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Full Length Research Paper

Bacterial contamination: A comparison between rural and urban areas of Panipat District in Haryana (India)

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A randomized sampling from open air of the kitchens in rural vs urban households to determine bacterial contamination of Haryana (India) were carried out by taking 80 samples between July to September 2009. 40 samples of each in rural and urban area were collected in culture plates. The inoculation procedures were varied from direct inoculation of the kitchen air into the nutrient agar medium. Identification by bacterial taxonomy key, different morphological and biochemical tests in rural households, numbers of bacteria revealed *Salmonella* spp., *Acinetobacter* spp., *Pseudomonas* spp. and *Paenibacillus* spp. with 9 different strains and in urban households, numbers of bacteria revealed *Bacillus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Paenibacillus* spp. *and Acinetobacter* spp. with 27 strains. Among the isolates, *Salmonella* spp. (80%) followed by *Acinetobacter* (63%), *Pseudomonas putida* (38%) and *Paenibacillus polymyxa* (30%) were observed in rural areas. In urban areas *Bacillus* spp. (88%), *Pseudomonas* spp. (75%), *Micrococcus* spp. (70%), *Paenibacillus* spp. (38%) and *Acinetobacter* spp. (30%) were observed. The bacteriological quality of air of kitchens in rural households was found to be more pathogenic and virulent as compared to that of kitchen in urban households. These opportunistic pathogens may be harmful, especially in immunocompromised host. In this setting, there is a constant risk of contamination and transfer to willing host. Hence, better quality of air can be achieved by manipulating sanitation and hygiene within houses, kitchens and surrounding areas.

Key words: Air of kitchens, households, bacteriological quality, sanitation and hygiene.

INTRODUCTION

Bacteria are naturally everywhere: in the water we drink; air we breathe; in our skin; and inside our body. However, they are not visible to the naked eyes. Tiny organisms like bacteria can only be examined under a compound light microscope. The structure or morphology of bacterial cells includes the shape, size and morphological arrangement. The best way to utilize this study guide is to understand the characteristics by description and be familiar with the visual representations (Kristina, 2010). Bacteria are one-celled microorganisms or unicellular microscopic living organisms. They are the smallest organisms which have all the needed protoplasmic equipment for self-multiplication and growth. They are classified as prokaryotes because the cell does not contain a nucleus. Microorganisms such as bacteria that can cause disease are known as pathogenic and those that are harmless are considered as non-pathogenic microorganisms. Many living rooms in urban areas of Meerut district favor carpeting over vinyl flooring, table top cover, carpeting and curtains improves aesthetics, reduces noise, and helps prevents slips and falls. But the possible spread of infectious diseases and odors caused by micro organisms, and the treat of allergies resulting from inhibited growth of microorganisms is a concern. (Jaakkola et al., 2006).

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Airborne microbes, allergens and chemicals cause respiratory disease, inflammation in the nose, throat, sinuses, upper airway and the lung. Many infections are acquired by inhalation of pathogens that may remain in the respiratory system but also invade the rest of the body through lymphatic and blood circulations. Under air-borne diseases, by a somewhat loose usage, we may include a long list of diseases in which the channels of entrance and exit are the air passages. The medium of transfer is the air, in which the bacteria, usually breathed, coughed, or sneezed out in droplets, pass from person to person. This group of infections includes some of the most important diseases which affect mankind. In some of these diseases we do not know the actual germ, but it has been shown that they are carried from person to person in the air such as:

Typhoid fever: This is an infectious disease which is caused by the *Salmonella typhimurium*. It is also spread by air (By Roger I. Lee, The Air-Borne Diseases, Part I). Tuberculosis: This is an infectious disease which is caused by the tubercle Bacillus. The disease may infect any tissue of the body and may assume a wide variety of manifestations, but the most common form of tuberculosis is that of the lungs, consumption or phthisis (By Roger I. Lee, The Air-Borne Diseases, Part I). Influenza: True influenza is a distinct disease and is not an ordinary cold or "grippe." The disease is due to a specific Bacillus which can be isolated readily and which, of course, is entirely distinct from the microorganism which causes the cold or "grippe." (By Roger I. Lee, The Air-Borne Diseases, Part I).

Pathogenic organisms continuously enter the home with foods (foodborne) or through water (waterborne), through foods prepared in the home by an infected person (person to person spread), through the air (airborne), by the insects or via pets (Beumer et al., 1999). These are considered as a primary source of potential harmful microorganisms in the home. In the domestic environment, the kitchen is particularly important in spreading infectious diseases. Bryan *(*1988) indicated that a colonized person handling the implicated food was the most frequently identified factor that contributed to staphylococcal food poisoning, shigellosis and typhoid fever. Several studies on bacterial contamination in the kitchen were carried out in the past decades (Finch et al., 1978; Speirs et al., 1995). Bacterial load of hand towels, dishcloths, tea towels, steel sinks and working surfaces were implicated to be the frequent sites (Finch et al., 1978; Borneff et al., 1988; Josephon et al., 1997; Ikawa and Rossen, 1999; Kusumaningrum et al., 2002). Foodborne diseases associated with foods prepared in contaminated kitchen include salmonella as the most common culprit (Holah and Thorpe, 1990; Dufrenne et al., 2001; Kusumaningrum et al., 2004). Some other bacterial infections associated with contaminated kitchen environment are caused by Campylobacter, Listeria*, Staphylococcus aureus, Bacillus cereus and Escherichia coli* (Dufrenne et al., 2001; Regnath et al., 2004). Infectious diseases are known as a serious health risk for many centuries. The mortality rate due to these was a great concern even as in the late 18th and early 19th centuries. About 80% of the diseases prevalent in India can be attributed to rate of safe drinking water, poor sanitation and unhygienic practices followed by inhabitants.

The first well known bacterial transmission in the kitchen was documented in the early part of the 20th century, when Mary Mallon, who worked as a cook in private New York households, was identified as a healthy chronic carrier of the typhoid fever bacterium. She had been spreading typhoid fever through the food she prepared. Due to poor personal sanitary habits, she caused more then 30 cases of typhoid fever with three deaths, while Mallon herself had never been sick with typhoid fever (Olsen et al., 2000; Lerner et al., 1996; Porter et al., 1996). This through epidemiological discovery work and the finding of typhoid bacterium in Mallon's stool, proved the significant role of household environment in transmission of food borne diseases and had a great impact on the science of microbial hygiene.

Historically, salmonella has caused the largest proportion of reported food borne diseases outbreak associated with private homes in Europe and other bacterial infections associated with this environment are caused by campylobacter*, S. aureus, B. cereus and E. coli* (Olsen et al., 2000). Salmonella and campylobacter contamination rates of poultry product are found to be up to 60 and 80% respectively (Dufrenne et al., 2001; Scott et al., 1982). Indeed both clean air and water are the basic necessities of human life. In many Indian villages supply of safe drinking water is a serious problem and the same holds true for air in urban areas. Any carelessness in adoption of routine hygienic measures can lead to transmission of infection through air droplets. The present research work revealed that the status of air of kitchens and living rooms have been found repeatedly contaminated with a variety of bacterial contaminations. In India and specially Haryana states of India very few literatures have been identified of bacterial contamination in the air of kitchens and living rooms in rural and urban areas and their comparison. This study therefore aimed to investigate, identify, and comparison of the bacterial contamination in air of kitchens and living rooms of 160 different samples from 80 homes in rural and urban areas of Panipat district.

METHODOLOGY

One hundred and sixty samples of open air of kitchens and living rooms were collected from Panipat district and its surrounding villages between July to September 2009. In this study, we randomly selected four villages from rural areas namely Israna, Balana, Naultha, Mehrana and four urban sites namely Modal Town, HUDA Sector, NFL Colony and Sukhdev Nagar. 160 air

samples from 80 homes were collected for bacteriological analysis from 40 homes (kitchens and living rooms) in each of the rural and urban areas. All these samples were analyzed by conventional techniques as described by Buchanan and Gibbons (1974) and Carter and Cole (1995). Some samples were identified by IMTECH (Institute of Microbial Technology) Chandigarh, India (CSIR Institute, Govt. of India).

Study area

Randomly selected 80 different homes (kitchens and living rooms) in Panipat district of Haryana were surveyed for potentially harmful pathogens in the domestic kitchens and living rooms of rural and urban areas between July to September 2009.

Sample collection

The 160 samples were collected from kitchens and living rooms of rural and urban areas in Panipat district. The samples were aseptically collected in already clean prepared culture plates of Nutrient Agar. The samples were taking from the open air from kitchens and living rooms. Nutrient agar plate (NA) were using for the collection of samples. For each site two replicates were place on the kitchens and living rooms, left open for 1 to 2 h, and then incubated for 24 h at 30 to 34°C.

Sample analysis

All samples were analyzed by conventional techniques as described by Buchanan and Gibbons (1974); Carter and Cole (1995). After collection of samples, culture plates were incubated in BOD incubator at 30 to 34°C for 24 h. After incubation samples were analyzed by morphological or biochemical methods. Microbiological direct analysis of air requires quantitative determination, that is, total population of microorganisms. The densities of cells, spores/conidia of microorganisms were measured in the laboratory through several methods of direct microscopic or colonies counter. In the direct microscopic counts, a known volume of liquid is added to the slide and the numbers of microorganism are counted by examining the slide with the bright field microscope. For colony counter Neubauer or Petroff-Hausser counting chamber, breed smears or electric cell counter (or Coulter counter) were used. The samples were again analyzed by 13 different biochemical tests for kitchens sample and 12 biochemical test for living rooms such as catalase test, oxidase test, hydrogen sulphide production test, nitrate reduction test, indole production, MR reaction, VP reaction, citrate use test, urease test, litmus milk test, lactose fermentation, sucrose fermentation, dextrose fermentation.

Identification of isolates

After 24 h of incubation, the colonies that appeared morphologically dissimilar were chosen, counted, subcultured to fresh appropriate culture media and incubated at 30 to 34°C for 24 h. Identification of microorganisms did not commence, due to the fact that inhibition was evident that a pure culture had been obtained. Colonies identifiable as discrete on the different agar medium (EMB, Blood agar, MacConkey agar, XLD etc) will carefully examined macroscopically for culture characteristics such as the shape, color, size texture and hemolytic reactions. Colonies are gram stained and individual bacterial cell were observed. The bacteria were speciated using there isolated colonies (Beumer et al., 1996). Further identification of enteric organisms was done using different taxonomical methods given by Aneja (2003). Anaerobes and many traditional morphological and biochemical test were selected for this study.

RESULTS AND DISCUSSION

A total of 160 samples from 80 homes (40 kitchens and 40 living rooms of each rural urban area) were collected and analyzed for bacterial contamination and their comparisons. Samples obtained from rural and urban kitchens from near dustbins, sink, washing-up areas, food shelf, cutlery and crockery, refrigerator, vegetables racks, floor, back side of door and near gas cylinder. Samples obtained from in rural and urban living rooms near carpets, tabletop, curtains, dressing tables and ceiling fans etc. The higher positive bacterial growth was observed 98% in kitchens of rural areas and 95% in kitchens of urban areas. On the other hand, in living rooms 97.5% of rural areas and 92.5% in urban areas are observed in Panipat district of Haryana.

Bacterial contamination in kitchens of rural and urban area

After bacterial isolation from kitchens, they were subjected to various tests and the results were obtained and summarized in Table 1. On the basis of primary characterization, the samples were subjected to morphological and biochemical analysis to confirm the identify bacteria. The presence of bacteria was discerned in 77 samples of air of kitchens in rural and urban households out of 80 samples. Only three samples (one in rural and two in urban) of air of kitchens were found to be bereft of bacteria. In urban areas isolated bacteria on nutrient agar from open air reveled growth of the *Bacillus* spp. (10), contributed the major fraction of bacteria in kitchen air followed by *Pseudomonas* spp.(7), *Micrococcus* spp. (6), *Paenibacillus* spp. (3) and *Acinetobacter* spp. (1). However, in rural areas the *Salmonella* spp. (4) contributed the major fraction of bacteria in the kitchen air followed by *Acinetobacter* spp. (3) and (1) in both *Pseudomonas putida* and *Paenibacillus polymyxa.* The total numbers of bacterial isolates from the air of kitchens in rural and urban areas were 52 and 55 respectively with the notable fact that *S. typhimurium* was found only in the kitchens in rural households. On the other hand *Micrococcus luteus and Bacillus flavus* were seen only in the kitchens of urban households (Table 2).

In the air of kitchens in rural areas, the presence of *Salmonella* spp. which is virulent and pathogenic in nature was recorded, whereas in urban areas it was not seen in any of the samples. Notably, a reverse trend was observed with respect to *Bacillus* spp., which is generally harmless and causes food spoilage only. The pathogenic and non pathogenic status of all the isolated Bacteria are shown in Table 2, which indicates that the bacteria

Table 1. Bacterial analysis in the air of 80 domestic kitchens in rural and urban areas in Panipat district of India.

Type of samples	Source of samples	Total no. of samples processed	No. of samples devoid of bacteria	Total no. of bacteria isolated	Number of genus isolated	Bacteria identified
Kitchens of rural households	Israna	10	Nil	11	2	[1]
	Balana	10	1	15	2	[2]
	Naultha	10	Nil	17	1	[3]
	Mehrana	10	Nil	09	2	[4]
Kitchens of urban households	Model town	10	Nil	10	3	[5]
	Huda colony	10	1	19	4	[6]
	NFL colony	10	1	12	4	[7]
	Sukhdev Nagar	10	Nil	14	3	[8]

Kitchens of rural households: [1] *Salmonella* spp.- 2 strains, *Pseudomonas* spp. [2] *Salmonella typhimurium*, *Acinetobacter* spp.- 2 strains, [3] *Paenibacillus polymyxa*, [4] Acinetobacter*, Salmonella typhimurium.* Kitchens of urban households: [5] *Bacillus* spp.- 5 strains, *Micrococcus* spp.-3 strains, *Pseudomonas* spp.-4 strains [6] *Bacillus* spp.-3 strains, *Paenibacillus polymyxa, Micrococcus luteus, Pseudomonas putida* [7] *Bacillus flavus, Paenibacillus* spp. -2 strains, *Micrococcus luteus, Pseudomonas* spp. -2 strains [8] *Bacillus flavus, Micrococcus luteus,* Acinetobacter.

Table 2. The pathogenic status of bacteria and number of colonies isolated from the air of kitchens of rural and urban households.

S. No.	Culture medium	Sampling	Bacteria identified (Genus)	Sources	{No. of colonies (%)} / 40 plates in each (rural and urban areas)	Pathogenic/non Pathogenic
1	NA	Open air	*Bacillus* spp.	Urban	35 (88)	Generally harmless, causes food spoilage
2	NA	Open air	*Paenibacillus* spp.	Rural Urban	12 (30) 15 (38)	Plant growth promoter
3	NA	Open air	*Micrococcus* spp.	Urban	28 (70)	Harmless
4	NA	Open air	*Pseudomonas* spp.	Rural Urban	15 (38) 30 (75)	Saprophytic soil bacteria
5	NA	Open air	*Acinetobacter* spp.	Rural Urban	25 (63) 12 (30)	Causes nosocomial infections
6	NA	Open air	*Salmonella* spp.	Rural	32 (80)	Human pathogen cause typhoid fever

NA: Nutrient agar.

isolated from air of kitchens in rural areas are more virulent and show higher pathogenic activity as compared to the bacteria isolated from air of the kitchens in urban areas. The total number of 6 genus isolates from kitchens in rural and urban areas. Table 2 shown in among these isolates, in rural areas *Salmonella* spp. for 80% of isolates (32 colony types from 40 plates), followed by *Acinetobacter* spp. (63%), *P. putida* (38%) and *P. polymyxa* (30%). In urban areas, *Bacillus* spp. accounted for 88% of isolates (35 colony types from 40 plates) and allowed by *Pseudomonas* spp. (75%), *Micrococcus* spp. (70%), *Paenibacillus* spp. (38%) and *Acinetobacter* (30%). The morphological identification of the bacteria based on agar slant culture characteristic and preliminary characterization of bacteria is given in Tables 3 and 4 respectively, in which the morphological characteristics such as shape, size, colour, texture, and hemolytic growth are help to identify the bacteria. Some preliminary characters such as motility, gram positive, gram negative and growth in broth are again help to identify the bacteria.

Table 3. Morphological identification of the bacteria based on agar slant culture characteristics.

S.No	Agar slant culture character	Probable bacteria
1	Abundant, opaque, White waxy growth	*Bacillus* spp.
2	Whitish, grayish, slightly transparent glistening appearance	*Paenibacillus* spp.
3	Soft, smooth, yellow growth	*Micrococcus* spp.
4	Abundant thin, white growth, media turning green	*Pseudomonas* spp.
5	Rough surface growth, paper like	*Acinetobacter* spp.
6	Thin, even, grayish growth	*Salmonella* spp.

Table 4. Preliminary characterization of based on following parameters.

S.No.	Characters	No. of strains
1	Motility	25
2	Gram(+)	22
3	Gram(-)	14
4	MacConky agar	14
5	Shape	7 round, 29 rod
6	Anaerobic growth	4
7	Oxidative fermentation	19
8	Acid production by glucose	22

Table 5. Biochemical characterizations based on 13 different tests for a total 36 strains of gram positive and gram negative bacteria.

S.No.	Test	No. of strains (+)	No. of strains (-)
1	Catalase test	32	4
2	Oxidase test	6	30
3	Hydrogen sulphide production test	4	32
4	Nitrate reduction test	29	7
5	Indole production	-	36
6	MR reaction	5	31
7	VP reaction	14	22
8	Citrate Use test	12	24
9	Urease test	8	28
10	Litmus milk test	36	-
11	Lactose fermentation	4	32
12	Sucrose fermentation	19	17
13	Dextrose fermentation	19	17

The biochemical characterization based on 13 different tests for both gram positive and gram negative strains in the total number of 36 strains is given in Table 5.

Bacterial contamination in living rooms of rural and urban area

After bacterial isolation from living rooms, they were subjected to various tests and the results were obtained and summarized in Table 6. On the basis of primary characterization, the samples were subjected to morphological and biochemical analysis to confirm the identified bacteria. The presence of bacteria was discerned in 76 samples of air of living rooms in rural and urban areas out of 80 samples. Only 04 samples (one in rural and three in urban areas) of air of living rooms were found to be bereft of bacteria. In rural living rooms the

Table 6. Bacterial analysis in the air of 80 living rooms in rural and urban areas in Panipat district of Haryana, India.

Type of samples	Source of samples	Total no. of samples processed	No. of samples devoid of bacteria	Total no. of bacteria isolated	Number of genus isolated	Bacteria identified
Living rooms of rural households	Israna	10	Nil	16	7	[1]
	Balana	10	Nil	13	7	[2]
	Naultha	10	1	12	5	[3]
	Mehrana	10	Nil	14	7	[4]
Living rooms of urban households	Model Town	10	Nil	12	4	[5]
	Huda colony	10	1	12	4	[6]
	NFL colony	10	Nil	10	3	[7]
	Sukhdev Nagar	10	2	07	4	[8]

Living rooms of rural households: [1] *Staphylococcus* spp., *Proteus* spp., *E. coli, Shigella* spp., *Klebsiella* spp., *Alcaligenes* spp., *Bacillus* spp. [2] *Bacillus* spp., *Klebsiella* spp., *Salmonella* spp., *Micrococcus* spp., *Proteus* spp., *E. coli, Staphylococcus* spp. [3] *E. coli, Micrococcus* spp., *Salmonella* spp., *Proteus* spp., *Shigella* spp. [4] *Klebsiella* spp., *Micrococcus* spp., *Shigella* spp., *E. coli, Bacillus* spp., *Salmonella* spp., *Proteus* spp. Living rooms of urban households: [5] *Streptococcus* spp., *Staphylococcus* spp.*-2 strains, Acinetobacter* spp.*-2 strains, Bacillus* spp.*- 2 strains.* [6]*Micrococcus* spp.*-2 strains, Paenibacillus* spp.*-2 strains, E. coli, Lactobacillus* spp.*-2 strains.* [7] *Bacillus* spp*-2 strains, Streptococcus* spp.*-2 strains, Pseudomonas* spp. [8] *Staphylococcus* spp.*-2 strains, Micrococcus* spp.*-2 strains, E. coli, Paenibacillus* spp.*-2 strains.*

Proteus spp. and *E. coli* (04) contributed the major fraction of bacteria in the living rooms air followed by *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *Bacillus* spp. and *Micrococcus* spp. (03), *Staphylococcus* spp. (02), *Alcaligenes* spp. (01). However, in urban living rooms, isolated bacteria on nutrient agar from open air revealed growth of the *Staphylococcus* spp., *Bacillus* spp., *Paenibacillus* spp., *Micrococcus* spp. (04) and contributed the major fraction of bacteria in living rooms air followed by *Streptococcus* spp.(03), *Acinetobacter* spp., *E. coli, Lactobacillus* spp.(02) *and Pseudomonas* spp.(01),. The total number of bacterial isolates from the air of living rooms in rural and urban areas was 54 and 41 respectively. The total numbers of genus identified 14 in both living rooms of rural areas and living rooms of urban areas. It is notable fact that the more pathogenic bacterial genus were found only in the living rooms of rural areas such as *Proteus* spp., *Salmonella* spp., *Shigella* spp., *Klebsiella* spp. and non-pathogenic genuses such as *Staphylococcus* spp., *Bacillus* spp., *E. coli, Micrococcus* spp and *Alcaligenes* spp. On the other hand, the pathogenic bacterial genuses were found in the living rooms of urban areas such as *Acinetobacter* spp. and *Streptococcus* spp. and non-pathogenic bacterial genuses *Pseudomonas* spp. *and Paenibacillus* spp., *Klebsiella* spp., *Bacillus* spp., *Micrococcus* spp., *E. coli, Lactobacillus* spp. *and Staphylococcus* spp. (Table 7).

In the air of living rooms of rural areas, the maximum fraction of *Proteus* spp. (04), *Salmonella* spp., *Shigella* spp., *Klebsiella* spp (03). Which is virulent and pathogenic in nature was recorded whereas; in urban areas it was not seen in any of the samples. In urban areas living rooms generally harmless and causes food spoilage bacterial genus are observed. The pathogenic and non-pathogenic statuses of all the isolated bacteria from living rooms are shown in Table 7. The present result shows that, the bacteria isolated from air of living rooms in rural areas are more virulent and show higher pathogenic activity as compared to the bacteria isolated from air of the living rooms in urban areas with lower pathogenic activity. Table 7 shows among these isolates, in rural areas the bacterial growth on the basis of colonies forming /plate are observed in *E. coli* for 80% of isolates (32 colony types from 40 plates), followed by *Proteus* spp. and *Salmonella* spp. (60%), *Micrococcus* spp. (55%), *Klebsiella* spp. (50%), *Bacillus* spp. (50%), *Staphylococcus* spp. (30%), *Shigella* spp. (22.5%), *Alcaligenes* spp. (15%). In urban areas, *Staphylococcus* spp. accounted for 60% of isolates (24 colony types from 40 plates), followed by *Bacillus spp.* (45%), *Paenibacillus* spp. and *Streptococcus* spp. (40%), *Micrococcus* spp. (37.5%), *E. coli* (30%), *Acinetobacter* (20%), *Lactobacillus* spp. (17.5%), *Pseudomonas* spp. (10%).

The maximum number of bacterial growth with pathogenic bacterial growth is 60% in *Proteus* spp. and *Salmonella* spp. are observed in rural areas. On the other hand, the maximum number of bacterial growth with non-pathogenic 60% is observed in *Staphylococcus* spp. in urban areas. The present results show that, the rural living rooms are highly contaminated as compared to urban living rooms. The morphological identification of the bacteria isolated from living rooms based on agar slant culture characteristic and preliminary character-rization of bacteria is given in Tables 8 and 9 respectively, in which the morphological characteristics such as shape, size, colour, texture, and hemolytic growth help to identify the bacteria. Some preliminary characters such as motility, gram positive, gram negative and growth in broth, again help to identify the bacteria. The biochemical characterization based on 12 different tests for both gram positive and gram negative strains in the total number of 52 strains is given in Table 10.

Table 7. The pathogenic status of bacteria and number of colonies isolated from the air of living rooms of rural and urban areas.

S.No.	Culture medium	Sampling	Bacteria identified (Genus)	Sources	{No. of colonies (%)}/ 40 plates	(Pathogenic / non-pathogenic) effect
1	N.A	Open air	*Staphylococcus* spp.	Rural Urban	12 (30) 24 (60)	Harmless
2	N.A	Open air	*Proteus* spp.	Rural Urban	24 (60) 16 (40)	Cause human Urinary Tract Infections
3	N.A	Open air	*Bacillus* spp.	Rural Urban	20 (50) 18 (45)	Human pathogen causes Typhoid fever.
4	N.A	Open air	*E. coli*	Rural Urban	32 (80) 12 (30)	Cause Dysentery and Hemolytic Uremic Syndrome.
5	N.A	Open air	*Micrococcus* spp.	Rural Urban	22 (55) 15 (32.5)	Generally harmful, cause food spoilage
6	N.A	Open air	*Klebsiella* spp.	Rural	20 (50)	Cause Pneumonia, UTI, Septicemia.
7	N.A	Open air	*Shigella* spp.	Rural	09 (22.5)	Harmless
8	N.A	Open air	*Salmonella* spp.	Rural	24 (60)	Harmless
9	N.A	Open air	*Alcaligenes* spp.	Rural	06 (15)	Harmless
10	N.A	Open air	*Acinetobacter* spp.	Urban	08 (20)	Cause nosocomial infections
11	N.A	Open air	*Lactobacillus* spp.	Urban	07 (17.5)	Harmless
12	N.A	Open air	*Pseudomonas* spp.	Urban	04 (10)	Saprophytic soil bacteria
13	N.A	Open air	*Paenibacillus* spp.	Urban	16 (40)	Plant growth promoter
14	N.A	Open air	*Streptococcus* spp.	Urban	04 (10)	Pain on swallowing, tonsillitis, high fever, headache.

NA: Nutrient agar.

A comparison between kitchen vs. kitchen of both rural and urban areas, more contaminated kitchens of rural areas with pathogenic virulent bacteria as compared to urban kitchens. In concern of living rooms the same trends are observed. The rural living rooms are more contaminated with pathogenic virulent bacteria as compare to urban living rooms. The status of kitchen vs. living rooms of both rural and urban areas, the kitchen are more contaminated with pathogenic virulent bacteria compared to living rooms. The pathogenic and non-pathogenic bacterial contamination spread out through air and by other factors into kitchen to living rooms because the same bacterial contamination are observed in both kitchen and living rooms in both rural and urban areas.

Domestic kitchen environment are potential places for harboring and spreading pathogenic bacteria including *pseudomonas* spp., *Bacillus* spp., *Paenibacillus* spp., *Micrococcus* spp., *Acinetobacter* spp., *Salmonella* spp. according to Kusumaningrum et al. (2002); Tumwine et al. (2003); Borneff et al. (1985,1989) these pathogen survive on the surface for hours or days, depending on the species. They also stated that wiping of surfaces (physical removal) tends to transfer and spread microorganisms from one surface to the other (Ojima et al., 2002a). Bacteria are readily spread from cloths and sponges during wiping (Cogan et al., 2002; Ojima et al., 2002b; Gorman et al., 2002). *Pseudomonas* spp., an opportunistic pathogen causes UTI, respiratory tract infection, dermatitis, soft tissue infection, bacteremia, bone and joint infection, gastrointestinal infections and a variety of systemic infections. Ragnath et al. showed that, *Pseudomonas* spp. can also be found in households drains of showers and kitchens (Regnath et al., 2004). Its prediction to moist environment makes it more possible to exist in kitchen surfaces, dustbins and used sponges. Once infection with Pseudomonas is established, it is hard to control since this organism is frequently resistant to many commonly used antibiotics (Qarah et al., 2006; Humphrey, 2001).

Generally, *Bacillus* species are neither morphologically nor phylogenetically indistinguishable from each other. Though most of the members of this genus is considered

Table 8. Morphological identification of the bacteria based on agar slant culture characteristics of kitchens and living rooms of rural and urban households samples.

S.No.	Agar slant culture characteristics	Probable bacteria
1	Abundant, opaque, white waxy growth	*Bacillus spp.*
2	Whitish, grayish, slightly transparent, glistening appearance	*Paenibacillus spp.*
3	Soft, smooth, yellow growth	*Micrococcus spp.*
4	Abundant thin, white growth, media turning green	*Pseudomonas spp.*
5	Rough surface growth, paper like	*Acinetobacter spp.*
6	Thin, even, grayish growth	*Salmonella spp.*
7	Abundant, opaque, golden growth	*Staphylococcus spp.*
8	White, moist, glistening	*Escherichia coli*
9	Slimy, white, translucent, raised growth	*Klebsiella spp.*
10	Thin, even, grayish growth	*Shigella spp.*
11	Thin, blue-gray, spreading growth	*Proteus spp.*
12	White, irregular, big circular	*Lactobacillus spp.*
13	Irregular, white, rough surface	*Alcaligenes spp.*
14	Thin, even growth, white	*Streptococcus spp.*

Table 9. Preliminary characterization of based on following parameters of living rooms.

S.No.	Characters	No. of strains of living rooms
1	Motility	22
2	Gram(+)	29
3	Gram (-)	23
4	MacConkey agar	23
5	Shape	16 round, 36 rod
6	Anaerobic growth	41
7	Oxidative fermentation	48
8	Acid production by glucose	37

Table 10. Biochemical characterization based on 12 different biochemical tests of living rooms samples.

S.No.	Biochemical tests	No. of strains(+) of living rooms	No. of strains(-) of living rooms
1	Catalase test	29	23
2	Oxidase test	09	43
3	Hydrogen sulfide production test	07	45
4	Nitrate reduction test	44	08
5	Indole production test	10	42
6	MR reaction	29	23
7	VP reaction	16	36
8	Citrate use test	15	37
9	Urease test	14	38
10	Lactose fermentation	18	34
11	Sucrose fermentation	31	21
12	Dextrose fermentation	39	13

contaminants, there are 2 members which are of significant medical importance, *Bacillus anthracis* and *B. cereus. B. anthracis cause* anthrax *and B. cereus* causes food poisoning *(*Cunha, 2006). Salmonella infections are zoonotic and can be transferred between human and non human animals. Many infections are due to ingestion of contaminated food. A distinction is made between enteritis Salmonella and typhoid/paratyphoid

Salmonella, due to special virulence factor and a capsule protein (virulence antigen) can cause serious illness, such as *Salmonella enterica* subspecies or typhi, *Salmonella typhi* is adapted to human and does not occur on animals (www.wikkipedia.com). Oberoi et al. (1994) observed that bacterial quality of household's air was found to be better in large farm size category. It might be due to better cleanliness maintained in such houses as well as their kitchens. The households' air was mostly loaded with non-pathogenic bacteria in these cases. A survey conducted by Dhillon et al. (1990) in five colonies of Ludhiana city and five adjoining villages of Ludhiana district revealed that, a lack of family finance was the root cause of households pollution, followed by other factors such as places of dwelling and defection, poor drainage systems, improper disposal of refuse and traditional style of living.

Staphylococcus epidermidis has become the most important cause of nosocomial infections in recent years. Its pathogenicity is mainly due to the ability to form biofilm on indwelling medical devices. In a biofilm, *S. epidermidis* is protected against attacks from the immune system and against antibiotic treatment, making *S. epidermidis* infections difficult to eradicate (Vuong and Otto, 2002). *S. aureus is* ubiquitous and may be a part of human flora, however, the organism may cause disease through invasion and toxin production such as abscess, pneumonia, diarrhea and the most feared toxic shock syndrome (Tolan, 2007). *Klebsiella pneumonia* can cause pneumonia, septicemia, wound infection, burn infection, UTI and ankylosing spondylitis. Like Pseudomonas, it is an opportunistic pathogen. Pneumonia caused by Klebsiella has around 50% mortality, due to the underlying disease but may reach 90% in untreated cases (Umeh and Berkowitz, 2006).

The bacteria isolated from the air of kitchens samples in rural areas were found to be virulent with pathogenic activity. On the other hand, the bacteria isolated from the air of kitchens in urban areas were generally harmless and caused food spoilage only except *Acinetobacter* spp. Our study showed that, potentially harmful pathogens are easily accessible to every individual through contaminated sources present in kitchens. Similar results with those of Joeshson and Rubino (1997), Scott et al. (1982), and De Boer and Hahne (1990). Bacterial contamination in kitchen spread out through sponges and washcloths use in normally in kitchens were similarly reported (Suaad, 2007). Bacterial contamination spread out into kitchens to living rooms and it surrounded areas through air and other factors such as dustbin, dusting cloth, utensils etc. were similarly reported (Tyagi et al., 2011).

The results of our study have several implications on the preference for floor, carpet, tabletop in living rooms and unwashed hands, damaged vegetables, dust beans, sink, washing-up areas, food shelf, cutlery and crockery, refrigerator, vegetables racks, floor, back side of door and near gas cylinder in kitchens. The primary sources of these bacteria are kitchens in which the food spoilage and stored dustbin contain for many days and directly entered vegetables (some infected with higher pathogens). After sometime, bacteria spread out to its surrounding areas which is more suitable for growth. In living rooms such as carpet, curtains, toilet doors, table top, dressing tables and ceiling fans etc. are the best places in which the bacterial growth are more conditionable and when the favorable conditions start (seasonal variation) these bacteria infected the individuals. This explains why most people experience a lot of respiratory symptoms from acute allergic rhinitis to pneumonia during climate changes. Avoiding these infections, we have made some arrangement in our kitchens, living rooms and its surrounding areas. When possible, floor carpeting in homes should be minimized or avoided, since this serves as habitat for opportunistic infection agents that pose harm to one's health.

Proper ventilation and sanitation in both kitchens and living rooms should be provided. Renewable kitchen sponges should be dried after use or immersed in boiling water for 5 to 10 min. Furthermore, hygienic measures and precaution in the kitchen should be well maintained to reduce harmful bacteria levels. Vegetables are entered in the kitchen after proper washing and checking. Hence, the better quality of air can be achieved by manipulating sanitation and hygiene within houses, kitchens and its surrounding.

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