). In Tamil Nadu specifically, concerns about milk quality have persisted, with studies highlighting the presence of adulterants such as detergents, starch, neutralizers and watered-down samples circulating in local markets (Rajarajan, 2021). Given the scale of this problem and the limited availability of recent region-specific data, this study was undertaken as part of a food safety and public health initiative to systematically assess the prevalence and types of adulteration in milk samples collected from the Cauvery delta region of Tamil Nadu.

MATERIALS AND METHODS

Thanjavur district is geographically located within the fertile Cauvery delta of Tamil Nadu positioned between 9° 50' and 11° 25' north latitude and 78° 45' and 79° 25' east longitude. The area is distinguished by its notably flat landscape which gently slopes eastward towards the Palk Strait primarily bordered by the Kollidam River to the north and the Bay of Bengal to the south. This region often referred to as the "Rice Bowl of Tamil Nadu" is interlaced with a complex system of rivers and canals including the Vennar and Vadavaru which support its intensive agricultural and dairy-based economy. With an average elevation of around 58 meters above sea level the district’s distinctive deltaic topography offers optimal environmental conditions for the extensive livestock rearing that is prevalent.The current cross-sectional study was carefully carried out within the geographical confines of the Thanjavur district in Tamil Nadu, India over sixduration from July 2025 to January 2026.

Map 1. Shows the distribution of samples in district (Taluk wise)



In order to enable a comprehensive assessment a total of 45 raw milk samples were systematically collected from a range of prominent retail establishments situated in both urban and peri urban areas. The sampling methodology was specifically crafted to compare different levels of the informal dairy supply chain leading to the collection of 20 samples from well established local tea shops and 25 samples directly from mobile street vendors.

Each sample was collected using aseptic procedures to avert any external environmental contamination that could distort the analytical findings. Only high quality, pre-sterilized and chemically inert containers were employed for the collection process to guarantee the preservation of the milk chemical and biological characteristics. Immediately after collection each container was sealed and carefully labeled with specific information including the type of source, precise location and time of collection. In order to maintain the original state of the milk and to avert any possible microbial proliferation or enzymatic degradation during transportation the samples were placed in specially designed insulated cool boxes that ensured a consistently low temperature. Following this the samples were swiftly transported under rigorously controlled hygienic conditions to a specialized laboratory facility for comprehensive analysis. The subsequent laboratory procedures were structured to investigate the samples for a wide array of chemical adulterants while simultaneously evaluating the overall hygienic quality and freshness of the milk. Detection of adulterants was carried out using standard laboratory-prepared chemical reagents following established colorimetric methods as outlined by FSSAI (2015), Sharma et al. (2021) and Darwesh et al. (2025). Detection of adulterants including gelatin, neutralizers, urea, detergent, annatto, formalin, hydrogen peroxide, boric acid, cane sugar, starch, skim milk powder, sodium chloride and added water was carried out using standard laboratory prepared chemical reagents based on colorimetric colour change reactions as per the methods outlined in the

Manual of Methods of Analysis of Foods: Milk and Milk Products (FSSAI, 2015), the Qualitative Tests for Detection of Common Adulterants in Milk (FSSAI, 2021) and the Detection of Adulterants in Milk A Laboratory Manual (Sharma et al., 2021). Physicochemical and microbiological quality was assessed through pH measurement, alcohol test, clot on boiling test and methylene blue reduction test following the methods of Moosavy et al. (2019) and Desye et al. (2023).

RESULT AND DISCUSSION:

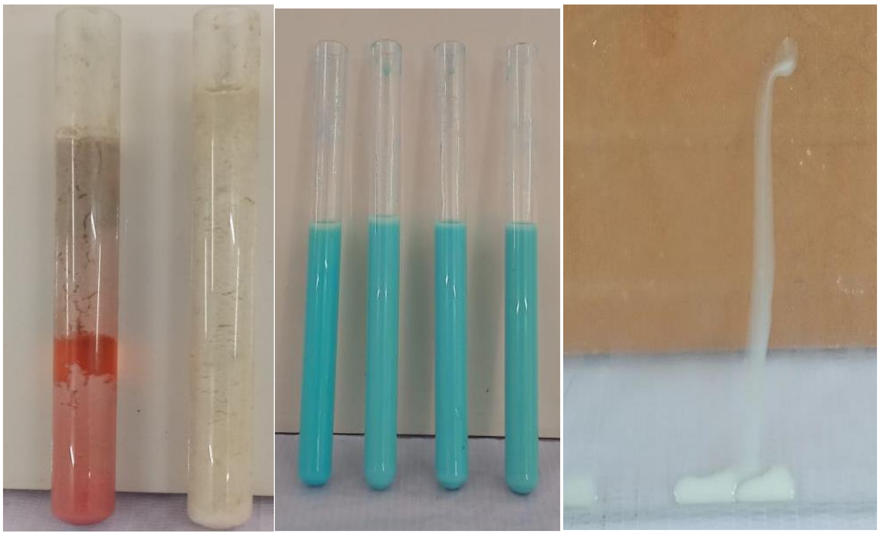
A total of 45 milk samples were gathered from various sources in the Thanjavur region which included street vendors (n = 25) and tea shops (n = 20). The findings revealed that milk adulteration was widespread in the study area although the types and frequencies of adulterants differed between the two sources. Water emerged as the most prevalent adulterant identified in this study with 76% of samples from street vendors and 55% from tea shops testing positive. The practice of adding water to milk is one of the most common forms of adulteration as it increases the volume of milk and thus enhances the economic return for vendors. However this dilution significantly diminishes the nutritional quality of milk by reducing the levels of fat, protein, lactose and minerals. Furthermore if contaminated water is utilized it may introduce harmful microorganisms and pathogens that pose serious health risks to consumers. Previous research has also recognized water as the most frequently detected adulterant in milk within developing countries. (Azad and Ahmed, 2016; Patari*et al.*, 2022). Similar results were noted in research carried out in Tamil Nadu and various other areas of India where water contamination was identified as a prevalent practice among informal milk suppliers. This issue arose due to insufficient regulatory oversight and the economic pressures faced by vendors (Dhanalakshmi *et al.*, 2017; Rajarajan, 2021).

In the current study it was found that sugar adulteration occurred in 28% of samples from street vendors and 20% from tea shops whereas starch adulteration was identified in 25% of street vendor samples. The incorporation of sugar and starch is typically linked to efforts to conceal the effects of dilution with water. Sugar amplifies the sweetness of diluted milk while starch enhances viscosity and provides the milk with an appearance akin to that of natural milk. Carbohydrate adulterants including starch and sugar are commonly noted in studies on milk adulteration due to their low cost and easy availability. As noted by Chauhan et al. (2019) starch is frequently utilized to augment the thickness of diluted milk while sugars may be introduced to enhance flavor and density. These adulterants compromise the nutritional quality of milk and can lead to digestive issues when ingested in significant amounts. In this research it was found that skim milk powder was present in 10% of samples from tea shops while it was absent in samples collected from street vendors. Skim milk powder is sometimes incorporated into diluted milk to enhance the solid not fat (SNF) content improve viscosity and augment the visual appeal of milk used for making tea or coffee. Previous studies have also reported the prevalent use of skim milk powder as an adulterant in diluted milk. Such practices are frequently observed in small food establishments where milk serves as an ingredient for beverages rather than being sold directly to consumers (Singuluri and Sukumaran, 2015; Paramasivam, 2021). Despite the fact that skim milk powder is derived from milk its addition to fresh milk modifies the natural composition and may diminish the overall nutritional value. The present study identified urea in 10% of samples from tea shops while none of the samples from street vendors contained these adulterants. Urea is occasionally introduced into milk to enhance the perceived protein content especially in the manufacturing of synthetic milk. The detection of urea in milk may also be linked to environmental pollution from fertilizers utilized in agriculture. Overconsumption of milk tainted with urea can lead to kidney damage, gastrointestinal issues and metabolic disturbances (Chitra, 2021; Patari*et al*., 2022).

Sugar Positive; Pink colour

Starch Positive; Blue colour

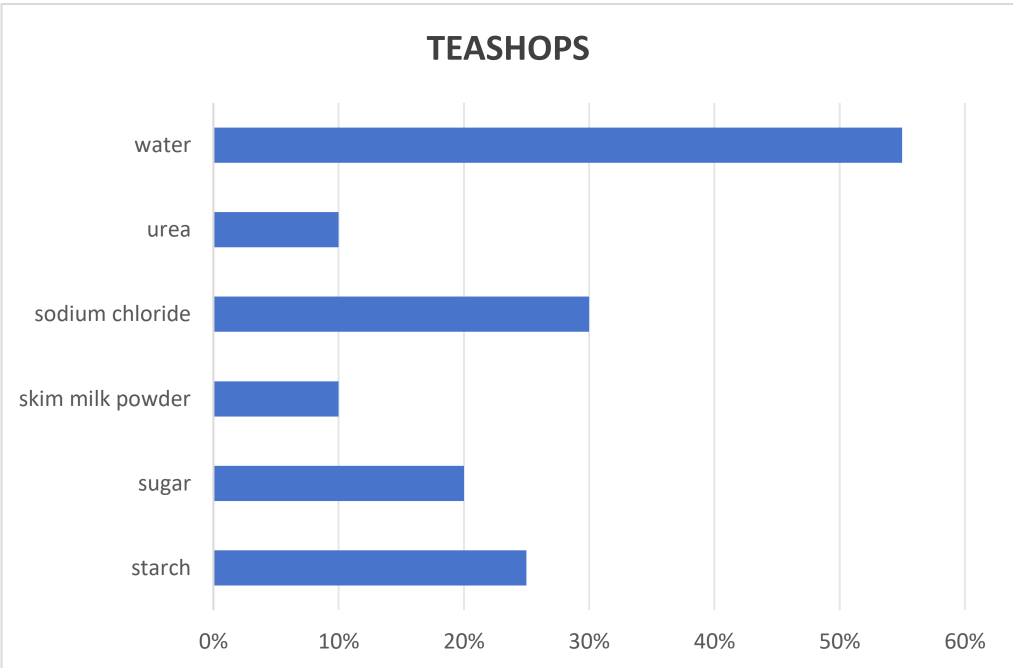
Urea Positive; Yellow colour



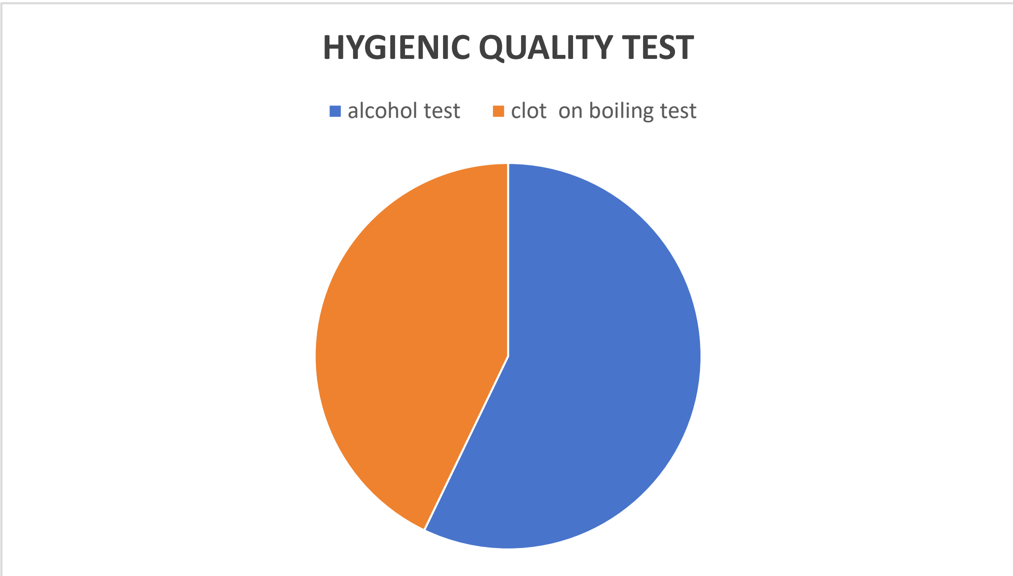
Skim milk powder positive ; Orange

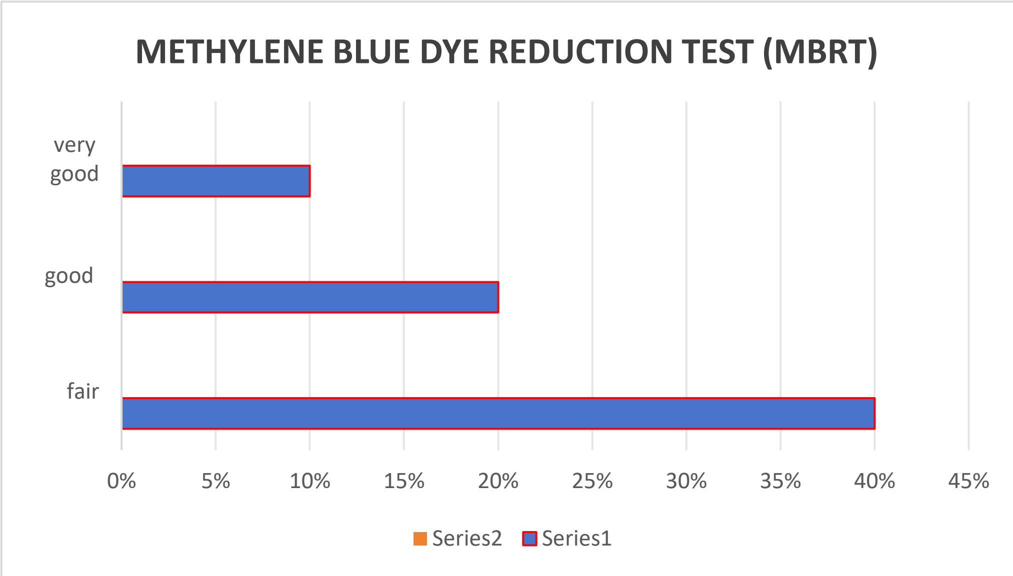
Methylene Blue Dye Reduction Test (MBRT)

Clear Line ; Without Adulteration Of Water




STREET VENDORS
sugar
water


HYGIENIC QUALITY TEST
alcohol test
clot on boiling test



The microbial quality of raw milk samples procured from street vendors in Tamil Nadu was evaluated through a combination of physicochemical and rapid microbiological tests including pH measurement, alcohol test, clot-on-boiling (COB) test and methylene blue reduction test (MBRT). These field applicable methods serve as reliable cost effective proxies for assessing milk freshness, protein stability, acidity development and overall microbial load particularly in informal marketing channels where advanced laboratory infrastructure is limited (Sharma et al., 2025; Mukhopadhyay et al., 2024). The mean pH of the collected samples was 6.9 which lies within the typical range for fresh bovine milk (generally 6.6–6.8). This value indicates that the majority of samples had not undergone extensive lactic acid fermentation at the time of analysis. However even minor elevations or fluctuations in pH can signal incipient microbial activity, mastitis conditions or adulteration. Comparable pH values (6.62–6.66) were documented in raw standardized milk from northern India where samples largely complied with regulatory standards under controlled handling (Sharma et al., 2025). Deviations from this narrow physiological window often correlate with increased bacterial proliferation especially in tropical climates that accelerate lactose fermentation by mesophilic lactic acid bacteria. The alcohol test revealed coagulation in approximately 40% of the samples pointing to protein instability linked to elevated acidity or elevated microbial enzymatic activity. This test detects early proteolytic or acid induced destabilization of casein micelles which is common when milk is subjected to prolonged ambient storage or contaminated milking equipment. Studies on informally marketed milk have similarly attributed such instability to inadequate refrigeration and extended holding times during distribution leading to partial denaturation and reduced suitability for further processing (Desye et al., 2023). Positive COB reactions were observed in nearly 30% of samples indicating heat instability and early stage spoilage. The COB test is highly sensitive to developed acidity (typically above 0.18–0.20% lactic acid equivalent) which arises primarily from microbial fermentation of lactose. In regions like Tamil Nadu high ambient temperatures (often exceeding 30°C) combined with delays in transportation and the absence of cold-chain facilities create ideal conditions for rapid bacterial multiplication exacerbating positive COB outcomes. Microbiological grading via MBRT provided deeper insight into viable microbial populations. Of the 25 samples 10 (40%) were graded as fair quality, 5 (20%) as good quality and 10 (40%) as very good quality with no samples falling into the poor category. The MBRT relies on the respiratory activity of bacteria which deplete dissolved oxygen and reduce the methylene blue dye to its colorless leuco form. Shorter reduction times directly reflect higher metabolic activity and contamination levels (Roy et al., 2024). The notable proportion of fair quality samples underscores moderate to substantial microbial ingress consistent with reports from other regions evaluating raw milk in unorganized sectors (Sharma et al., 2025). Several interconnected factors likely contributed to the observed contamination profile. Unhygienic milking practices use of improperly sanitized utensils, environmental dust exposure during open vending and lack of immediate cooling allow diverse microbiota including coliforms, staphylococci and lactic acid bacteria to proliferate. Milk's nutrient rich matrix (high lactose, proteins and water activity) makes it an excellent growth medium enabling rapid increases in total viable counts under tropical conditions. Pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp and *Listeria monocytogenes* pose heightened food safety risks in such scenarios particularly for vulnerable consumers (Mukhopadhyay et al., 2024). A clear linkage emerged between potential water adulteration and microbial deterioration. Dilution with untreated water not only reduces nutritional density but introduces exogenous microorganisms and elevates coliform loads. Recent investigations confirm that adulterated samples frequently exhibit compromised physicochemical parameters and accelerated spoilage (Patel et al., 2025). Encouragingly the present study detected no hydrogen peroxide or detergent adulteration suggesting that vendors in the sampled area rely primarily on simpler adulterants like water rather than chemical preservatives. This aligns with observations in certain Indian dairy systems where traditional practices predominate over synthetic interventions. Comparative data from controlled versus ambient storage highlight the critical role of temperature. Milk held at room temperature in informal chains shows markedly higher total plate counts and coliform levels than refrigerated counterparts accelerating quality decline during marketing (Sharma et al., 2025). The absence of samples with extremely poor MBRT grades may reflect relatively short vendor to consumer turnover times in the study area yet the fair quality segment still signals a need for intervention.

Collectively these results demonstrate that while a subset of street vended milk maintains acceptable microbial standards a substantial fraction exhibits early deterioration, reduced keeping quality and potential safety concerns. The findings reinforce the necessity for targeted interventions: adoption of hygienic milking protocols, routine utensil sanitation, implementation of affordable cooling systems (e.g., bulk chillers or insulated transport) and regular training for street vendors on good hygienic practices. Enhanced regulatory surveillance coupled with simple rapid tests like MBRT and COB at collection points could significantly elevate safety standards in informal dairy sectors without imposing prohibitive costs. Future studies integrating culture-based enumeration, PCR detection of specific pathogens and whole genome sequencing would further elucidate contamination sources and resistance patterns supporting evidence based policy for consumer protection.

Conclusion

The present investigation clearly demonstrated that milk adulteration remains a widespread issue in the informal milk marketing system of the Thanjavur region particularly among street vendors and tea shops. Water emerged as the predominant adulterant among street vendors (76%) while tea shop samples exhibited higher use of sodium chloride, starch, sugar, skim milk powder and urea. This study uniquely evaluated both adulteration status and microbial quality simultaneously across two major informal retail sectors providing a comprehensive understanding of milk safety in the local supply chain. Street vendors primarily relied on simple low cost adulterants such as water, sugar and starch whereas tea shops employed additional substances to enhance consistency, appearance and shelf life of milk used in beverage preparations. Notably none of the samples tested positive for hydrogen peroxide, detergents, boric acid, chromate, dichromate or pulverized soap indicating distinct regional patterns of adulteration practices within the Cauvery delta zone. Physicochemical and microbiological assessments revealed that a considerable proportion of milk samples exhibited reduced heat stability, increased acidity and moderate microbial contamination. These findings reflect poor hygienic handling, inadequate storage and lack of cold chain facilities during transportation and sale. The methylene blue reduction test further classified

40% of samples as fair quality underscoring early microbial deterioration and the pressing need for improved sanitation practices. The detection of hazardous adulterants such as urea in tea shop samples is particularly concerning as chronic consumption may lead to gastrointestinal disturbances, renal complications and other long term toxic effects. Overall informal milk marketing systems continue to remain highly vulnerable to both adulteration and microbial contamination due to insufficient regulatory surveillance, inadequate infrastructure and limited awareness among handlers. Regular quality monitoring using rapid economical field tests such as pH analysis, alcohol test, clot-on-boiling test and methylene blue reduction test can serve as practical tools for on site assessment. Strengthening consumer awareness programs, promoting hygienic milk handling practices, improving cold chain infrastructure and enforcing stringent food safety regulations are essential steps toward minimizing adulteration and ensuring safe milk availability to the public. The present study provides valuable baseline scientific data on milk adulteration and microbial quality in the Thanjavur region that can serve as a reference for future food safety surveillance programs, public health interventions and policy development in unorganized dairy sectors. Further studies employing advanced analytical techniques and molecular approaches are recommended for detailed identification of emerging adulterants, microbial pathogens and antibiotic resistant contaminants in milk marketed through informal channels.

adulterants	Source of sample	Number of positive	Percenrtage of positive adultertion
starch	Street vendors - 25 Tea shops - 20	5 0	25%
Sugar	Street vendors - 25 Tea shops - 20	7 4	28% 20%
Skim milk powder Formalin	Street vendors - 25 Tea shops - 20	0 2	 10%
Hydrogen peroxide	Street vendors - 25 Tea shops - 20	0 0	
Carbonate or bicarbonate	Street vendors - 25 Tea shops - 20	0 0	
Pulverized soap	Street vendors - 25 Tea shops - 20	0 0	
Chromate or dichromate	Street vendors - 25 Tea shops - 20	0 0	
detergent	Street vendors - 25 Tea shops - 20	0 0	
annato	Street vendors - 25	0	

	Tea shops - 20	0	
Boric acid	Street vendors - 25 Tea shops - 20	0 0	
Sodium chloride	Street vendors - 25 Tea shops - 20	0 6	 30%
water	Street vendors - 25 Tea shops - 20	19 11	76% 55%
urea	Street vendors - 25 Tea shops - 20	0 2	 10%
	Street vendors - 25 Tea shops - 20		

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