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Original Article

Comparative Account of Microbial Load Assessment in a University Cafeteria

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ABSTRACT

The physical environment of a cafeteria may influence some factors such as hygiene and food preparation, and both factors can play a crucial role in the transmission of infectious disease among students, hostellers and staff using the cafeteria. Food contact surfaces are a major concern for food service facilities in the cafeteria for controlling the spread of various kinds of pathogenic infections.
The Microbiological analysis of main cafeteria of a University, in Noida was taken for the study. Random sampling was done taking sample from food storage area, food preparation area, washing area, serving area and eating area of the cafeteria. Samples were later, assessed using standard microbiological methods.
Various genera of bacteria and fungus were isolated and identified, they were mainly Gram positive, Gram negative bacteria such as Staphylococcus aureus, E. coli,Streptococcus along with Aspergillus, Penicilium and yeast colonies were also observed. These are some of the infectious agents which cause diarrhoea, typhoid, abdominal pain, microbial food poisoning etc. for which no clinical or laboratory findings are provided.
In general, it has been observed that the level of personal hygiene of the food handlers in food establishment was unsatisfactory due to poor sanitation and wrong practices. This can be enhanced by regular cleaning & monitoring of the cafeteria by the staff, process owner/authorities as per food safety practices. Regular training/workshops on personal hygiene will increase the awareness and good practices in cafeteria.

Keywords: personal hygiene, bacterial load, cafeteria microbial load assessment, food handler.

1. INTRODUCTION

Proper supply of safe, complete and healthy food is essential for the health and well - being of the humans (Adak et al., 2005). [2] Consumption of contaminated or unsafe foods may result in illnessand can lead to food borne diseases (WHO,2000; Bryan, 1997). Food hygiene is essentially aimed at producing food which is

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safe for human consumption and is required for good health (Scheule, 2001). [25]

Biological contaminants such as bacteria, viruses, fungi, protozoa and helminthes constitute the major cause of concern ranging from mild infection to life threatening illness or both. Diseases such as cholera, campylobacterosis*E. coli* gastroenteris, salmonelosis, shigellosis, typhoid fever, brucellosis, amoebiasis are being reported due to unhygienic food preparation and storage conditions in most of the developing countries. (Edema, 2005). [11]

Lacking awareness and personal hygiene amongst food handlers is one of the most commonly reported practices contributing to food – borne illness. Dirty hand of the workers and work surface hygiene also adds to the problem (WHO,2000; Bryan, 1997). The risk of food borne illness due to contact with hands or surface depends on level of contamination and transmission of the disease causing vector during food preparation and storage till its consumption.

The presence and absence of pathogenic microorganisms in food materials, food preparation surfaces, equipments and utensils has led to a high degree of chronic illness (Egonu and Alan, 2000).Food safety need to be ensured during preparation, production, processing, storage, distribution and preparation of food to minimize the contamination and to maintain its safety for human consumption (Edema*et al.*,2005).[11] Effective cleaning is of prime importance since it does not only remove gross contamination but also residues that could support the subsequent survival and growth of microorganisms (Bean *et al.*,1990). [3]

Few reasons which predominates the contamination of the food and outbreak of food borne diseases are subsequently identified as unsafe sources, contaminated raw food items, improper food storage, and poor personal hygiene during food preparation, inadequate cooling and reheating of food items and a prolonged time lapse between preparation and consumption of food items (Linda du and Irma, 2005).

In large scale cooking, specially, in cafeteria, restaurants, hotels and dhabas, food passes through many hands, thereby increasing the chances of food contamination due to improper handling which might endanger the health of consumers (Omaye,2004). [21]

In the year 1998, Zhao et al., [28] reported that contamination from food handlers usually results due to inadequately washed hands, improper food preparation techniques, incorrect cleaning procedures of food preparation surfaces, chopping boards and tables. Bacteria have been reported to survive on chopping-boards for more than three hours, especially when boards are not properly cleaned (Zhao et al., 1998; Salo et al., 2000) [24, 28]

In addition, Salo and colleagues (2000) reported that wet items such as dishcloths, hand towels, Apron, and sponges, as well as sink drain areas with leaking pipes, uncovered drains, garbage, leftovers might also serve as continuous reservoirs that harbor potentially harmful microorganisms, which may end up settling on kitchen surfaces (Zhao et al., 1998; Salo et al., 2000). [24, 28] Improper food hygiene practices and unclean surfaces have been associated with opportunistic pathogenic microorganisms such as *Staphylococcus aureus* (Andargie et al., 2008; Garcia, 2007). [2, 14] Improperly cleaned surfaces along with deficient food handling practices have led to an increase in microbiological hazards in food preparation areas (Nkhebenyane, 2010). [20]

2. MATERIAL AND METHODS

2.1. Study Area

As per the guideline of FSME (Food Safety Management System) of the University, study was carried out in theUniversity cafeteria in Noida, Uttar Pradesh. Various samples were collected from student's cafeteria during working hours. The duration

of the study was during the month of March 2014 till March 2015

2.2. Sample Collection

Random Sampling was done. Sterilized swab sticks were used to collect the samples. The samples were taken from the hands of food handlers, tables, apron, utensils (storage, cooking), washed utensils (includes spoons, plates, trays, bowls), juicer, fridge, food storage area, food making area, washing area, serving area and eating area. Swab sticks were dipped in 1ml of double distilled water tubes and brought to the laboratory for further examination.

2.3 Sample Processing

50 µL of sample were plated onto Luria Bertuni agar (LA) for bacterial count and on Potato Dextrose agar (PDA) for fungal count using spread plate method. LA and PDA plates were incubated at 37°C and 25°C for 24 - 72hrs respectively.

2.4 Identification of Microbial Isolates

After incubation period, the bacterial and fungal colonies were counted; the morphological characteristics were observed. Later the gram staining procedure was performed for the identification of the gram positive and gram negative strains of Bacteria.Fungal/ Bacterial/Yeast isolates were also observed under the microscope. Biochemical test were performedsuch as oxidase, catalase, coagulase, indole, urease, citrate, sugar utilization as described by Speck (1986) [26] and Cheesebrough (2004) to identify the bacterial species.

3. RESULT

The microbial quality assessment of hands of food handlers, tables, apron, utensils (includes storage, cooking), washed utensils (includes spoons, plates, trays, bowls), juicer, fridge, food storage area, food making area, washing area, serving area and eating area were examined using standard method. The total bacterial and fungal counts were done on Luria Bertuni agar (LA) and Potato Dextrose Agar (PDA) respectively. Occurrence of microbial isolates of specimens obtained from students' cafeteria in the University is presented in **Table 1& 2.** The results reveal that, on PDA plates the average bacterial colonies present were 3.78×10^3 CFU/ml,mainly in fridge, wash basin and spoon. The highest bacterial colonies were ranging from 10 -25×10^3 CFU/mlincluding chopping vegetables, worker's hands and juicer. Whereas the least bacterial colonies were ranging from 0.2 -7.2×10^3 CFU/ml including tray, plates and eating table.21 fungal colonies including Aspergillus andPenicilliumwere present in worker's hand, worker's clothes, salad chopper, cooked food storing area, eating table, etc. and one sample (fridge) showed pink coloured yeast colonies. On LA plates, plates the average bacterial colonies present were 2.44×10^5 CFU/ml, mainly in salad chopper, worker's hand, fridge, juicer, wash basin, spoon and eating tables. The highest bacterial colonies were 1×10^8 CFU/ml including roti area, chopping vegetables, cooked food storing area, washed cooker, storage containers and utensils. Whereas the least bacterial colonies were ranging from 2×10^2-9.4×10^3 CFU/ml including worker's clothes, washed utensils, tray, serving area and billing counter.Two samples of washed utensils also showed pink colored yeast colonies. Preliminary analysis of microbes was done with the help of biochemical test**(Table3)**

4. DISCUSSION

In the present study, nine genera of bacteria were isolated and identified. They were identified as *E.coli*, *Clostridium sp, Micrococcus* sp, *Staphylococcus* sp, *Streptococcus* sp, *Pseudomonas* sp, Shigellasp, *Bacillus* sp, *Salmonella sp* by comparing their morphology and biochemical characteristic**(Table 3)** with standard reference organisms.

The presence of organism such as *E. coli, Salmonella sp, Clostidiumsp* and other organisms in this study is of special concern and perhaps the greatest danger associated with the water for food processing and drinking purpose (Lynch et al., 2003). [18] Qualitative hand swab results showed that a high fraction of the personnel's hand were contaminated by *E. coli, shigellasp, micrococcus sp* even though the source of those contaminants was not determined they are highly indicative of inadequate hand sanitation (Collins et al., 1989; Brown et al., 2000). [5, 9] However the workers serving food were not found contaminated as they were wearing gloves. Large number of the *Staphylococcus* sp and *Streptococcus* sp were isolated although they are normal commensal on human which reflect improper hygiene practice such as pocking nose with fingers (Collins 2001). It was observed that there was no hand sanitizer/soap available for the workers to clean their hand after using the toilet or handling food/raw foods. It has also been observed that the common practice after washing is to dry their hands in their apron, garment which could probably serve as source of further contamination, which has been reported by Moyo and Baudi (2004). [19] From these assessments, the food handlers personal hygiene standard and food handling practices were unsatisfactory, the tables and plates used for eating could also be a source of spread of food borne diseases unless corrective sanitary measures are put in place.

In this study, the presence of *Staphylococcus* sp, *E. coli, Pseudomonas* sp, *Bacillus sp* in the plates shows the existing poor sanitary qualities of food utensils, ineffective washing techniques, improper handling and storage of clean utensil. Repeated usage of water for cleaning utensils and their hand increases the severity of the infection. Clothes and mops used for wiping and drying plates and tables are also improperly cleaned.

The presence of Bacillus, fungi such as Aspergillus, Penicilliumspp in the foods could be due to the fact that they are spore formers. These heat-resistant spores may have survived processing while vegetative cells were eliminated. Contamination of foods could have resulted from inappropriate processing, incomplete heating, or secondary contamination via contact with contaminated hand, equipments and utensils (Oranusi et al., 2013). [22]

Yeasts, including Candida albicans, Rhodotorula rubra, Torulopsis andTrichosporoncutaneum, have been found living in between people's toes as part of their skin flora (Oyeka and Ugwu 2002). Yeasts are found to be present in the gut flora of mammals and some insects such as flies, cockroaches, thus the worker's hand and bad hygienic condition of the cafeteria can lead to food contamination. Yeasts are able to grow in foods with a low pH (5.0 or lower) and in the presence of sugars, organic acids, and other easily metabolized carbon sources which results in food spoilage as reported by Kurtzman (2006). [15]

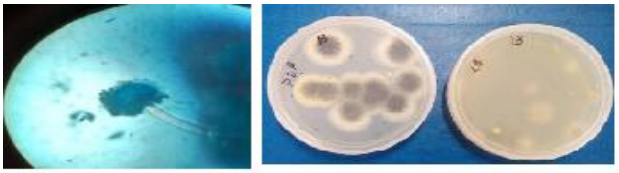


Fig. 1a and 1b: Media plates and microscopic image are showing Aspergillus colonies isolated from worker's clothes (chopping area).

Fig. 2a and 2b: Plates are showing bacterial and yeast colonies isolated from fridge; microscopic view of isolated yeast.

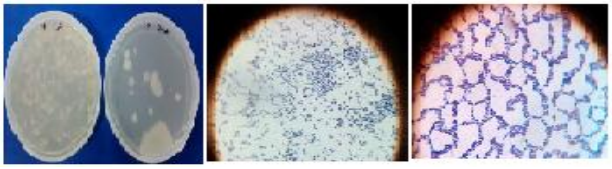


Fig. 3a, 3b and 3c- Plates are showing bacterial colonies isolated from washed spoon; microscopic view of isolated gram positive bacteria.

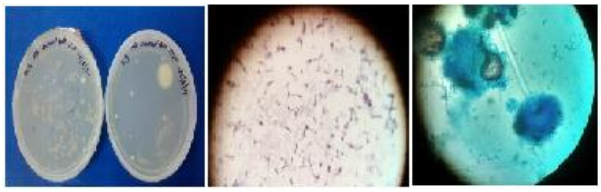


Fig. 4a, 4b and 4c-LA plate is showing gram positive bacterial colonies and PDA plate,Aspergillus colony with their microscopic view isolated from clean eating table

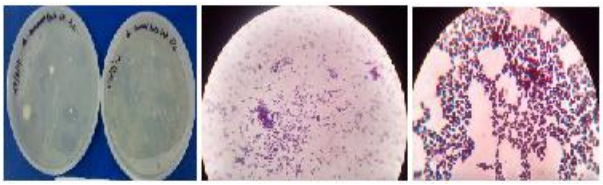


Fig. 5a, 5b and 5c- plates are showing gram positive bacterial and yeast colonies with its microscopic view isolated from washed bowl

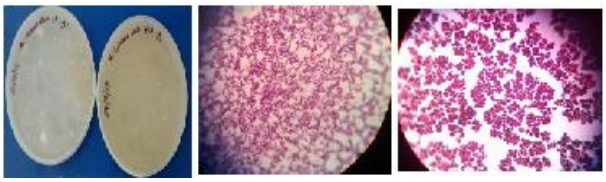


Fig. 6a, 6b and 6c- Media plates are showing gram negative bacterial and yeast colonies with its microscopic view isolated from washed utensils.

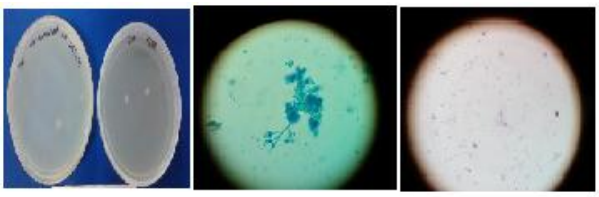


Fig. 7a, 7b and 7c- LA plate is showing gram negative bacterial colonies and PDA plate penicillium colony with their microscopic view isolated from papad tray.

Food handlers with skin lesion, respiratory infection, eyes and nose discharge could have served as the source of *Staphylococcus aureus* on the plate. As *Staphylococcus aureus* lives and flourishes in the human nose, eyes, skin and throat, the likely hood of recontamination of cleaned plate by infected food handlers is quite high. This has also been observed that the workers are not aware of these infectious agents and their harmful effects. Many researchers all over the world have reported bacterial and fungal infection of the food in restaurants, hotels and cafeteria. However, no concrete plan of action has been found in place to handle such contamination at public place. There is an urgent need to maintain the workers' health chart along with the immunization detail by the cafeteria owners so that the infective people could not be employed at such places.

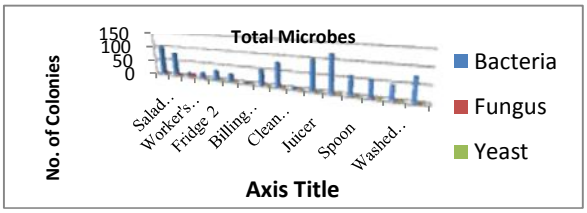
Globally some of the reported research such as Collins and Brown in the year 2000 has shown that bacterial count of a food reflect the hygienic and unhygienic condition of the food outlets and the food handlers. Bryan in 1997 [7] has emphasized on the importance of training and awareness about the infectious diseases and its mode of action in the human so that each worker feels the responsibility of minimizing it at the worksite.Study of Abdullahi et al., [1] in the year 2004 has reported that the most of the harmful disease causing bacteria are found in the hand of worker such as *Staphylococcus*, *E. coli*, *Pseudomonas* , *Klebsiellaetc*so, proper awareness will definitely help in the minimization of the these agents.

As we see that the contamination is within the premises of food preparation and processing so precautionary measures such as hand sanitizers, hand dryer, clean garments, aprons will help to further minimize the contamination. Moyo and Baudi, in the year 2004 has also emphasized on the clean working environment. Feglo and Sakyi, 2012 [13] has reported that most ready-to-eat foods in Kumasi (Ghana) were contaminated with enteric bacteria and other potential food poisoning organisms with bacterial counts higher than the acceptable levels. Utensils and equipment used in food preparation and processing need to be properly cleaned to stop the cross contamination. Disinfecting equipment, hands, surface, and utensils have been advocated by Linda et al., in the year 2005. Storage area is more prone to spore producing bacilli and lactic acid bacterium as mentioned by Lucyna et al., 2013. [17]

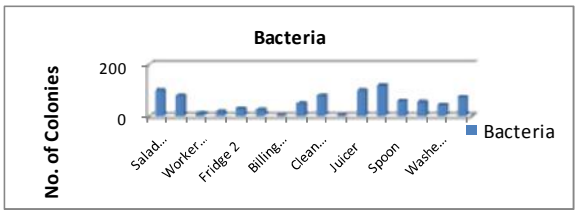
5. CONCLUSION

Conclusively, it should be noted that the working surface or any surface which comes under direct contact with food shall not contain more than 100 viable microorganisms /gram during the analysis. The

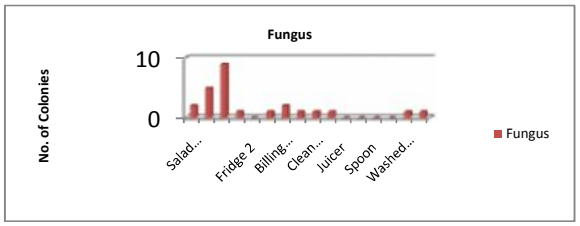
total microbial count of the hand should be considered as negligible. Process owners has to take the responsibility of providing personal hygiene/sanitation training to the staff and should also bear the moral responsibility of developing tactics to motivate food handlers to practice food hygiene and implement a regular screening of food outlet for microbial load.



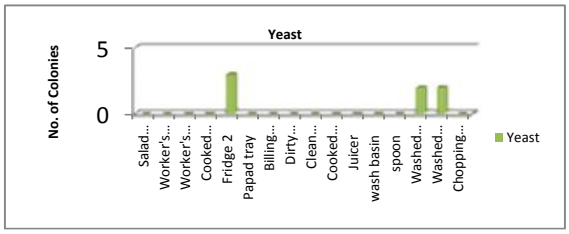
GraphA 1: Comparative analysis of microbes in highly contaminated samples after 24 hrs, 48hrs of incubation on LA plates and 72hrs of incubation on PDA plates.



Graph A 2: Comparative analysis of Bacterial colonies in highly contaminated samples after 24hrs of incubation on LA plates.



Graph A 3: Comparative analysis colonies of Fungus in highly contaminated samples after 72hrs of incubation on PDA plates.



Graph A4: Comparative analysis of Yeast colony in highly contaminated samples after 48hrs of incubation on PDA Plates

6. ACKNOWLEDGEMENT

Authors acknowledge Amity University for providing necessary facilities and infrastructure to perform the tests.

Table 1: Comparative account of Fungal load from the sample collected area on PDA plate.

S. no.	Sample Area	Sample size	No. of colonies grown		Physical characteristics	Lactophenol cotton blue stain
			Bacterial count (Cfu/ml)	Fungal count		
1	Roti area	50µl	3.6×10^3	0		
2	Noodles area	50µl	0.2×10^3	0		
3	Chopping vegetables 2*	50µl	15×10^3	1	White color with black spores.	Aspergillus*
4	Washed cooker	50µl	0.2×10^3	0		
5	Salad chopper*	50µl	10×10^3	2	White color with black spores.	Aspergillus*
6	Worker's hand* (chopping)	50µl	20×10^3	5	White color with black spores.	Aspergillus*
7	Worker's clothes* (chopping)	50µl	0	8	White color with black spores.	Aspergillus*
8	Rice area*	50µl	2.2×10^3	1	White color with black spores.	Aspergillus*
9	Fridge 1	50µl	6.6×10^3	0		
10	Fridge 2*	50µl	7.2×10^3	0	Pink color	Yeast*
11	Juicer	50µl	25×10^3	0		
12	Wash basin	50µl	3.8×10^3	0		
13	Spoon (washing area)	50µl	2.6×10^3	0		
14	Papad tray*	50µl	0.2×10^3	1	Green colored colonies	Penicillin*
15	Billing counter*	50µl	0.6×10^3	1		Aspergillus*
16	Dirty eating table*	50µl	0.2×10^3	1		Aspergillus*
17	Clean eating table*	50µl	1.8×10^3	1		Aspergillus*
18	Washed plates (serve to students)	50µl	0.2×10^3	0		
19	Front serving area	50µl	0.2×10^3	0		
20	Apron	50µl	0.2×10^3	0		
21	Roti container	50µl	0.4×10^3	0		
22	Cooked food storing area*	50µl	1×10^3	1	White colored colonies with black spores.	Aspergillus*
23	Jalebi tray	50µl	3×10^3	0		
24	Washed bowl	50µl	14×10^3	0		
25	Washed utensils (in washing area)	50µl	0.4×10^3	0		
26	Washing slab containing utensils	50µl	6×10^3	0		

Note:* shows higher concentration of Fungal contamination in the specified area. Mean Bacterial count= 3.78×10^3 and Standard deviation= 6.27×10^3

Table 2: Comparative account of Bacterial load from the sample collected area on LA plates.

S. no.	Sample Area	Sample size	No. of colonies grown	Physical characteristics	Gram stain
			Bacterial count (Cfu/ml)		
1	Roti area	50µl	1×10^8	Cream coloured lawn	G+ve
2	Roti area 2	50µl	1×10^8	White isolated colonies	G-ve
3	Noodles area	50µl	1×10^8	Cream coloured lawn	G+ve
4	Chopping vegetables 1	50µl	1×10^8	Cream lawn with some isolated colonies	G-ve
5	Chopping vegetables 2	50µl	1×10^8	Cream coloured lawn	G+ve
6	Storage utensil container	50µl	1×10^8	Cream coloured lawn	G+ve
7	Large storage container	50µl	1×10^8	Bacterial lawn	G-ve
8	Washed cooker	50µl	1×10^8	2 Bacterial lawns	G+ve
9	Storage area utensils	50µl	1×10^8	Cream coloured lawn	G+ve
10	Salad chopper*	50µl	2.2×10^4	Many Cream coloured colonies	G+ve
11	Worker's hand (chopping)	50µl	1×10^6	2 types of Bacterial lawns	G+ve
12	Worker's clothes (chopping)*	50µl	2.4×10^3	Yellowish colony	
13	Rice area	50µl	1.6×10^3	White coloured colonies	G+ve
14	Fridge 1	50µl	7×10^4	White coloured colonies	G+ve
15	Fridge 2	50µl	4×10^2	White coloured colonies	G-ve,G+ve
16	Juicer*	50µl	1×10^6	Bacterial lawn with isolated colonies	G+ve
17	Wash basin*	50µl	4×10^4	Brown and cream coloured colonies	G+ve
18	Spoon (washing area)*	50µl	1.4×10^4	Brown and cream colonies	G+ve
19	Papad tray*	50µl	4.8×10^3	White and cream colonies	G-ve
20	Billing counter*	50µl	2×10^2	White coloured mycelium	
21	Dirty eating table*	50µl	1.1×10^4	Brown and cream coloured colonies	G+ve
22	Clean eating table*	50µl	1.4×10^4	Brown and cream coloured colonies	G+ve
23	Washed plates (serve to students)	50µl	6×10^2	White coloured colonies	G+ve
24	Front serving area	50µl	4.4×10^3	Cream coloured colonies	G+ve
25	Apron	50µl	1.4×10^3	Cream coloured colonies	G+ve, G-ve
26	Roti container	50µl	8×10^2	White coloured colony	G-ve, G+ve
27	Cooked food storing area	50µl	1×10^8	Cream coloured lawn	G+ve, G-ve
28	Jalebi tray	50µl	1×10^8	Cream coloured lawn	G-ve, G+ve
29	Washed bowl*	50µl	2×10^3	White coloured isolated colonies	G+ve, G-ve yeast*
30	Washed utensils (in washing area)*	50µl	9.4×10^3	White and brown coloured isolated colonies	G-ve, yeast*, G+ve
31	Washing slab containing utensils	50µl	1×10^3	White colonies	G-ve, G+ve
32	Serving hand	50µl	0		

Note:* shows higher concentration of Bactrial contamination in the specified area. Mean Bacterial count= $2.44\times10^5$and Standard deviation= 2.32×10^5

Table 3: Biochemical Identification of bacterial colonies

S.No.	Suspected microorganism	Gram	Oxidase	Catalase	Coagulase	Citrate	Urease	Indole	Glucose	Lactose	Sucrose	Mannitol	Motility
1	*E.Coli*	-	-	-	-	-	-	+	+	+	+	+	+
2	*Salmonella sp.*	-	-	-	-	+	-	-	+	-	d	+	+
3	*Shigella sp.*	-	-	-	-	-		+	+	-	d		-
4	*Clostridium sp.*	+	-	-		-	-	-	+	+	+		+
5	*Pseudomonas sp.*	-	+	+	-	+	-	-	+	-	-	+	+
6	*Streptococcus sp.*	+	-	-	-	-	-	-	+	d	d	+	-
7	*Staphylococcus sp.*	+	-	+	+	-	-	-	+	-	-	+	-
8	*Bacillus sp.*	+	+	-	-	-	-	-	-	-	d	+	+
9	*Micrococcus sp.*	+	-	+	-	+	+	-	-	-	-	-	-

***d-differential result**

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