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Biodiversity of microbiota in cephalosporin-manufacturing environments at T3A factory, Assiut, Egypt

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Abstract: The diversity of airborne bacteria and fungi inside and outside the T3A cephalosporin production factory was studied using different microbiological techniques. Results of indoor aeromicrobiota showed complete absence of microbial units in the environment of aseptic filling machine (class A). The counts of microbes in the aseptic filling room (class B) as well as in gowning room (class C) and in the rooms designed for preparation and filling of non-sterile cephalosporin capsules and dry mix (class D) were within the limits recommended by WHO guidelines. The count of outdoor airborne microbes using the air sampler technique was about 7-folds that of indoor air (6240 versus 854 colonies). The number of species isolated from the outdoor air was higher than that of indoor air (17 versus 10 species). Microbiological analysis of water showed much lower counts of microbes in purified water than in city water samples (80 versus 761 colonies). Microbes were also isolated from 60% of non-sterile finished products but they were completely absent in all samples of sterile finished products. The majority of microbial species recovered from the non-sterile finished products were found in both indoor and outdoor air of the cephalosporin-manufacturing factory but only four of these microbes were obtained from the purified water samples. Common microbial contaminants, namely species of *Bacillus, Staphylococcus, Aspergillus, Penicillium,* and *Cladosporium* were encountered.

Key words: T3A, cephalosporin factory, air and water bacteria and fungi.

Introduction

T3A is an Egyptian company, which began marketing of pharmaceutical products in 1997. The manufacturing facility in Assiut was opened in the year 2000. The manufacturing complex comprises two separate factories: a dedicated cephalosporins (sterile and non-sterile) plant, and a multipurpose facility that produces solids, semi-solids and liquids. T3A is operating in the production, marketing, distribution and selling of pharmaceutical products with primary focus on ethical generic drugs. It has 539 product forms belonging to 14 therapeutic classes including analgesics, anti-infective (oral and injectable), cardiovascular, psychotropic, alimentary, anesthetics, respiratory, dermatological, etc.

Understanding the diversity of microbiota is important for the pharmaceutical microbiologist (Jimenez 2004). Knowing the species of microorganisms can provide invaluable information to its origin. For example, many Gram-positive cocci are part of human skin biota. Gram-positive rods can be transferred into clean areas via equipment and on footwear, and Gram-negative rods are often linked to water sources. Such characterizations are important for data determining if contamination could have arisen from personnel intervention, or understanding if a sterility test failure is due to product contamination or is a false positive (Sandle 2011).There are many sources of contamination within clean rooms. The atmosphere contains dusts, microorganisms, condensates, and gases. People in clean environments are the greatest contributors to contamination, emitting body vapours, dead skin, microorganisms, skin oils, and so on. A level of protection is afforded by personnel wearing masks, gloves and clean room suits and through protective airflow barriers from unidirectional airflow cabinets (Cundell 2004). Of the resident skin biota, *Staphylococcus aureus* is perhaps the most undesirable isolate to be found in pharmaceutical products. Other bacteria present on skin are the micrococci, *Sarcina* spp. and diphtheroids but occasionally Gram-negative rods such as *Acinetobacter* spp. and *Alcaligenes* spp. achieve resident status. In the fatty and waxy sections of the skin, lipophilic yeasts are often present. Various dermatophyte fungi such as species of *Epidermophyton, Microsporon* and *Trichophyton* may also be present (Somerville-Millar and Noble 1974). The second greatest contamination source is from air, unfiltered and uncontrolled air contains a high number of particles within clean rooms. Physical controls include the use of unidirectional airflow units, and the isolation of the operator through the use of barriers, which act to separate the filling area from the operator (Whyte 1986). The third most important source of contamination in clean rooms is water. The most likely types of microorganisms traceable to water are Enterobacteriaceae, including *Escherichia coli* and *Salmonella* spp. as the most readily recognizable types in domestically contaminated waters, and *Pseudomonas* spp., which are always found in natural, potable and pharmaceutical waters (Halls 2004). With the fourth source of contamination, equipment poses a risk in relation to the way that it is introduced into the clean room; this can range from trolley wheels, which are not correctly sanitized, to cardboard used to hold

reagents, to non-sterile items in critical areas (Prout 2009).

The present work was designed to study the incidence of bacteria and fungi in the cephalosporin-manufacturing environments at T3A pharmaceutical company in Assiut Governorate, Egypt.

Materials and Methods

Sampling location

This work was carried out at T3A pharmaceutical company at the Industrial Zone, Arab El-Awamer, Abnoub, about 20 km north Assiut City Egypt. Samples were taken from different sites covering the air inside clean rooms, open air outside the factory as well as city and purified water and the finished products.

1- **Indoor environment:** Samples were taken from the 4 representative different classes and locations inside the cephalosporin factory for one year as shown in Table (1).
2- **Outdoor environment:** A total of 96 samples were taken. Two sites were selected for isolation, site 1 about 12 meters towards the east of the building (48 samples) and site 2 about 12 meters westward (48 samples).
3- **Water samples:** A total of 120 samples were analyzed which included 24 city water samples (2 samples / month) from water station before any treatment process, and 96 purified water samples (8 samples / month) from use point in the production area (vials washing machine for aseptic filling machine).
4- **Finished products:** Fifteen samples from each of sterile and non-sterile cephalosporin finished products were selected as shown in Table (2).

Table 1: Description of different grades of clean rooms, location of sampling and numbers of indoor air samples taken by different methods.

Class	Description and sampling location	Sampling method and number of samples				
		Air sampler	Settle plates	Contact plates	Swabs	Total
A	Highest grade of cleanliness (aseptic filling machine)	240	240	--	96	576
B	High grade of cleanliness (aseptic filling room)	96	96	96	96	384
C	Intermediate measure of cleanliness (gowning room for aseptic filling room)	48	48	48	48	192
D	Low measure of cleanliness (preparation and filling of capsule and dry-mix)	48	48	48	48	192

Table 2: List of sterile (using membrane filtration method) and non-sterile finished products (using pour plate method) tested for sterility.

No	Sterile finished products			Non-sterile finished products		
	Product name	Concentration / vial	Batch No.	Product name	Concentration	Batch No.
1	Cefotax T3A	1.0 g	120002	Cepodem drymix	40 mg / 5 ml	120006
2	Cefazone	1.0 g	120020	Cepodem drymix	40 mg / 5 ml	120008
3	Wincef	1.0 g	120096	Cepodem drymix	40 mg / 5 ml	120447
4	Oframax	1.0 g	120042	Cepodem drymix	40 mg / 5 ml	120118
5	Cepimax	1.0 g	120273	Cepodem drymix	40 mg / 5 ml	120446
6	Cefotax T3A	1.0 g	120242	Medicefalxin drymix	250 mg / 5 ml	M21205Z
7	Cefotrix T3A	1.0 g	120278	Medicefalxin drymix	250 mg / 5 ml	M21206Z
8	Peractam	1.5 g	120444	Medicefalxin drymix	250 mg / 5 ml	M21207Z
9	Cefepime	1.0 g	120478	Speczil drymix	250 mg / 5 ml	1306116
10	Taximodel	2.0 g	120099	Speczil drymix	250 mg / 5 ml	1306117
11	Cepimax	1.0 g	120051	Speczil drymix	250 mg / 5 ml	1306118
12	Cefotax T3A	1.0 g	120419	Medicefalxin Capsule	500 mg /capsule	M21201LM
13	Wincef	0.5 g	120640	Medicefalxin Capsule	500 mg /capsule	M31201LM
14	Cetazime	1.0 g	120499	Medicefalxin drymix	250 mg / 5 ml	M21202Z
15	Cefepime	1.0 g	120509	Medicefalxin drymix	250 mg / 5 ml	M21203Z

Microbiological analysis

1- **Airborne microbiota:** Four different techniques were used: **a)** Active air sampling using volumetric air samplers (Ljungqvist and Reinmüller 2000), **b)** Passive air sampling (Andon 2006) using settle plates and Tryptone Soy Agar medium (TSA) whose composition is as follows (g/l): Pancreatic digest of casein, 15; Agar, 15; Papaic digest of soybean meal, 5, and NaCl 5.0, pH 7.3 ± 0.2 (Oxoid), mixed with penicillinase enzyme which inactivates any residue of cephalosporin antibiotics / liter medium, **c)** Swabs for irregular surface sampling (Favero *et al.* 1968), and **d)** Contact plates using Tryptone Soy Agar medium (TSA) mixed with penicillinase for flat surfaces (Cundell 2004).
2- **Water samples:** The samples were microbiologically analyzed using membrane filtration method and TSA medium as described by Rompré *et al.* (2002); all plates were incubated at 32ºC for 5 days. The volume of sample should not be less than 100 ml.
3- **Sterile finished products:** The samples were analyzed using membrane filtration method according to Kastango and Bradshaw (2004). Twenty vials from each of 15 batches were used for checking their sterility. Two types of media were employed, first Tryptic soy broth (TSB) for aerobic microorganisms whose composition is as follows (g/l): Pancreatic digest of casein, 17; K_2HPO_4, 2.5; Papaic digest of soybean, 3; and NaCl 5.0, pH7.3 ± 0.2 (Oxoid), and second Fluid Thioglycolate Medium (FTM) for anaerobic microorganisms whose composition is as follows (g/l): Pancreatic digest of casein, 15; Glucose, 5.5; Yeast extract, 5; NaCl, 2.5; L-Cystine, 0.5; Sodium thioglycolate, 0.5, and Resazurin, 0.001, pH 7.3 ± 0.2 (Oxoid). TSB were incubated at 22ºC whereas FTM tubes at 32ºC for 14 days.
4- **Non-sterile finished products**
Products were microbiologically analyzed using the pour plate method (Gad *et al.* 2011). Ten g of each sample were dissolved in 100 ml sterile 0.1% peptone water of pH 7.2, then 1 ml of the sample mixture was inoculated into TSA for bacteria, and Sabouraud's dextrose agar (SDA) for fungi; the latter's composition is as follows (g/l): Mycological peptone, 10; Agar, 20 and Glucose, 40; pH 7.3 ± 0.2.

Identification of microbial isolates

Bacterial isolates

A- Phenotypic identification
Bacterial isolates were purified, stained with Gram stain and examined under microscope (Cowan *et al.* 2003).

B- Genotypic identification
Some bacterial isolates were selected and sent to SolGent Company (Daejeon, South Korea) for RNA gene sequencing. The sequence of the 16s rRNA gene has been widely used as a phylogenetic marker to study genetic relationships between different strains of bacteria. Bacterial DNA was extracted and isolated using SolGent purification beads. Prior to sequencing, the ribosomal rRNA gene was amplified using the polymerase chain reaction (PCR) technique in which two universal bacterial primers 27F (forward) and 1492R (reverse) were incorporated into the reaction mixture (White *et al.* 1990). Primers used for gene amplification have the following composition: 27 F (5'- AGA GTT TGA TCM TGG CTC AG - 3'), and 1492R (5'- TAC GGY TAC CTT GTT ACG ACT T- 3'). Sequences were further analyzed using BLAST from the national Center of biotechnology information website (NCBI), and phylogenetic analysis of sequences was done with the help of MegAligen (DNA star) software version 5.05

Fungal isolates: Fungi were identified on the basis of their macro- and microscopic features with the aid of the following references: Raper and Fennell (1965), Pitt (1979), Moubasher (1993) and Domsch *et al.* (2007).

Results and Discussion

The present study showed marked variations among the counts of total and individual microbial species depending on the level of environmental cleanliness at the sampling sites, the type of samples and the seasons during which these samples were collected.

Indoor aeromicrobiota

The total counts of indoor aeromicrobiota using the two methods of sampling exhibited regular decrease with the increase in the degree of cleanliness inside the different classes of clean rooms. In this study two bacterial genera were recovered from indoor environment, namely *Staphylococcus* and *Bacillus* which are gram positive (Table 3). These results are similar to those obtained by Wu and Liu (2007) who reported that the most common microorganisms in clean rooms are Gram-positive bacteria. The possible reason that Gram-positive bacteria predominate, because the skin is generally dry environment, and any fluids present on the surface generally have a high osmotic pressure, thus Gram-positive bacteria (especially the staphylococci and micrococci) may be better adapted for such environments. Favero *et al.* (1966), Hyde (1998) and Costello *et al.* (2009) reported that the common genera of bacteria isolated from clean room included *Micrococcus,*

Staphylococcus, Corynebacterium and *Bacillus*. Halls (2004) recorded that *Bacillus* species present in clean rooms might be transferred into them via personnel, dust, and material transfer. In addition, there are, in fewer numbers, certain fungi associated with clean rooms.

Where cephalosporin products were filled and packaged the highest microbial number (495 CFU) was obtained from class D and the lowest (177 CFU) the class B by using air sampler method. It is worthy to mention that class A was consistently free of microbes. Bacterial counts in class B, C and D were regularly dominating those of fungi (93.7% versus 6.3% of total microbes).

From class B and by using volumetric air sampler technique, four bacterial species were identified genotypically (Fig. 1), *Staphylococcus arlettae* was the most common appearing in 61.5 % of air samples and matching 47.4 % of the total catches. *Bacillus subtilis*, *B. simplex* and *B. pumilus* occurred in 43.8%, 22.9% and 11.5% of air samples accounting for 29.9%, 12.4% and 6.2% of total microbial catch respectively (Table 3). These bacterial species were deposited in the culture collection of Assiut University Mycological Centre and assigned AUMC Nos. b-152 (*B. subtilis*), b-153 (*S. arlettae*), b-154 (*B. simplex*) and b-157 (*B. pumilis*). In the present study, *Rhodotorula mucilaginosa* was the only representative yeast fungus in class B contaminating 7.3% of the samples matching 3.9 % of the microbial count (Table 3). Using volumetric air sampler technique the average microbial counts per sample ranged from 0.07 to 0.88 CFU/m^3 as shown in Table (4), that lie within limits recommended by WHO guidelines (10 CFU/m^3 for class B). Seasonal monitoring of indoor aeromicrobes in class B showed regular increase in their counts from 18 CFU in the winter season of 2011 to 40 CFU and 65 CFU in the spring and summer of 2012 respectively, and the count slightly dropped to 54 CFU in the autumn of 2012 as shown in Table (5).

Surveying the indoor aeromicrobiota in class C environment showed that all samples taken by air sampler method were positive for microbes (Table 5). The average microbial counts in the individual samples using volumetric air sampler technique ranged from 0.02 to 1.27 CFU/m^3 as shown in Table (4), and these counts are within the limits recommended by WHO guidelines (100 CFU/m^3 for class C). Eight species of microorganisms were identified including four bacteria (*S. arlettae, B. subtilis*, *B. simplex* and *B. pumilus*) and four fungi *(A. flavus*, *P. oxalicum, C. cladosporioides* and *R. mucilaginosa*) as shown in Table (4). Summer months were the richest in bacterial colonies (56 CFU), whereas winter months were the poorest (26 CFU). On the other hand fungi prevailed in winter (6 CFU) as shown in Table (5).

In class D, all samples taken by volumetric air sampler were heavily contaminated with microbes, yielding a total of 495 CFU during the whole period of study (Table 5). Four bacterial species were isolated with the most dominant being *S. arlettae, B. subtilis*, *B. simplex* and *B. pumilus* (100 % - 66.7 % of samples) matching 25.4 % - 15.3 % of total microbial count as shown in Table (3). The Average microbial counts in the individual samples ranged from 0.08 to 2.75 CFU/m^3 as shown in Table (4), and these counts are within the limits recommended by WHO guidelines (200 CFU/m^3 for class D). Unidentified bacterial species are also detected in 39.6 % of air samples accounting for 8.7 % of total counts of microbes. The prevalence and count of fungal species were generally low ranging from 8.3% to 22.9 % of samples representing 0.9% to 2.4% of total microbial count. Six fungal species belonging to four genera were identified as shown in Table (3). *A. flavus*, *P. oxalicum* and *C. cladosporioides* were also found in class C clean room. However, *A. niger*, *P. citrinum* and *Acremonium curvulum* appeared in class D but were absent in class C. In class D the seasonal distribution of both bacteria and fungi was almost similar to that in class C where the highest count of bacteria (163 CFU) was estimated in summer and the lowest (73 CFU) in winter. In case of fungi, winter season was the richest (20 CFU) and summer was the poorest (2 CFU).

Aspergillus species were the most frequently isolated fungal isolates in the present study. Utescher *et al.* (2007) reported that species of *Cladosporium, Aspergillus, Scopulariopsis, Fusarium, Alternaria,* and *Mycelia Sterilia* were found in low frequencies and predominantly in class D environments. In a similar study, Kim *et al.* (2010) isolated *Aspergillus, Penicillium, Rhizopus* and *Alternaria* species. Wilson *et al.* (2007) conducted studies on mould contamination and clean room air handling, and identified common mould species in a total of 566 tape lifts and 570 swab samples collected from the various locations of air handling units. Sandle *et al.* (2012) reported that the species of *Aspergillus, Cladosporium, Penicillium, Alternaria, Curvularia* and *Fusarium* were the most predominant fungal isolates from pharmaceutical processing environments. They also stated that there are very few available articles regarding mould contamination incidences or identification from pharmaceutical clean room environments, therefore it is difficult to compare aerobiocontamination data in different regions.

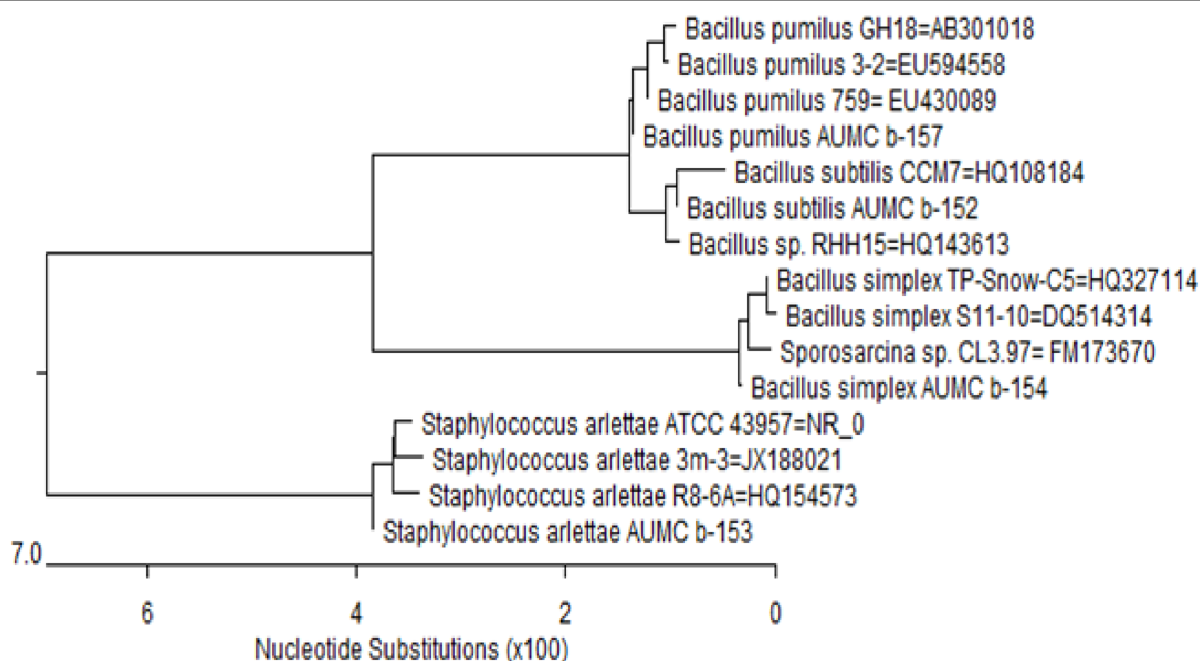

Fig 1: Phylogenetic tree for some bacterial species isolated from pharmaceutical clean room.

Microbiota of walls

The microbiota of walls was surveyed using the swab and contact plate methods (Table 6). The results revealed that all samples taken from class A and B by the swab method were free from any microbial colonies. In class C and D the average total counts of bacteria were 1.55 CFU and 3.02 /swab. Fungi were recovered in Class D only with average total count of 0.10 / swab

When using contact plate method, the average total counts of bacteria increased regularly from class B, C to D giving values of 0.02, 1.88 and 4.36 / plate respectively. Fungi were absent in class B, and counting 0.02 and 0.25/ plate in class C and D respectively. All the above records by the two methods are within the limits recommended by WHO guidelines.

Four species of bacteria were encountered by the two methods, of which *S. arlettae* followed by *B. subtilis* were the most dominant in class C and D. Five species of fungi were recovered by the two methods, *A. flavus* was the dominant species in class D, and the only organism recovered in class C when the contact plate method was used, but was completely absent in class D and was replaced by *A. niger* and *P. oxalicum* in case of swab method.

Outdoor airborne microbiota

Surveying the aeromicrobiota outside the T3A factory using volumetric air sampler technique revealed that all samples were positive yielding 3529 colonies in site 1 and 2699 colonies in site 2 (Table 7). The average total count of bacterial colonies outnumbered those of fungi (115.3 colonies / m^3 versus 31.76 colonies / m^3). In the individual samples, the bacterial counts fluctuated between 15.63 and 55.50 colonies / m^3 and those of fungi ranged from 0.17 to 6.75 colonies / m^3. The outdoor airborne bacteria comprised different species of *Staphylococcus, Bacillus and Pseudomonas*. Eight fungal genera comprising 14 species and 1 variety were also identified (Table 7). *Aspergillus* and *Penicillium* were the most dominant genera recorded from 48 and 42 air samples matching 313 and 101 colonies (Table 3). Five species of *Aspergillus* were recovered, of which *A. flavus* and *A. niger* were the most prevalent (48 samples accounting for 313 – 224 CFU). The remaining species of *Aspergillus* (*A. ochraceus*, *A. parasiticus* and *A. sydowi*) were less frequently encountered. Out of 4 *Penicillium* species, *P.citrinum* was the most common (35 samples matching 101 CFU) (Table 3). The outdoor aeromicrobiota showed seasonal variations with the highest total count being obtained in summer in sites 1 and 2 (1061 and 851 colonies respectively) whereas the lowest in autumn (728 and 554 colonies in sites 1 and 2 respectively) as shown in Table (5). Variations in bacterial counts followed the same trend but in case of fungi, winter season was the richest (236 colonies) and summer was the poorest (29 colonies) as shown in Table (5). In Benin City, Nigeria, Ekhaise and Ogboghodo (2011) studied the microbiological indoor and outdoor air quality of two major hospitals and isolated 6 bacterial and 10 fungal species from the different units studied. The bacterial species included *Staphylococcus aureus, S. epidermidis, Bacillus cereus, Bacillus sp., Serratia marcescens and Micrococcus* sp. while the fungal isolates included *Aspergillus niger, A. flavus, Botryodiplodia acerina, Rhizopus stolonifer, Mucor* sp., *Penicillium* sp., *Candida* sp. and *Trichoderma viride*. In Finland, Rintala *et al.* (2008) reported a clear distinction of the

effect of seasons on airborne microbiota, where the total concentration of cultureable microorganisms in indoor air was highest in summer and fall than in winter. The dry atmosphere and high temperature in the months of dry season influence the movement of airborne microbial particles and thus support evidences for the concentration of fungal species within the period.

Microbiota of water samples

The microbiological analysis of city water (untreated) revealed that all samples were contaminated with different species of bacteria and fungi (Table 8). The dominant bacteria belong to the genera *Bacillus* and *Staphylococcus*, which appeared in 100% of samples comprising 29.6 % and 50.6 % of total microorganisms. *A. sydowii* was the dominant fungal species, recovered from 58% of samples contributing 8.7 % of total microbial count. Other fungal species, namely *A. flavus*, *A. ochraceus, P. citrinum*, *P. brevicopactum* and *Acremonium strictum* were less frequent. The microbial counts ranged from 18 – 46.5 colonies / 100 ml in the individual samples, much lower than those recommended by WHO (50000 CFU/100 ml for city water).

In purified water 63 out of 96 samples (65 .6 %) were positive. However, the count of microbes per 100 ml was very low ranging from 0.13 – 1.75 CFUs / 100 ml in the individual samples, extremely much lower than those recommended by WHO (10000 CFU/100 ml for city water). The microbial species in purified water were the same as in city water except for the absence of *A. ochraceus* in purified water (Table 9). The process of water purification involved reverse osmosis technique and UV treatment, which are more efficient in bioburden control of water and could be considered as the major reason for lower proportion of positive samples of purified water compared with untreated city water. The results are in agreement with those obtained by Florjanic and Kristl (2006) in Slovenia, that the purified water that entered into the distribution system had low microbial counts.

Microbiota of finished products

All samples of sterile finished products were completely free of microbes with no evidence of bacterial or fungal growth after cultivation on TSB and FTM media. On the other hand, 9 out of 15 non-sterile finished products were contaminated with some species of bacteria and fungi and the total microbial load per sample ranged from 100-220 CFU/g (Table 10). Counts of bacteria were mostly higher than those of fungi (30-150 versus 40-90 per gram respectively). Species belonging to *Staphylococcus* and *Bacillus* were respectively isolated from 9 and 6 batches of non-sterile products. Seven fungal species identified as *Aspergillus flavus, A. niger, A. ochraceus, Penicillium brevicompactum, P. oxalicum, Cladosporium cladosporioides* and *Acremonium curvulum* were recovered from the non-sterile products. It is worthy to mention that the microbial load in positive batches analyzed during the present study is much lower than that recommended by WHO guidelines (10^3 CFU/g for bacterial count and 10^2 CFU/g for fungal count).
Previous studies on microbial contamination of non-sterile pharmaceutical preparations revealed that *Bacillus* species are ubiquitous, but undesirable because of their spoilage potential, and their presence in a product suggests poor environmental hygiene during processing or heavily contaminated or adulterated raw materials (Christenson *et al.* 1999 and Soriano *et al*. 2000). *Bacillus* and *Aspergillus* species were the most common isolated microbes from finished pharmaceutical products analyzed by Baird *et al.* (1979). Akerele and Ukoh (2002) were able to isolate *Staphylococcus aureus* and some species of Gram-positive cocci and other bacteria from 1977 pharmaceutical preparations in Nigeria. In Tanzania, Mugoyela and Mwambete (2010) found that all tested samples of capsules, syrup and tablets were microbiologically contaminated, and the isolated aerobic bacteria were mainly *Bacillus* spp, while the fungal contaminants comprised *Candida* spp. and *Aspergillus* spp. They also reported a bioburden up to 10-fold of the acceptable limits in these final products. In Egypt, Gad *et al.* (2011) evaluated the microbial contamination of five non-sterile pharmaceutical preparations which included syrup, suspension, oral drops, nasal drops and tablets from one or more of the tested preparations, where bacterial species belonging to *staphylococcus* and *Bacillus* as well as fungal species related to *Aspergillus* (*A. flavus, A. fumigatus* and *A. niger*), *Penicillium, Cladosporium* and *Alternaria* were isolated.

A plausible explanation for microbial contamination of pharmaceutical products lies in the improper handling, poor hygienic procedures during repackaging into smaller packs, and dispensing (Akerele and Ukoh 2002). Moreover Denyer *et al.* (2008) suggested that the microbiologic quality of non-sterile solid dosage forms, like tablets, is dependent on the bioburden of the raw materials, both in the active ingredients and excipients. Hence, the failure of strict observation of good manufacturing practice at any stage of production may greatly affect the microbiological quality of the products.

Table 3: Total counts (CFU) and percentages (%), number of cases of isolation (NCI) and percentages (%) of indoor and outdoor airborne bacteria and fungi isolated by air sampler method.

Species	**Class B ***		**Class C ****		**Class D ****		**Outdoor ****	
	CFU (%)	NCI (%)	CFU (%)	NCI (%)	CFU (%)	NCI (%)	CFU (%)	NCI (%)
Staphylococcus arlettae Schleifer	84 (47.4)	59 (61.5)	61 (33.5)	40 (83.3)	126 (25.4)	48 (100)	----	----
Staphylococcus spp.	-----	-----	----	----	----	----	1816 (29.2)	48 (100)
Bacillus subtilis Cohn	53 (29.9)	42 (43.8)	31(17.0)	22 (45.8)	132 (28.8)	48 (100)	---	----
B. simplex Meyer and Gottheil	22 (12.4)	22 (22.9)	39 (21.4)	30 (62.5)	90 (19.6)	37 (77.1)	---	----
B. pumilus Meyer and Gottheil	11 (6.2)	11 (11.5)	24 (13.2)	19 (39.6)	70 (15.3)	32 (66.7)	---	---
Bacillus spp.	-----	-----	----	----	----	----	2140 (34.4)	48 (100)
Pseudomonas sp.	0	0	0	0	0	0	874 (14)	39(81)
Unidentified bacteria	0	0	18 (9.9)	18 (37.5)	40 (8.7)	19 (39.6)	0	0
Total counts of bacteria	**170**		**173**		**458**		**4830**	
Acremonium curvulum W. Gams	0	0	0	0	4 (0.9)	4 (8.3)	33 (0.5)	15 (31)
Aspergillus flavus Link	0	0	6 (3.3)	6 (12.5)	11 (2.4)	11 (22.9)	313 (5.0)	48 (100)
A. flavus var. *columnaris* Raper and Fennell	0	0	0	0	0	0	223 (3.5)	48 (100)
A. niger van Tieghem	0	0	0	0	5 (1.1)	5 (10.4%)	224 (3.6)	48 (100)
A. ochraceus Wilhelm	0	0	0	0	0	0	88 (1.4)	48 (100)
A. parasiticus Speare	0	0	0	0	0	0	65 (1)	39 (81)
A. sydowii Bainier	0	0	0	0	0	0	32 (0.5)	35 (72)
Cladosporium cladosporioides (Fresenius) de Veries	0	0	1 (0.55)	1 (2.1)	6 (1.3)	6 (12.5)	72 (1.2)	35 (72)
Emericella nidulans (Eidam) Vuillemin	0	0	0	0	0	0	18 (0.3)	10 (20)
Epicoccum nigrum Link	0	0	0	0	0	0	4 (0.1)	1 (2)
Penicillium citrinum Thom	0	0	0	0	5 (1.1)	5 (10.4)	101 (1.6)	35 (72)
P. brevicompactum Dierckx	0	0	0	0	0	0	38 (0.6)	42 (87)
P. oxalicum Currie and Thom	0	0	2 (1.1)	2 (4.2)	6 (1.3)	6 (12.5)	97 (1.6)	33 (69)
Rhodotorula mucilaginosa (Jorgensen) F. C. Harrison	7 (3.9)	7 (7.3)	0	0	0	0	51 (0.8)	31 (64)
Setosphaeria rostrata (Drechsler) Subram. and Jain	0	0	0	0	0	0	35 (0.6)	29 (60)
Total counts of fungi	**7**		**9**		**37**		**1398**	
Total counts of bacteria and fungi	177		182		495		6228	

* Out of 96 samples ** Out of 48 samples

Table 4: Average counts of indoor airborne species of bacteria and fungi using air sampler and settle plate methods.

Species	Air sampler (count/m^3/sample)				Settle plate (count/plate/sample)			
	Class A*	Class B**	Class C***	Class D***	Class A*	Class B**	Class C***	Class D***
Staphylococcus arlettae	0	0.88	1.27	2.63	0	0.82	1.65	2.88
Bacillus subtilis	0	0.55	0.65	2.75	0	0.40	0.71	2.71
B. simplex	0	0.23	0.81	1.88	0	0.34	0.79	2.13
B. pumilus	0	0.11	0.50	1.46	0	0.21	0.79	1.73
Unidentified bacteria	0	0	0.38	0.83	0	0	0.44	0.81
Total counts of bacteria	**0**	**1.77**	**3.61**	**9.55**	**0**	**1.77**	**4.38**	**10.26**
Aspergillus flavus	0	0	0.13	0.23	0	0	0.15	0.13
A. niger	0	0	0	0.10	0	0	0	0.21
Penicillium oxalicum	0	0	0.04	0.13	0	0	0.04	0.19
P. citrinum	0	0	0	0.10	0	0	0	0.02
Cladosporium cladosporioides	0	0	0.02	0.13	0	0	0.02	0.19
Acremonium curvulum	0	0	0	0.08	0	0	0	0.06
Rhodotorula mucilaginosa	0	0.07	0	0	0	0.82	1.65	2.88
Total counts of fungi	**0**	**0.07**	**0.19**	**0.77**	**0**	**0.82**	**1.86**	**3.68**
Total counts of bacteria and fungi	0	1.84	3.79	10.32	0	2.59	6.24	13.94
Gross total count of all samples	0	177	182	495	0	185	222	530

* Out of 240 samples * *Out of 96 samples *** Out of 48 samples

Table 5: Seasonal distribution of airborne bacteria and fungi isolated from indoor and outdoor environments using air sampler method.

location		Season	Total number of samples	Number of positive samples	Number of bacterial colonies	Number of fungal colonies	Total count
Indoor	Class B	Winter	24	24	15	3	18
		Spring	24	24	39	1	40
		Summer	24	24	64	1	65
		Autumn	24	18	52	2	54
		Total	**96**	**90**	**170**	**7**	**177**
	Class C	Winter	12	12	26	6	32
		Spring	12	12	41	1	42
		Summer	12	12	56	1	57
		Autumn	12	12	50	1	51
		Total	**48**	**48**	**173**	**9**	**182**
	Class D	Winter	12	12	73	20	93
		Spring	12	10	127	6	133
		Summer	12	12	163	2	165
		Autumn	12	12	95	9	104
		Total	**48**	**46**	**458**	**37**	**495**
Outdoor	Site 1	Winter	6	6	429	236	665
		Spring	6	6	655	210	865
		Summer	6	6	1032	29	1061
		Autumn	6	6	629	99	728
		Total	**24**	**24**	**2745**	**574**	**3319**
	Site 2	Winter	6	6	465	310	775
		Spring	6	6	440	301	741
		Summer	6	6	809	42	851
		Autumn	6	6	358	196	554
		Total	**24**	**24**	**2072**	**849**	**2921**

Table 6: Average counts of indoor bacteria and fungi species isolated from walls using contact plates and swab methods.

Species	Contact plate (count/plate/sample)			Swab (count/swab/sample)			
	Class B*	Class C**	Class D**	Class A*	Class B*	Class C**	Class D**
Staphylococcus arlettae	0.02	0.33	1.27	0	0	0.46	0.96
Bacillus subtilis	0	0.29	1.04	0	0	0.23	0.85
B. simplex	0	0.40	0.98	0	0	0.21	0.42
B. pumilus	0	0.48	0.67	0	0	0.44	0.58
Unidentified bacteria	0	0.38	0.40	0	0	0.21	0.21
Total counts of bacteria	**0.02**	**1.88**	**4.36**	**0**	**0**	**1.55**	**3.02**
Aspergillus flavus	0	0.02	0.13	0	0	0	0
A. niger	0	0	0.02	0	0	0	0.02
Penicillium oxalicum	0	0	0.02	0	0	0	0.02
P. citrinum	0	0	0.02	0	0	0	0.04
Cladosporium cladosporioides	0	0	0.04	0	0	0	0.02
Acremonium curvulum	0	0	0.02	0	0	0	0
Rhodotorula mucilaginosa	0	0	0	0	0	0	0
Total counts of fungi	**0**	**0.02**	**0.25**	**0**	**0**	**0**	**0.10**
Total counts of bacteria and fungi	0.02	1.90	4.61	0	0	1.55	3.12
Gross total counts of all samples	2	91	221	0	0	74	150

Table 7: Average counts of outdoor airborne genera and species of bacteria and fungi using air sampler and settle plate methods.

Species	Air sampler (count/m^3/sample)		Settle plate (count/plate/sample)	
	Site 1*	Site 2*	Site 1*	Site 2*
Staphylococcus sp.	44.17	31.50	27.29	22.08
Bacillus spp.	55.50	33.67	36.46	45.67
Pseudomonas sp.	15.63	20.79	14.50	3.38
Total counts of bacteria	**115.3**	**85.96**	**78.25**	**71.13**
Acremonium curvulum	0.50	0.88	0.79	1.75
Aspergillus flavus	6.75	6.33	8.29	4.38
A. flavus var. *columnaris*	5.50	3.83	3.71	4.13
A. niger	4.21	5.13	3.29	2.17
A. ochraceus	2.29	1.38	1.04	1.58
A. parasiticus	1.88	0.83	1.38	0.58
A. sydowii	0.83	0.54	0.75	0.63
Cladosporium cladosporioides	1.33	1.67	0.54	1.08
Setosphaeria rostrata	0.75	0.71	0.00	0.00
Emericella nidulans	0.63	0.13	0.00	0.00
Epicoccum nigrum Link	0.17	0.00	0.46	0.00
Penicillium brevicompactum	0.92	0.67	0.50	0.08
P. citrinum	2.00	2.21	1.50	1.25
P. oxalicum	2.75	1.33	1.04	0.50
Rhodotorula mucilaginosa.	1.25	0.88	1.04	0.96
Total counts of fungi	**31.76**	**26.52**	**24.33**	**19.09**
Total counts of bacteria and fungi	147.06	112.48	102.58	90.22
Gross total counts of all samples	3529	2699	2462	2165

* Out of 24 samples

Table 8: Seasonal distribution of average counts (per 100 ml) of genera and species of bacteria and fungi isolated from city water during the period from December 2011 to November 2012 (out of 6 samples per season).

Microbial species	Winter	Spring	Summer	Autumn	Total (%)
Staphylococcus spp.	12.8	10.0	7.2	7.5	37.5 (29.6)
Bacillus spp.	13.2	14.3	15.5	21.2	64.2 (50.6)
Total counts of bacteria	**26.0**	**24.3**	**22.7**	**28.7**	**101.7 (80.2)**
Aspergillus sydowii	7.5	2.0	0	1.5	11 (8.7)
A. flavus	4.5	0.3	0	1.0	5.8 (4.6)
A. ochraceus	1.2	0	0	0.7	1.9 (1.5)
Penicillium citrinum	1.7	0	0	0.5	2.2 (1.7)
P. brevicompactum	1.0	0.2	0	0.2	1.4 (1.1)
Acremonium strictum W. Gams	2.2	0	0	0.8	3 (2.4)
Total counts of fungi	**18.0**	**2.5**	**0**	**4.7**	**25.2 (19.9)**
Total counts of bacteria and fungi	44.0	26.8	22.7	33.3	126.8

Table 9: Seasonal distribution of average counts (per 100 ml) of genera and species of bacteria and fungi isolated from purified water during the period from December 2011 to November 2012 (out of 24 samples per season).

Microbial species	Winter	Spring	Summer	Autumn	Total (%)
Staphylococcus spp.	0.3	0.1	0.3	0.1	0.8 (22.9)
Bacillus spp.	0.5	0.7	0.3	0.0	1.5 (42.9)
Total counts of bacteria	**0.8**	**0.8**	**0.6**	**0.1**	**2.3 (65.7)**
Aspergillus sydowii	0.4	0	0	0	0.4 (11.4)
A. flavus	0.1	0	0	0	0.1 (2.9)
Penicillium citrinum	0.3	0	0	0	0.3 (8.6)
P. brevicompactum	0.1	0	0	0	0.1 (2.9)
Acremonium strictum	0.2	0	0	0.1	0.3 (8.6)
Total counts of fungi	**1.1**	**0**	**0**	**0.1**	**1.2 (34.3)**
Total counts of bacteria and fungi	1.9	0.8	0.6	0.2	3.5

Table 10: Counts (per gram) of genera and species of bacteria and fungi isolated from non-sterile finished products.

Product name	Cepodem drymix			Medicefalxin drymix				Speczil drymix	Medicefalxin Capsule	NCI Out of 9 samples	Total count
Batch No / **Species**	**120006**	**120447**	**120446**	**M21206Z**	**M21205Z**	**M21202Z**	**M21203Z**	**1306116**	**M31201LM**		
Staphylococcus sp.	60	70	90	80	20	60	30	60	20	9	490
Bacillus sp.	40	30	60	0	20	0	0	60	10	6	220
Total counts of bacteria	**100**	**100**	**150**	**80**	**40**	**60**	**30**	**120**	**30**	**----**	**710**
Aspergillus flavus	20	10	30	10	10	0	0	0	10	6	90
A. niger	10	20	0	0	30	20	0	20	20	6	120
A. ochraceus	0	0	30	30	30	0	0	0	0	3	90
Penicillium brevicompactum	0	10	0	20	0	0	20	0	0	3	50
P. oxalicum	10	0	0	0	0	20	30	20	0	4	80
Cladosporium cladosporioides	10	20	10	0	0	0	10	10	0	5	60
Acremonium curvulum	0	0	0	0	20	10	10	10	10	5	60
Total counts of fungi	**50**	**60**	**70**	**60**	**90**	**50**	**70**	**60**	**40**	**----**	**550**
Total counts of bacteria and fungi	150	160	22	140	130	110	100	180	70	---	1260

*NCI= number of cases of isolation

Conclusion: Finished cephalosporin products of the T3A factory in Assiut are either completely free of microbial contamination (sterile products) or loaded in non-sterile products with a few number of microbes whose counts are much lower than those recommended by WHO guidelines. The source of microbial contamination of some non-sterile products could be attributed to the air outside and inside the factory as well as the water used for washing vials, bottles and filling machine parts. It is highly recommended to follow the perfect hygienic conditions to avoid microbial contamination in the pharmaceutical factory environment. Utilization of fresh and microbial-free raw materials is very important in avoiding or reducing the microbial contamination in finished products.

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