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Microbiological evaluation of the environmental quality of a pharmaceutical plant in Lagos, Southwest Nigeria

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Abstract

The evaluation of predominant microorganisms distributed in the air of the critical rooms in the production plant of a pharmaceutical company in Agege Local Government area, Lagos-Nigeria was investigated using settle- plate method. A total of seven critical rooms in the production facility that is, Dispensary, Granulation, Blending, Blistering, Tableting, Syrup, and Syrup filling rooms were sampled respectively under aseptic conditions. These samples were processed in duplicates on Sabouraud Dextrose Agar (SDA) and Tryptone Soya Agar (TSA) and incubated at 25ºC and 37 ºC respectively. Thereafter, microorganisms were isolated and identified using standard and conventional methods. The data obtained were analyzed to find the microbial distributions in the environment of the pharmaceutical facility. Eight bacterial species were isolated from different locations in the pharmaceutical facility, they were of the following genera; *Staphylococcus* (12.5%), *Micrococcus* (50%) and Bacillus (37.5%). Most of the microorganisms found in this study belong to the genus *Micrococcus* (four species) and three *Bacillus* species and one *Staphylococcus* species. Despite the compliance of this company with international standards, the above mentioned microbial species were still found within the facility. Hence, compliance with the international specified standard alone, that is, Good Manufacturing Practice (GMP) does not guarantee sterile pharmaceutical product from the industry. Therefore, other microbial quality assurance procedures must be strictly adhered to in order to have products that comply with international specifications. The microbial isolates from the pharmaceutical facility include: *Bacillus subtilis, Bacillus badius, Staphyloccocus aureus, Proteus mirabilis, Micrococcus sp. Pseudomonas aeruginosa, Enterobacter aerogenes, Citrobacter sp. Talaromyces sp. Aspergillus tamari.*

Keywords: Air; Environmental monitoring; Microbial contaminants; Pharmaceutical plant; Settle - plate method

1. Introduction

Pharmaceuticals are manufactured in clean rooms to minimize contamination by both microorganisms and inert particles. A factory contains different locations that require varying environmental conditions such as temperature, humidity control or air extraction facilities. However, it has been reported that the personnel working in the manufacturing plant, air and surfaces require frequent monitoring to guarantee safety of the finished products [1]. In order to achieve products with compendia requirements, monitoring the environment for probable microbial contaminants is important [2]. Controlling measures for quality control of pharmaceutical manufacturing facility begins from the initial design of the industrial plant [3]. Environmental monitoring is a program for scrutinizing microbial and particulate contaminants of clean rooms and associated clean zones for aseptic processing [4].

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Regulatory authorities recommend the monitoring of all manufacturing areas to control viable and non-viable particles. Where aseptic operations are performed, monitoring should be frequent, using a variety of methods including contact plates (for work surfaces and personnel monitoring), especially before and after critical operations [5]. The results should be reviewed and assessed prior to product release in order to analyze trends, set appropriate alert and action limits for the outcome of environmental monitoring, and where these limits are exceeded, there should be an established plan of corrective measures. Surface sampling will indicate the effectiveness of cleaning and disinfection policies. Moreover, monitoring the environment and the surface samples using contact plates is critical to verifying microbiological cleaning efficacy of the surface as well as to understand the success of the operations. The methodology used in microbiological quality control is simple, fast and not expensive, which provides insight for the evaluation of the process efficiency and highlights the limiting factors and the drawbacks that affect the quality of cleaning [3,6].

In the pharmaceutical industry today, the quality by design approach has become essential for the continuous improvement of product quality. This combined concept and strategy for product quality management contributes to assuring safety and effectiveness of drugs administered to patients who generally cannot directly determine the quality of drugs [7].

Microbiological environmental monitoring of air quality is a key tool to determine the appropriateness of manufacturing area in clean rooms for microbiologically safe drug production. Since aerial microbial distributions are highly dynamic and show significant variability from time to time depending on several influential variables, a monitoring system would be the most appropriate strategy in order to establish control on trends, major sources of risks and defects [8].

Microbiological assessment of non-sterile products is important in view of the fact that microbial contamination can reduce or even eliminate the therapeutic effect of drugs or cause drug-induced infections. Microbes present in drugs not only makes them hazardous from the infectious standpoint, in addition may change the chemical, physical and organoleptic properties of the drugs or change the contents of active ingredients. Furthermore, microorganisms can convert drugs to toxic products. The presence of even a low level of pathogenic microorganisms, higher levels of opportunistic pathogens or bacterial toxic metabolites, which persist even after the death of the primary contaminants can make the product ineffective. Not only the presence of microorganisms, which cause undesirable bacterial infections is harmful, but also the presence of metabolites or toxins may alter the drug composition even if they are included in small amounts. Some of these toxin – related diseases include diarrhea, acute gastroenteritis or abdominal pain. Symptoms vary from mild distress of stomach to death, depending on the individual sensitivity to toxin, amount of ingested toxin and victim's general health. Severe infections in immunocompromised people have been assigned to *Klebsiella* sp., and *Bacillus* sp. Several hospital - acquired and some outpatient - acquired infections, particularly pneumonia, are also assigned to *Klebsiella* sp. [9].

Ashour *et al*. [10] had reported that regular environmental monitoring of samples for microbiological quality of different locations in pharmaceutical facility are by far a very critical measure in controlling clean area and environment which have direct impact on the microbiological quality of the final pharmaceutical products. Environmental monitoring describes the microbiological testing undertaken in order to detect changing trends of microbial counts and microflora growth within clean rooms 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 [11].

In order to be in compliance with current Good Manufacturing Practices (cGMP) the environment in which a pharmaceutical product is manufactured must be monitored. Constant monitoring of contaminant sources and identification of the predominant contaminant microorganism is usually necessary to ensure a clean room [12].

The current study was therefore, designed to evaluate the quality of air of a pharmaceutical plant in compliance with the international cleanroom standards proposed by the World Health Organization (WHO) for the production of quality biological products.

2. Material and methods

2.1. Sampling procedure

The air of critical area including syrup manufacturing room, syrup filling room, granulation room and compression tableting room were sampled according to the guideline for microbiological monitoring of manufacturing areas in WHO guideline on Quality Assurance of pharmaceuticals [12]. Sampling of air was carried out using settle-plate method. All

media including Tryptone Soya Agar (TSA), Nutrient agar (NA), Mannitol salt agar (MSA), Simmon citrate agar and normal saline were prepared as directed by the manufacturer and sterilized with the autoclave at 121°C for 15 minutes at 15lbs for growth promotion test [3,13].

2.2. Growth promotion test

Growth promotion test was carried out on all the media used for this research to ensure that the media would support the growth of microorganisms. Plates were prepared aseptically in triplicates.

The plates were labeled accordingly and properly dated. One for negative control used for verifying, testing condition and the other two plates served as positive control. The plates were allowed to set after which they were dried for 1 h. Standard organism (*Bacillus subtilis)* was inoculated onto the two plates for positive control and then incubated at 37°C for 24 h.

The positive plates gave rise to emergence of the standard organism indicating a positive result. Hence, the media were now used for sampling and sub-culturing. The prepared media were allowed to cool to about 45°C and plates were poured in duplicates. Thereafter, plates were allowed to solidify and pre-incubated for 24 h at 37°C to ensure that the plates were not contaminated before use. After 24 h, the plates were exposed at each location in duplicates with appropriate labeling and date for 4 h. The plates were covered and transported to the microbiology laboratory for incubation. The plates were incubated in an inverted position at 37°C for 72 h for bacterial species while fungal plates were incubated in upright position at 25ºC for 3-5 days [4, 13].

2.3. Sub-culturing of pure culture of isolates

Each isolates from a mixed culture were sub-cultured onto a nutrient agar and sabouraud dextrose agar plates for bacterial and fungal isolates respectively under the Laminar Air Flow (LAF). This was done aseptically and incubation was at 37°C for 24h for bacterial isolates and 25ºC for fungal isolates. Microscopic examination using ×100 objective lens with oil immersion for bacterial isolates and fungal isolates observed ×40 objective lens for the morphological characteristics of the isolates was done. Thereafter, the colonies were picked and streaked on agar slant and incubated. Agar slope was then stored in the refrigerator for further biochemical tests for identification [13, 14].

2.4. Biochemical characterization of isolates

This was carried out using 24 h culture. The tests include Gram reaction, motility, catalase, oxidase, Voges proskauer, Methyl red, indole, starch hydrolysis, citrate, gelatin liquefaction, nitrate reduction, urease and sugar fermentation. [13, 15]

3. Results

A number of bacterial species were isolated in the routine microbiological monitoring processes for clean rooms of the pharmaceutical plant. The cultural, cellular and biochemical characteristics of the isolated microorganisms from the critical area of the pharmaceutical plant were listed in Table 1 and 3. The colonial and cell morphology, as well as the distribution of the isolates were shown in Table 1. The cell morphologies were further observed under binocular microscope (×100 - oil immersion for bacterial isolates and ×40 objectives for fungal isolates). A total of 145 bacterial isolates and 70 fungal isolates were obtained from the pharmaceutical plant. Micromorphology and biochemical analysis gave the identity of the isolates (Tables 2 and 3). The Trend analysis of Total Aerobic Microbial Count (TAMC) over a period of 6 months showed that the incubation room had the highest microbial contaminations (Fig. 1). Although, the high personnel traffic in this room may have caused this development.

The sterility room (microbiology laboratory) had no contaminants detected with the settle-plate method, this may be due to extremely low traffic of personnel in the laboratory and the readily available hand sanitizer at the entrance. The fungal isolates were obtained in the rooms with high traffic of personnel and close to the doors that were frequently opened as well as close to the changing rooms. The micromorphology of fungal isolates were compared with standard cultures by Smith (1981).

Table 1 Air monitoring for microbial contaminants

S/N	Class/Location	No. of Plates Per Room	Bacteria (CFU/4hrs)	Fungi (CFU/4hrs)
1	Sterility Room (Microbiology lab)	2	Nil	Nil
2	Wet Chemistry Lab	2	22	14
3	Excipient Dispensary Room	2	1	Nil
4	Granulation Room 1	2	4	12
5	Compression Room 2	2	6	4
6	Blistering Room	2	50	4
7	Solution Preparation Room	2	7	11
8	Oral Liquid Manufacturing	2	44	19
9	Oral Liquid Filling	2	10	2
10	Coating Room 1	2	1	4
11	Control	2	Nil	Nil

Table 2 Morphological and Biochemical Characterization of isolates

Isolate Code	Gram Reaction & Morphology	Pigmentation	Motility	Citrate	Catalase	Indole	Methyl Red	Voges Prauskauer	Starch hydrolysis	Oxidase	Lactose	Glucose	Sucrose	Manitol	Urease	Galatin liquefaction	Nitrate reduction	Most probable organism
SR1	(+) rods in cluster	-	+	+	+	-	+	+	+	-	+	+	-	+	-	+	+	*Bacillus subtilis*
SR2	(+) cocci singles and cluster	-	+	+	-	-	+	+	-	+	+	+	+	-	+	+	+	*Staphylococcus aureus*
SR3	(-) rods clusters	-	+	+	+	-	+		-	-	-	+	-	-	+	–	+	*Proteus mirabilis*
SF4	(+) cocci single	-	-	-	+	-	+	-	-	+	+	+	+	+	+	-	+	*Micrococcus Species*
GR5	(-) rods singles	+	-	+	+	-	-	-	+	+	-	-	-	-	+	+	–	*Pseudomonas aeruginosa*
GR6	(-) rods singles	-	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	*Enterobacter aerogenes*

BL1	(-) rods	-	+	+	+	-		-	+	-	+	+	+	+	–	–	+	*Citrobacter Species*

Table 3 Morphological characterization of fungal isolates

Isolate code	Colonial appearance on SDA	Microscopy	Identity
GR1	Fast growing light greyish colonies of lower aerial mycelium with light green border. Reverse side was with tan pigments at the centre of about 2-3cm	Ascospores with stripes bearing terminal were biverticillate or less commonly monoverticillate	Talaromyces species
GR2	Green with brownish top. Reverse was slightly brown	Conidiophores were of different sizes in length and were smooth. Sterigmata are single, radiate columnar	Aspergillus tamari

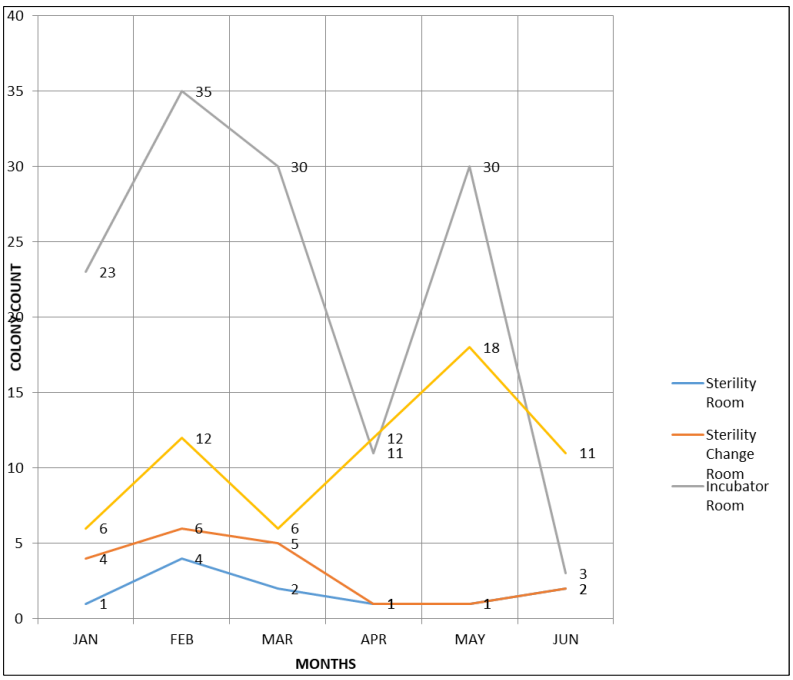

Figure 1 Environmental monitoring trend in microbiology laboratory (TAMC)

4. Discussion

The suspension of particulate matter in the air surrounding the plant would be one of the significant source of the microbial contamination, since inefficient air - filtration into the clean rooms and other sections of the plant could serve as the predisposing factor. This corroborated the findings of Dharan & Pittet [16]. Clean rooms are essential in aseptic pharmaceutical processes. However, monitoring, identifying and evaluating microbial contamination is part of Good Manufacturing Practice and it aids in finding a more effective environmental monitoring and quality management system for the pharmaceutical plant. The frequency with which people enter and exit specific area may also increase the number of microorganisms in indoor environments, this could be the reason for the observations of the Trend analysis (Fig. 1) which agreed with the findings of [17]. Airborne droplets usually carry microorganisms such as Gram-positive cocci and Gram-negative rods, whose presence is considered undesirable in pharmaceutical products [18]. The presence of commensals and potentially pathogenic bacteria were found in the sampled rooms. Contrary to previous report that Gram negative organisms were principally responsible for contamination of pharmaceutical products [19], this study found that Gram positive rods were more frequently isolated from the clean rooms and the plant environment

than any other microbial groups. Indoor air contamination is linked with inappropriate environmental control measures of the buildings, including materials-of-construction, heating, ventilation and air conditioning (HVAC), and the other sources are related to humans, such as inappropriate behavior and numbers of people in constrained spaces [20, 21, 22]. This was evident in the results obtained using settle – plate technique for sampling of the various clean rooms in the plant. The quality of personal hygiene of personnel influenced the environmental air quality of the plant which subsequently would affect the quality of the pharmaceutical products from the plant. The design approach for the plant as well as frequency of monitoring and the degree of application of appropriate treatment to eliminate microbial contamination may be the reason for the observations in Fig.1. This corroborated the report of [11] and [12]. The possibility of crowded incubation room and inappropriate behavior by employees who may sneeze or cough in the room may form the basis for presence of fungal spores and bacteria in the room as suggested by [21].

5. Conclusion

The currently applied settle - plate technique for assessment of air-quality in a pharmaceutical plant is simple and rapid yet it is convenient in identifying sources of microbial presence and trend analysis. The study outlined that passageways e.g. corridors and airlocks are areas of high probability of failure in microbiological air quality samples and the personnel activities in the clean rooms have significant impact on aerial distribution of microbes in the production environment which may have aggravate microbial counts.

Recommendation

The results of this study graduated into the following recommendations;

- Environmental monitoring controls at all stages of product development and supply chain production ought to be executed consistently in pharmaceutical plants.
- An extensive and comprehensive environmental control program that includes adequate fumigation of the plant and sanitation ought to be scheduled at specified intervals.
- Training and re-training of the entire factory personnel on personal hygiene should be prioritized.
- Heating - ventilating air - conditioned system should be checked periodically for efficiency.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflict of interest to disclose because this study was self-sponsored by all the listed authors as a collaborative study. The authors did not receive support from any organization for the conduct of the study and the submitted manuscript.

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