Original Article

Mahidol Univ J Pharm Sci 2016; 43 (3), 115-126

Novel rapid method in ecological risk assessment of air-borne bacteria in pharmaceutical facility

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Abstract

Environmental monitoring (EM) of microbiological air quality in clean rooms of pharmaceutical plants is not critical only for the control of good manufacturing practice (GMP) but also for assessing the risk associated with aerial bioburden to the final customer through medicinal products. Extensive EM samples were taken from newly established pharmaceutical facility including 59 active and 41 passive air samples of classified area in production area and its associated microbiology laboratory. The gathered samples were incubated then examined for bacterial count populations. Bacterial colonies were then subjected to further identification using BBL™ Crystal™ Identification System and a distribution profile was made from which Pareto chart was constructed which showed that the following bacterial families: *Micrococcaceae*, *Staphylococcaceae*, *Bacillaceae*, *Streptococcaceae*, *Microbacteriaceae* and *Corynebacteriaceae* contributed by more than 80% of air samples. A quantitative risk index was adopted to reflect the potential impact of the environmental aerial bacteria type on the possible health hazard of the final consumers through the medicinal dosage form. Interestingly, this risk index showed that the greatest risk came from *Enterobacteriaceae* followed by *Streptococcaceae* then *Staphylococcaceae* with more than 99% contribution to the total risk provided that the all other parameters are constant such as batch size, clean area classification, preservation power and the reduction factor of microorganisms by the manufacturing process such as heating and compression. The quantitative risk index used in this study provides milestone in the assessment of the possible hazard that could arise from environmental contamination of the medicinal product to the final consumer.

Keyword: environmental monitoring, clean rooms, good manufacturing practice, Pareto chart, quantitative risk index.

1. INTRODUCTION

Microbial contamination that is originated during pharmaceutical products manufacturing may contribute to potential risk that could be raised due to the microorganisms' load that is carried by medicinal product to the patients. This is evident by reports of Center for Drug Evaluation and Research (CDER) to the nation on drug safety and quality, there were 401 prescriptions and 101 over-the counter (OTC) drug recalls in the fiscal year of 2005. Out of the top 10 reasons for the recalls in 2005 by the FDA microbial contamination of non-sterile products was listed as number three[1].

Transfer of microbial contamination to the drug can be easily disseminated as particles in air have significant role because they can enter a product and contaminate it physically or, by acting as a vehicle for microorganisms, biologically[2]. Monitoring of total particulate count in controlled environments, even with the use of electronic instrumentation on a continuous basis, does not provide information on the microbiological content of the environment. The basic limitation of particulate counters is that they measure particles of 0.5 μm or larger. Monitoring the environment for non-viable particulates and microorganisms is an important control function because they both are important in

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achieving product with compendial requirements[3]. An understanding of the sources and anticipated distribution of microbial contaminants is crucial in developing an effective microbiological control program[4].

Microbiological risk assessments enable pharmaceutical companies to identify the hazard that may emerge from microorganisms dispersed in the environment on pharmaceutical products which affect finally patients' health and life. Several techniques which are qualitative in nature are used in the risk assessments in the pharmaceutical field including the use of ranking to assess the score from which the degree of the risk could be identified. This was discussed by Frank *et al*., 2008[5]. On the other hand, quantitative risk was implemented in the field of food industry and in the evaluation of drinking city water using other techniques primarily quantitative microbial risk assessment which has been defined by Haas & Eisenberg, 2001[6]. Risk based approaches include FMEA (Failure Mode and Effects Analysis); FTA (Fault Tree Analysis) and HACCP (Hazard Analysis Critical Control Points), all of which employ a scoring approach (Sandle, 2000)[7]. At present, no definitive method exists and the various approaches differ in their process and the degree of complexity involved. However, the two most commonly used appear to be HACCP (which originated in the food industry) and FMEA (which was developed for the engineering industry)[8].

Since the currently used risk approaches in pharmaceutical industry field is subjective in nature and environmental monitoring programs relay mainly on the microbiological quantity rather than the quality to assess the degree of compliance of clean rooms in the pharmaceutical manufacturing area, the current study aimed to approach for new quantitative risk assessment of aerially distributed bacteria in clean area - screened from newly installed pharmaceutical facility in Egypt – was established in order to determine their impact on the final consumer through manufactured medicinal drugs. Moreover, the current study aimed to develop a method for controlling and improving the quality of clean area in order to deliver microbiologically safe medicinal products to the patients. Better control could be achieved by identifying microbes and hence their potential sources to restrict their intrusion into clean area. Meanwhile, Improvement can be accomplished by spotting and recognizing microorganisms that possess the greatest risk to consumers health through product contamination. Hence, quality team would be able to focus on eliminating the major contributors of the deterioration of pharmaceutical products quality.

2. MATERIALS AND METHODS

Random active and passive air samples were taken from newly established pharmaceutical plant (solid oral manufacturing facility (class D), Mouth wash and liquid oral product plant and semisolid production facility (class C)) with their associated microbiology laboratory facility (class C) using methods described by Mostafa, 2014[9]. Heating ventilation air conditioning (HVAC) were supplied with high efficiency particulate air (HEPA) filters. Sampling was done in classified manufacturing and microbiology laboratory areas class C and D which followed WHO requirements and conditions for working in clean area. The standard and guidelines for air sampling method were followed as described in details by WHO, 2011[10]. The total number of samples was 100 (59 active and 41 passive air samples). Isolates were obtained from the microbiology laboratory in the quality control department after incubation in Series BD 115 Incubators with natural convection (BINDER GmbH, Im Mittleren, Ösch 5, 78532 Tuttlingen, Germany). Digital colony counter (Digital Colony Counter Model: 361, Laxman Mahtre Rd. Navagaon, Dahisar West, Mumbai)was used for enumeration. The bacterial environmental isolates were isolated and identified using miniaturized biochemical identifications kits BBL™ Crystal™ Identification System purchased from BD (Becton Dickinson Microbiology Systems, Cockeysville, Md.) as described by Ashour *et al*., 2011[11]. All the nutrient media and chemicals were purchased from OXOID (Basingstoke, Hampshire) and Sigma-Alrich (St. Louis, MO 63103), respectively. All media were sterilized by autoclaving in steam sterilizer (FEDEGARI FOB3, Fedegari

Autoclavi SpA, SS 235 km 8, 27010 Albuzzano (PV), Italy). Active air samples were taken using Biotest RCS Plus and Biotest RCS High Flow air sampler (Biotest, AG Landsteinerstr., 5 63303 Dreieich, Germany). Plastic 9 mm sterile plates were purchased from Sterilin Limited (solaar house, 19 mercers row, Cambridge, UK). All microbial processing was made under validated and calibrated biological safety cabinet (Jouan MSC 9 Class II A2 BioSafety Cabinet, Thermo Fisher Scientific Inc.81 Wyman Street, Waltham, MA, USA 02451). Isolated colonies were obtained after growth on BBL™ Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) as specified in the instruction manual of the biochemical identification kits.

THEORY/CALCULATION: Numerical approach for the risk assessment and evaluation was used as described by Sandle, 2006 for both active and passive air sampling but with modification to extent the risk impact study from air in clean area to the final customer through medicinal product[12]. The modification primarily included the addition of the following parameters in combined equation: infectious dose of the microbe, antimicrobial effect of the product (if any), exposure time and area of the product to the surrounding air in the clean manufacturing rooms, bulk volume of the manufactured product, the average settling velocity of the microbial particle, contribution factor of the microbe from the bioburden, reduction factor of microbial population by harsh manufacturing processes and maximum dose could be ingested by the patient. Two simplified equations were derived taking in consideration as the value of risk index decreases the associated hazard or risk will decrease accordingly and vice versa:

1. For passive air sample:

$$RIs = Cs \times \frac{Ap}{As} \times \frac{Tp}{Ts} \times \frac{Fm}{Vp} \times \frac{Rf}{ID} \times \frac{Sd}{Pp} \quad \text{Eq.1}$$

2. For active air sample:

$$RIa = Ca \times Sm \times Ap \times Tp \times \frac{Fm}{Vp} \times \frac{Rf}{ID} \times \frac{Sd}{Pp} \quad \text{Eq.2}$$

Where:

RIs and RIa = Risk Index for passive and active air samples, respectively (dimensionless values).

Cs and Ca = Settle plate (CFU) and active air-borne bacteria count (CFU/m^3), respectively.

Ap and As = Product area exposed to air and settle plate surface area, respectively (m^2).

Ts and Tp = Exposure time of settle plate and product (hours), respectively.

Fm = Fraction contribution of specific bacterial family from total trended results.

Vp = Total product bulk volume (m^3).

Rf = Reduction factor of microorganisms by manufacturing process such as heating, filtration and compression pressure.

ID = Minimum microbial dose required to start infection in the host (CFU).

Sd = Maximum single dose that could be administered to the patient (m^3).

Pp = Preservation power measured by the reduction ratio of microbial count of the initial to the final value.

Sm = Velocity (speed) of microorganisms-bearing-particle deposition (m/hr).

Sm value was taken as 41.67 m/hr as the average size of microbial particle will deposit, by gravity, onto surfaces at a rate of approximately 1 cm/s[13]. Provided that Cs value was about half that of Ca, then RIs would have about the half value of RIa. This means that if both methods of microbial recovery give the same risk value (the sensitivity or detectability of passive and active air sample were the same) provided that other testing conditions are constant and the same, when Cs = Ca. Table 1 shows the sources from which data could be obtained. All Pareto charts were constructed using Minitab® v17.1.0 while complex calculations and the derived bar graphs were generated using Microsoft Office Excel 2007.

Table 1. Parameters for gathering of data for the assessment of the risk encountered from air-borne bacteria to the pharmaceutical products.

Parameter	Method of obtaining values of data
Cs and Ca	Count enumeration after incubation from the microbiology laboratory. Values were taken at the action limit of 50 and 100 CFU, respectively of the most critical manufacturing site i.e. class C.
Ap and As	Obtained from data sheet or direct measurement. Form the production record of liquid products Ap= 1353 m^2 and As which was obtained from measurement of the plate diameter = 0.006 m^2.
Ts and Tp	Measurable time obtained from both production and quality control. Settle plate time is fixed = 4 hours while product exposure time = 0.016 hours.
Fm	Obtained from initial intensive sampling at the initial assessment after then by trending coupled with identification. Determined from the frequency of microbial detection in the current study.
Vp	Estimated and from batch production record (BPR). The bulk volume of the batch = 5 m^3.
Rf	Obtained from literatures and/or validation studies. In the current study, the worst case was assumed, where no microbial reduction was induced by the manufacturing processes i.e. Rf = 1.
ID	Available from literatures for common pathogens or studies done on experimental animals[13].
Sd	Obtained from medical literatures and/or pamphlet of specific drug. For the current product = 0.000015.
Pp	Preservative efficacy tests (PET) performed by pharmaceutical companies. The estimated reduction times for Gram-positive and Gram-negative were 29512 and 70795, respectively.
Sm	Obtained from literatures or from derived equation $y = 0.108x^2 - 0.0015x + 0.0092$[14,15] where: y is the settling rate (m/hr) and x is the particle size in range 0.1μm to 40 μm

3. RESULTS

The contribution of Gram-negative and Gram-positive bacteria from total environmental air samples was 6% and 94% (73% cocci and 21% bacilli), respectively. General characteristics of isolated bacteria from air samples were shown in Table 2, 3 and 4. Generally, the results showed the greater abundance and diversity of Gram-positive cocci over Gram-positive bacilli and Gram-negative rods. Fig. 1 demonstrates the major contributors for aerial bacterial contamination in the clean rooms with *Micrococcaceae*, *Staphylococcaceae* and *Bacillaceae* showing 66% of the total bacteria. After identification of the microorganisms, the possible sources of contamination and the participation of each could be deduced as illustrated using stacked bar in Fig. 2. More than 60% of the bacterial contamination originated from human with the major contribution by more than 70% from Gram-positive cocci from the total samples with the human source being the main possible origin.

Table 2. Gram-positive cocci found in environmental monitoring samples of air, their family, colonial morphology on general culture media and general biochemical characteristics.

Family	Bacteria	Colony Morphology on General Culture Media	General Biochemical Reactions
NA**	Unidentified Gram-Positive Cocci	Large, round, entire, convex and white Small, round, entire, raised and transparent	0677544443 2344210054
Micrococcaceae	*Micrococcus spp.*	Small to large, smooth or rough, entire, convex, raised or flat and yellow	Oxidase and Catalase positive
	Micrococcus luteus	Small to moderate, round, smooth, convex or raised, entire and white, yellow or light brown	
	Micrococcus lylae	Small to large, round, entire, convex or raised and yellow	
	Kocuriarosea	Small to moderate, entire, convex, round, smooth and orange to red	Catalase positive and Coagulase negative
	Kocuria kristinae	Small, round, smooth, convex and white	
	Stomatococcus mucilaginosus	Small, round, entire, raised and pink	?*
Dermacoccaceae	*Kytococcus sedentarius*	Moderate to large, entire, convex, raised or flat white or orange	Oxidase and Catalase positive
Staphylococcaceae	*Staphylococcus hominis*	Small, round, entire, convex and white	Catalase positive
	Staphylococcus heamolyticus	Small to moderate, round, smooth, onvex, entire and white	Oxidase negative
	Staphylococcus equorum	Small to large, round or irregular, smooth, convex, flat or raised, entire and white	
	Staphylococcus vitulinus	Small, smooth, entire, convex and white	Coagulase negative
	Staphylococcus aureus	Small to round, smooth, convex, round, entire and light buff to orange	Catalase and Coagulase positive
	Staphylococcus lugdenensis	Moderate, round, entire, convex and yellow	Coagulase negative
	Staphylococcus saprophyticus	Small or large, round, smooth, convex, entire and white	
	Staphylococcus capitis	Moderate, round, entire, convex and white	
	Staphylococcus epidermidis	Small to moderate, round, smooth, convex or flat, entire and white	Catalase positive and Coagulase negative
Streptococcaceae	*Streptococcus sanguinis*	Fine to moderate, round, smooth, entire, convex or flat and light pink or buff	Oxidase and Catalase negative
	Streptococcus parasanguinis	Small, round, smooth, convex and light brown	
	Streptococcus agalctiae	Moderate, round, convex and white	
	Streptococcus mitis	Small, round, smooth, convex and dark orange	
	Streptococcus intermedius	Small, smooth, flat, entire and light orange	
	Streptococcus constellatus	Moderate, round, smooth, convex, entire and white	
Carnobacteriaceae	*Alloiococcus otitidis*	Small to moderate, round, entire, convex or raised and orange or light rose	Catalase positive and oxidase negative
Nocardiaceae	*Rhodococcus equi*	Round, smooth, convex and orange	Catalase positive

*=Biochemical reaction results were not supplied from the laboratory.
**=Not applicable as the identification system could not identify the microorganism."

Table 3. Gram-positive bacilli found in environmental monitoring samples of air, their family, colonial morphology on general culture media and general biochemical characteristics.

Family	Bacteria	Colony Morphology on General Culture Media	General Biochemical Reactions
NA*	Gram-Positive Bacilli	Moderate to large, wavy, flat, wrinkled and white	2074777713
Microbacteriaceae	*Leifsoniaaquaticum*	Small to moderate, round or wavy, smooth or wrinkled, entire, convex or raised and yellow to buff	Catalase positive
Corynebacteriaceae	*Corynobacterium pseudotuberculosis*	Small, round, entire, convex and yellow	Catalase positive and Oxidase negative
	Corynobacterium genitalium	Large, irregular, wrinkled, raised and yellow	
	Corynobacterium bovis	Large, round, entire, convex and buff	Catalase positive
Bacillaceae	*Bacillus subtilis*	Small to large, irregular, wrinkled, raised and white to dark buff	Catalase positive
	Bacillus cereus	Large, irregular or wavy, flat and white to grey or buff	?**
	*Bacillus licheniformi*s	Large, round, wavy or irregular, raised, wrinkled and white to grey with waxy texture or not	Catalase positive
	Bacillus megaterium	Large, round, raised, entire and yellow	Catalase positive
Actinomycetaceae	*Rothia dentocariosa*	Small ,round, smooth, entire, convex and creamy	Catalase positive
	Rothia mucilaginous	Small ,round, smooth, entire, raised and pink	?**
Bifidobacteriaceae	*Gardinella vaginalis*	Small to moderate, entire, convex and white	Catalase and Oxidase positive
Erysipelotrichidae	*Erysipalothrix rhosiopathia*e	Small, round, raised and light brown	Oxidase and Indole negative

*= Not applicable as the identification system could not identify the microorganisms.
**= Biochemical reaction results were not supplied from the laboratory.

Table 4. Gram-negative rods found in environmental monitoring samples of air, their family, colonial morphology on general culture media and general biochemical characteristics.

Family	Bacteria	Colony Morphology on General Culture Media	General Biochemical Reactions
NA*	Gram- Negative Rods	Large, round, entire, convex and white	0677544443
		Small, round, entire, raised and transparent	2344210054
Moraxellaceae	*Acinetobacter lwofii*	Small to moderate, entire, raised	Oxidase and Indole negative
Flavobacteriaceae	*Chrysobacterium indologenes*	small, round, smooth, convex and buff	Oxidase positive, Indole negative and Catalse positive
Enterobacteriaceae	*Shigella spp.*	?**	Oxidase and Indole negative

*= Not applicable as the identification system could not identify the microorganisms.
**= Colonial morphology of the microorganism was not indicated in the final report from the laboratory.

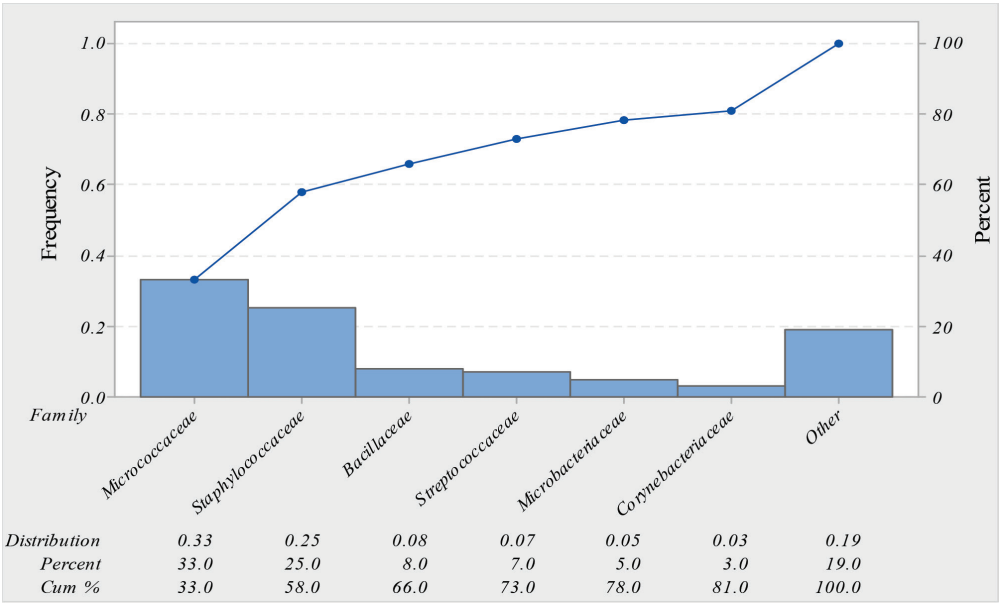

Figure 1. Pareto chart showing the 80% contribution of bacterial families from the total environmental monitoring samples of air in the pharmaceutical facility. (Graph was generated using Minitab® v17.1.0)

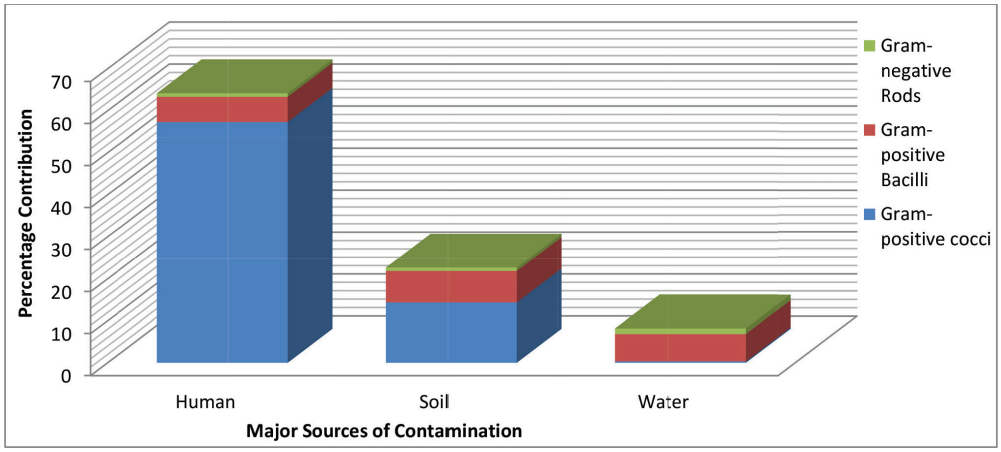

Figure 2. Major sources of microbial contamination in clean area in relation to bacterial type showing the major contributors based on the possible origin of each microorganism according to the miniaturized biochemical identification system. (Figure was generated using Microsoft Office Excel 2007)

However, when applying the quantitative risk index in the same ecological distribution a different order of priorities emerged as illustrated in Fig. 3 using Pareto chart. *Enterobacteriaceae* followed by *Streptococcaceae* (usually from mouth, throat and nasopharynx origin) on the other hand were the major source of the health risk to the final customers through the manufactured drugs. Both risk indices for active and passive air sampling showed the same order of bacterial risk distribution profile with approximately the same values when calculations performed considering action limits for passive and active air counts in class C at 50 and 100 CFU, respectively

and under the same conditions and parameters. However, the risk is doubled when considering action limits of class D.

Interestingly, applying the quantitative risk assessment showed difference in prioritization order which signified that reliance on the environmental abundance only for determining microbial risk may be misleading. The main microbial risk began with *Enterobacteriaceae*– represented by Shigella species – then *Streptococcaceae* and *Staphylococcaceae* as the greatest contributor to the hazard risk (about 99%) showed that combination of other factors should be included.

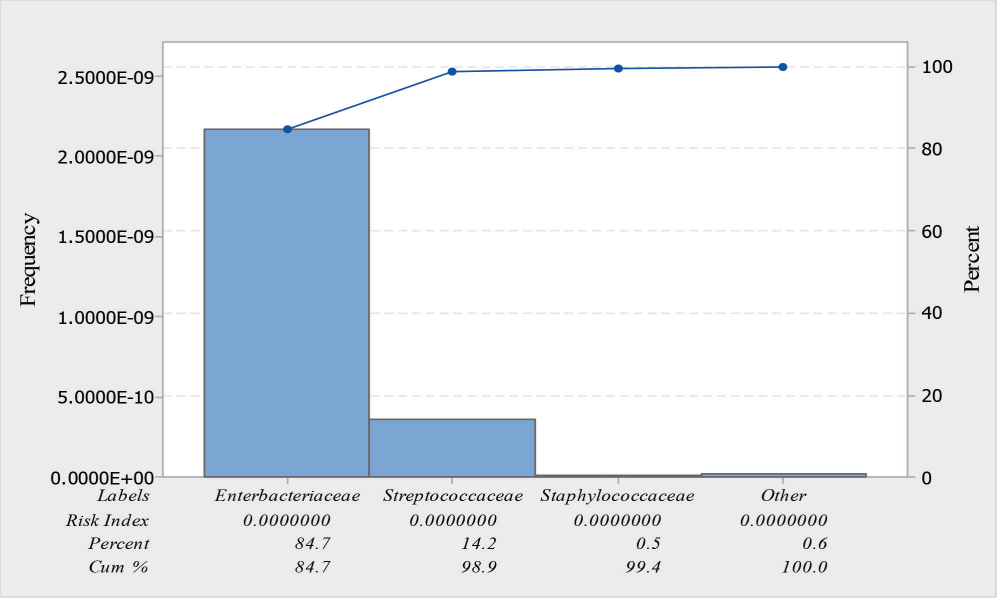

Figure 3. Pareto chart showing the 99% contribution of bacterial families from the total identified environmental monitoring samples of air in the RI at the pharmaceutical facility. (Graph was generated using Minitab® v17.1.0)

4. DISCUSSION

The bacterial distribution profile in the current study showed agreement with other researchers' findings[11]. The applied Pareto chart was helpful in identifying the major influential bacterial families in this distribution. Predominant contaminant bacteria in the clean rooms air of pharmaceutical facility were a group of Gram-positive bacteria: either spore-forming *Bacillus species*, or non-sporulating *Staphylococcus species* and *Microbacterium species*[16]. Many gram-negative species, such as *Acinetobacter species*, *Escherichia coli*, *Klebsiella species*, *Serratia marcescens* and *Shigella species*, can also survive for months[17].

However, identification of bacterial species is very important and not only the total bioburden with regard to their origin or source from which they spread in clean area and hence can be stopped or minimized. People are the major source of microbial contamination in a manufacturing environment; companies should focus on effective aseptic technique training and clean rooms behavior and establish personnel flows and maximum number of people for their various manufacturing suites [1]. The most commonly occurring microorganisms come from human skin (either commensurable or transient) includes Gram-positive microorganisms which include the following: *Staphylococcus aureus, Micrococcus species* and *Bacillus species.* Whereas those associated with eyes, ears and mucus include Gram-negative microorganisms[18].

Based on these finding, the main target to improve air quality in clean rooms of drug manufacturing facility is achieved by applying strict control in addition to the review of training process over operators gowning, attitudes, movements and behavior should decrease the potential risk of air contamination and hence the manufactured product. In wet areas such as sinks and drains, particularly where stagnant water accumulates, typical waterborne species of *Pseudomonas* and *Acinetobacter* can readily survive and grow. Airborne contamination is mainly associated with dust and skin scales and is again mostly bacterial spores and skin cocci. Contamination from process operators must be considered a significant hazard. During normal activity, loss of skin scales by shedding is about 10^4/min; a large proportion of these skin scales will be contaminated with species of the normal skin flora. These are mainly nonpathogenic micrococci, diphtheroids, and staphylococci but may also include *Staphylococcus aureus* as part of the normal skin flora. Other organisms (belonging to *Enterobacteriaceae*), not part of the resident skin flora, may also be carried transiently on the skin surface where poor hygienic practices exist among operators, and may be shed into the product via skin scales or direct contact. Cross-contamination from the outer packaging of raw materials is possible in the dispensary, although in practice, with good air handling and dust extraction, it is minimal. *Bacillus spp.* and micrococci and staphylococci from skin scales are the commonest contaminants at this stage. Because water is involved in the production of liquids, creams, and ointment products and in the cleaning of the plants, there is a potential for microbiological contamination of the production environment. The greatest danger lies in cross contamination from the manufacturing environment to the product. Water on the floor, in the drains and gullies of the manufacturing environment, and the wash areas, enables Gram-negative bacteria to grow profusely. Counts of 10^6 to 10^7 CFU/ml are easily attained in the wet areas if regular disinfection and drying is not carried out. However, the total elimination of contamination can never be effected, as there is always a reservoir of contamination in the drains. In addition, the feet of the operators and the wheels of pallet trucks are probably a major vector for transferring contamination from one area to another and it is, therefore, important to keep the floors and surfaces as dry as possible[4].

The presence of *Enterobacteriaceae* in the environmental samples is indicative to the improper sanitary behavior of the operators and possible water aerosol generated from splashes from drain or water on the floor. This finding required from quality responsible to enforce proper gowning with mouth mask and to ensure appropriate training for operators about standard hygiene procedure for work in classified area supplemented with appropriate knowledge in addition to GMP. The presence of Gram-negative bacilli is almost invariably associated with water in the production process. Good manufacturing practices are particularly important in preventing cross contamination from the plant environment to the product. Particularly at risk are those operations that may have to be carried out in close proximity to the floor, such as hose connections[4]. Other microorganisms which do not have clinical significance are assumed to have very high infective dose that approach ∞ theoretically and hence, the risk values in eq. 1 and 2 will equal zero value i.e. those microorganisms do not impose health risk even if they were much higher in number than those pathogenic and pharmaceutically objectionable ones under normal conditions.

The appearance of *Enterobacteriaceae*– represented by Shigella species – followed by *Streptococcaceae* then *Staphylococcaceae* presented the greatest contributor to the hazard risk (more than 99%) which showed that combination of other factors should be included. This is not strange in view of the long list of objectionable microorganisms published by The FDA Center for Food Safety and Applied Nutrition and this list is continuously growing [19]. Due to this situation and in order to not complicate the risk, the grouping was made in reference to bacterial family and choosing the most significant pathogen within this family (if applicable or present) as indicator or reference in the risk. This situation aroused due to the lack of specific data on the increasingly list of

microorganisms of concern. Howard *et al*., 2006 demonstrated similar situation in which completing a Quantitative microbial risk assessment (QMRA) for every pathogen that may be transmitted by water would be time-consuming and the necessary information is currently not available for many pathogens[20]. To overcome this difficulty, WHO (2004) recommended using a suite of 'reference pathogens'[21]. Average microbial count from passive air samples was approximately 26 CFU/4 hr. While the mean recovery of aerial bioburden from active air samples was about 54 CFU/m^3 in about ten minutes (about twice the result of passive air samples). This finding highlighted that the risk prioritization did not change in active air than that of passive air samples. Moreover, the values may be the same for both active and passive results at operation condition in the production facility, if both showed the same count provided that Sm = 41.67 m/hr. On the other hand, some researchers on aerial bioburden distribution in clean area concluded that if the air sampling performed during operation is carried out to monitor the risk of microbial contamination by settling, passive measurement is better than volumetric sampling at predicting the likely contamination rate at the surgical site, as it allows a direct measure of the number of microorganism settling on surfaces[22,23,24]. On the contrary, if the sampling is performed to obtain information on the concentration of all inhalable viable particles, the active method should be preferred[21].

So, it is not surprising that the value of the risk index of active air samples is higher than that of passive air, meaning that it captures more particles. The comparative sensitivity was 50 times greater for active air sampling (5.40 CFU/min.) versus passive air sampling (0.11 CFU/min.). The current attempt to develop quantitative risk assessment was similar to QMRA which is a technique that has been developed for calculating the burden of disease from a particular pathogen. The major tasks of a QMRA have been defined by Haas & Eisenberg (2001)[6]. In the current risk, bacterial air count was established on average to be at 50 CFU at operation in order to simplify the analysis for the newly established companies especially at the absence of previous historical trending of data for environmental monitoring. However, by the time a trend can be established, control charts generated and the exact risk can be assessed for each area, rooms and condition. Napoli *et al*., 2012 have demonstrated that when a strict protocol is followed results of active and passive sampling correlate in a comparable way with the quality of air for both *at rest* and *in operational* sampling [25]. In the meantime, it is possible to conclude that both methods can be used for general monitoring of air contamination, such as routine surveillance programs. Under standard conditions, the mean deposition rate in active air sampling equation is 41.67 m/hr for the equivalent average viable particle diameter 19.64 µm. This is not strange in the view of that Tham and Zuraimi 2005 demonstrated that the main contributor of viable bacteria was humans and postulated that bacteria viable particles of size >7.5 µm may be due to rafting of microorganisms on skin scales shed by the subjects[26]. Also at 26 °C, bacteria of size >7.5 µm correlated with exhaled carbon dioxide indicating nasal carriers. Airborne microorganisms are not free-floating or single cells, they frequently associate with particles of 10 to 20 µm. Particulate counts as well as microbial counts within controlled environments vary with the sampling location and the activities being conducted during sampling[3]. Whyte, 1986 demonstrated that most air borne microorganisms can be carried on physical particles of 12 µm or larger which are heavy enough to settle out of air by gravity [27].

5. CONCLUSION

The conclusion that could be drawn is that both the microbial ecological distribution and the quantitative RI are complementary and should be used together to identify the major factors affecting the quality of the pharmaceutical products with subsequent effect on the final consumers. The current study highlighted that the core source microbial contamination is human. Thus, appropriate behavior, attitude and

training have major rule in limiting the hazard of such contamination. The study indicated that if good personnel monitoring and hygiene were implemented microbiological air quality would be positively improved. Both active and passive air sampling risk assessment indices are important in determining microbial hazard to the final customers through medicinal products but with different prospective. However, their values may vary according to their difference in capabilities to recover aerial microorganisms under identical experimental conditions. This is the first time a quantitative microbiological risk assessment based on ecological quality and quantity for the distributed of bacteria was used within EM programs in pharmaceutical facility.

6. ACKNOWLEDGEMENT

This work was supported partially financially by HIKMA Pharma pharmaceutical company – 2nd Industrial zone - 6th of October city. Reference and writing style review was performed by Dr. Engy Refaat Rashed. Microbiological sampling and data gathering and collection were performed by the microbiology laboratory team of the quality control department.

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