Microbiological Environmental Monitoring in Pharmaceutical Facility

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ABSTRACT

Regular environmental monitoring samples for microbiological quality of different locations in pharmaceutical facility are by far very critical measure in controlling clean area and environment which have direct impact on the microbiological quality of the final pharmaceutical products. Different methods were used to collect samples from different locations. These samples were processed and followed by isolation and identification. The obtained data were analyzed to find microbial distribution in the environment of pharmaceutical facility. Twenty one bacterial and fungal species were isolated from different locations in the pharmaceutical facility. Group of genus *Staphylococci* contributes to 38.4% of total positive samples while genus *Micrococci* contributes to 22.4% and *Gemella morbillorum* represents 1.1%. Genus *Bacilli* represents 35.0%, *Candida albicans* 1.3%, *Klebsiella pneumonia* 1.0% and *Stenotrophomonas maltophilia* 0.01%. Most microorganisms found among 7 identified genuses were belonging to *Staphylococcus*, *Micrococcus* and *Bacillus species*.

Key words: Environmental monitoring, settle plate, reuter centrifugal sampler, miniaturized identification system, contact plate.

INTRODUCTION

Environmental monitoring describes the microbiological testing undertaken in order to detect changing trends of microbial counts and micro-flora growth within clean room or controlled environments. The results obtained provide information about the physical construction of the room, the performance of the heating, ventilation, and air-conditioning (HVAC) system, personnel cleanliness, gowning practices, the equipment, and cleaning operations (Sandle, 2006).

The design and construction of clean rooms and controlled environments are covered in Federal Standard 209E. This standard of air cleanliness is defined by the absolute concentration of airborne particles. Methods used for the assignment of air classification of controlled environments and for monitoring of airborne particulates are included. This federal document only applies to airborne particulates within a controlled environment and is not intended to characterize the viable or nonviable nature of the particles (USP, 2001).

Although there is no direct relationship established between the 209E controlled environment classes and microbiological levels, the pharmaceutical industry has been using microbial levels corresponding to these classes for a number of years; and these levels have been those used for evaluation of current GMP compliance (Mashburn and Whitfield, 1967).

Adequately separating areas of operation is an important part of contamination prevention. To maintain air quality in areas of higher cleanliness, it is important to achieve a proper airflow and a positive pressure differential relative to adjacent less clean areas. Rooms of higher air cleanliness should have a substantial positive pressure differential relative to adjacent rooms of lower air cleanliness. For example, a positive pressure differential of at least 12.5 Pascals should be maintained at the interface between classified and unclassified areas (FDA, 2002).

Clean rooms are normally designed as functional units with specific purposes. A well-designed clean room is constructed with materials that allow for ease of cleaning and sanitizing. Examples of adequate design features include seamless and rounded floor to wall junctions as well as readily accessible corners. Floors, walls, and ceilings are constructed of smooth and hard surfaces that can be easily cleaned (CDER, 2003).

The most likely microflora found in pharmaceutical manufacturing can be hard to come by a definite source. The most commonly occurring microorganisms come from human skin (either commensurable or transient) are Gram positive microorganisms and common environmental fungi which include the following:
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus hominis, Micrococcus species, Bacillus species and *Candida species*. Whereas those associated with eyes, ears and mucus include Gram-negative microorganisms, which can arise on rare occasions directly from the operator and include the following: *Pseudeomonas aeruginosa,* and *Escherichia coli* (Jiminez, 2004).

Strains of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were isolated from pharmaceutical effluent (Lateef, 2004).Water that entered into the distribution system has low heterotrophic plate counts (Florjanič and Kristl, 2006). *Stenotrophomonas maltophilia* was the most commonly isolated bacteria in samples taken from water supplies (Penna *et al.,* 2002).

Identification was made for gram-negative non-fermenting bacteria, which were isolated from drinking water and water purification systems, since *Pseudomonas* genera represents opportunistic pathogens which disperse and adhere easily to surfaces, forming a biofilm which interferes with the cleaning and disinfection procedures in industrial environments. Seventy eight isolated colonies were identified as the following bacteria genera: *Pseudomonas, Flavobacterium* and *Acinetobacter.* According to the miniature kits used in the identification, there was a prevalence of isolation of *P. aeruginosa* 32.05%, *P. picketti (Ralstonia picketti)* 23.08%, *P. vesiculares* 12.82%, *P. diminuta* 11.54%, *F. aureum* 6.42%, *P. fluorescens* 5.13%, *A. lwoffi* 2.56%, *P. putida* 2.56%, *P. alcaligenes* 1.28%, *P. paucimobilis* 1.28% and *F. multivorum* 1.28% (Penna *et al.,* 2002).

This study shows the importance of properly designed sampling technique, identification tools and careful intepretation and analysis of the results obtained in order to discover sources of contaminations in controlled environment to make immediate actions to prevent spreading of contaminant which may finally influence the microbiological quality of the final products which may lead to sever financial losses for pharmaceutical company. This study provides a new approach for detecting source of fault in controlled environment using trend and type of microorganisms.

MATERIALS AND METHODS
Sampling locations:

Samples were taken from representative different classes and locations in pharmaceutical facility including:

1- Facility water system: City water and purified water were obtained from water treatment and distribution system.

2- Class A1 (the highest grade of cleanliness): Microbiology laboratory laminar air flow units.

3- Class A2 (the highest grade of cleanliness): Sterility test isolator.

4-Class B (high grade of cleanliness): Safety cabinet, sampling and dispensing laminar air flow units.

5-Class C (intermediate measure of cleanliness): Microbiology laboratory.

6-Class D1(low measure of cleanliness): Ointment preparation and primary packaging, sampling room, dispensing room, oral liquid dosage form preparation and filling and suppositories preparation room.

7-Class D2 (low measure of cleanliness): Mixer and granulator room, drying oven room, tablet compression rooms, coating rooms, kneader room, capsule filling rooms, blistering lines I, II and primary tablet and capsule packaging rooms.

8-Class E (the least stringent cleanliness measures): Secondary capsules and tablets packaging, liquid formulation air lock, ointment primary packaging and suppositories air lock, blister lines corridor, ointment preparation air lock, powder preparation air lock, production entrance corridor, main material corridor and washing room air lock.

Specimen collection:
Water sampling (USEPA, 2002 & Bordner *et al*., 1978):

1-The whole process of water sampling was performed under strict aseptic conditions. Water samples were collected in sterile polypropylene sample containers with leak proof lids. When collecting the sample, enough air space was left in the bottle to allow for proper mixing before examination. Samples representative of the water being tested were collected. Sample ports were flushed and disinfected and aseptic techniques were used to avoid sample contamination. The sample bottle was kept closed until it had to be filled. Cap was removed carefully to avoid contaminating the inner surface of the cap and neck of the bottle. Container was filled without rinsing. Cap was replaced immediately. The volume of sample should be sufficient to carry out all tests required (not less than 100 ml) (ASTM, 1993).

2-Residual chlorine in city water (or chlorinated effluent) samples were neutralized with sodium thiosulfate (1 ml of a 10% solution per liter of water) at the time of collection.

3-Water samples were kept at a temperature of 1-4°C during transit to the laboratory except when transportation time is very short.

4-Using a flamed forceps, a membrane filter (pore size 0.45 µm) was placed, with gridded-side up, on the porous plate of the filter base.

5-The funnel was attached to the base of the filtration unit. This allowed the membrane filter to be located between the funnel and the base.

6-Approximately 30 ml of sterile phosphate-buffered solution water was poured in the bottom of the funnel.

7-The sample container was shaken vigorously 25 times. An appropriate volume (100 ml for water) was measured in graduated cylinder, and poured it into the funnel. The vacuum was turned on, and leave it on while rinsing the funnel twice with about 30 ml sterile dilution water.

8-The membrane filter was held at its edge with a flamed forceps, gently lift and place the filter grid-side up on the tryptone soya agar plate.

9-The agar petridish was incubated for five days at 30ºC - 35ºC.

Active air sampling (Pitt and Hocking, 1999):

1-Air sampler was placed in the center of each room at a height of approximately 1 meter above the floor.

2-The sampler was disinfected with 70% ethanol before use at each location.

3-One thousand liters of air sampling were obtained by Reuter centrifugal sampler.

4-The agar strips TCI-γ were incubated for 48 hours at 30ºC - 35ºC and then for 72 hours at 20ºC - 25ºC.

5-After incubation, the colonies were counted and recorded in specific protocols.

Passive air sampling (Pasquarella *et al*., 2000):

1-Passive air sampling was performed by exposing two 9 centimeters Petri dishes containing tryptone soya agar medium to the air according to the 1/1/1 scheme (for one hour, at a height of one meter at least one meter from walls or any obstacle) for each round and sampling room in areas where there was little air movement (i.e. "dead spaces") and where airflows converge were excessively turbulent and areas within the clean room where there was personnel activity or where specific operations were carried out.

2-All Petridishes were covered with the lid right after sampling, sealed with parafilm and incubated for 48 hours at 30ºC - 35ºC and then for 72 hours at 20ºC - 25ºC.

Surface monitoring:

I- Contact plate method (Kastango and Douglass, 2001):

1-Surface sampling was performed with raised RODAC plates. The tryptone soya agar in contact plates was mixed with polysorbate 80 and lecithin, which inactivate many residual disinfectants. Polysorbate 80 neutralizes phenols, hexachlorophene, and formalin, and lecithin inactivates quaternary ammonium compounds.

2-During sampling, a contact plate was pressed onto the area to be tested. Any microorganisms on the surface of the area tested (which should ideally be flat) were transferred onto the contact plate.

3-After the sample had been obtained; the area tested had been wiped down with isopropyl alcohol 70 % to remove any residue left by the contact plate.

4-The contact Plates were placed upside down in the incubator for 48 hours at 30ºC - 35ºC and then for 72 hours at 20ºC - 25ºC.

II-Swabbing method (Sanderson *et al*., 2002):

1-Swab samples were collected by removing a sterile swab from a sterile tube, moistening it by inserting it into a second tube which contained a sponge soaked with sterile 1.5 ml of phosphate-buffered saline (PBS) at pH 7.2.

2- The selected surface was swabbed by moving the swab back and forth across the surface with several horizontal strokes, then several vertical strokes. The swab was rotated during sampling to ensure that the entire surface of the swab was used.

3-After sampling, the swab was returned to its prelabeled sampling tube containing appropriate amount of liquid media.

Identification using miniaturized biochemical systems:

BBL CRYSTAL identification systems:

Principle:

The tests used in the BBL CRYSTAL E/NF identification system are based on microbial utilization and degradation of specific substrates detected by various indicator systems. **Gram-positive ID kit** includes tests for fermentation, oxidation, degradation and hydrolysis of various substrates. In addition, there are chromogen and fluorogen linked substrates to detect enzymes that microbes use to metabolize various substrates (Hartman, 1968; Maddocks and Greenan, 1975; Killian and Bulow, 1976; Edberg and Kontnick, 1986;

Manafi *et al*., 1991; Moncia *et al*., 1991 & Mangels *et al*., 1993). Enzymatic hydrolysis of fluorogenic substrates containing coumarin derivatives, results in increased fluorescence that is easily detected visually with a UV light source (Maddocks and Greenan, 1975; Manafi *et al*., 1991 & Moncia *et al*., 1991). Chromogenic substrates upon hydrolysis produce color changes that can be detected visually.

Identification is derived from a comparative analysis of the reaction pattern of the test isolate to those held in the data base (Murray *et al*., 1995).

Procedure:

Prior to BBL CRYSTAL E/NF panel set-up, oxidase and indole tests should be performed from a nonselective isolation plate no more than 24 hours old. The following steps were followed for microorganisms inoculation in the kit for both BBL CRYSTAL E/NF and BBL Crystal GP ID system:

1. Lids were removed from pouch. Desiccants were discarded. Once removed from the pouch, covered lids should be used within 1 hour.

2. An inoculum tubes were taken and labelled with specimens data and numbers. Using aseptic technique, with the tip of a sterile cotton swab or a wooden applicator stick or disposable plastic loop, well isolated large colonies were picked from a blood plate and suspended in a tube of BBL CRYSTAL Enteric/non fermenter or Gram positive inoculum fluid.

3. Tubes were recaped and vortexed for approximately 10-15 seconds. The turbidity should be equivalent to a McFarland standard no. 0.5.

4. Entire contents of inoculum fluids were poured into target area of base.

5. Bases were held in both hands and inocula were rolled gently along the tracks until all of the wells were filled.

6. The lids were aligned so that the labeled end of the lid is on top of the target area of the base.

7. The lids were pushed down until a slight resistance was felt.

8. Using a sterile loop, a small drop from the inoculum fluid tubes were recovered either before or after inoculating the base and an agar slants or plates (any appropriate media) were inoculated for purity check. The slants or plates were incubated for 18-24 hours at 35-37°C.

9. Inoculated panels were placed in incubation trays. Ten panels can fit in one tray (5 rows of 2 panels). All panels should be incubated face down with 40-60% humidity. The incubation time for panels is 18-20 hours at 35-37°C.

10. All panels should be read using the BBL CRYSTAL light box or panel viewer.

11. The resulting profile number and off-line test results (indole and oxidase) should be entered on a PC in which the BBL CRYSTAL ID System Electronic Codebook has been installed, to obtain the identification.

Identification of the yeast isolates using API 20 C AUX (Schuffenecker *et al*., 1993):

Preparation of the strip:

- The incubation boxes (trays and lids) were prepared and about 5 ml of distilled water were distributed into the honey-combed wells of the tray to create a humid atmosphere.
- The isolate reference was recorded on the elongated flap of the tray.
- The strips were removed from its individual packaging and placed it in the incubation tray.

Preparation of the inoculums:

• Ampules of suspension medium were opened.

• Using a pipette, a portion of a yeast colonies were picked up by suction and a suspensions were made with a turbidity equal to McFarland standard no. 2.

• A single drops from each yeast isolates were dispensed onto the corn meal agar tween.

• Ampules of C Medium (Ammonium sulphate 5 g/L, Monopotassium phosphate 0.31 g/L, Dipotassium phosphate 0.45 g/L, Disodium phosphate 0.92 g/L, Sodium chloride 0.1 g/L, Calcium chloride 0.05 g/L, Magnesium sulphate 0.2 g/L, Histidine 0.005 g/L, Tryptophane 0.02 g/L, Methionine 0.02 g/L, Agar-agar 0.5 g/L) were opened and 100 μl (2-4 drops) of the previous suspensions were transferred into it. Gentle homogenization with pipettes was made and introduction of air bubbles were avoided.

Inoculation of the strip:
• The cupules of the strips were filled with the suspension obtained in the ampule of C Medium.
• The lids were placed on the tray and incubate at 30°C for 48-72 hours.

Reading the strip:
After 48 or 72 hours of incubation if the tests, in particular glucose, were not clearcut after 48 hours, a check for growth (the 0 cupule serves as a negative control). A cupule more turbid than the control indicates a positive reaction to be recorded on the result sheet.

Identification:
Identification can be obtained:
    Using the analytical profile index: the pattern of the reactions obtained must be coded into a numerical profile. On the result sheet, the tests are separated into groups of 3 and a number 1, 2 or 4 is indicated for each. By adding the numbers corresponding to positive reactions within each group, a seven-digit number was obtained which constitutes the numerical profile. The presence of hyphae (mycelium) or pseudohyphae (pseudomycelium) constitutes the 21st test and has a value of 4 when positive. Using the identification software by manually entering the 7-digit numerical profile via the keyboard.

RESULTS
Results of environmental monitoring samples taken from different locations in pharmaceutical facility:
Water sampling: City water samples were taken from water station while purified water samples were taken from different use points in production facility and quality control laboratory (chemistry, microbiology and washing rooms). Results were shown in Table (1). The total numbers of city water samples were 216 samples among which 62 samples (28.7%) showed positive growth while 154 samples (71.3%) showed no growth. The total numbers of purified water samples were 984 samples among which 130 samples (13.2%) showed positive growth while 854 samples (86.8%) showed no growth. City water showed greater number of positive samples in comparison with purified water.

Table 1: Water analysis of water plant samples taken from different use points.

Type of samples	Specified limit	Sampling frequency	Total no. of samples	Positive samples		Negative samples	
				No.	%	No.	%
City water	< 50.000 CFU/100 ml	once/month	216	62	28.7	154	71.3
Purified water	< 10.000 CFU/100 ml	once/week	984	130	13.2	854	86.8

Surface monitoring: All samples were taken using contact plates from production area and microbiology laboratory in facility except sterility test isolator samples were taken by swab technique. Results were shown in Table (2). All samples of classes A1 (3456 samples), A2 (1008 samples), B1 (144 samples) and B2 (1728 samples) showed no growth. Out of 2496 samples of class C 1700 samples (68.1%) showed positive growth and 796 samples (31.9%) showed no growth. All samples of class D1 and D2 showed positive growth.

Table 2: Surface monitoring in pharmaceutical facility using either swab technique or contact plate method.

Classes	Specified limit	Sampling frequency	Total no. of samples	Positive samples		Negative samples	
				No.	%	No.	%
Class A1	< 1 CFU/plate (wall and benches)	Once/shift	3456	0	0.0	3456	100.0
Class A2	< 1 CFU/swab	Once/shift	1008	0	0.0	1008	100.0
Class B1	< 5 CFU/plate (wall) < 10 CFU/plate (bench)	Once/month during activity	144	0	0.0	144	100.0
Class B2	< 5 CFU/plate (wall) < 10 CFU/plate (bench)	Once/shift	1728	0	0.0	1728	100.0
Class C	<25 CFU/plate (wall) <50 CFU/plate (floor)	Twice/week	2496	1700	68.1	796	31.9
Class D1	<50 CFU/plate (wall and floor)	Once/month during activity	408	408	100.0	0	0.0
Class D2	<50 CFU/plate (wall and floor)	Once/3 months during activity	232	232	100.0	0	0.0

Class A1: Microbiology laboratory laminar air flow units.
Class A2: Sterility test isolator.
Class B1: Sampling and dispensing LAF walls and benches.
Class B2: Safety cabinet.
Class C : Microbiology laboratory.
Class D1: Ointment preparation and primary packaging, sampling room, dispensing room, oral liquid dosage form preparation and filling and suppositories preparation room.
Class D2: Mixer and granulator room, drying oven room, tablet compression rooms, coating rooms I and II, kneader room, capsule filling rooms, blistering lines and primary tablet and capsule packaging rooms.

Viable passive air sampling: Samples were taken from microbiology laboratory and production area in facility using settle plate technique. Results were shown in table (3). All samples of class A which was composed of 1152 samples showed no growth while all samples of class C (72 samples), class D1 (408 samples) and class D2 (160 samples) showed positive growth.

Table 3: Viable passive air monitoring using settle plate technique.

Classes	Specified limit	Sampling frequency	Total no. of samples	Positive samples		Negative samples	
				No.	%	No.	%
Class A	< 1 CFU/2 hour	Once/shift	1152	0	0.0	1152	100.0
Class C	< 50 CFU/4 hours	Once/month	72	72	100.0	0	0.0
Class D1	< 100 CFU/4 hours	Once/month	408	408	100.0	0	0.0
Class D2	< 100 CFU/4 hours	Once/3 months	160	160	100.0	0	0.0

Class A: Microbiology laboratory laminar air flow units.
Class C: Microbiology laboratory.
Class D: Ointment preparation and primary packaging, sampling room, dispensing room, oral liquid dosage form preparation and filling and suppositories preparation room.
Class D2: Mixer and granulator room, drying oven room, tablet compression rooms, coating rooms I and II, kneader room, capsule filling rooms, blistering lines and primary tablet and capsule packaging rooms.

Viable active air sampling: Samples were taken from microbiology laboratory and production area in facility using air sampling apparatus (reuter centrifugal sampler) for quantitative determination of microorganisms**.** Results were shown in Table (4). All samples of class A1 (48 samples) and class A2 (144 samples) showed negative growth in contrast to all samples of class C (576 samples), class D1 (192 samples), class D2 (12 samples) and class E (80 samples) which showed positive growth. Thirty five samples (48.6%) out of 72 samples of class B show positive growth while 37 samples (51.4%) showed no growth.

Table 4: Viable active air monitoring using reuter centrifugal sampler (RCS) technique.

Classes	Specified limit	Sampling frequency	Total no. of samples	Positive samples		Negative samples	
				No.	%	No.	%
Class A1	< 1 CFU/m^3	Once/month during activity	48	0	0.0	48	100.0
Class A2	< 1 CFU/m^3	Once/shift	144	0	0.0	144	100.0
Class B	< 10 CFU/m^3	Once/month during activity	72	35	48.6	37	51.4
Class C	< 100 CFU/m^3	Twice/week during activity	576	576	100.0	0	0.0
Class D1	< 200 CFU/ m^3	Once/month during activity	192	192	100.0	0	0.0
Class D2	< 200 CFU/ m^3	Once/3 months during activity	12	12	100.0	0	0.0
Class E	<500 CFU/m^3	Once/3 months	80	80	100.0	0	0.0

Class A1: Microbiology laboratory laminar air flow units.
Class A2: Sterility test isolator.
Class B: Safety cabinet, sampling and dispensing LAF units.
 Class C: Microbiology laboratory.
Class D1: Ointment preparation and primary packaging, sampling room, dispensing room, oral liquid dosage   form preparation and filling and suppositories preparation room.
Class D2: Mixer and granulator room, drying oven room, tablet compression rooms, coating rooms I and II, kneader room, capsule filling rooms, blistering lines and primary tablet and capsule packaging rooms.
Class E : Secondary capsules and tablets packaging, liquid formulation air lock, ointment primary packaging and suppositories air lock, blister lines corridor, ointment preparation air lock, powder preparation air lock, production entrance corridor, main material corridor and washing room air lock.

Distribution of identified microorganisms from the positive samples of environmental monitoring specimens:

Twenty one bacterial and fungal species were isolated from different locations in the pharmaceutical facility.

Group of genus *Staphylococci* contributes to 38.4% of total positive samples while genus *Micrococci* contributes to 22.4% and *Gemella morbillorum* represents 1.1%. Genus *Bacilli* represents 35.0%, *Candida albicans* 1.3%, *Klebsiella pneumonia* 1.0% and *Stenotrophomonas maltophilia* 0.01%. Results were shown in Table (5).

The abundance of the environmental isolates was in the following descending order: genus *Staphylococci,* Genus *Bacilli,* genus *Micrococci, Candida albicans, Klebsiella pneumonia* and *Stenotrophomonas maltophilia.*

Table 5: Distribution of microorganisms between different samples.

Microrganisms	Sample type								Percentage of organism from total positive samples
	Water		Contact plate		Settle		RCS*		
	No. of positive samples	Percentage from total positive samples	No. of positive samples	Percentage from total positive samples	No. of positive samples	Percentage from total positive samples	No. of positive samples	Percentage from total positive samples	
Staphylococcus aureus	0	0.0%	64	2.752%	0	0.0%	0	0.0%	1.226%
Staphylococcus capitis	0	0.0%	164	7.009%	328	18.303%	50	5.587%	10.38%
Staphylococcus cohnii	0	0.0%	88	3.752%	0	0.0%	40	4.469%	2.452%
Staphylococcus epidermidis	1	0.52%	205	8.761%	0	0.0%	0	0.0%	3.947%
Staphylococcus haemolyticus	1	0.52%	163	7.008%	530	29.575%	53	5.922%	14.313%
Staphylococcus saprophyticus	0	0.0%	82	3.504%	317	17.689%	162	18.101%	10.749%
Staphylococcus schleiferi	0	0.0%	90	3.846%	8	0.446%	0	0.0%	1.877%
Staphylococcus simulans	0	0.0%	65	2.777%	0	0.0%	0	0.0%	1.245%
Staphylococcus vitulinus	0	0.0%	76	3.252%	0	0.0%	0	0.0%	1.456%
Staphylococcus warneri	0	0.0%	0	0.0%	0	0.0%	49	5.475%	0.938%
Micrococcus luteus	0	0.0%	208	8.888%	356	19.866%	303	33.855%	16.612%
Micrococcus lylae	0	0.0%	86	3.675%	191	10.658%	179	20%	8.737%
Candida albicans	0	0.0%	58	2.478%	0	0.0%	10	1.117%	1.302%
Klebsiella pneumonia	0	0.0%	64	2.752%	0	0.0%	0	0.0%	1.226%
Stenotrophomona maltophilia	12	6.25%	0	0.0%	0	0.0%	0	0.0%	0.229%
Gemella morbillorum	0	0.0%	0	0.0%	0	0.0%	43	4.804%	0.823%
Bacillus cereus	158	82.291%	94	4.017%	18	1.004%	6	0.007%	5.288%
Bacillus circulans	0	0.0%	96	4.12%	0	0.0%	0	0.0%	1.839%
Bacillus licheniformis	14	7.291%	123	5.256%	0	0.0%	0	0.0%	2.625%
Bacillus pumilus	6	3.125%	327	14.017%	0	0.0%	0	0.0%	6.380%
Bacillus subtilis	0	0.0%	287	12.265%	44	2.455%	0	0.0%	6.342%

*: Reuter centrifugal sampler.

DISCUSSION

City water showed higher proportion of positive samples if compared with purified water although it contains chlorine yet purified water disinfection by ozone was more efficient in bioburden control of water and this factor may be one of the major reasons

account for lower proportion of negative samples for purified water if compared to city water. The results were in agreement with that of Florjanič and Kristl (2006) who found that the purified water that entered into the distribution system has low heterotrophic plate counts. Purified water storage and distribution systems at ambient temperature are highly susceptible to microbial contamination and formation of biofilm but the specified ozone level prevented microbial growth and formation of biofilm in the distribution system that might otherwise endanger the water quality by sporadic release of microbes.

In the environmental monitoring results *Staphylococcus species* were the predominant microorganism isolated (38.4% from total positive samples) followed by *Bacillus species* (25.8% from total positive samples) then *Micrococcus* species (22.4%). Although the results showed that *Staphylococcus* and *Micrococcus species* were very sensitive microorganism compared to others in this study yet they were very common in the environmental samples especially reuter centrifugal sampler (RCS), contact plate samples by replicate organism detection and counting (RODAC) method and settle plates. *Staphylococcus* and *Micrococcus species* were common isolates of different air samples were effect of disinfectants were nil and most of these microorganisms are human flora which comes from operators in these area. Mancinelli and Shulls (1978) found that the most frequently isolated organisms were *Micrococcus* (41%), *Staphylococcus* (11%), and *Aerococcus* (8%) in urban air and *Micrococcus* was frequently found in air sampling because it produces pigments that protect it against the radiation of sunshine. During the collection of airborne bacteria in a museum in England some bacterial strains were isolated which due to their fatty acid profiles were clearly identified as members of the genus *Staphylococcus* (Wieser and Busse, 2000).

One species belonging to *Staphylococcus* group (*Staphylococcus haemolyticus*) was found in water. This result was in agreement with that obtained from the study of Lateef (2004).

On the other hand *Bacillus species* were widely distributed among different types of samples but their occurrence in air samples was minimum. Being spore forming microorganisms, *Bacillus species* were able to survive different unfavorable environmental conditions and this may explain their distribution among all samples and this can be explained by endospore formation, a property universally found in the *Bacillus* group, is thought to be a strategy for survival in the soil environment, where in these bacteria predominate. Aerial distribution of the dormant spores probably explains the occurrence of aerobic spore formers in most habitats examined (Todar, 2009).

Candida albicans were found in air and surfaces. *Klebsiella pneumonia* was found in surface samples. *Gemella morbillorum* was found in air samples only. Kramer *et al.* (2006) found that *Candida albicans* as the most important nosocomial fungal pathogen can survive up to 4 months on surfaces and they reported also that many gram-negative species, such as *Acinetobacter species*, *Escherichia coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Shigella species*, can also survive for months.

Stenotrophomonas maltophilia was found in water samples. Penna *et al.* (2002) found that the most commonly isolated bacteria were *Stenotrophomonas maltophilia* in samples taken from water supplies. This result was in agreement with that of Lateef (2004) who found *Pseudomonas species* in the effluent of a pharmaceutical company.

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الرصد البيئي الميكروبيولوجي في المنشأة الصيدلانية

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عينـات الرصد البيئي المنتظمـة للجـودة الميكروبيولوجيـة من مواقـع مختلفـة في المنشـأة الصيدلانية هـى عنصـر معيـارى مهـم جـدا فـي السـيطرة علـى المنطقـة والبيئـة النظيفـة التـي لهـا تـأثير مباشـر علـى نوعيـة الميكروبيولوجية للمنتجات الصيدلانية النهائية. واستخدمت أساليب مختلفة لجمع عينـات من مواقـع مختلفـة. وتم تجهيز هذه العينات وتلاها عزل وتحديد هوية الميكروبات. وقد تم تحليل البيانات التي تم الحصـول عليها للعثـور على توزيع الميكروبات في بيئة المنشأة الصيدلانية.وقد وجد أن معظم الكائنات الحية الدقيقة تنتمي إلى الجراثيم العنقودية، الميكروكوكس والأنواع العصوية.