

Air Bacterial Contamination from Hydro debridement of Wounds.

Bowling F L*

Stickings D S*

Edwards-Jones V**

Armstrong D G***

Boulton A J M*

From the Department of Medicine, Manchester Royal Infirmary, University of Manchester*. Manchester Metropolitan University Department of Clinical Microbiology** United Kingdom and the Southern Arizona Limb Salvage Alliance (SALSA), Department of Surgery, University of Arizona College of Medicine,*** Tucson, Arizona, USA

Address correspondence to Frank L Bowling, University Department of Medicine, Manchester Royal Infirmary, Manchester, M13 9WL, United Kingdom

+44 161 276 4406

frank.bowling@manchester.ac.uk

Key words: wounds, debridement, micro-organisms, air sampling.

Abstract

Aims: The purpose of this study was to assess the level of air contamination with bacteria after hydro surgical debridement and to determine the effectiveness of hydro surgery on bacterial reduction of a simulated infected wound.

Methods: Four porcine samples were scored then infected with a broth culture containing a variety of organisms and incubated at 37°C for 24 hours. Fallout (settle) culture plates were located at set distances around the clinic room area for passive sampling. The infected samples were then debrided with the hydro surgery tool (Versajet, Smith and Nephew, Largo, Florida, USA). The air was sampled using the SAS-Super 90 air sampler. Samples of sample were taken for microbiology, histology and scanning electron microscopy pre-infection, post infection and post debridement.

Results: No significant reduction in bacterial load was observed post hydro debridement. Analysis of the passive sampling showed a significant ($P < 0.001$) increase in microbial counts post-hydro debridement. Levels ranging from 950 cfu/m³ to 16780 cfu/m³ were observed with active sampling of the air whilst using hydro surgery equipment. During removal of the wound dressing, a significant difference was observed relative to basal counts ($P < 0.05$). Microbial load of the air samples was still significantly raised 1 hour post-therapy.

Conclusions: The results of this study suggest significant microbial fallout after hydro debridement of colonized tissue. We believe that action might be taken to mitigate fallout in the settings in which this technique is used.

Introduction

Treatment of chronic wounds frequently requires a combination of medical and surgical therapy to effect successful healing. Non-viable tissue may serve as a source of infection and thereby retard wound healing or increase the risk for complications. ¹⁻³

Therefore by removing necrotic tissue and reducing the bacterial load on the wound surface, wound debridement may assist in healing. ^{4, 5}

There are numerous wound debridement techniques available to the clinician.⁶ Surgical (also known as sharp) debridement using a scalpel or a biopsy is considered the optimal method for rapidly cleaning the ulcer and converting it to an acute wound; however it can be painful and not all practitioners are trained or permitted to perform such procedures. Other mechanical forms of “sharp” debridement include pulsed lavage, ultrasound disruption of debris, and high-pressure water jet dissection of the wound surface.⁷ These alternative techniques may possibly serve to reduce biofilm prevalence and local bacterial burden thereby stimulate the repair process. Additionally, they may better be able to debride superficial slough than traditional biopsy or scalpels. Over the past several years, these alternate mechanical methods of debridement have become increasingly commonplace both in operating rooms and in clinical settings worldwide and are generally well-regarded by clinicians that employ them. We are, however, unaware of prior reports that have evaluated the potential for aerosolization of particulates, namely bacteria, into the peri-operative environment whilst using these modalities.

The purpose of this study was therefore to evaluate the potential for aerosolization of microbes during hydro-debridement therapy and additionally determine the effectiveness of hydro surgery in reducing the amount of bacteria in a simulated infected wound.

Method

Four porcine joints with skin intact were purchased on day 1 of the study and disinfected with 90% alcohol. Artificial wounds were created with a scalpel blade to produce three wound sites per specimen; a superficial wound (site 1), a deep wound without a sinus (site2) and a deep wound with a sinus (site3).

Baseline sampling

Biopsies were taken from site one of each porcine specimen using a 6mm sterile cutter. Samples were collected in normal saline for histology and scanning electron microscopy (SEM). Three swabs were taken from each site of each specimen.

Specimen Inoculation

On completion of baseline sampling the artificial wounds on each of the four specimens were inoculated with various pathogens. Specimen one was infected with Oxford Staphylococcus, specimen two with Pseudomonas Aeruginosa, specimen three with Escherichia coli. Specimen four was infected with 1ml of an overnight polymicrobial broth culture derived from a patient with an MRSA colonised wound. The specimens were then incubated in a sterile container overnight at 37 °c. Swab and biopsy samples were taken after incubation and again following debridement using the same method described for baseline sampling.

Swabs were immersed in 1 ml of phosphate buffered saline (PBS) and vortex mixed to promote equal bacterial suspension. 0.1ml of suspension was removed and added to 9.9ml of PBS to produce a 10⁻² dilution. Culture plates were inoculated with 50 ul using a Spiral Plater. Mannitol salt agar was used for detection and enumeration of Staphylococcus Aureus, Nutrient Agar for P. Aeruginosa and MacConkey Agar for E.coli. Plates were incubated for 24 hours at 37 degrees centigrade after which further dilution was achieved by adding 0.1ml of the incubated sample to 9.9ml PBS for 10⁻⁴ dilution. Plates were again inoculated and incubated as above. The resulting colony forming units (CFU's) were counted using an image analyser.

Biopsies taken for microbiology were placed in 1ml of PBS and weighed then vortex mixed. The contents were ground in a sterile grinder until the tissue was evenly homogenised then transferred to a sterile universal container for five minutes of further vortex mixing. This was then processed as for the swabs.

Biopsies for electron microscopy were fixed in 10% neutral buffered formalin for 48 hours to kill any bacteria. The formalin was removed by washing in distilled water then passing through various concentrations of alcohol to remove residual water before allowing drying. Gold was used as a sputter coating before being mounted for viewing on the SEM.

Hydro-debridement

All specimens were debrided consecutively on the same day and in the same treatment room. The room was approximately 3 metres by 5metres in size and representative of a typical outpatient clinic. In keeping with operating room requirements there was no controlled air flow. The clinical room was disinfected after each debridement and a two hour rest period followed when the proceeding treatment was a different specimen. This approach was in accordance with local infection control policy to allow for dispersal of any pathogens.

The Versa – jet (VJ) operator had undergone training in the theory and practise of the Versa – jet in a clinical setting. During debridement the operator wore gloves, plastic apron, bonnet, visor and mask for protection against contamination and injury.

Evaluation of air bacterial contamination- active sampling

Air sampling took place at three stages in the treatment process using the SAS-Super 90 air sampler (SAS). One hour before debridement the air was tested to provide baseline data. Specimens were presented for treatment with a dressing over the artificial wound which was removed immediately prior to debridement using a sterile non touch technique at which point another air sample was taken. This was representative of clinical treatment sessions allowing for the possibility of aerosolisation of bacteria from the wound.

Specimens were debrided until “surgically” clean which took approximately five minutes to complete for the three sites on each specimen. During the procedure the air was

sampled at 100 litres per minute on the right hand side of the VJ operator. The SAS was positioned 2.5 metres from the operator with a horizontal distance of 2m and a vertical height of 1.5m (where, $2.5 = \sqrt{2^2 + 1.5^2}$). Final samples were collected following treatment completion after 5,15,30 and 60 minutes. All samples were analysed for microbial content.

Evaluation of air bacterial content – passive sampling

Settle plates containing Tryptone soya agar (TSA) were placed at floor level then 1,2 and 3 metres from the active sampling area. Plates were 90mm diameter.

Statistical analysis was by Minitab v15. Significance testing for the parametric data was performed with a two sample T test.

Results

Microbiology

No surface contaminating organisms were identified from the pre-inoculation sampling.

Following debridement with the Versa jet all wound sites in all specimens appeared clean and free from visible signs of decay.

Bacterial counts obtained from specimens before and after VJ treatment showed no significant difference (Table 1). Five of the twelve samples (42%) showed a non-significant reduction in bacteria with a 1-1.5 log reduction in the post debridement bacterial count.

The biopsy samples yielded up to 1 log reduction in bacterial counts with the E.Coli specimens showing the greatest decrease.

Wound type did not have an affect on bacterial numbers obtained pre and post treatment.

Figure 2 illustrates Staphylococcal counts from Specimen 1 pre and post Versa-jet for all three wound sites.

Results for active air sampling

Air samples taken during dressing removal showed a significant increase ($P < 0.05$) in microbial counts relative to baseline.

During the actual debridement process the infecting organism was isolated from the air and in the case of specimen 2 air samples contained *Staphylococcus aureus* from the previous debridement. (Table 2)

Figure 3 illustrates *P. Aeruginosa* isolated from active sampling during Versa-jet use.

Microbial levels ranged from 950cfu/m³ to 16780 cfu/m³ (n=3) (Table 4) during treatment and although counts decreased after treatment cessation the microbial load of air samples was still significantly raised one hour post therapy at 850 cfu/m³ (Figure 4) compared to background levels.

Results from passive air sampling

The results from the settle plates showed a higher number of cfu's in the 1m and 2m zones when compared to the 3 m zone (Table 3). Statistical analysis of the plates showed a significant ($P < 0.001$) increase in average microbial counts from 50.4 cfu/plate/time (cfu/p/t) pre-treatment to 90.2 cfu/p/t post treatment.

During debridement of specimen 1 the Versa-jet became temporarily blocked and there was a large increase in cfu's on the plates to the extent that they were too numerous to count. This is shown in figure 5.

Figure 6 shows the settle of bacteria from the left side of the peri-operative environment up to 3 metres away from the treatment trolley. The high number of cfu's is visible to the naked eye.

Results from electron microscopy showed adhesion of bacteria to the specimen surfaces. Figure 7 illustrates Methicilin Resistant Staphylococcus aureus.

Conclusions

There is substantial empirical evidence that wound healing can be improved with surgical debridement and a general consensus among clinicians that debridement creates a favourable wound bed.⁴⁻⁶ Positive outcomes reported include an increase in the percentage of granulation tissue and a marked decrease in slough.

One aim of this study was to examine bacterial load following hydro-surgery debridement. No significant differences were found between bacterial counts of wound swabs or biopsies obtained pre and post hydro-surgery independent of bacteria or wound type. Although wounds had an improved appearance after treatment there is no significant reduction in bacterial load.

However, it is clear from the cfu's illustrated in figure 2 that hydro-surgery can decrease the quantity of bacteria resident in a wound to some extent but not reaching significance. One wound site actually saw an increase in bacterial count post debridement. This occurred in a site designed to simulate a deep wound with a sinus. We suggest that this could be due to inaccuracies arising from the use of swabs to collect material from a deep seated, irregular and undermined wound with a sinus.

Despite not achieving statistical significance the results still demonstrate a decrease in the quantity of bacteria present in the wound but the question of where the organisms go needs to be addressed. Tables 2 and 3 provide evidence of aerosolization of bacteria

both during and following debridement. Furthermore, this fallout appears to be displaced throughout much of the peri-operative environment as illustrated in Figure 6.

Of grave concern are the extreme bacterial quantities recorded when the debridement tool becomes blocked as seen in figure 5. The results showed irregular displacement of pathogens especially in the front right settle plates. The only area to avoid significant fallout was 3 meters in front and to the right of the clinician. It is not possible to account for equal or unequal fallout, due to the nature of high pressure spray but we can postulate that the SAS may have obscured the front right 3meter settle plate.

During the active sampling process a count of 16780 cfu/m³ was obtained which is extremely high. A possible explanation would be that the cfu's visible during imaging had originated from more than one cell thus when the plate becomes crowded the actual number of visible cfu's does not represent a true figure. A statistical factor has been applied to allow for this.

Despite a two-hour time delay between debridement of different specimens cross contamination occurred. The samples taken from specimen two, inoculated with *Pseudomonas*, also contained *Staphylococcus Aureus* from the previous specimen.

Our results clearly demonstrate that there is a potentially high risk of contaminating the peri-operative environment during the process of hydro-debridement making cross infection a real possibility. Careful consideration of clinical location is necessary prior to using such debridement tools, particularly as hydro-surgery consoles are becoming

increasingly used in community clinic settings globally. This is especially true in a climate where hospital acquired infections are under increasing scrutiny. The fallout recorded from dressing removal deserves the same consideration independent of hydro-surgery and has implications for clinicians on a daily basis at every level.

The results from this study should not dissuade the clinician from utilising hydro-surgery as an adjunct to other treatments but it is vital that action be taken to mitigate the bacterial fallout associated with its use. A transparent hood to cover the cutting tool and seal the affected area may reduce potential fallout, thus reducing bacterial contamination. Similarly, an improved cutting tool designed with bacterial fallout in mind could diminish contamination.

Acknowledgements

We have not received any financial support for the study.

Bowling F L* No conflict

Stickings D S* No conflict

Edwards-Jones V** No conflict

Armstrong D G*** No conflict

Boulton A J M* No conflict

References

1. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS: Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 39:885-910, 2004.
2. Armstrong DG, Lipsky BA: Diabetic foot infections: stepwise medical and surgical management. *Int Wound Journal* 1:123-132, 2004.
3. Brem H et al, Molecular Markers in Patients with Chronic Wounds to Guide Surgical Debridement. *Molecular Medicine* 2007;13:30-39.
4. Attinger CE, Bulan E, Blume PA: Surgical Debridement: the Key to Successful Wound Healing and Reconstruction. *Clin Podiatr Med Surg* 17:599-630, 2000.
5. Brem H, Sheehan P, Rosenberg HJ, Schneider JS, Boulton AJ: Evidence-based protocol for diabetic foot ulcers. *Plast Reconstr Surg* 117:193S-209S; discussion 210S-211S, 2006.
6. Falabella AF: Debridement and wound bed preparation. *Dermatol Ther* 19:317-325, 2006.
7. Granick M, Boykin J, Gamelli R, Schultz G, Tenenhaus M: Toward a common language: surgical wound bed preparation and debridement. *Wound Repair Regen* 14 Suppl 1:S1-10, 2006

Table 1
Mean Bacterial Count (n=3) Pre-versajet and Post versajet.

	Site 1	Site 2	Site 3	
	Swab cfu/ml	Swab cfu/ml	Swab cfu/ml	Biopsy cfu/ml
Specimen 1 (Staph aureus)				
Pre-versajet	5.57 x 10 ⁸	2.57 x 10 ⁸	3.53 x 10 ⁸	4.43 x 10 ⁷
Post versajet	9.03 x 10 ⁸	7.97 x 10 ⁶	2.65 x 10 ⁷	2.13 x 10 ⁶
Specimen 2 (Pseudomonas aeruginosa)				
Pre-versajet	5.54 x 10 ⁷	2.12 10 ⁸	7.68 x 10 ⁷	5.60 x 10 ⁷
Post versajet	5.38 x 10 ⁷	3.17 x 10 ⁸	3.07 x10 ⁸	8.20 x 10 ⁶
Specimen 3 (E.coli)				
Pre-versajet	1.10 x 10 ⁸	1.57 x 10 ⁸	4.00 x10 ⁷	3.00 x 10 ⁷
Post versajet	3.20 x10 ⁶	6.47 x10 ⁶	5.20 x 10 ⁶	5.67 x 10 ⁶
Specimen 4 (Mixed wound organisms)				
Pre-versajet	1.17 x 10 ⁷	7.70 x 10 ⁶	4.57 x 10 ⁶	4.74 x 10 ⁶
Post versajet	N/A	2.43 x 10 ⁶	1.73 x 10 ⁶	4.07 x 10 ⁶

Note: site 1 = Small deep cut, site 2 = small deep cut with a sinus, site 3 = a superficial wound, N/A – not available.

Table 2

The number of Cfu/100litres of air during debridement process.

	Cfu/ 100 litres of air	Bacteria isolated
Specimen 1 Staph aureus		
1 min	23	No SA
2min	72	50 SA
3min	TNTC****	SA +++
4min	TNTC****	SA+++
Specimen 2 P. aeruginosa		
1 min	10	1 SA
2min	36	5SA
3min	20	1 SA, 2 PAE
4min	9	2 SA, 5 PAE
Specimen 3 E. coli		
1 min	9	
2min	9	
3min	15	
4min	10	2 ECO
Specimen 4 Mixed wound bacteria		
1 min	4	4 MRSA
2min	0	
3min	0	
4min	7	

Note: **** = Versajet blocked SA = S. aureus; PAE= P. aeruginosa; ECO= E.coli ;
MRSA= methicillin resistant S. aureus; TNTC = To numerous to count.

Table 3

Settle of bacteria in the air during a one hour period whilst the Versajet was used to debride sample samples 1-4.

	Settle plates (back right)	Settle plates (front right)	Settle plates (back left)	Settle plates (front left)
1m	85 ***	45	80	180**
2m	46***	106**	80	47
3m	44	0	72	32

Note: *** colonies include *S. aureus* and *E. coli* ** predominantly *P. aeruginosa*

Table 4

The results of active sampling with SAS (cfu / m³) during debridement of four infected porcine samples.

Specimen	Remove Dressing (cfu / m ³)	During VJ (cfu / m ³)
1	650	950
2	580	16780
3	630	3560
4	570	6880
Average	607.5	7042.50

Figure Legend

Figure 1. Schematic diagram (plan view) of the treatment room layout illustrating position of VJ, settle plates and SAS (not to scale).

Figure 2. This figure shows the staphylococcal count from specimen 1. The top row shows pre-versajet staphylococcal counts from the three sites and the lower row is the post Versajet.

Figure 3. Shows the air sampling during debridement using Versajet on specimen infected with *P. aeruginosa*

Figure 4. The aerosolization effect of Versajet therapy pre, during and post Versajet debridement and dressing removal.

Figure 5. Shows the air sampling during debridement using Versajet on specimen infected with *S. aureus*.

Figure 6. Shows the settle plates whilst debriding using Versajet on the left side of the room (Front and Back) at 1m, 2m and 3 meters from the trolley.

Figure 7. Shows the adhesion of MRSA on the sample after infection with wound bacteria x 7000 magnification.

Figure 1.

Schematic diagram (plan view) of the treatment room layout illustrating position of VJ, settle plates and SAS (not to scale).

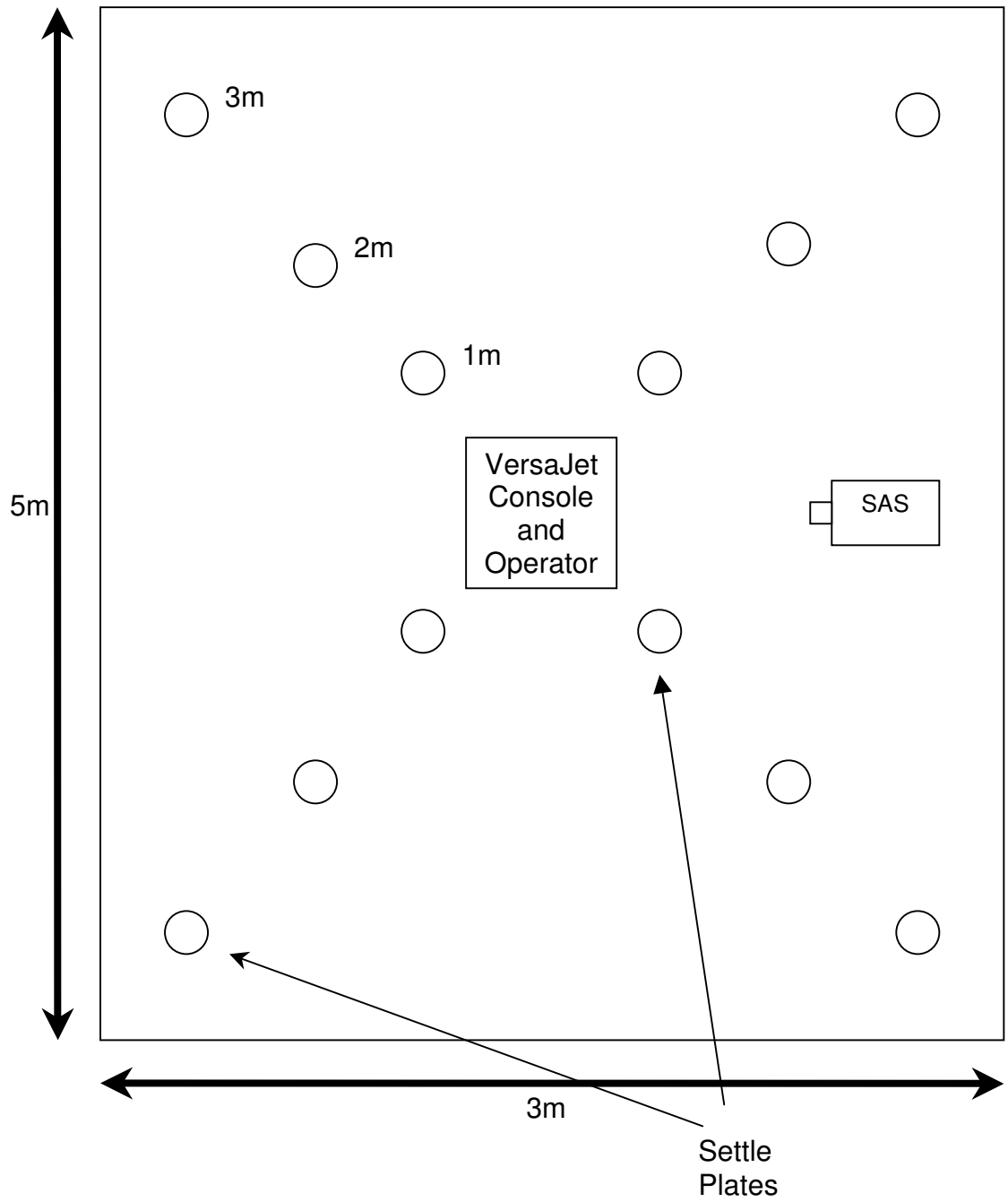


Figure 2.

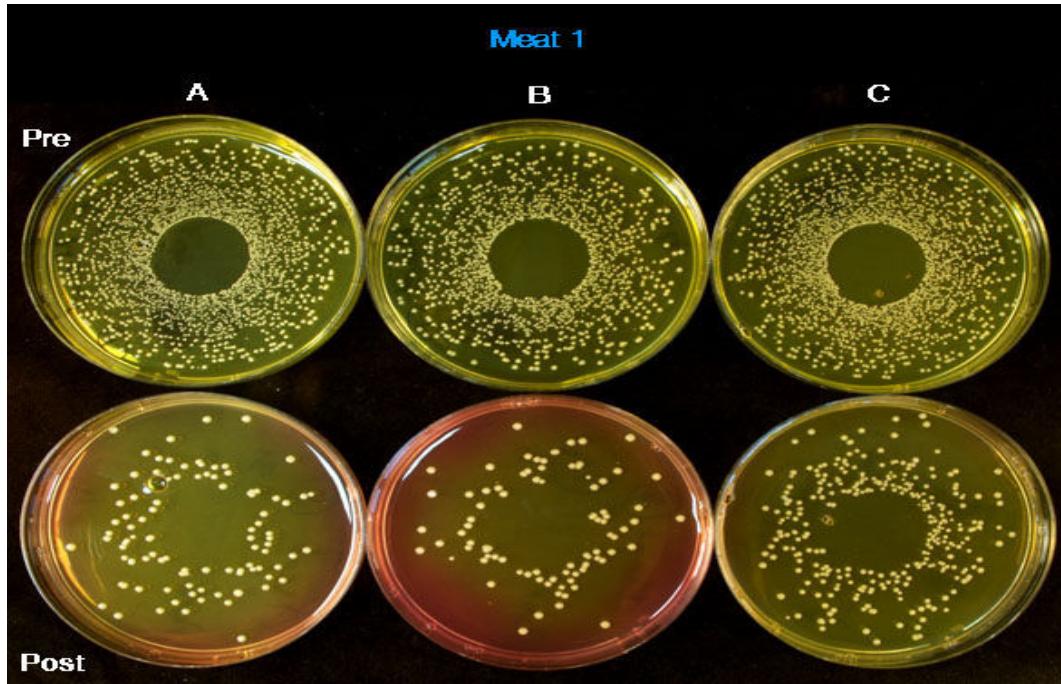


Figure 3.

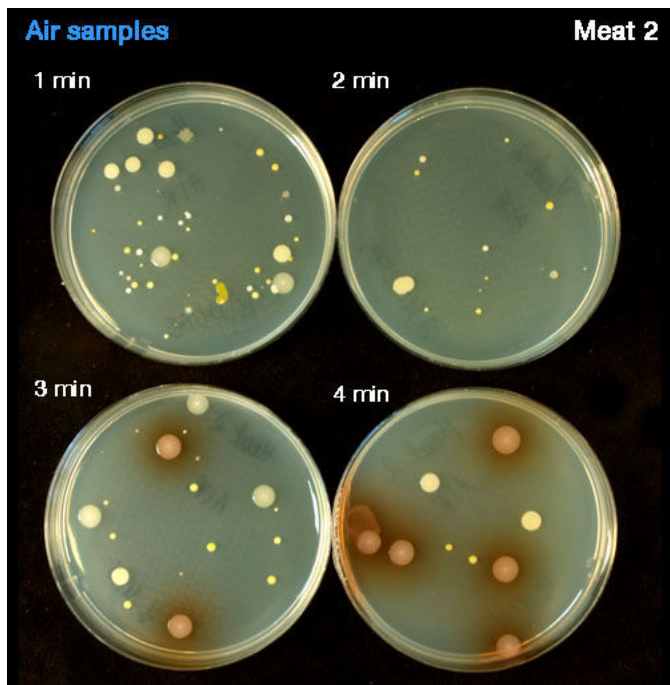


Figure 4.

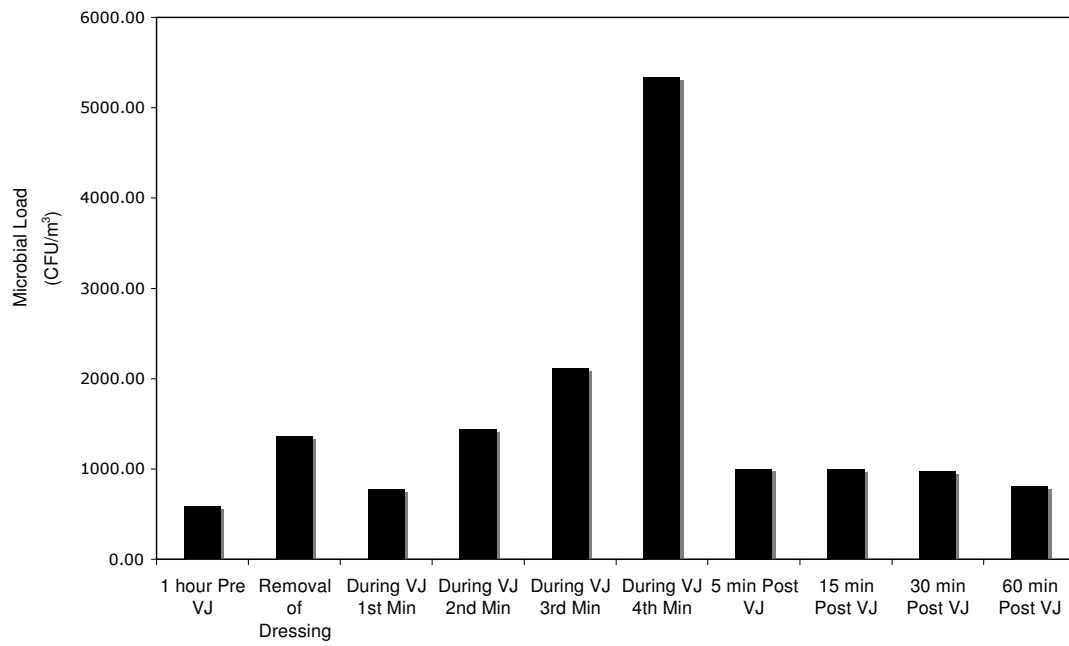


Figure 5.

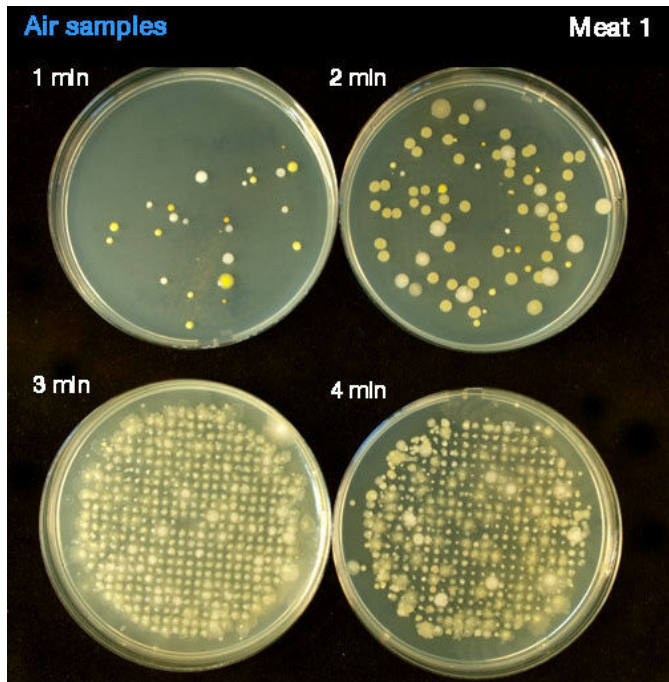


Figure 6.

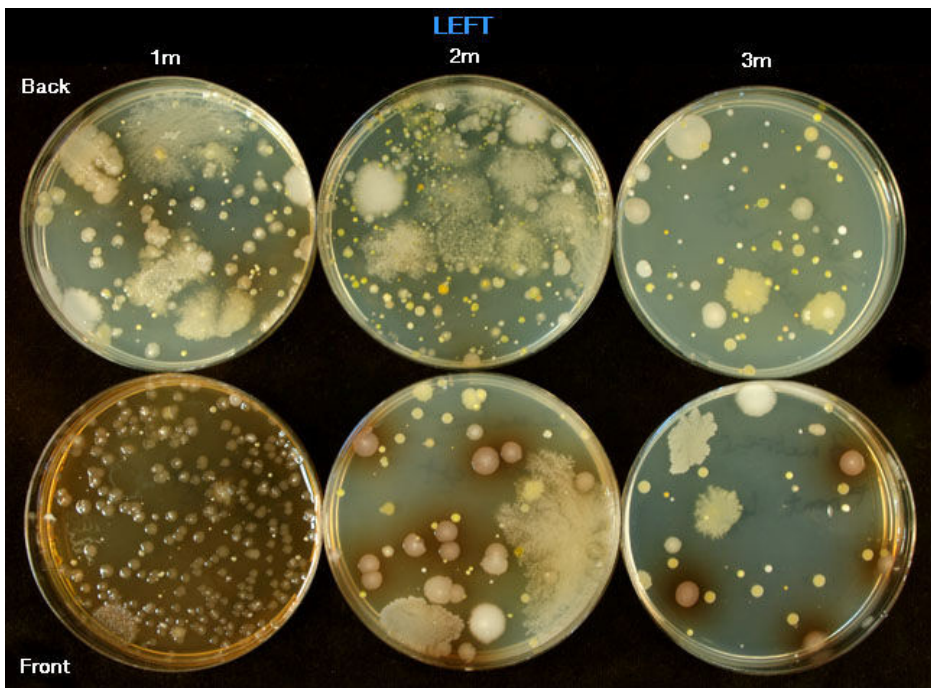


Figure 7.

