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CHARACTERIZATION OF THE CLEAN ROOM ENVIRONMENT

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Abstract– Maintenance of appropriate quality of the pharmaceutical product is considered vital for achieving success in the global trade. Monitoring microbial distribution and identifying the predominant isolates is part of good manufacturing practice. Therefore, Clean rooms are essential in maintaining an aseptic environment in the pharmaceutical Industry. In this study, we have evaluated the dominant/ abundance and occurrence of Environmental micro flora which were identified from clean room over a period of time. Clean room microorganisms were isolated using Soybean Casein Digest Agar Media by various microbiological techniques like Plate (Passive Monitoring), Air Sampling (Active Monitoring) and Contact plate Method, which are used for surface monitoring. Over the period of study around 155 isolates are isolated, out of which 150 were bacterial isolates and 5 were fungal isolates. These isolated microorganisms were identified by applying Vitek 2 identification system and 16S- RDNAribotyping. The data generated by Vitek 2/16S RDNA ribotyping wereanalyzed in the following manner. 1. The isolated microorganisms were identified and segregated on the basis of the Gram nature of microorganism. Out of 150 isolates, 137 (91.33%) are Gram positive cocci, 10 (6.67%) are Gram positive rods and remaining 3 (2%) are Gram negative rod. 2. The isolated microorganisms were also analyzed for predominance and it was found that the most predominant isolate is *Micrococcus luteus*. This study concludes that periodical validation of a clean room is necessary as in pharmaceutical industry long production runs keeps contaminating the production area and surroundings.

INTRODUCTION

Clean rooms are essential in maintaining aseptic environment in industries like pharmaceutical or food production. Monitoring microbial distribution and identifying the predominant isolates is part of good manufacturing practices (Akers, 1997).

Maintenance of appropriate quality of pharmaceutical product is considered vital for achieving success in the global trade. But one of the major problems in the pharmaceutical industry, especially during long production runs, is keeping contaminant microorganisms out of the production area. Contaminant bacteria, it seems, always multiply fast and rapidly turn a clean production run into a nasty mess. Moreover, when rogue bacteria go on a rampage, high clean-up cost, disrupted production schedule and most important-lost of revenue will be faced. For this type of microbiological concerns in pharmaceutical product, manufactures continue to challenge those associated with their production. The microbiological quality of pharmaceutical products is greatly influenced by the environment in which they are manufactured (Hossain *et al*., 2004)

Environmental monitoring represents a critical process in the manufacture, especially those manufactured within highly controlled clean room conditions. The programme includes monitoring of air, personnel and surfaces and to meet regulatory authority requirements. Air monitoring is a critical component of the environmental monitoring programme of any pharmaceutical manufacturer. It is also a Good Manufacturing Practice (GMP) requirement that provides information on the quality of the processing environment during manufacturing and enables the study of microbiological air quality trends. So, air contamination control is an essential part of a global environmental monitoring approach. Surfaces also

contain many microorganisms that can contaminate manufacturing process. Surface monitoring is performed to assess the level of microbial load on floors, walls, equipment, etc. Antiseptics and disinfectants are used extensively in pharmaceuticals and other health care settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of infections (Larson, 1996 and Rutala, 1995).

The microbiological quality of drugs and biologics is necessary for their efficacy and patient safety, because microbialcontamination of drugs causes immediate adverse effects on patient health in terms of morbidity and mortality, aswell as long-term adverse effects, such as cancer, autoimmune, and other diseases. Additionally, microbes can alter thechemistry and pharmacology of drugs, with a potential adverse impact on their effectiveness due to the breakdown ofthe active ingredients as well as on their safety due to the toxicity of potential degradant products. Therefore, controlof microbes in drugs is essential, either by assuring absence of microbes in sterile drugs that are administered parenterally and applied to sensitive tissues or by controlling microbial bioburden to appropriate levels for non-steriledrugs that are administered to regions rich in microbial flora with physical or immunological barriers to infections (Matthews, 2002 and Smith, 2013).

MATERIALS AND METHODS

Growth media

Soyabean Casein Digest Agar (SCDA) (90 mm) and Soyabean Casein Digest Agar (SCDA)(55 mm RODAC) plate were selected as a growth mediam.

Sampling procedure

Microbial Monitoring by Settle Plate Method (Passive Air Sampling)

Soyabean Casein Digest Agar (SCDA) plates is Expose at designated location by placing the plate on exposure stand at working height in upright position and slowly removing the lid and placing it in such a way that the inner surface of the lid is facing downward position. Plate was Exposed for not more than 4 hr. After exposure the plates were collected and incubated the plate at 20-25 °C for atleast 72 hours followed by 30-35 °C for 48 hours in inverted position.

Microbial Monitoring By Microbial Air Sampling Method (Active Sampling)

Soyabean Casein Digest Agar (SCDA) plates were kept in Slit of Agar Airsampler (Make: AES, Biomeriux) and about 1000 liters (1 m3) of air was collected. After exposure the plates were incubated at 20-25 °C for atleast 72 hours followed by 30-35°C for 48 hours in inverted position.

Monitoring of Surfaces by contact plate (RODAC) method

The even surface was monitored by using 55mm RODAC (Replicative Organism Detecting and Counting) plate The lid of RODAC plate has to be opened and gently press in such a way that agar surface of the plate gets contact on a selected location. Later on the plates were rolled in such a way that the entire convex surface of agar plate comes in contact with the surface. After exposure the plates were incubated at 20-25 °C for about 72 hours followed by 30-35°C incubationfor 48 hours in inverted position.

Estimation of total microbial count

After completion of incubation the number of colonies were estimated by colony counter. The estimation will be the number of counts in form of cfu (Colony forming unit).

Identification of the bacterial strains

The isolated microorganisms were collected from environment and identified up to species level by using VITEK-2 identification system. The isolates which were not identified by VITEK-2 identification system and fungal colonies were identification by ribotyping methodology.

RESULTS AND DISCUSSION

The isolated and identified microorganism data was collected and verified. First the isolated microorganisms were identified and segregated on the basis of Gram nature of microorganism. Secondly, species occurrence was identified on the basis of number of times occurrence of each species. Then the predominant micro flora were identified by percentage wise, which infers which microorganism is dominant among the collected data of isolated micro flora of environment. The pie chart was applied for percentage of dominance

among the isolated micro flora.

Isolates identified from environment from January 2019 to December 2019 are reviewed as mentioned below

A) Total Isolates
B) Review of Bacterial Isolates- Gram nature wise
C) Predominance of Bacterial Isolates

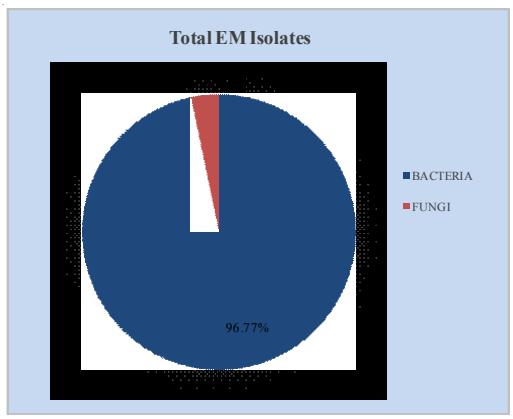
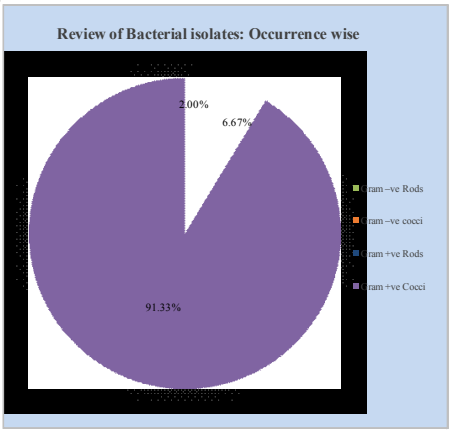


Table 1. A. Total isolates: EM Isolates

Microorganism	Number of times identified	Percentage
Bacteria	150	96.77%
Fungi	5	3.23%



B. Review of Bacterial isolates: Gram nature wise (Occurrence wise)

Types of Microorganism	Total No. of	Percentage times identified
Gram –ve Rods	3	2.00 %
Gram –ve cocci	0	0%
Gram +ve Rods	10	6.67%
Gram +ve Cocci	137	91.33%
Total times identified	150	100%

C. Predominantly Identified Bacterial Isolates

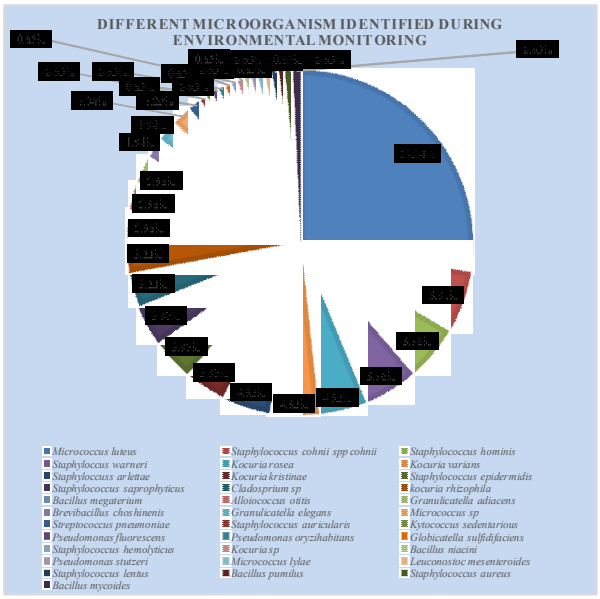
Types of Microorganism	Total No. of times identified	Percentage	Cumulative % of Top 10 isolates are 69.04%
Micrococcus luteus	43	27.74	
Staphylococcus cohniisppcohnii	9	5.81	
Staphylococcus hominis	8	5.16	
Staphyloccuswarneri	8	5.16	
Kocuria rosea	7	4.52	
Kocuriavarians	7	4.52	
Staphyloccussarlettae	7	4.52	
Kocuriakristinae	6	3.87	
Staphylococcus epidermidis	6	3.87	
Staphylococcus saprophyticus	6	3.87	
Cladospriumsp	5	**Remaining isolates**	
kocuriarhizophila	5	**are 31.06%**	
Bacillus megaterium	4		
Alloiococcus otitis	4		
Granulicatellaadiacens	4		
Brevibacilluschoshinenis	3		
Granulicatella elegans	3		
Micrococcus sp	3		
Streptococcus pneumoniae	2		
Staphylococcus auricularis	1		
Kytococcussedentarious	1		
Pseudomonas fluorescens	1		
Pseudomonas oryzihabitans	1		

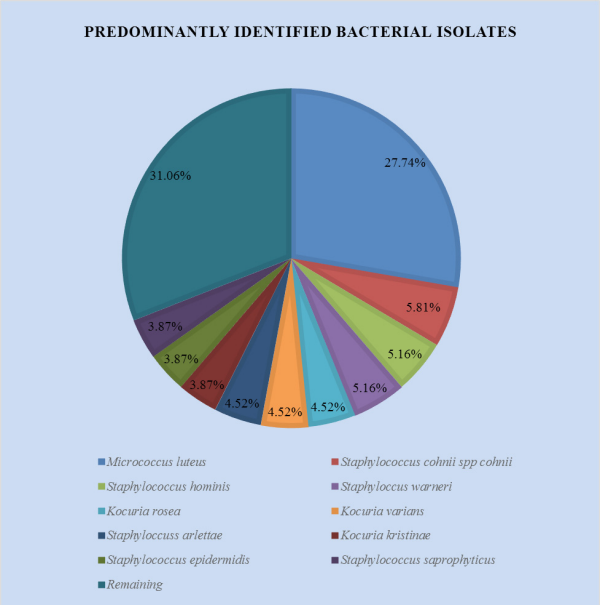
C. Predominantly Identified Bacterial Isolates

Types of Microorganism	Total No. of times identified	Percentage	Cumulative % of Top 10 isolates are 69.04%
Globicatellasulfidifaciens	1		
Staphylococcus hemolyticus	1		
Kocuriasp	1		
Bacillus niacini	1		
Pseudomonas stutzeri	1		
Micrococcus lylae	1		
Leuconostocmesenteroides	1		
Staphylococcus lentus	1		
Bacillus pumilus	1		
Staphylococcus aureus	1		
Bacillus mycoides	1		
Total	**155**	**100.1~ 100 %**	

Table 2. Different Microorganism identified during Environmental Monitoring

Sr No.	Types of Microorganism	Total No. of times identified	Percentage
1	*Micrococcus luteus*	43	27.74
2	*Staphylococcus cohniisppcohnii*	9	5.81
3	*Staphylococcus hominis*	8	5.16
4	*Staphyloccuswarneri*	8	5.16
5	*Kocuria rosea*	7	4.52
6	*Kocuriavarians*	7	4.52
7	*Staphyloccussarlettae*	7	4.52
8	*Kocuriakristinae*	6	3.87
9	*Staphylococcus epidermidis*	6	3.87
10	*Staphylococcus saprophyticus*	6	3.87
11	*Cladospriumsp*	5	3.23
12	*kocuriarhizophila*	5	3.23
13	*Bacillus megaterium*	4	2.58
14	*Alloiococcus otitis*	4	2.58
15	*Granulicatellaadiacens*	4	2.58
16	*Brevibacilluschoshinenis*	3	1.94
17	*Granulicatella elegans*	3	1.94
18	*Micrococcus sp*	3	1.94
19	*Streptococcus pneumoniae*	2	1.29
20	*Staphylococcus auricularis*	1	0.65
21	*Kytococcussedentarious*	1	0.65
22	*Pseudomonas fluorescens*	1	0.65
23	*Pseudomonas oryzihabitans*	1	0.65
24	*Globicatellasulfidifaciens*	1	0.65
25	*Staphylococcus hemolyticus*	1	0.65
26	*Kocuriasp*	1	0.65
27	*Bacillus niacini*	1	0.65
28	*Pseudomonas stutzeri*	1	0.65
29	*Micrococcus lylae*	1	0.65
30	*Leuconostocmesenteroides*	1	0.65
31	*Staphylococcus lentus*	1	0.65
32	*Bacillus pumilus*	1	0.65
33	*Staphylococcus aureus*	1	0.65
34	*Bacillus mycoides*	1	0.65
	Total	**155**	**100.1 ~ 100 %**





CONCLUSION

All of the isolates were collected and were characterized.In Environmental monitoring program total 155 isolation was performed and 34 different isolates were identified during the period January 19 to December 19. Out of these 155 isolates, 150 are bacterial isolates and remaining 5 are fungal isolates. All the Bacterial isolates were reviewed for their gram nature and distribution and out of this 150 isolates, 137 are Gram + ve cocci, 10 are Gram + ve rods and remaining 3 are Gram – ve rod. 5 isolates are identified as fungus. Occurrence wise Gram + ve cocci was observed 91.33% and are Gram + rods was 6.67% and remaining 2% is Gram – veRod. Bacterial isolates were reviewed for Predominance and it was found that the most predominant isolate is *Micrococcus luteus*.

This study concludes that periodical validation of a clean room is necessary as in pharmaceutical industry as long production runs keeps contaminating the production area and surroundings.

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