Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [11] October 2018 : 07-13

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Analysis of Microbial Quality of the Air in Meat and Dairy Plants by Impaction Technique

Vinayananda, C O*[1], S.J.Deepak[2], Rongsensusang[1], A.Elango[2], K.Porteen[2], V.Apparao[1], and B.Dhanalakshmi[3]
[1]Department of Livestock Products Technology(Meat Science),
Madras Veterinary College, Chennai-600 007
[2]Department of Veterinary Public Health and Epidemiology, Madras Veterinary College,Chennai-600 007
[3]Department of Livestock Products Technology(Dairy Science),
Madras Veterinary College, Chennai-600 007
* **E-mail:** vinvet22@gmail.com

ABSTRACT
The air quality in dairy and meat processing plants often goes neglected, yet they exert a great influence over the shelf life and quality of final product. The present study was designed to analyze the microbiological quality of air in institutional meat and dairy processing plants of Madras Veterinary College. The air sampling was done using impaction technique for meat plant in slaughter hall, processing room, chilling room and further processing room. Similarly, for dairy plant it was done in the milk reception area, pasteurization unit and packaging rooms. The enumeration of aerobic count,psychrophilic count, yeast and mold count and occurrence of Staphylococcus aureus in slaughter house and dairy plant was carried using air sampler (impaction method). On analysis the microbial counts of slaughter house revealed that, slaughter hall has significantly high number of aerobic count followed by processing room, further processing rooms and chilling room. Yeast and mold count was significantly more in slaughter hall. In dairy plant, reception area has shown significantly high aerobic count and yeast and mold count was significantly low in the pasteurization area compared to reception and packaging area. There was no significant difference in the psychrophilic counts among the different sections of slaughter house and dairy plant units. The occurrence of Staphylococcus aureus was observed in slaughter hall, processing and further processing section of slaughter house and only in reception section of dairy plant. The results of air microbes obtained in the present study for all the sections of slaughter house and dairy plant by air sampler technique were well within the recommendations given by APHA for food processing plants. With respect to dairy and meat processing industry this study on bio-aerosols mainly helps us to implement the clean room practices for good manufacturing practices.
***Keywords*:** *Aerobic count, Psychrophilic count, Yeast and molds counts, Staphylococcus aureus, Hygiene*

Received 22.06.2018 Revised 22.07.2018 Accepted 06.10.2018

INTRODUCTION

The quality of air isoften neglected factor in dairy and meat industry processing which contributes to the quality of the final product. The air is an agglomeration of physical, chemical and microbial components which affect the quality of air in processing plants. Indoor microbial air quality depends on several factors, such as ambient air, soil, human activity, microclimatic factors, geographic location, hygienic practices and ventilation type [1].Bio-aerosols are particles of biological origin suspended in the air, e.g. bacteria, fungi, virus, toxins and plant debris [2].The size of aerosols varies between 0.02 to 100 µm in diameter [3]. These microorganisms could affect air quality, ecosystem and human health [4]. They have potential of spreading airborne infectious, spoilage and pathogenic microbial agents through droplets and bio-aerosols [5,6].

The demand for safe meat and dairy products with extended shelf life have been put increased importance on the microbial quality of air in processing environments. The potential transfer of microbes through air can affect the quality and food safety objective. Contaminated air is often involved in reduction of shelf life and may serve as vehicle for transmitting spoilage and pathogenic organisms [7]. Contamination of meat and dairy products by microorganisms is a major public health and economic

problem for the industry [8]. In meat industry, unscientific slaughter process can lead to microbial contamination of carcass by hide or gastrointestinal tract of the animal or from the slaughter plant environment, including facilities and personnel. Few studies have shown the involvement of air and slaughter hall practices leading to contamination of carcass[9], beef carcass[10], poultry plants [11] and pork processing [12]. Air quality in the reception, processing and packaging area is the critical control points in production of good quality meat and dairy products and should be monitored on a regular basis. Few studies have shown the characteristics of air in dairy plant [13]. Every precaution should be taken to prevent airborne contamination of the product during and after processing [14, 15].

Air sampling in food manufacturing environments can be undertaken for three main reasons. Firstly, during process development, it is useful to record the number of microorganisms in the air throughout the production day to establish whether events occur that lead to high microbial counts. Secondly, when a process has been established, the contribution of the air in terms of microbial cross-contamination to the product can be determined. This may have an effect on determining any additional controls necessary, particularly if the contribution is significant compared to other cross contamination vectors (food contact surfaces, operative's hands, etc.). Thirdly, air sampling can be used to verify the performance of specific prerequisites designed to control airborne microbial levels, such as air filtration systems or assess the ongoing risk of potential microbial aerosol sources such as evaporative condensers. There are various techniques are available for determining the number of microorganisms present in the air of food processing environment[16,17]. Microorganisms retain their virulence in the air, thus it is necessary to control air contamination in food production areas in order to facilitate detection and elimination of potential health hazards resulting from their presence [18]. There are several techniques for estimation of microbial load of air, among those impaction technique is better to recover higher number of microbes in air at faster rate and more sensitive compared to settling plate method. But initial cost is more compared to other technique This technique is classified as method B by American Public Health Association(APHA)[19]. The aim of present study was to assess the air microbial quality of selected production facilities/sections of a meat and milk processing plants by impaction method using air sampler.

MATERIALS AND METHOD

Study Design

The evaluation of air for presence of microorganisms in meat and dairy plants ofMadras Veterinary College, Chennai was carried out using impaction technique using air sampler during December-March, 2017. The sampling for meat plant was as follows slaughter hall, processing room, chilling room and further processing room were evaluated separately in triplicates during a working hour of the plant. Similarly, for dairy plant milk reception area, pasteurization unit and packaging rooms were evaluated separately in triplicates during a working hour of the plant. The enumeration of total viable bacterial count (aerobic and psychrophilic), yeast and mold count and the occurrence of *Staphylococcus aureus* were studied according to APHA[14].

Air Sampling

Air sampler system used for evaluation of the microbial quality of different study environment. Air sample was collected using HiMedia. No LA002air sampler system, which works based on the principle of Centrifugal impaction (Maximum sample volume is 2520 liters and flow rate air is 280 lit/min). The air sampler's cup was sterilized at 121 °C for 15 minutes, sanitized with 70 % ethyl alcohol, before and after each sampling.

Sterile plastic air sampler strips were aseptically filled with sterile molten respective agar medium and same strips were used for air sampling after solidification. For air sampling one such strip was carefully inserted into the slot in the metal cup without touching the agar surface of the strip. After loading the strip, the timer of the control box set for 3 minutes. After collecting the sample, the strips were placed back into the wrappers and incubated. The sampling was in duplicate for aerobic, psychrophilic, yeast and mold counts and consisted of 1 cubic meter of air per sampling.

Air Microbiology

The Plate count agar (HiMedia,India) was used for aerobic and psychrophilic counts,sabrouds dextrose agar (HiMedia,India)was used for yeast and mold count; whereas for detection *Staphylococcus aureus,*standard cultural method followed by biochemical and molecular confirmation was carried. The experiment was conducted with a threefold repetition for each microbiological parameter. After air sampling strips were kept back in their respective wrappers and incubated at 37 °C for 48 hours for determination ofaerobic /TVC(Total Viable Count) and 8°C for 48hours for determination of psychrophilic count. For, yeast and mold count incubation was at 25 °C for 5 days. The level of bacterial load in air per cubic meter was calculated by using the following formula[20]

$B = 1000N/RT$ cfu/m^3

Where, B is Bacterial load
N is the number of colonies counted on the sample plate,
T is the duration of test in minute,
R is the air sampling rate in liters/minute,
cfu- colony forming units.

Identification of *Staphylococcus aureus*

The Air sampler strips with plate count agar after sampling were transferred to brain heart infusion (BHI) (HiMedia, India) broth and incubated at 37°C for 24hours.Selective plating was carried with a loop full of broth on Mannitol salt agar (HiMedia, India) and incubated at 37°C for 24 hours based on morphological characteristics and further confirmation was done by biochemical tests according to Bacteriological Analytical Manual[21]. The molecular confirmation was done using polymerase chain reaction (PCR) assay with primers for organism specific thermonuclease (nucA) gene as described by Brakstad[22] with slight modification. DNA was extracted from 2-3 suspected colonies employing hot boiling and snap chill techniques as described by Zahrei and coworkers [23]. The nucleotide sequences of the forward and reverse primers were 5'- GCGATTGATGGTGATACGGTT -3' and 5'- AGCCAAGCCTTGACGAACTAAAGC -3', respectively with amplicon size of 267bp.PCR amplification were initial 94°C denaturation step for 5min followed by 30 cycles, with each cycle consisting of 30s at 94°C for denaturation, 30s at 55 °C for primer annealing, 30s at 72°C for strand elongation and the final cycle at 72°C for 5 min. The PCR products were electrophoresed on 1.5% agarose gel pre-stained with ethidium bromide (0.5ug/mL) and viewed under UV light using a UV trans illuminator with the DNA bands sized by extrapolation based on mobility of 100 bp DNA markers co-electrophoresed.

Statistical analysis

The samples were collected and analyzed on three separate occasions. The data were subjected to one-way analysis of variance as per Snedecor and Cochran[24] and Tukey's multiple range test using SPSS (SPSS version 20.0 for windows; SPSS Inc., Chicago, IL) for comparing the air microbial load means to find significant ($P < 0.05$) differences among the different sections of meat and milk processing plants.

RESULT AND DISCUSSION

Meat and milk products are not only highly nutritious foods of animal origin but also good medium for microbial multiplication and source of food borne pathogens. In the production process, farm to fork involves many critical points, in which the most neglected critical point is atmospheric air of the manufacturing facility. Microbial contamination results in spoilage of meat, reduced shelf life of meat and public health hazards. It is generally accepted that microbial loads on surfaces and equipment vary in different food plants depending on the microbial quality of the raw material and plan of processing plant[25].

In the present study, air sampling and analysis was done for different sections of meat and dairy institutional demonstration plants. The enumeration of aerobic count, psychrophilic count, yeast and mold count and occurrence of *Staphylococcus aureus* in slaughter house and dairy plant was carried using air sampler strip (Figure 1).The results of the microbial analyses concerning aerobic count, psychrophilic count, yeast and mold counts by the impaction method are presented in Table 1.The PCR gel image of *Staphylococcus aureus* confirmed (Figure 2). The aerobic count, psychrophilic count, yeast and mold counts were highest in slaughter hall of meat plant and reception area of dairy plant. The lowest microbial counts at meat plant were observed for aerobic count, psychrophilic count, yeast and mold counts in chilling room, processing room and further processing room, respectively. Similarly, lowest counts for dairy plant observed in packaging section for TVC and psychrophilic count but pasteurization unit for yeast and molds count respectively. On statistical analysis of microbial counts of slaughter house revealed that, slaughter hall has significantly high number of aerobic count followed by processing room, further processing rooms and Chilling room. Yeast and mold count was significantly more in slaughter hall and there was no significant difference among the Processing, Chilling and Further processing sections. Among the three section examined in dairy plant, reception area has shown significantly high aerobic count and there was no significant difference among the pasteurization and packaging sections. The yeast and mold count was significantly low in the pasteurization area compared to reception and packaging area. There was no significant difference in the psychrophilic counts among the difference sections of slaughter house and dairy plant units. Air microbial contamination varied and depended on the sampling site, the time of sample collection and the methodology used to assess the contamination level [26].

The skin and internal organs of slaughtered animals has been the important source of air borne bacteria in slaughter houses [27]. The reception of milk and personnel involvement increases the bacterial load. The realization claim that bio-aerosols transport bacteria contributes to the further contamination of

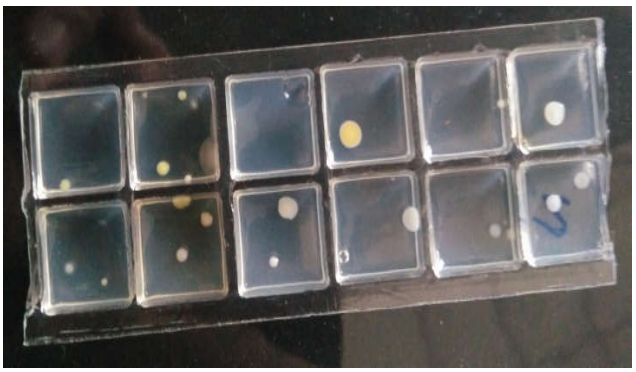
dairy and meat products. And, various practices involved in carcass dressing and slaughter lead to formation of aerosols have potential to spread the infectious or spoilage organism [28, 29].The mean total plate count of different slaughter houses and mutton stalls at different locations in Bangalore city was found to be 45 cfu (colony forming unit) for procurement area and 134.5 cfufor degutting area with sedimentation technique of air sampling[30](Ahmed and Sarangi, 2013). Similarly, in dairy plant Salustianoand co-workers[19] reported, aerobic bacteria to be 313cfu/m^3 for milk reception, 161cfu/m^3 for pasteurization section and 100cfu/m^3 for milk packaging unit with impaction technique of air sampling. In another study by Radha and Lakshmi [31] atUniversity dairy plant of Kerala Veterinary and Animal Sciences University found that mean total aerobic counts to be 32.66 cfu/m^2, 25.32 cfu/m^2 and 33.36 cfu/m^2 for raw milk reception dock, pasteurization room and product preparation room, respectively. Salustiano *et al.*[19] reported yeast and mold count in dairy plant by was found to be 111 cfu/$m^3$for milk reception area, 176 cfu/m^3 for pasteurization section and 184.4 cfu/m^3 for milk packaging unit with impaction technique of air sampling. The APHA recommendation of aerobic count and yeast and mold count to be less than 100 cfu/m^3. In addition to APHA others proposed recommendation are according to Kang and Frank [7] maximum levels of mesophilic aerobic bacteria of air could be 180-360 cfu/m^3 and yeast and molds could be 70-430 cfu/m^3 for in milk processing plants. According to Krzysztofik [32], the permissible level of aerobic count of air in meat industry should be less than 500 cfu/m^3 by settle plate technique. The results of aerobic, psychrophilic, yeast and molds counts obtained in the present study for all the sections of slaughter house and dairy plant by air sampler technique were well within the recommendations given by APHA for food processing plants.The results obtained in the present study were in complete agreement with the results reported by other workers[19,33,34].

The occurrence of *Staphylococcus aureus* was observed in slaughter hall, processing and further processing sections of slaughter house and only in the reception section of dairy plant, which may be attributed to the higher personnel activity in these areas of processing plants. Salustiano *et al.*[19] reported 1-4.3 cfu/m^3 of *Staphylococcus aureus* count in the milk packaging room. Radha and Lakshmi [31] observed significantly high *staphylococcus* count in product preparation room than raw milk reception dock and pasteurization room. Even regular activities of working personnel like speaking, breathing, sneezing and coughing were also the major reason for aerosol production in a processing plant along with that water spraying system and drains contribute for aerosol production interns associated with the significant increase in *Staphylococcal* count, this validates the importance of controlling airborne contamination in the meat and milk processing plants [31,35, 36].

Table 1: Enumeration of aerobic count, psychrophilic count, yeast and mold count and occurrence of *S. aureus* in air samples analyzed in different section of meat and dairy plant.

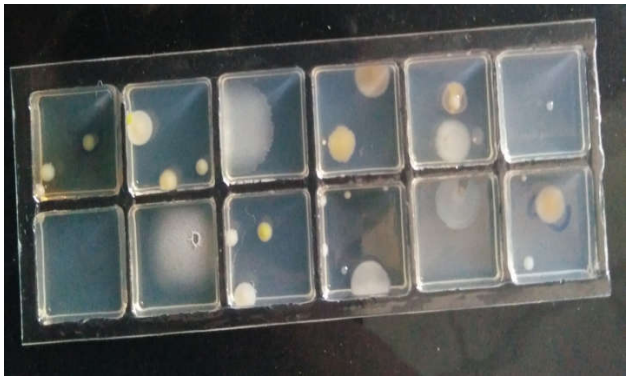
Study area	Aerobic count (TVC)	Psychrophilic count	Yeasts and molds count	Occurrence of *S. aureus*
Slaughter House				
Slaughter hall,	56.67±2.60^a	2.67±1.40^a	32.00±2.50^a	+
Processing room	35.67±1.45^b	1.33±0.88^a	14.33±2.33^b	+
Chilling room	16.00±2.64^c	1.67±0.88^a	13.33±0.88^b	-
Further processing	27.67±2.02^d	1.67±0.88^a	9.33±2.33^b	+
Dairy Plant				
Reception	58.33±4.91^a	2.67±0.33^a	38.67±2.72^a	+
Pasteurization section	27.67±2.40^b	2.33±0.33^a	16.67±2.03^b	-
Packaging section	21.0±01.73^b	1.67±0.33^a	28.00±2.51^a	-

Note: Superscript (^{a,b}) indicates significance difference for the corresponding parameter



A

B



C

Figure 1: Pictorial representation of air sampler strip after incubation: (A) Aerobic count (TVC), (B) psychrophilic count, (C) Yeast and mold count.

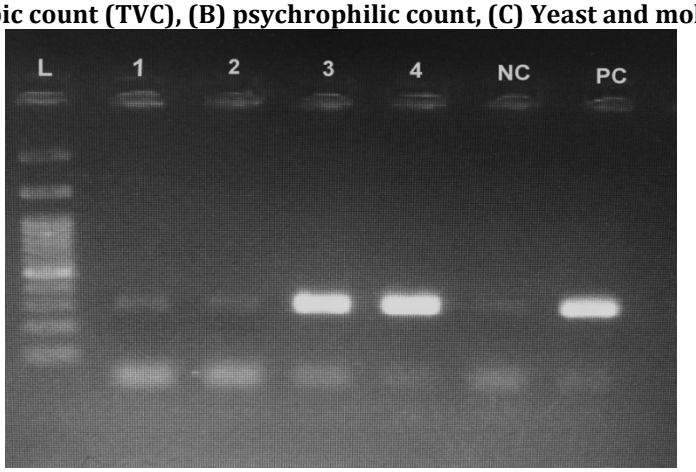


Figure 2: PCR pictorials representation of *Staphylococcus aureus* with product size of 267bp (L: ladder, 1-4: samples, NC: negative control, PC: positive control)

CONCLUSION

It is revealed that, the results of aerobic, psychrophilic, yeast and molds counts obtained in the present study for all the sections of slaughter house and dairy plant by air sampler technique were well within the recommendations given by APHA for food processing plants. Further, the occurrence of *Staphylococcus aureus* in meat and dairy plant, may be attributed to higher personnel activity in theareas examined.In this regard, it is important to practice effective personal and air hygiene to prevent possible adverse human health. An investigation on air quality in dairy and meat processing plants for institutional demonstration setup helps us to understand the scientific design and importance of hygienic practices employed.

ACKNOWLEDGEMENT

Authors like to thank the support and cooperation provided by Department of Livestock Products Technology(Meat Science), Department of Livestock Products Technology(Dairy Science), Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.

CONFLICT OF INTEREST: None

REFERENCES

1. Jung, C., Wu, P., Tseng, C. & Su, H. (2015). Indoor air quality varies with ventilation types and working areas in hospitals. Build.Environ.,85:190–195.
2. Haig, C.W., Mackay, W.G., Walker, J.T. & Williams, C.(2016).Bioaerosol sampling: sampling mechanisms, bio-efficiency, and field studies. J. Hosp. Infect.,93:242–255.
3. Maier, R., Pepper, I. &Gerba, C.H.(2009).Environmental Microbiology, 2nd ed., Academic Press: New York..
4. Despres, V.R., Huffman, J.A., Burrows, S.M., Hoose, C., Safatov. A.S.&Buryak, G.(2012). Primary biological aerosol particles in the atmosphere: a review. Tellus. B. Chem. Phys.Meteorol.,64:58.
5. Fernstrom, A. & Goldblatt, M. (2013). Aerobiology and its role in the transmission of infectious diseases. J.Pathog..http://dx.doi.org/10.1155/2013/493960.
6. Luongo, J.C., Fennelly, K.P., Keen, J.A. Zhai, Z,J., Jones, B.W. & Miller, S.L. (2016). Role of mechanical ventilation in the airborne transmission of infectious agents in buildings (Review). Indoor Air, 26(5): 666–678.
7. Kang, Y.I& Frank, F.J. (1989) Biological aerosols: A review of airborne contamination and its measurement in dairy processing plants. J. Food Prot., 52:512-524.
8. Lin, J., Kuang-Sheng, Y., Hsueh-Tao, L. &Jiunn-Horng Lin. (2009)*Staphylococcus aureus* Isolated from Pork and Chicken Carcasses in Taiwan: Prevalence and Antimicrobial Susceptibility. J. Food Prot.,72(3):608–611.
9. Rahkio, T.M.&Korkeala, H.J. (1997) Airborne bacteria and carcass contamination in slaughterhouses, J. Food Prot., 60:38-42.
10. Sofos, J.N., Kochevar, S.L., Reagan, J.O. & Smith, G.C. (1999). Extent of beef carcass contamination with Escherichia coli and probabilities of passing regulatory criteria. J. Food Prot., 62:234-238.
11. Lutring, K.R., Linton, R.H., Zimmerman, N.J., Peugh, M. & Heber, A.J. (1997) Distribution and quantification of bioaerosols in poultry slaughtering plants. J. Food Prot., 60:804-810.
12. Kotula, A.W., Semswiler-rose, B. (1988). Airborne microorganisms in a pork processing establishment. J. Food Prot. 12:935-937.
13. Kang, Y.J. & Frank, J.F. (1990). Characteristics of biological aerosols in dairy processing plants. Dairy Sci. Technol. 73:621-626.
14. Hickley, P.K.,Beckeheimer, C.E. &Parrow, T. (1992). Microbiological tests for equipment, containers, water and air.Standard methods for the examination of Dairy products. 16th Ed. American Public Health Association, Washington DC, 397-412.
15. Mostert, J.F. & Jooste, P.J. (2002). Quality control in the dairy industry. In: Robinson R.K. editors. Dairy Microbiology Handbook. 3rd ed. John Wiley and Sons, New York, 655–736.
16. Miettinen H. (2005) Improving air sampling. (eds.Lelieveld, H.L.M., Mostert, M.A., Holah, J.)In: Handbook of hygiene control in the food industry. Woodhead Publishing Series in Food Science, Technology and Nutrition No. 116. Cambridge, 619–640.
17. Wirtanen, G., Miettinen, H., Pahkala, S., Enbom, S. &Vanne, L. (2002). Clean air solutions in food processing. VTT Publications 482, Espoo, Finland.
18. Kaiser, K.&Wolski, A. (2007) Control of air microbial quality. Refrigeration & Air- Conditioning Engineering. 4:159-162.
19. Salustiano, C.V., Andrade, J.N., Brandao, S.C., Azeredo, R.&Mandlima, S.M. (2003). Microbiological air quality of processing area in a dairy plant as evaluated by the sedimentation technique and a one stage air sampler. Braz. J. Microbiol.,34:252-259.
20. Senior, B.W. (2007). Examination of water, milk, food and air. (edsColleeJ. G.; Fraser A. G; Marmion B. P. Simmons A Mackie & Mc Cartney)In:Practical medical microbiology. 14th ed. Elsevier India: Churchill Livingstone, 908-910.
21. BAM (Bacteriological Analytical Manual). (2010). U.S. Food & Drug Administration. 8th Ed (Revision A /1998). Centre for Food Safety & Applied Nutrition.

22. Brakstad, O.G., Aasbakk, K. &Maeland, J.A. (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. J. Clin. Microbiol., 30:1654-1660.
23. Zahrei, S.T., Mahzounish, M. &Saeedzadeh. (2005).The isolation of antibiotic resistant Salmonella from intestine and liver of poultry in Shiraz province of Iran. Int. J.Poult. Sci., 4(5): 320-322.
24. Snedecor, G.W. & Cochran, W.G.(1999). Statistical methods. 8th ed. The lowa state university, Ames, Lowa, 4313.
25. Evans, J.A., Russell, S.L., James, C. & Corry, J.E.L. (2004). Microbiological contamination of food refrigeration equipment. J. Food. Eng., 62:225-232.
26. Konieczny, P.I., Cegielska-Radziejewska, R.I., Mroczek, E.I. &Dziedzic, J.I. (2016). Analysis of Air Quality in Selected Areas of a Poultry Processing Plant with the Use of a Microbiological Air Sampler. Rev. Bras.Cienc.Avic., 8(3):401-406.
27. Nottingham, P.M., Penny, N. & Harrison, J.C. (1974). Microbiology of beef processing, I. Beef dressing hygiene, N.Z.J. Agris. Res.:17:79-83.
28. Burfoot, D., Reavell, S., Tuck,C. &Wilkinson, D. (2003). Generation and dispersion of droplets from cleaning equipment used in the chilled food industry. J. Food Eng., 58:343–353.
29. Prendergast, D.M., Daly, D.J., Sheridan, J.J., Mcdowell, D.A. & Blair, I.S. (2004). The effect of abattoir design on aerial contamination levels and the relationship between aerial and carcass contamination levels in two Irish beef abattoirs, Food Microbiol.,21:589–596.
30. Azhar, S.A&Sarangi, S.K. (2013). Comparative Studies on the Air Microflora in Some Slaughtering Houses of Bangalore City. Int. J. Pharm. Sci. Invent., 2(9): 11-14.
31. Radha,K. & Lakshmi, S.N. (2014). Studies on the air quality in a dairy processing plant. Indian J.Vet. Anim. Sci. Res.43(5):346 – 353.
32. Krzysztofik, B. (1992).Mikrobiologiapowietrza. Wyd. PolitechnikiWarszawskiej, Warszawa, Polska; 19-20. Polish.
33. Lück,H. &Garvon. (1990). Quality control in the dairy industry(Eds..Robinson, R.K)In:Dairy Microbiology- the Microbiology of Milk products, 2nd Ed. Elsevier Applied Science, London, 2:345-392.
34. Belestioids, E, Ghikas, D. &Kalantzi. (2011). Incorporation of microbiological and molecular methods in HACCP monitoring scheme of moulds and yeasts in a Greek Dairy Plant: A case study -11th International congress on Engineering and Food (ICEF11), Procedia. Food Sci.,1051-1059.
35. Ren, T.J. & Frank, F.J. (1992). A survey of four fluid milk processing plants for airborne contamination using various samping methods. J. Food Protect.,55:38-42.
36. Srikanth, P., Sudharsanam, S. & Steinberg. R. (2008). Bio-aerosols in indoor environment: composition, health effects and analysis. Indian J. Med.Microbiol. 26:302-312.

CITATION OF THIS ARTICLE

Vinayananda, C O, S.J.Deepak, Rongsensusang, A.Elango, K.Porteen, V.Apparao, and B.Dhanalakshmi. Analysis of Microbial Quality of the Air in Meat and Dairy Plants by Impaction Technique. Bull. Env. Pharmacol. Life Sci., Vol 7 [11] October 2018: 07-13