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A Comparative Study of Bacterial Contamination in Kitchens of Meerut Region of Uttar Pradesh, India

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A B S T R A C T

Microbial populations in indoor environments, where we spent our maximum time are indeed essential for public health. Several microbial species found in the kitchens of rural and urban area, can be a prominent source of air borne diseases. During present study, we have selected several samples from kitchens of district Meerut (UP-India). The results suggested that kitchens have a higher level of bacterial growth represented by total 117 positive samples for bacterial contamination out of 200 samples from different sites. Our results are agreed with Shruti *et al.*, (2011) and we have also found the contamination more pronounced in rural region than in urban.

Introduction

Environmental pollution is one of the most important issues in the world today. Environmental pollution includes outdoor pollution and indoor pollution. For many decades the scientists have been studied outdoor pollution. This area of interest includes the pollution of ambient air, the pollution of water, soil, housing and the effects of ionizing and non-ionizing radiation. The indoor environment has several aspects that are quite important. One aspect is linked to the chemical pollution of the indoor air. Other aspects can be linked to the biological contamination of air and surfaces or to the radiation pollution of indoor air linked especially to the presence of radon and radon daughters. Health can be negatively affected by all types of environmental pollution. Both the outdoor and the indoor environments are linked together. In order to increase the efficiency of the life style as well as the hygienic conditions of the people residing in

different areas, various efforts have been made by the scientists in the field of medicine and science. Today, there is drastic decline in the epidemic diseases like polio, tuberculosis, DPT etc (Schlipköter and Flahault, 2010). Although the focus has been shifted to other diseases like asthma, neurological disorders which have led to decline in the research area of hygiene maintenance in the house hold area along with the surrounding environment. The problem is worsening by other type of opportunistic infections also. These air borne diseases may include measles, chickenpox, mumps and rubella which can easily be spread from the diseased person via secretions exhaled by them or may also be transferred via close contact (Morens and Fauci, 2013). These microorganisms remain in the air until they are inhaled by population and proliferate in the biological system to increase their population size. According to Lal (2011), there are approximately 4 million deaths per year due to acute respiratory infection worldwide which harbors up to 30% of all under-five deaths in India and most of these deaths are preventable. There are several factors which bring about this disastrous situation. They are not only the climatic conditions but also the poverty, poor nutrition, poor housing conditions, indoor air pollution such as parental smoking, absence of ventilation, overcrowding, industrialization, social cultural values, overuse and misuse of antibiotics, lack of basic health services and lack of awareness. Most of our time is spent indoors where we are exposed to a wide array of different microorganisms living on surfaces and in the air of our homes. In many human activities micro-organisms in the environment represent a hidden but dangerous risk factor. Concern has increased with the introduction of advanced technologies in hospitals, industries and agricultural field. In recent years, many studies have been carried out on this topic, and nowadays the evaluation of the level of air microbial contamination in places at risk is considered to be a basic step toward prevention. However, there are still problems to be solved relating to methodology, monitoring, data interpretation and maximum acceptable levels of contamination (Charnley and Eftekhar, 1969). According to Consumer Product Safety Commission and the American Lung Association, 1990.), the Biological Pollutants in Your Home are Dirty air conditioner, Dirty humidifier and/or dehumidifier, Bathroom without vent or window, Kitchen without vent or window, Dirty refrigerator drip pan, Laundry room with unvented dryer, Unventilated attic, Carpet/rug on damp floor, Bedding, Closet on outside wall (cold wall), Dirty heating/air conditioning system, Dogs or cats, Water leaks and/or damage (around windows, the roof or the basement). People in today's world is only concerned how to generate advanced resources by destroying the natural resource without thinking of the consequences generated from their cruel act to the environment.

This is not only generating unhygienic environment but also leading to the development of resistant species which are prevailing everywhere in the society and reproducing without any therapeutic agent that can inhibit their growth. This leads to have major impact on the health issues of the population for the respiratory, gastro-intestinal tract, urinary tract and other infections (Smith *et al.,* 2005; USEPA 2013). Air borne infectious diseases are the major cause of the mortality among all the infectious diseases. The problem is worsening by other type of opportunistic infections also. These air borne diseases may include measles, chickenpox, mumps and rubella which can easily be spread from the diseased person via secretions exhaled by them or may also be transferred via close contact (Morens and Fauci, 2013). These microorganisms remain in the air until they are inhaled by population and proliferate in the biological system to increase their population size. In order to reduce the effect of this

infectious organism it is recommended to follow various hygienic practices like remain in isolated area during the period of illness so as to avoid contact with healthy person, cover the facial area to avoid contamination through sneezing, coughing, use of proper disinfectant to reduce the microbial population from exposed parts of body. Infectious diseases are generally passed from person to person through physical contact. Some bacteria and viruses circulate through indoor ventilation systems, particularly if there is a moisture problem in the system. Inhaling these viruses or bacteria can spread coughs, colds, influenza, tuberculosis and other infectious agents.

Therefore, the present study carried out some experimental survey to investigate the bacterial contamination of air of kitchens in rural and urban areas of selected district of Uttar Pradesh i.e. Meerut.

Materials and Methods

Sample procurement

A total of 200 different samples from kitchens of rural and urban areas of Meerut district surveyed from potentially harmful pathogens in the domestic kitchens. The urban and rural areas cover 5 sites namely: Jawaher quarter, Inder lok, Begum Bagh, Rajan Kunj, Defence Colony, and Dorli, Palheda, Sofi Pur, Putha, Pawali Khas respectively.

Nutrient agar media preparation

Nutrient agar powder (12.6g) was mixed in 450ml of cold demineralised water in an 800ml beaker and gently stirred. After addition of agar mixture was autoclaved and allowed to cool to 50 °C. The prepared agar was then poured into clean Petri dishes, cooled to caste and stored at 4°C until use (Arulanantham *et al.,* 2012).

Incubation

Incubation of the inoculated culture media plates was done in incubator at 28-30˚C for 24 hours. The growth was observed on the successive day and it was different biochemical analysis were made positive samples. These tests were carried out to categorize the type of infection in a particular area and also the level of infection. The level of drug resistance parasites/infection was also determined using by biochemical techniques using different parameters (Sivashanmugam *et al.,* 2009).

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, 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 their 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 200 samples from 200 houses (100 each samples of rural and urban living room respectively) were collected and analyzed for bacterial contamination and their comparisons (Table 2–4 and Fig. 1–7).

Table.1 Bacterial contamination analysis in the air of kitchens in rural and urban area

Types of samples	Experimental site	Total no. of samples processed	No. of samples devoiod of bacteria	No. of samples with bacterial growth	Total no. of bacterial genus isoolated	Bacteria identified
Kitchens Rural KR	Dorli	20	3	17	11	**[1]**
	Palheda	20	5	15	7	**[2]**
	Sofipur	20	8	12	6	**[3]**
	Putha	20	10	10	5	**[4]**
	Pawli khas	20	6	14	8	**[5]**
	Total	**100**	**32**	**68**	**17**	
Kitchens Urban KU	Jawahar quarter	20	11	9	9	**[6]**
	Inderlok	20	10	10	8	**[7]**
	Begum bagh	20	8	12	9	**[8]**
	Rajan kunj	20	11	9	6	**[9]**
	Defence colony	20	11	9	5	**[10]**
	Total	**100**	**51**	**49**	**13**	

1. *E. coli, Micrococcus spp., Bacillus spp., Alcaligenes spp., Lactobacillus spp., Brevibacterium lines, Proteus spp., Salmonella spp., Clostridium spp., Streptococcus spp., Pseudomonas spp.,*
2. *E. coli, Alcaligenes spp., Lactobacillus spp., Paenibacillus spp., Streptococcus spp., Pseudomonas spp., Corynebacteria spp.,*
3. *Lactobacillus spp., Staphylococcus spp., Bacillus spp., Proteus spp., Salmonella spp., Pseudomonas spp.,*
4. *E. coli, Alcaligenes spp., Lactobacillus spp., Streptococcus spp., Pseudomonas spp.,*
5. Entero*coccus spp., Aeromonas spp., E. coli, Micrococcus spp., Bacillus spp., Proteus spp., Salmonella spp., Pseudomonas spp.,*
6. *E. coli, Micrococcus spp., Bacillus spp., Lactobacillus spp., Proteus spp., Salmonella spp., Clostridium spp., Streptococcus spp., Pseudomonas spp.,*
7. *E. coli, Micrococcus spp., Bacillus spp., Lactobacillus spp., Leuteococcus spp., Haemophilus spp. Campylobacter, Shigella spp.,*
8. *E. coli, Micrococcus spp., Bacillus spp., Enterococcus spp., Aeromonas spp., Proteus spp., Salmonella spp., Shigella spp., Streptococcus spp.,*
9. *E. coli, Micrococcus spp., Bacillus spp., Lactobacillus spp., Shigella spp., Streptococcus spp.,*
10. *Micrococcus spp., Bacillus spp., Lactobacillus spp., Proteus spp., Streptococcus spp.,*

Table.2 Morphological identification based on agar slant culture characteristics and number of colonies of the bacteria isolated from the air of kitchen in rural and urban households

Bacterial genus in kitchen	No. of colonies(%)/ 200 sample	Bacteria found in kitchen (urban/ rural)
Micrococcus spp.	40	(N.P) rural Urban
Bacillus spp.	46	(N.P) rural Urban
Paenibacillus spp.	8	(N.P) rural
Lactobacillus spp.	46	(N.P) rural Urban
Proteus spp.	37	(P) rural Urban
Clostridium spp.	13	(P) rural Urban
Streptococcus spp.	48	(P) rural Urban
Pseudomonas spp.	32	(P) rural Urban
Escherichia coli	48	(N.P) rural Urban
Corneybacterium spp.	8	(P) rural
Salmonella spp.	32	(P) rural Urban
Alcaligens spp.	21	(N.P) rural
Enterococcus spp.	7	(N.P) rural Urban
Aeromonas spp.	7	(N.P) rural
Shigella spp.	16	(P) rural Urban
Haemophilus spp.	5	(N.P) urban
Camphylobacter spp.	5	(P) urban
Staphylococcus spp.	6	(N.P) rural
Brevibacterium spp.	9	(N.P) rural
Leuteococcus spp.	5	(N.P) urban

Table.3 Morphological identification of the bacteria based on agar slant culture characteristics of kitchens of rural and urban household samples

S. NO	Morphological characteristics based on NA slant culture	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.	Small to medium sized and typically grey to grey-yellow and translucent.	*Clostridium spp.*
6.	Thin, even, grayish growth	*Salmonella spp.*
7.	Abundant, Opaque, Golden growth	*Staphylococcus spp.*
8.	White, Moist, glistening	*Escherichia coli*
9.	Pinpoint to small, smooth, entire colonies	*Enterococcus spp.*
10.	Thin, even, grayish growth	*Shigella spp.*
11.	Thin, blue-gray, spreading growth	*Proteus spp.*
12.	White, irregular, big circular	*Lactobacillus spp.*
13.	Convex, smooth, pale, grey or transparent **colonies**.	*Haemophilus spp.*
14.	White, circular, thin, pin drop like growth	*Aeromonas spp.*
15.	Flat, droplet-like, glistening	*Campylobacter spp.*
16.	Pinpoint to small, smooth, entire colonies	*Luteococcus spp.*
17.	Thin, even growth, white	*Streptococcus spp.*
18.	Irregular, white, rough surface	*Alcaligenes spp.*
19.	Thin, white, smooth, regular	*Corynebacterium spp.*
20.	Gray white to yellow, opaque convex and smooth	*Brevibacterium spp.*

Table.4 Biochemical tests used for testing the samples

S. No.	Biochemical test	No. of positive strains (%) in kitchens of rural region	No. of positive strains (%) in kitchens of urban region
1	Gram positive	61.7	53.1
2	Catalase test	63.9	87.7
3	Citrate test	40	29.9
4	VP test	60.6	66.7
5	Indole test	29.5	26.2
6	H_2S test	60.7	66.7
7	Nitrate test	42.7	61.9
8	Lactose test	54.1	61.9
9	Sucrose test	70.5	61.9
10	Urease test	19.7	30.9

Fig.1 Showing bacterial genus found in kitchens in urban and rural area

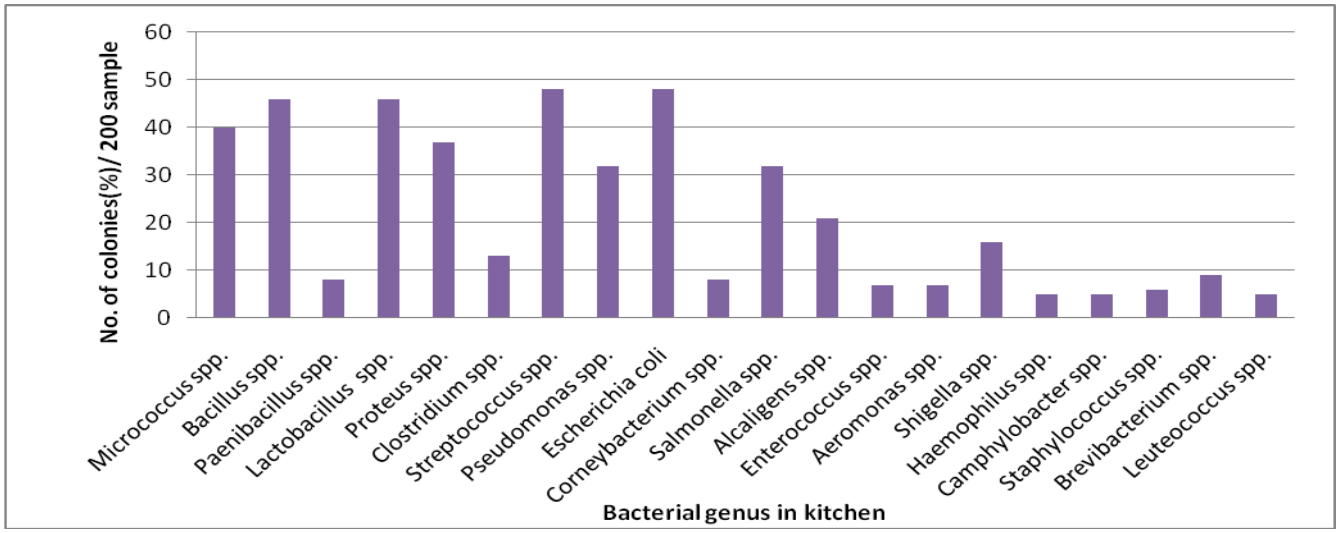


Fig.2 Showing pathogenic bacteria found in kitchens in rural area

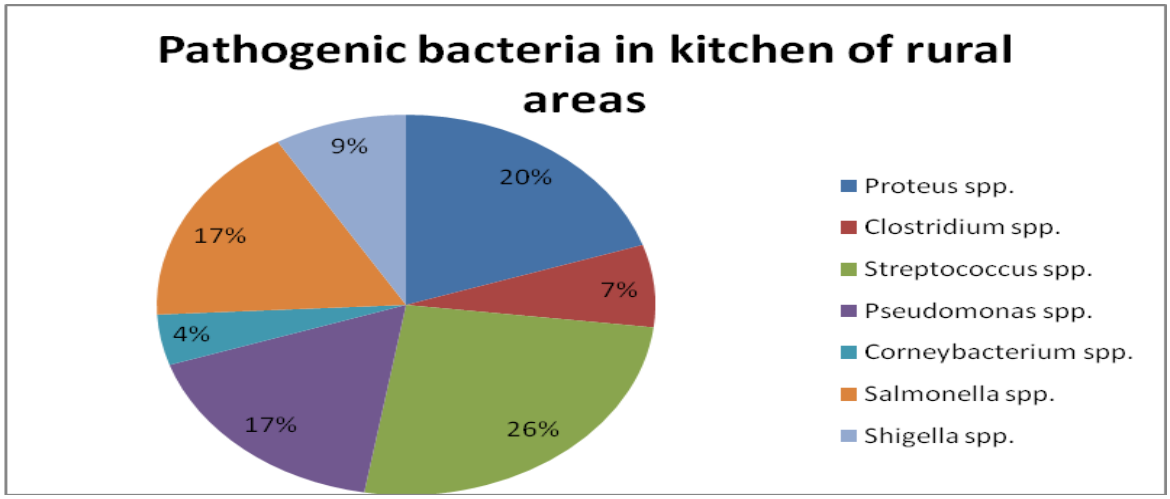


Fig.3 Showing non-pathogenic bacteria found in kitchens in rural area

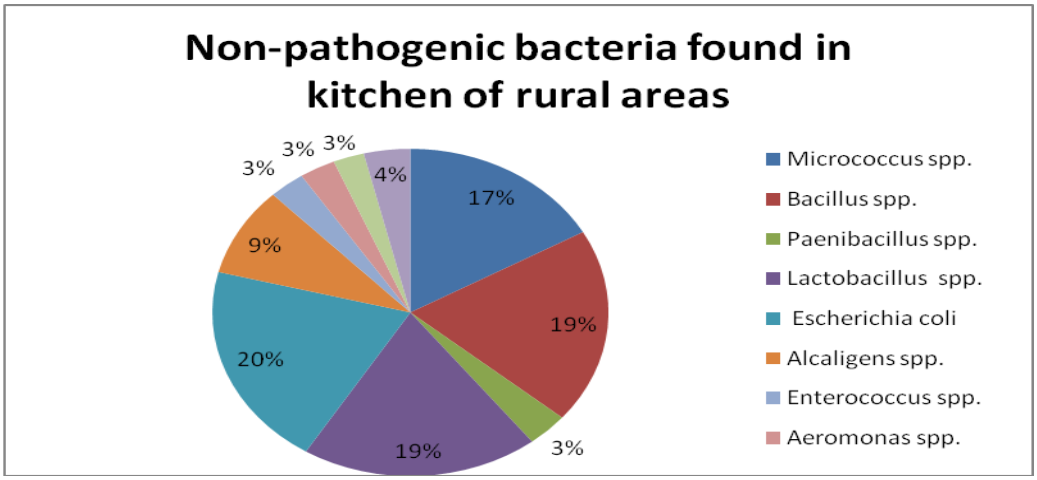


Fig.4 Showing pathogenic bacteria found in kitchens in urban area

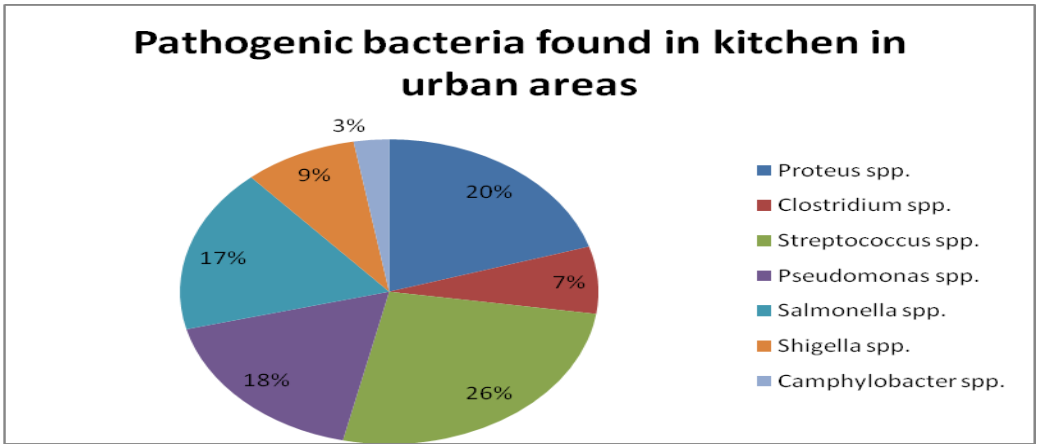


Fig.5 Showing pathogenic bacteria found in kitchens in urban area

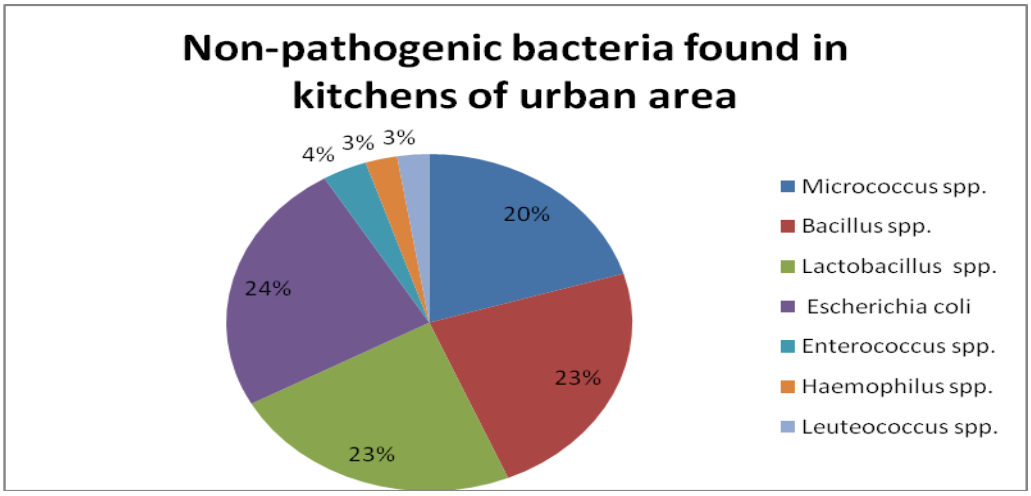
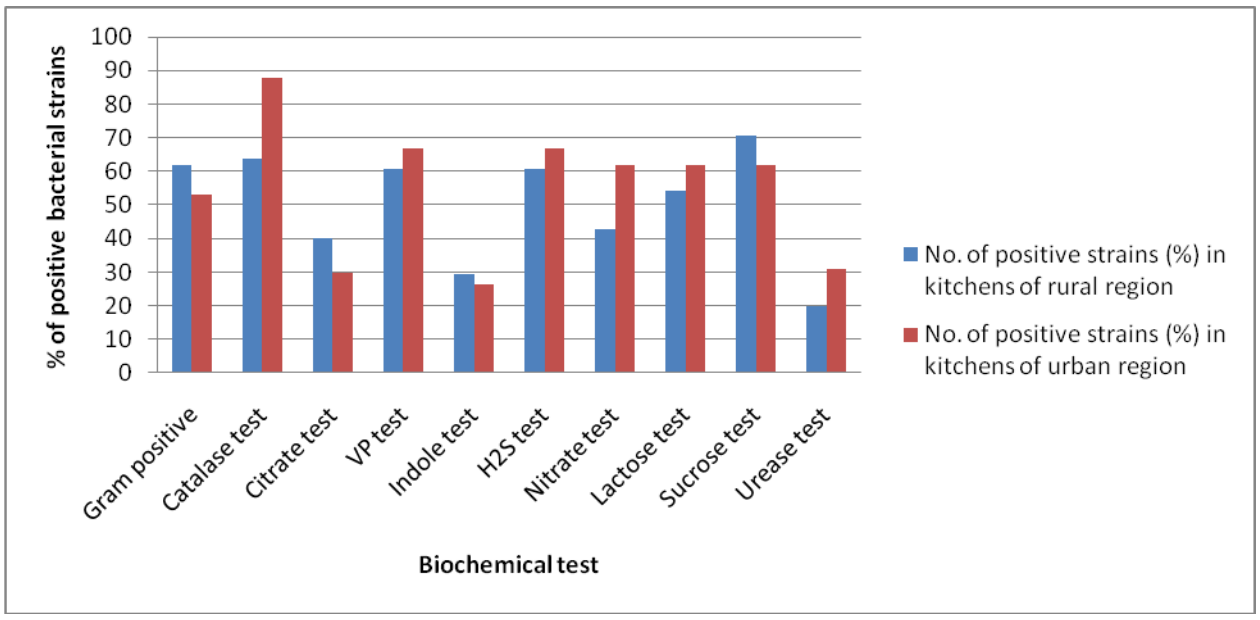


Fig.6 showing positive strains with the help of biochemical tests



The numbers of bacterial genus identified in kitchens of rural areas are 16 and in kitchen of urban areas are 15. In rural area kitchens, *Streptococcus* spp. and *E. coli* contributed the major fraction of bacteria followed by *Pseudomonas spp., Lactobacillus spp., Bacillus spp., Proteus spp., Salmonella spp., Alcaligenes spp., Micrococcus spp., Brevibacterium spp., Clostridium spp., Paeniobacillus spp., Corynebacterium spp., Enterococcus spp., Aeromonas spp.and Staphylococcus spp.* However, in urban area kitchens, *Bacillus spp. and Micrococcus spp.* contributed the major fraction of bacterial genus followed by *E.coli spp., Streptococcus spp., Lactobacillus spp., Proteus spp., Shigella spp., Salmonella spp., Enterococcus spp., Aeromonas spp., Leuteococcus spp., Haemophilus spp., Campylobacter spp., Clostridium spp. and Pseudomonas spp.* It is a notable fact that 6 pathogenic bacterial genus such as *Proteus spp., Salmonella spp., Clostridium spp., Streptococcus spp., Pseudomonas spp., Corynebacterium spp.* were found in kitchen of rural areas with 10 non-pathogenic bacterial genus such as *E.coli spp., Micrococcus spp., Bacillus spp., Alcaligenes spp., Lactobacillus spp., Brevibacterium spp., Paenibacillus spp., Staphylococcus spp., Enterococcus spp., Aeromonas spp.* On the other hand, 7 pathogenic bacterial genus such as *Proteus spp., Salmonella spp., Clostridium spp., Streptococcus spp., Pseudomonas spp., Campylobacter spp., Shigella spp.,* were found in kitchens of urban areas with 8 non-pathogenic bacterial genus such as *E.coli spp., Micrococcus spp., Bacillus spp., Lactobacillus spp., Leuteococcus spp., Haemophilus spp., Enterococcus spp., Aeromonas spp.* As shown in table 1, pathogenic bacteria were found common in both kitchens of rural and urban areas such as *Proteus spp., Salmonella spp., Clostridium spp., Streptococcus spp., Pseudomonas spp. whereas Corynebacterium spp.* was found in rural areas kitchen. On the other hand, *Campylobacter spp. and Shigella spp.* were found in urban areas kitchen. The present result shows that bacterial genus isolated from kitchens in rural areas are more in percentage as compared to kitchens in urban area and rural area kitchens are more pathogenic as compared to urban area kitchens.

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