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ANALYZING THE BACTERIOTA (RESIDENTIAL BACTERIA) FROM THE SURFACES OF FOOD PROCESSING UNITS

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ABSTRACT

Environmental sampling including the swabs from the surface of various locations in food processing units are considered as the major area thereby the overall hygiene is get disturbed leads to complete contamination in the surface hygiene. The main objective of this study is to analyze the presence of residential bacterial population from the surface areas of different locations of food processing units including restaurants and fresh juice preparation shops. The swabs were taken from five different locations of the food processing units before and after cleaning and disinfection mainly of two time with two days differences. Microbial enrichment media was used for the immediate culturing and transport to the laboratory. General, selective and differential culture media were used to determine the bacterial population. As a result, before cleaning and disinfection, gram negative bacteria including *Pseudomonas aeruginosa*, *Escherichia coli* dominated followed by *Micrococcus* sp and *Acinetobacter* sp whereas *Staphylococcus aureus* was found largely in the places where more milk and its products used. The second samples after two days after cleaning and disinfection, *Pseudomonas* and *Acinetobacter* remains due to its biofilm forming nature and tolerance to biocides. Predominantly both the samples showed the same set of bacterial species to be grown due to its residential nature on the particular nutrition rich environment. Thus cleaning and disinfecting before starting the morning jobs in these environment is suggested. Same way, the type and concentration of the disinfectant and method of disinfection should be changed regularly in order to reduce or avoid the colonization of the residential bacteria that may or may not cause mild to severe infections to the consumers.

KEYWORDS: Residential bacteriota, Food processing unit, Biofilm, Biocide tolerance.

INTRODUCTION

Microorganisms are found abundant in environment, where it creates and adjust with their own environment for survival. For growth and multiplication of such invisible creatures, the common factors and requirements are nutrients (organic materials), water, oxygen, humidity, pH, temperature, pressure and climate (season).[1,2] In general compared to other places in residential areas, kitchen and toilet are the prone places where microbes multiply and survive; but in home kitchen, the cleaning is properly done and the modes of nutrient spillage or deposit are very less.[3] But in commercial food restaurants and fruit stalls are spilling lot of nutrient substrates and waste foods and fruit peels for the enhancement of microbial growth.[4,5]

Food borne illnesses are largely noticed from these types of restaurants and fruit stalls thereby the microbial growth are enormous and scrupulous. [6] The presence of microbial entity in these environments are visceral and very tough to understand the types and nature. The cleaning procedures of restaurants and fruit stalls are not highly regulated and the usage of disinfectants also not standardized.[7] Development and implementation of better guidelines for cleaning has the potential to reduce microbial burden on these surfaces and therefore reduce the risk of food borne illness.[8,9]

Generally, the microbiological assessments of the restaurants and fruit stalls are not done as a part of the inspection process. The major reasons behind this exclusion are:

1. Improper execution of the health care evaluation.
2. Traditional microbiological analyses take 48 hours to provide result after sample collection.
3. Mobile microbiology analysis system is not yet introduced.

All the food processing units have a lawful responsibility for producing safe food. Based on the principles of Hazard Analysis of Critical Control Point (HACCP) and good hygiene practice (GHP), the implementation of food safety management system and surveillance have to be done in order to achieve the food safety at permissible limit.[10,11] Now a days, the surface of the food processing unit are made of various materials including plastic, stainless steel, glass, wood etc and these surfaces may encourage the microorganism to form biofilm.[12,13,14] In most times, eventhough after using the disinfectants to clean the surface of food processing units, the microbial contamination are found due to various intrinsic factors like:

1. Improper cleaning like drying the wet cleaned surfaces; left the food debris in the corners etc.
2. Usage of same disinfectants for long time (resistance may occur).[15,16]
3. Unhygienic cleaning cloth (non-sun dried repeated usage clothes).
4. Not providing appropriate time for chemical – microbe interactions.

In many food borne infections' outbreak situations, the surface contamination of the food processing unit play a vital role. Working surfaces are also responsible for cross-contamination of various products by contact with product specific microorganism like *Salmonella* from poultry meat, *E. coli* from ground beef etc.[17] The HACCP based process is now widely used for the control of microbial hazards to ensure food safety. To adopt that, microbiological analyses of surfaces of the food processing unit has appeared as one of the routinely performing tool for checking good hygienic practices and to maintain a high level of producing safe foods.

In most of food processing units, residues of several kinds of chemical, biological, organic, inorganic food debris or substrates accumulated in the surface of the tables, vessels and other equipments.[18] This will cause severe and serious health problems by microbial growth with direct and indirect (microbial toxins) causes. The food industry related microbes have dual system of action.

1. ***Economic issues:*** Saprophytic nature of microbes affect the food leads to food spoilage.
2. ***Health issues:*** Pathogenic microbes cause acute to severe food borne infections and intoxications to the consumers.

Due to the high and repeated resistance of microbes towards various surface cleaning disinfectants, the frequent changes of disinfectant and increasing the concentration of the same disinfectants may be useful.[19] The surfaces of the food processing sites are not only the prime source for food spoilage, contamination and infections, some other known and unknown intrinsic and extrinsic factors also play a vital role for the same.[20] The important reasons for the need of awareness of residential microbes in food processing environments are:

1. The transfer of microbes from production site to storage unit is easy because of same type of factors maintained in both environments.
2. The pathogenic microbes affect the growth of non-pathogenic (need based microbes).
3. The strong adhesion of the residential microbes on the surface, corners, screws that increase the food spoilage.
4. The surface microbes persist and spread the antimicrobial resistant genes to the progeny.

By this study, we try our level best to explore the types of residential bacteriota in the surfaces of three different food processing units and fresh juice preparation shops by standard bacteriological methods. This study not only providing the knowledge about the types of residential bacterial community but also understands the bacterial physiology, food processing, sanitation and microbial sampling.

MATERIALS AND METHODS

Units and shops

A total of three food processing units and three fresh fruit juice preparation shops were included in this study. Before collecting the samples, the formal written consent was obtained from the owners and gave assurance that their shops' and units' name should be used else and we also promised them to provide the results to them to improve their quality. This study was also approved by institutional ethics committee of Trichy SRM Medical College Hospital and Research Centre, Tiruchirapalli, India.

Sample collection

The swab samples were taken from three major sites of the production unit. The samples were also collected for two times on the same site by giving two days time gap between two samples. On the same day three samples were collected from all the three sites (total of nine samples per day; for two days total of 18 samples obtained from one unit). In this study, three food production units and three fresh fruit juice preparation shops were included, thus, total of 108 swabs were processed bacteriologically. The samples, locations, number of times and sampling plan were depicted in table 1.

The three sites of the units included in this study are regular working table/ surface, lower corner of the

surface and the place where screw or bolt located. The three sites of the fresh juice preparation shops are surface, lower corner and inner region of the blender/ juicer. The three times of sampling are:

1. Time 1 - After cleaning
2. Time 2 - After cleaning and disinfection
3. Time 3 - After cleaning, disinfection and drying

The special care should be taken for timing especially to the hygienic regime of the food production unit. The sampling at time points where sporadic bacteria are present and that should be avoided. The samples of recontamination after cleaning, insufficient cleaning and before drying of surfaces should be avoided.

Table 1: Sample collection plan.

Units/ Shops	No. of Units/Shops	No. of sites/day	No. of times/day	No. of days	Total No. of samples
Food processing units	3	3	3	2	54
Fresh fruit juice preparation shops	3	3	3	2	54
Total samples included in this study					***108***

Culturing of the bacteria in swabs

All the swabs collected were aseptically put inside the test tubes that contain sterile peptone water and transferred to the microbiology laboratory for further selective and differential processing. All the preliminary nutritive swabs were inoculated by surface plating method in nutrient agar, MacConkey agar, blood agar and other selective agar medium including Mannital salt agar, Eosin methelene blue agar, *Salmonella-Shigella* agar, *Pseudomonas* isolation medium and *Campylobacter* enrichment medium. All the plates were incubated at appropriate temperature, time and other specific growth requirements.

Biochemical and Antibiotic sensitivity test

After appropriate incubation, all the culture plates were analyzed for the colony morphology (color, colony shape etc). Further, the colonies were subjected to direct and indirect confirmatory tests. The direct tests including microscopy (motility, Gram's staining) and instant biochemical tests like catalase, oxidase, coagulase tests. The indirect confirmatory tests like bacterial specific biochemical tests were also performed. Finally all the bacterial isolates were subjected to antibiotic susceptibility test to determine the resistant nature of the isolates.

RESULTS

Food preparation unit surface verses bacterial colony forming units (CFUs)

There are eighteen swabs were collected from work table of food processing units thereby high bacterial load was observed in the lower corner of the working table than the centre work tables in all the three units. The overall CFUs found among the surface of the three units was depicted in figure 1.

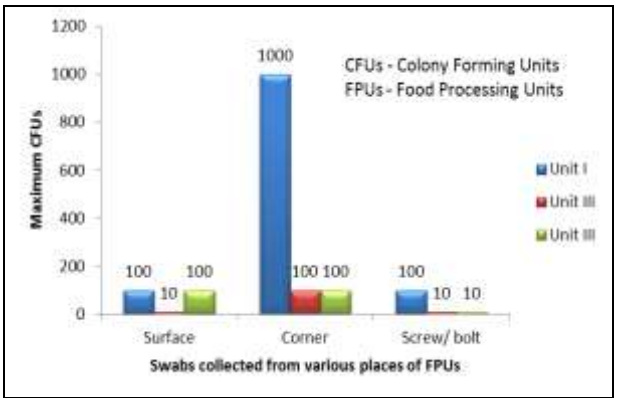

Figure 1: The overview of CFUs in surfaces of various food processing units.

Fresh Juice preparation unit surface verses bacterial colony forming units (CFUs)

Same like the food processing units, from the fresh juice preparation shops, a total of 18 swabs were collected and the bacterial isolates determined the high rate of bacterial load was found on the surface of the blades located inside the kitchen blender/ juicer. This may be mainly due to the improper cleaning on that site due to the presence of sharp and unpositioned blades. The detailed bacterial load was analyzed and depicted in figure 2.

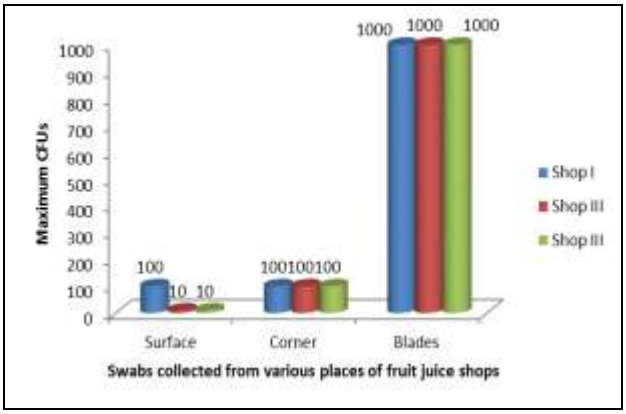

Figure 2: Overview of CFUS in various places of fresh juice preparation shops.

Aerobic colony count details

By analyzing the aerobic colony forming units in all the three units of food processing and fresh juice preparation shops, it was noticed that more colonies were determined immediate after cleaning with water only. While disinfectant is used for cleaning, then the colonies were reduced by half and most of the time, the colonies were not found after drying. The street based food stalls have more bacterial colonies than home based food preparation unit and middle level restaurants. The places where the bolt and screws were noticed have more colonies due to less priority to clean deeply on it. The same was observed in the blades of kitchen blender/ juicer; thereby plenty number and types of bacterial species identified. The complete descriptive analysis of CFUs of food processing units and fresh fruit juice preparation shops were impregnated in table 2 and table 3 respectively.

Comparison of surface verses more colony count

This section defines the importance and comparative analysis of bacterial colony count verses surfaces of the food processing units and fresh juice preparation stalls. In both the cases, the bolt and screw located in food processing units and blades of kitchen blender/ juicer had more colonies followed by lower corner of the processing areas compared to middle surfaces (Figure 1 and 2). Thus, cleaning of these areas by giving top priority is important. By this, the microbial (bacterial food poisoners) load may be reduced with some reasonable counts. Usage of disinfectants and cyclic changes of type and concentration of disinfectants minimize the emergence of resistant bacterial pathogens.

Table 2: Aerobic colony count details of Group I (Food processing Units).

Sample Descriptions	Number of samples supported with aerobic colony counts (n=54) [Unit 1/ Unit II/ Unit III]								
	No colonies	**1 – 9 colonies**	**10 - <10^2 colonies**	**10^2 - <10^3 colonies**	**10^3 - <10^4 colonies**	**10^4 - <10^5 colonies**	**10^5 - <10^6 colonies**	**Test not performed**	**Total samples**
Day 1 - After the food processing									
Site 1: Working table/ Surface									
Time 1	-	-	3	2	-	-	-	-	9 samples
Time 2	-	-	2	-	-	-	-	-	
Time 3	-	-	2	-	-	-	-	-	
Site 2: Lower corner of the surface									
Time 1	-	-	3	3	-	-	-	-	9 samples
Time 2	-	-	1	1	-	-	-	-	
Time 3	-	-	1	-	-	-	-	-	
Site 3: Place where bolt and screws are present									
Time 1	-	-	3	2	-	-	-	-	9 samples
Time 2	-	-	2	1	-	-	-	-	
Time 3	-	-	1	-	-	-	-	-	
Day 3 - After the food processing									
Site 1: Working table/ Surface									
Time 1	2	1	2	1	-	-	-	-	9 samples
Time 2	-	1	1	-	-	-	-	-	
Time 3	-	-	1	-	-	-	-	-	
Site 2: Lower corner of the surface									
Time 1	1	2	1	1	-	-	-	-	9 samples
Time 2	-	1	1	-	-	-	-	-	
Time 3	-	1	1	-	-	-	-	-	
Site 3: Place where bolt and screws are present									
Time 1	1	2	1	-	-	-	-	-	9 samples
Time 2	1	2	1	-	-	-	-	-	
Time 3	-	1	-	-	-	-	-	-	

[**Unit I**: Home based bulk food preparation unit; **Unit II** – Middle level restaurant and **Unit III** – Street food cart]

[***Time 1*** - After cleaning; ***Time 2*** - After cleaning and disinfection and ***Time 3*** - After cleaning, disinfection and drying]

Table 3: Aerobic colony count details of Group II (Fresh fruit juice preparation Units).

Sample Descriptions	Number of samples supported with aerobic colony counts (n=54) [Unit 1/ Unit II/ Unit III]								
	No colonies	1 – 9 colonies	10 - $<10^2$ colonies	10^2 - $<10^3$ colonies	10^3 - $<10^4$ colonies	10^4 - $<10^5$ colonies	10^5 - $<10^6$ colonies	Test not performed	Total samples
Day 1 - After the juice processing									
Site 1: Working table/ Surface									
Time 1	1	1	3	1	-	-	-	-	9 samples
Time 2	-	-	2	-	-	-	-	-	
Time 3	-	-	1	-	-	-	-	-	
Site 2: Lower corner of the surface									
Time 1	-	1	3	1	-	-	-	-	9 samples
Time 2	-	-	2	-	-	-	-	-	
Time 3	-	-	2	-	-	-	-	-	
Site 3: Lower corner and inner region/ blade of the blender/ juicer									
Time 1	-	-	1	3	1	-	-	-	9 samples
Time 2	-	-	-	2	-	-	-	-	
Time 3	-	-	-	2	-	-	-	-	
Day 3 - After the juice processing									
Site 1: Working table/ Surface									
Time 1	1	1	1	3	-	-	-	-	9 samples
Time 2	-	-	-	2	-	-	-	-	
Time 3	-	-	-	1	-	-	-	-	
Site 2: Lower corner of the surface									
Time 1	-	2	3	1	-	-	-	-	9 samples
Time 2	-	1	1	-	-	-	-	-	
Time 3	-	1	-	-	-	-	-	-	
Site 3: Lower corner and inner region of the blender/ juicer									
Time 1	-	-	1	3	1	-	-	-	9 samples
Time 2	-	-	-	2	1	-	-	-	
Time 3	-	-	-	1	-	-	-	-	

[**Unit I**: High level (100 juices/ day); **Unit II** – Middle level (50 - 75 juices/ day) and **Unit III** – Low level (50 juices/ day)]
[***Time 1*** - After cleaning; ***Time 2*** - After cleaning and disinfection and ***Time 3*** - After cleaning, disinfection and drying]

Bacteria confirmed (Food processing unit)

Among 54 samples collected from different food production surfaces and screws, and bolt areas, it was possible to isolate total of one hundred and seventy two (172) bacterial strains. On comparing with other surfaces, the space where the screws and bolts are located showed high number of bacterial colonies than other two surface areas. The colonies were initially identified based on its morphology, direct microscopy and further confirmed by biochemical tests. Among them, *Salmonella typhimurium* dominated (n=64; 37.2%) isolates, followed by *Staphylococcus aureus* (n=41; 23.8%) and *Escherichia coli* (n=32; 18.6%) (Figure 3).

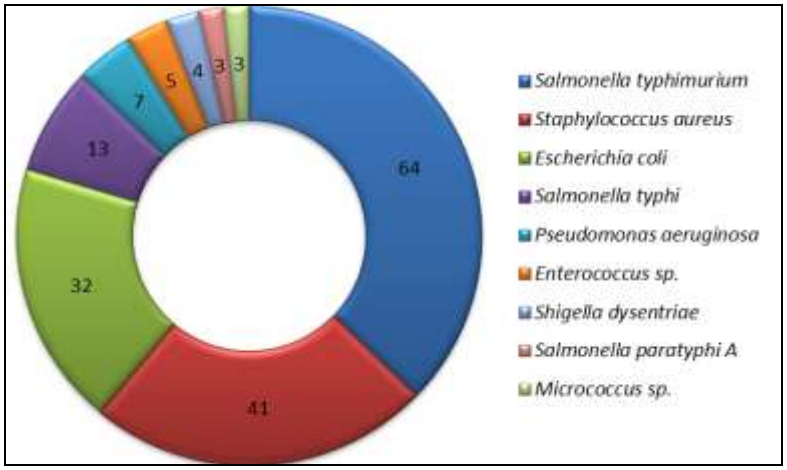

Figure 3: Bacterial isolates from food processing units (n=172).

Bacteria confirmed (Fresh fruit juice preparation unit)

From these areas, it was possible to isolate 202 bacterial colonies from 54 samples collected from different fruit juice preparation surfaces and blades of kitchen blender/ juicer areas. Same like food processing units, in fresh juice preparations shops also, the space where the blades of kitchen blender and juicer showed high number of bacterial colonies than other two surface areas. The results showed that *Escherichia coli* dominated followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* with 82 (40.6%), 41 (20.3) and 29 (14.4%) isolates respectively (Figure 4).

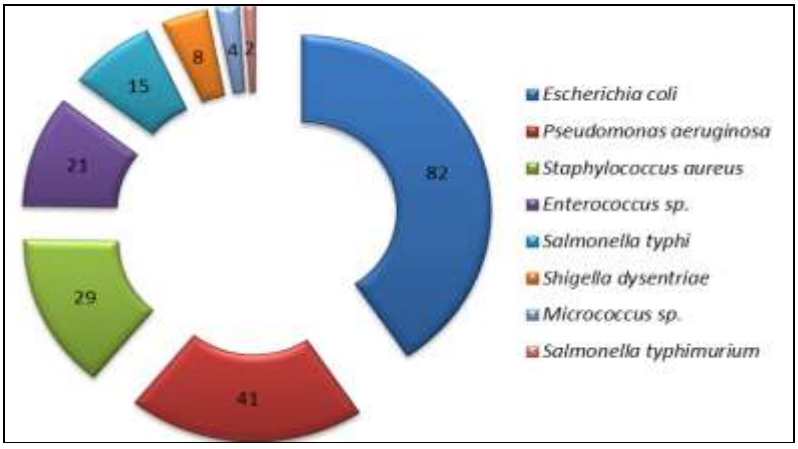

Figure 4: Bacterial isolates from Fresh juice preparation shops (n=202).

Antibiotic sensitivity pattern

The antibiotic susceptibility test was performed to understand the presence of antibiotic resistant bacterial strains in the surface of the food processing unit and fresh fruit juice preparation shops; thereby visceral resistant strains were identified. Among the bacterial isolates included in this study, *E. coli* showed maximum resistance to gentamycin (n=17; 14.9%) followed by ciprofloxacin and nitrofurantoin with 9 (7.9%) isolates each. By analyzing the antibiotic resistant pattern of *S. aureus*, the maximum resistance was found against amoxicillin, ampicillin, penicillin and gentamycin. *S. typhimurium* showed higher resistance to cefoperazine followed by ampiclin and trimethoprim. Ciprofloxacin, cefoperazone and amikacin are not having bactericidal nature towards *P. aeruginosa*. *S. typhi* showed resistance towards ciprofloxacin, amoxicillin, ampicillin etc. the remaining bacterial isolates also showed observable way of resistance towards various antibiotics. The detailed descriptive analysis of antibiotic sensitivity pattern of the bacterial isolates was impregnated in table 4.

Table 4: Antibiotic susceptibility pattern of the bacterial pathogens.

Antibiotic	Isolated bacterial strains (n=374) Vs Resistant pattern								
	***EC* (n=114)**	***SA* (n=70)**	**ST_1 (n=66)**	***PA* (n=48)**	**ST_2 (n=28)**	**EN (n=26)**	**SD (n=12)**	**MC (n=7)**	**SPA (n=3)**
Amikacin	8 (7.0)	23 (32.8)	-	16 (33.3)	-	2 (7.7)	-	3 (42.8)	-
Amoxicillin	-	27 (38.6)	-	-	17 (60.7)	-	4 (33.3)	-	2 (66.7)
Ampicillin	7 (6.1)	20 (15.4)	29 (43.9)	-	10 (35.7)	13 (50)	3 (25)	-	2 (66.7)
Azithromycin	-	-	-	-	9 (32.1)	7 (26.9)	5 (41.7)	-	1 (33.3)
Cefixime	8 (7.0)	-	12 (18.2)	-	-	-	7 (58.3)	-	-
Cefoperazone	-	-	36 (54.5)	17 (35.4)	-	-	-	2 (28.6)	-
Cefotaxime	-	-	14 (21.2)	-	11 (39.3)	-	5 (41.7)	-	1 (33.3)
Cefoxitin	-	9 (12.8)	-	-	9 (32.1)	-	-	-	1 (33.3)
Ceftriazone	-	-	-	-	8 (28.6)	-	-	-	2 (66.7)
Cefuroxime	-	-	-	-	4 (14.3)	-	-	-	1 (33.3)
Ciprofloxacin	9 (7.9)	15 (21.4)	19 (28.8)	19 (39.6)	17 (60.7)	21 (80.8)	7 (58.3)	-	3 (100)
Erythromycin	-	16 (22.8)	-	-	-	24 (92.3)	2 (16.7)	2 (28.6)	-
Gentamicin	17 (14.9)	19 (27.1)	-	8 (16.7)	-	21 (80.8)	1 (8.3)	2 (28.6)	-
Imipenem	-	-	-	11 (22.9)	7 (25)	4 (15.4)	3 (25)	-	2 (66.7)
Nitrofuratoin	9 (7.9)	-	-	-	-	2 (7.7)	-	-	-
Ofloxacin	-	-	-	-	-	2 (7.7)	3 (25)	2 (28.6)	-
Penicillin	-	20 (28.6)	-	-	-	21 (80.8)	-	-	-
Streptomycin	-	-	-	-	-	14 (53.8	-	2 (28.6)	-
Tetracycline	-	8 (11.4)	-	-	-	-	3 (25)	-	-
Trimethoprin	-	-	21 (31.8)	-	6 (21.4)	-	-	-	1 (33.3)

[**EC** – *Escherichia coli*; **SA** – *Staphylococcus aureus*; **ST_1** – *Salmonella typhimurium*; **PA** – *Pseudomonas aeruginosa*; **ST_2** –*Salmonella typhi*; **EN** – *Enterococcus* species; **SD** – *Shigella dysentriae*; **MC** – *Micrococcus* species and **SPA** – *Salmonella paratyphi* A] **[**Figure in parenthesis denotes percentage]

DISCUSSION

The safety of the food for consumption and re-exposure to the environment are highly associated with the quality of raw materials used and practice of hygiene (cleaning, disinfecting and maintaining good environmental sanitation) in the food processing unit.[9,11,21] In general, the type of raw materials used in the food processing units have an high impact of harboring microbial entities compared to other processes like processing, packaging etc. Thus, cleaning and sterilization of pre-food processing is quite important in food processing units in order to avoid the health issues to the consumers.[11,17,20] While interviewing the workers of the food processing units in this study, the same answers were given that the hygienicity of the raw materials and cleanliness of the environment play vital role in food spoilage and contamination.

The surfaces of the food processing units and fresh fruit juice preparation shops are the major reservoir for many number of food borne pathogenic bacteria and are responsible for food spoilage and food borne diseases including infections and intoxications.[4,9,22] Another study reported that the incidence of *S. aureus* with 12% was identified in the food contact surface of the food processing units;[2,6,12,17] but in this study *Salmonella typhimurium* dominated followed by *S. aureus* and *E. coli* (Figure 3); whereas in fresh juice preparation shops *E. coli* dominated followed by *P. aeruginosa* and *S. aureus* (Figure 4). In another study, *P. aeruginosa* dominated followed by *Acinetobacter*,[3,17,23] but in this study no single *Acinetobacter* was isolated.

Food related infections and illnesses are increased now a days are mainly due to increase in number of competitive restaurants including street vending foods, quality less packed local foods, laziness to prepare quality foods in home and bulk order food processing and preparations.[13,21,24]

The bacterial adherence on the surface of the processing unit during food production, after and before cleaning is different, which depends on the raw materials, type of food production, types of procedures, type of workers and workers related infections. Generally, a vast number of bacterial isolates were reported thereby *P. aeruginosa* dominated followed by enterobacteriaceae, *Acinetobacter* and lactic acid bacteria.[11,25] It was also determined that total of six specific bacterial genera was created more than 80% of food spoilage and contamination including *Pseudomonas, Acinetobacter*, Enterobacteriaceae, spore forming bacteria, *Staphylococcus* and lactic acid bacteria.[23,26]

Another study highlighted that the bacterial loads were high in the equipments, walls, floors, food raw materials, packaging and fabrication line sections. Among them, knifes, table spoons, tea spoons used for many activities are providing high risks of food spoilage and contamination leads to mild to severe food poisoning including food borne infections and intoxications. But in this study, we concentrated much of surface only not with the routine equipments used for the food processing except kitchen blender/ juicer, major equipment necessarily using in the fruit juice stalls. Few epidemiological outbreaks also found in various parts of the country that leads to more morbidity and mortality.[6,13,27]

This study has certain limitations of including less number of food processing units and fresh juice preparation stalls; inclusion of more and different units and shops provide better understanding of bacterial types and its physiology, and pathogenesis. The disinfectants routinely used in the units and shops are not examined for its effectiveness. The inclusion of specific areas of units and shops provide mere idea. In future, inclusion of more number and areas of food processing units, and disinfectants' evaluation will be done.

CONCLUSION

By this study, the knowledge about the types and composition of residential bacteriota was increased that implicate the workers in the food processing units to concentrate more in the food safety, quality and packaging. The strict hygienic measures are needed to ensure the control of pathogens that are involving in food spoilage and poisoning (both infections and intoxications). Changing the disinfectants frequently and variations in the concentration of the disinfectants used are also play a vital role in reducing the food spoilage by various bacteriota. Authorities are also involved in inspecting these units for their sanitation and hygiene for maintaining the quality and safety of the food products and consumers.

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