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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF DOMINATED ISOLATES FROM AIR BORN HOUSEHOLD CONTAMINATION

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ABSTRACT

In this study, we compare indoor microbial distribution pattern in rural vs urban areas of meerut region. A Very intensive bacterial growth was observed in the kitchen sections compare to living room area of both urban and rural area. The predominant bacterial group and moulds isolated from investigated air samples were: *Brevundimonas diminuta; Bacillus megaterium; Bacillus Subtilis; Bacillus ceres, Enterococcus, streptobacillus, Klebsiella pneumoniae; Pseudomonas aeruginosa, Citrobacter freundii* and *Alternaria* spp., *Mucur* spp., *Cladosporium* spp., *Aspergillus* Spp. This result indicates that neither proper cleaning was happened nor air ventilation was maintained in both urban and rural areas. Microbial dominance is depends on intrinsic or genetic adaptation. This work was extended to evaluate the antibiotic susceptibility pattern of dominated bacterial strains. Six bacteria (*Brevundimonas diminuta; Bacillus megaterium; Bacillus Subtilis; Klebsiella pneumoniae; Pseudomonas aeruginosa; Citrobacter freundii*) showed dominance on all other bacterial and fungal sp. Maximum resistant were found for Vanomycin (Va) and minimum resistance were observed for ciprofloxacin (Ci). *Citrobacter freundii* found sensitive against for Streptomycin, Ciprofloxacin and *Pseudomonas aeruginosa,* is found sensitive only for Amoxillin. Moreover, strain *Bacillus megaterium, Bacillus Subtilis, Klebsiella pneumoniae,* showed multiple drug resistant capability because it resist the entire antibiotic used in this study. The most sensitive strain *Brevundimonas diminuta* susceptible to antibiotic were negative for plasmid. The antibiotic resistance property was found to be lost in *Brevundimonas diminuta* indicated the above property is plasmid born.

KEYWORDS: Microbial Load, Bacteria, Fungi, Air Born Microbes.

INTRODUCTION

According to the U.S. Environmental Protection Agency (EPA), the average population spends nearly 90% of their time at indoors; consequently, the EPA considers indoor air pollution a high priority health risk. Microbial growth refers to the growth of fungi, bacteria and other microorganisms. The diversity of bacterial species present is wide, e.g., gram-positive cocci from *Staphylococcus* spp. and *Micrococcus* spp., which are abundant on human skin, pleomorphic organisms including diphtheroids, rods including *Bacillus* spp., as well as gram-negative *Pseudomonas* spp. and *Moraxella* spp. are common (Gallup et al., 1993). Humans are also as a source of bacterial pathogen exposure in the home. A number of laboratory-based studies demonstrate that drying of surfaces has a significant bactericidal effect. However, these data together with other studies (Scott and Bloomfield 1990, Humphrey *et al.*, 1994) indicate that many types of pathogenic bacteria can survive on inanimate surfaces long enough to allow transfer to others surfaces in sufficient numbers to represent an infection risk. That why indoor air born bacterial contamination is a serious problem.

Moreover, there has been a growing awareness in indoor microbe studies in recent years (Shruti *et al*., 2011; Shiferaw *et al*., 2013). Any carelessness in adoption of routine hygienic measures can lead to transmission of infection through air droplets. Here should not be any contamination in air, which can affect for the health. So monitoring of the microbial contamination in homes is important. Hence, purpose of this study was to enumerate and evaluate the distribution pattern of air borne bacteria and fungi in indoor air at kitchen and living area of rural and urban area. Microbial dominance is depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological changes of cells, as well as environmental modifications. This work was extended to evaluate the antibiotic susceptibility pattern of dominated strains.

MATERIAL AND METHODS

Chemicals

The chemicals were purchased from Sigma Aldrich. All solutions were prepared in double distilled water. All the culture media used in the work were procured from Hi-Media (Mumbai), Qualigens (Mumbai were), Sd-fine Chemicals (Mumbai).

Sampling, Isolation of microbial load

The samples were collected from kitchens (120) and living rooms (120) of selected urban (1. Ganganagar; 2. Pallavpuram; 3. Modipuram; 4. Indraprastha Estate; 5. Pushp Vihar) and rural (1. Palheda; 2. Siwaya; 3. Alipur; 4. Rajpura; 5. Baksar) areas of Meruth, U.P, India. In this study, a total of 240 samples from kitchen (60 urban and 60 rural areas, respectively) and living room (60 urban and 60 rural areas, respectively) were collected and evaluate the distribution pattern of indoor microbes. Samples were obtained from the living room of both urban and rural area near the bed, window, back of the door, below the table. In kitchen area samples collected from near sink, refrigerator, doors, floors, near gas cylinder. Samples were also collected after dusting or cleaning the kitchen and living area to see the effect of cleaning. The samples were aseptically collect in already clean prepared culture plates of nutrient agar media. The samples were taken from open air of kitchens and living rooms. Collected sample was brought to laboratory and store at field humidity in polyethylene bags until processing. Total bacterial count, total fungal counts were performed after incubation at 37°C for 24-48 hrs.

Analysis of microbial load

Analysis of the concentration of total bacterial, fungal strain and colony forming unit were performed mathematically. Air microorganisms were taken on the Petri plates filled with plate count agar media for bacterial sampling and potato dextrose agar media for fungus exposed in sampling points two times in a day before and after cleaning. The number of microorganisms expressed as CFU / m^3 was estimated. The dominated strains were subjected into biochemically characterization as per standard method described by Barrow and Feltham (1993).

Antibiotic Susceptibility Test

The sensitivity discs were carefully layered on the surface of Muller Hinton agar plates previously inoculated with an 18-h-old broth culture of the test organisms using sterile swab sticks. The plates were incubated aerobically at 37ºC for 24 h, after which zones of growth inhibition around each discs were measured. An isolate was regarded as resistant if there was no observed zone of growth inhibition around the antibiotic sensitivity disc. The diameter of inhibition zone was measured and interpreted against the recommendations proposed by Kirby and Bauer (Bauer et al. 1966). Agar plates inoculated only with bacterial test isolates without introduction of antibiotic disks served as controls. Analysis for each combination of

bacterial isolates and antibiotic disks were repeated three times. Data obtained were used to construct the phenotypic pattern of drug resistance for each organism. Concentration of antibiotics was decided on the basis of their minimum inhibitory concentration. (Gentamycin (30 µg/disc); Streptomycin (25 µg/disc); Ciproflaxin 30 µg/disc); Neomycin (30µg/disc); Norflaxin (10µg/disc); Amoxillin (10µg/disc) PenicillinG (10µg/disc); Vanomycin (30µg/disc).

RESULTS AND DISCUSSION

Microbial distribution in kitchen and living room in rural and urban area

The number of microorganisms (bacteria and fungi) in indoor air varied widely in the whole sampling period. The total number of bacteria before cleaning ranged from 153-260 cfu/m^3 and 122-188 in kitchen of rural and urban area respectively (Table 1). In living room bacterial load varies from 63-85 cfu/m^3 and 37-98 of rural and urban area, respectively. On other hand the total number of fungi ranged from 2-9 cfu/m^3 in kitchen and 1-7 cfu/m^3 in living room, respectively. A Very intensive bacterial growth before cleaning was observed in the kitchen sections of both urban and rural area. Moreover, an interesting result was also observed in kitchen section. The kitchen of urban area (With average 158 and Standard deviation 29) was significantly less contaminated than rural kitchen (With average 207 and Standard deviation 42.7).

Among the different places in the kitchen, water taps were found to be most contaminated followed by stove knob, towel & refrigerator handle. Water taps and stove knobs were often touched with unwashed hands during cleaning of raw food. Hence, high incidence of pathogens was found on them. On other hand living room of rural area (With average 74) was more contaminated than urban living room (With average 60) (Table 1). These results revealed that the domestic kitchens are more contaminated than living room of both rural as well as urban area. Tyagi and Tyagi, 2013 compare the bacterial load in kitchen of rural and urban areas. He found that about 97% of kitchen of rural area are more contaminated than urban kitchen. This is because of domestic kitchens are the possible place for development and spreading various type of microbes including *Bacillus sp. Enterococcus; Klebsiella pneumonia; Streptobacillus; fungal sp. etc.* According to literature these microbes can survive for hours or some days, depends upon the tolerance capability of sp. These microbes easily disperse from cloths and sponges during wiping (Luksamijarulkul et al., 2014). In the

face of a lack of any official reference limit for microbiological quality in indoor air it is difficult to fully interpret our results.

Moreover, the bacterial concentration at all investigated sites was significantly much higher than fungal concentration. Before cleaning bacterial and fungal air contamination was always higher than in after cleaning. Concentration of microorganism varies in the air not only in the course of a season but also throughout the day. Average number of bacteria and fungi present in different site before cleaning and after cleaning during sampling of rural and urban area is compared in Figures 1 and 2. Results of studies showed in cubic meter (m^3) of air after cleaning than before cleaning.

Identification of Microbial Isolates

For the bacterial identification colony morphology, Gram's staining and biochemical tests were used and the results are summarized in Table 2 and 3. On the basis of biochemical test isolated nine are identified as follows: *Brevundimonas diminuta; 2:Bacillus megaterium; 3: Bacillus Subtilis; 4: Bacillus ceres; 5:Enterococcus; 6: Klebsiella pneumonia; 7: Streptobacillus; 8: Pseudomonas aeruginosa; 9: Citrobacter freundii*.. There are four fungal species *Alternaria* spp., *Mucur* spp., *Cladosporium* spp., *Aspergillus* Spp were identified by performed by lectophenol cotton blue staining.

This work was extended to evaluate the antibiotic susceptibility pattern of dominated bacterial strains (**Figure 3**). In this study six bacteria (*Brevundimonas diminuta; Bacillus megaterium; Bacillus Subtilis; Klebsiella pneumoniae; Pseudomonas aeruginosa; Citrobacter freundii*) showed dominance on all other bacterial and fungal sp. That's why these six bacteria were subjected into antibiotic susceptibility test.

Antibiotic sensitivity pattern of dominating bacteria

In the past few decades the pathogenic bacteria especially multidrug resistant (MDR) is serious healthcare problem in our country because of low public awareness. At the present time the changing environment (polluted air, water, soil etc.) create a pressure in favour of bacteria possessing various types of genes has emerged from the abusive use of antibiotics. Due to this selected pressure the antibiotic resistant bacteria can be found in all different ecological niches. Different bacteria have genetic flexibility (capacity to acquire and transfer resistant genes) which are contributed to their continued existence in altered environmental.

The widespread emergence of multidrug resistance among bacterial pathogens has become one of the most serious challenge in clinical therapy.

In this study, dominated bacterial strains (*Brevundimonas diminuta; Bacillus megaterium; Bacillus Subtilis; Klebsiella pneumoniae; Pseudomonas aeruginosa; Citrobacter freundii*) were assayed for antibiotic resistance (Figure 4 & 5 and Table 4). Maximum resistant were found for Vanomycin (Va) and minimum resistance were observed for ciprofloxacin (Ci) (i.e maximum strains were found sensitive for ciprofloxacin). *Brevundimonas diminuta* is found sensitive against Gentamycin (Gn), Streptomycin (St), Ciproflaxin (Ci), Neomycin (Nr) and Penicillin (Pn). *Citrobacter freundii* found sensitive against for Streptomycin, Ciprofloxacin and *Pseudomonas aeruginosa*, is found sensitive only for Amoxillin. Our results are corroborated with Raj, 2012 and Shiferaw et al., 2013. Moreover, strain *Bacillus megaterium, Bacillus Subtilis, Klebsiella pneumoniae*, showed multiple drug resistant capability because it resist the entire antibiotic used in this study (Table. 4).

Plasmid distribution in bacterial isolates

Till date few attempt has been undertaken to isolation and distribution of plasmid DNA from bacteria isolated from kitchen and living room of urban and rural area. Keeping this point in view, the investigation was made in which isolated dominated strains were found resistant against antibiotics, and to know whether this property is genetic or plasmid borne, plasmid isolation is done.

In this study *E. coli* MTCC 131 was used as a source of standard plasmid marker for assessment of molecular weight of plasmids. *Escherichia coli* (MTCC 131) have harboured 8 diverse plasmids with known molecular weight viz., 35.8 MDa, 4.8 MDa, 3.7 MDa, 3.4 MDa, 2.6 MDa, 2 MDa, 1.8 MDa and 1.4MDa (Macrina et al., 1978). Plasmid isolation study revealed that all MDR isolates harboured a single plasmid of 54.4 kb in case of four bacteria *Bacillus megaterium, Klebsiella pneumoniae, Citrobacter freundii* which is equivalent to 35.8 MDa (1 MDa=1.52 kb) of known molecular weight of plasmid in *E. Coli* V517 (Figure 6).

Bacillus Subtilis and *Pseudomonas aeruginosa* have also a single plasmid but slightly higher molecular wt approximately 55 kb. Most of strains showed multi drug resistance. Multiple antibiotic resistances have been previously reported (Chaturvedi et. al., 2008). The most sensitive strain *Brevundimonas diminuta* susceptible to antibiotic were negative for plasmid

(Figure 6). The antibiotic resistance property was found to be lost in *Brevundimonas diminuta* indicated the above property is plasmid born. This is clear indication in relationship between plasmid content and antibiotic resistance pattern. This finding was also comparable with previous study Shahid et al (2003) and Oppegaard et al(2001), as they have isolated single plasmid of molecular weight 48.5 kb and 65 kb in multidrug resistant isolates of *Pseudomonas aeruginosa* and lactose-fermenting Coliform, respectively.

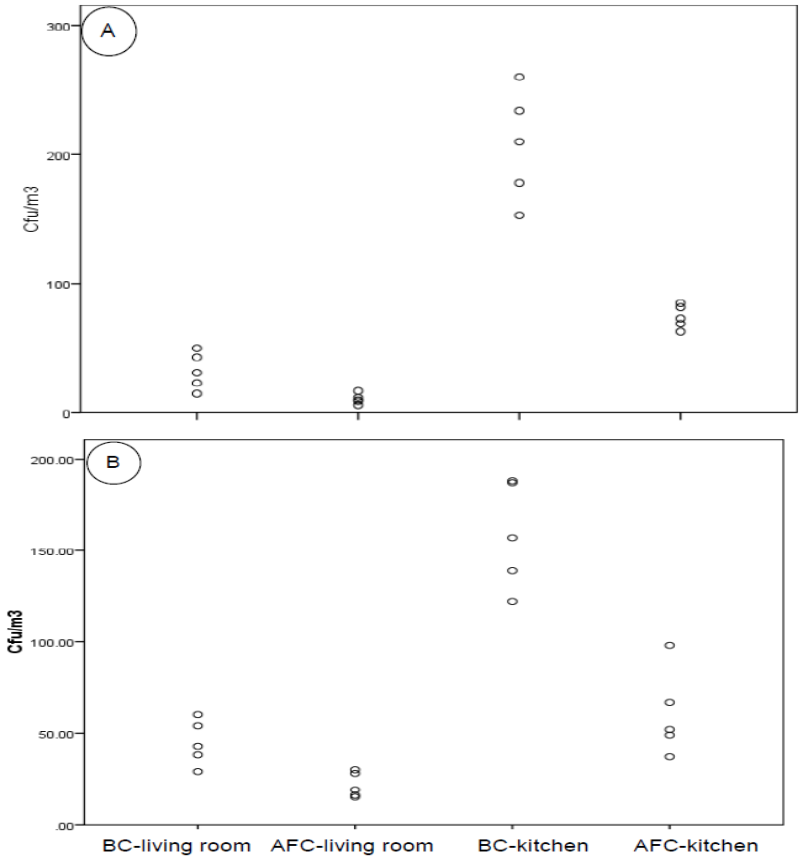
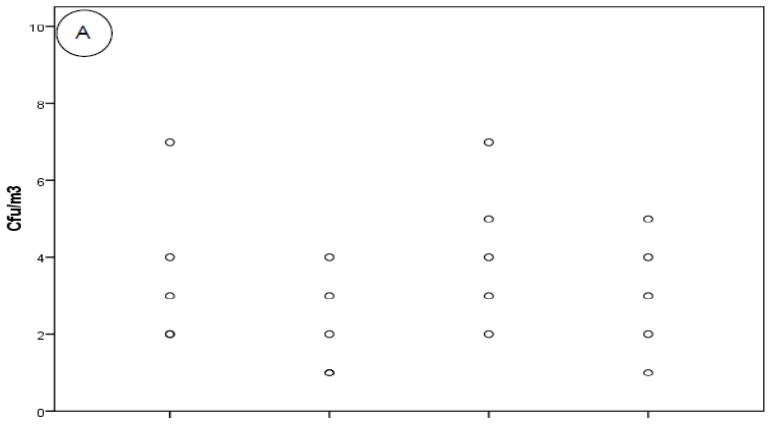


Figure. 1: Bacterial load before and after cleaning of rural area (A) and Urban area (B). BC: before cleaning; AFC: after cleaning.



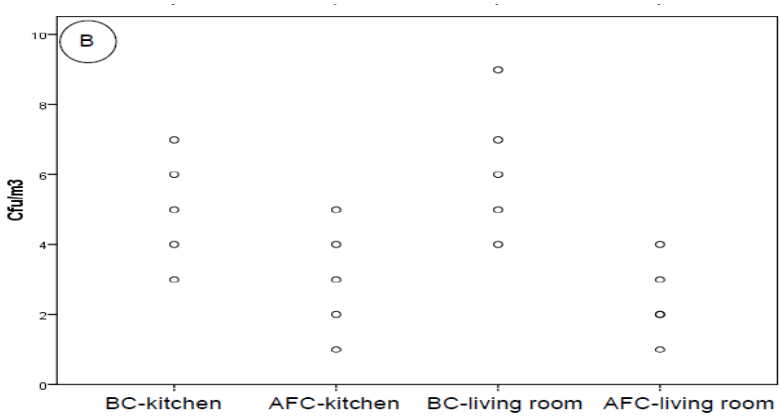


Figure. 2: Fungal load before and after cleaning of rural area (A) and Urban area (B). BC: before cleaning; AFC: after cleaning.

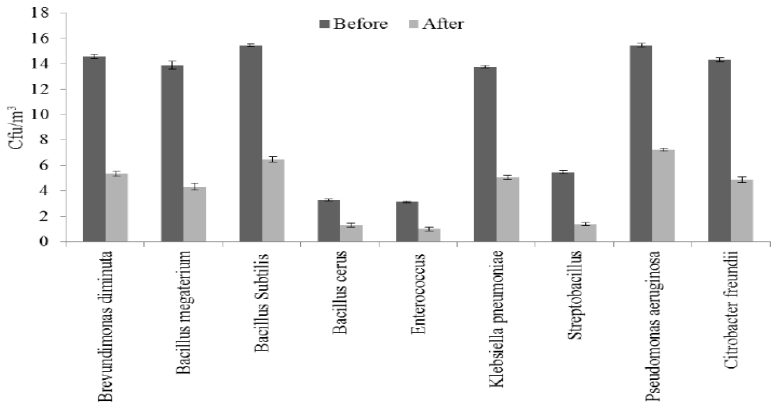


Figure. 3: Distribution pattern of dominated bacterial strains before and after cleaning.

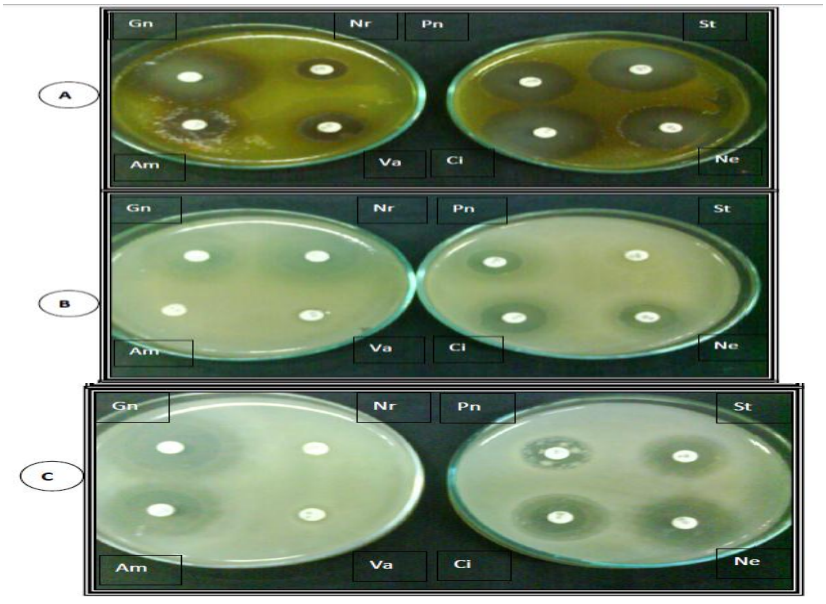


Figure. 4: Antibiotic sensitivity profile and zone size (mm) of *Brevundimonas diminuta (A); Bacillus megaterium (B)* and *Bacillus Subtilis (C).*

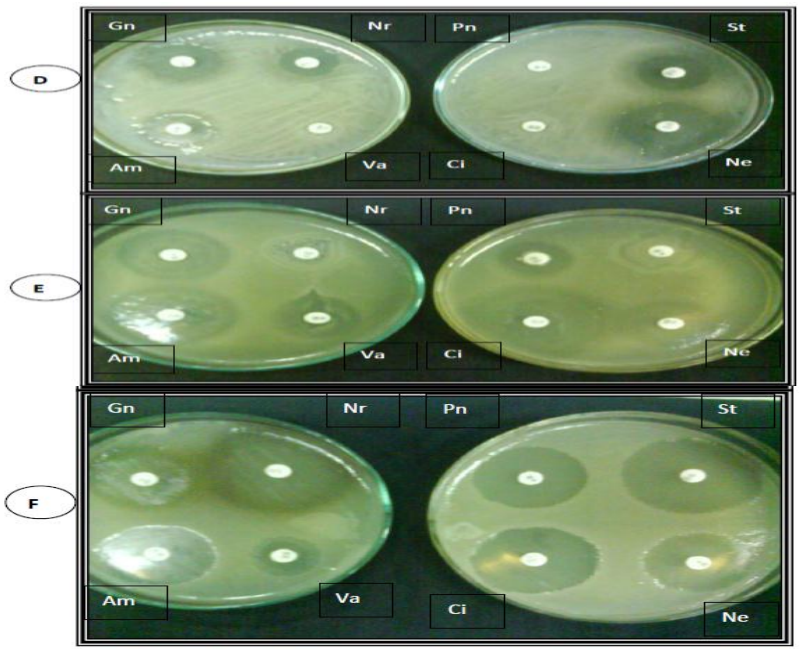


Figure. 5: Antibiotic sensitivity profile and zone size (mm) of *Klebsiella pneumonia (D); Pseudomonas aeruginosa (E) and Citrobacter freundi (F).*

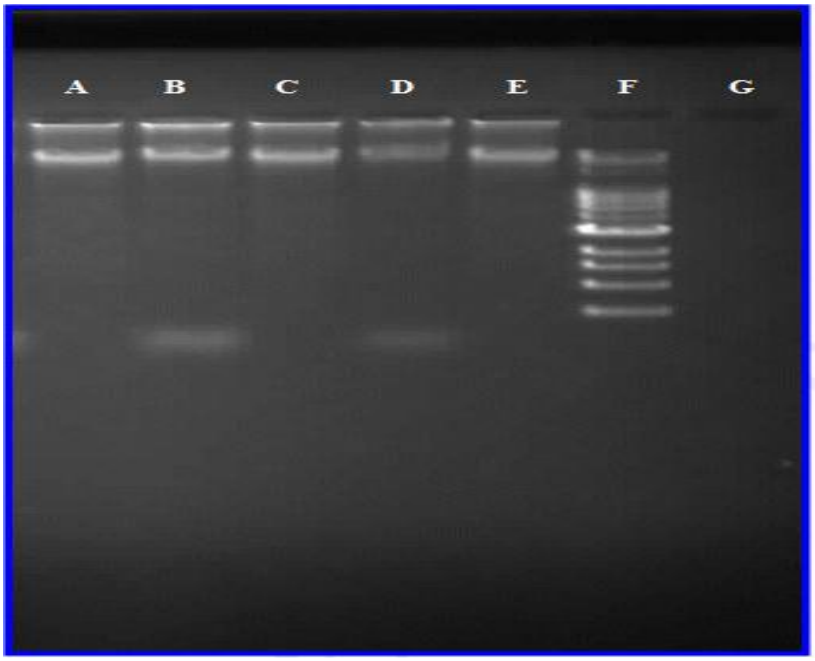


Figure. 6: Plasmid distribution of different bacterial sp. isolated from kitchen and bedroom in Meerut region. Lane A: *Bacillus megaterium*; Lane B: *Bacillus Subtilis*; Lane C: *Klebsiella pneumonia*; Lane D: *Pseudomonas aeruginosa*; Lane E: *Citrobacter freundii*; Lane F: *Escherichia coli* (MTCC 131). Lane G: *Brevundimonas diminuta.*

Table 1: Distribution pattern of Microbial (bacterial and fungal) load on different site location.

Site	Bacterial load (cfu/m^3)			
	Rural area		Urban area	
	Kitchen	Living	Kitchen	Living
1	153	85	139	52
2	178	73	157	37
3	210	69	122	49
4	234	82	188	98
5	260	63	187	67
Average	207	74.4	158.6	60.6
SD	42.73172	9.099451	29.14275	23.47978
Median	210	73	157	52
	Fungal load (cfu/m^3)			
	Rural area		Urban area	
	Kitchen	Living	Kitchen	Living
1	2	2	5	3
2	3	1	7	5
3	4	3	4	2
4	7	1	6	5
5	2	2	9	7
Average	3.6	1.8	6.2	4.4
SD	2.07	0.83	1.92	1.94
Median	3	2	6	5

Table 2: Detail of isolated bacterial stains.

Strain no.	No. of colony (%)	Gram strain	Shape at 100x	Edge	Color	Source
1	39	-	Rod	Entire	Yellow	Rural
2	47	+	Rod	Lobate	White	Urban & Rural
3	38	+	Rod	Erose	White	Urban& Rural
4	24	+	Rod	Undulate	Yellow	Urban
5	12	-	Coccus	Lobate	White	Urban
6	74	-	Rod	Rhyzoid	White	Urban& Rural
7	18	+	Rod	Erose	Yellowish	Rural
8	61	-	Rod	Lobate	White	Urban& Rural
9	46	-	Rod	Undulate	Yellow	Urban& Rural

+:positive; -:negative.

Table. 3: Biochemical Test of isolated Bacteria.

S. No.	Gram Reaction	MacConkey	Mortality	Starch Hydrolysis	H2S Production	Urea	MR/VP	Oxidase	Catalase	Indol Production	Glucose fermentation	Endospore	Gelatin	Growth in 10% NaCl	Growth at 50°C	Casein hydrolysis	Citrate utilization	OF test	Name of Organism
1.	-	+	+	-	-	-	+/-	+	+	-	-	-	-	-	-	-	-	+	*Brevundimonas diminuta*
2.	+	-	-	+	-	-	-/-	-	+	-	+	+	+	+	+	-	+	+	*Bacillus megaterium*
3.	+	-	-	+	-	-	-/-	+	+	-	+	+	+	+	+	+	+	+	*Bacillus Subtilis*
4.	+	-	-	+	-	-	-/-	+	+	-	+	+	+	+	-	+	-	-	*Bacillus ceres*
5.	+	-	-	-	-	-	+/-	-	-	-	+	-	-						*Enterococcus*
6.	-	+	+	-	-	-	+/-	-	+	+	+	-	-	+	+	-	-	+	*Klebsiella pneumoniae*
7.	+	-	-	+	-	-	-/-	+	+	-	+	+	+	-	-	-	-	-	*Streptobacillus*
8.	-	+	+	-	-	-	-/-	+	+		+	-	+	+	+	-	-	+	*Pseudomonas aeruginosa*
9.	-	+	-	-	-	-	+/-		+	+	+	-	-	-	+	-	+	+	*Citrobacter freundii*

+: Positive; -: Negative.

Table 4: Antibiotic sensitivity pattern of dominated bacterial isolates.

Antibiotics	µg/disc	*Brevundimonas diminuta*	*Bacillus megaterium*	*Bacillus Subtilis*	*Klebsiella pneumoniae*	*P. aeruginosa*	*Citrobacter freundii*
Gentamycin	30	S	R	R	R	R	I
Streptomycin	25	S	R	R	R	R	S
Ciproflaxin	30	S	R	I	R	R	S
Neomycin	30	S	R	R	R	R	I
Norflaxin	10	I	R	R	R	I	I
Amoxillin	10	I	R	R	R	S	I
Penicillin	10	S	R	R	R	I	R
Vanomycin	10	I	R	R	R	R	R

S = Sensitive; R = Resistant; I= Intermediate.

Table. 4.5 Multiple-drug resistance (MDR) patterns and plasmid distribution pattern of dominating strains.

Antibiotics	Antibiotic resistance pattern	
	No. of drug R/I/S	Plasmid Size (Kb)
Brevundimonas diminuta	-/3/5	-
Bacillus megaterium	8/-/-	54.4
Bacillus Subtilis	7/1/-	55
Klebsiella pneumoniae	8/-/-	54.4
P. aeruginosa	5/2/1	55
Citrobacter freundii	2/4/2	54.4

R = Resistant; I= Intermediate; S = Sensitive

CONCLUSION

In this study, domestic kitchens were more contaminated than living room of both rural as well as urban area. Moreover, the bacterial concentration at all investigated sites was significantly much higher than fungal concentration. Six bacteria (*Brevundimonas diminuta; Bacillus megaterium; Bacillus Subtilis; Klebsiella pneumoniae; Pseudomonas aeruginosa; Citrobacter freundii*) showed dominance on all other bacterial and fungal sp. *Brevundimonas diminuta* is found most sensitive strain. *Citrobacter freundii* found sensitive against for Streptomycin, Ciprofloxacin and *Pseudomonas aeruginosa*, is found sensitive only for Amoxillin. Moreover, strain *Bacillus megaterium, Bacillus Subtilis, Klebsiella pneumoniae,* showed multiple drug resistant capabilities**.**

CONFLICT OF INTEREST

None.

ACKNOWLEDGEMENTS

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