Vol. 13(1), pp. 1-10, January-June 2024
DOI: 10.5897/JBD2024.0060
Article Number: 705951B71856
ISSN 2141-2197

http://www.academicjournals.org/JBD

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Journal of Brewing and Distilling

Full Length Research Paper

Bacterial contamination and surface hygiene in the microbrewery environment

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Received 6 January, 2023; Accepted 6 February, 2024

Bacterial spoilage has a negative impact on the quality, stability and consumer acceptance of beer. The present study was conducted to determine bacterial surface and product contamination in the microbrewery environment. HybriScan™ *D* Beer rapid molecular testing kits for bacterial cell counts were used to evaluate three microbreweries of similar size at eleven different locations within each brewery. Analyses of HybriScan data showed the presence of spoilage bacterial species on all surfaces sampled after sanitation and bacteria were also present in liquid samples collected during production. The most highly contaminated locations were the racking arm and the blow off valves. Levels of spoilage bacteria differed significantly between microbreweries. These differences could be related to variations in sanitation protocols and floorplans. Some establishments allowed customer seating adjacent to production operations which could impact the contamination of surfaces and the quality of final products. Awareness of the presence of the organisms can facilitate proper sanitation, consumer awareness, and storage of these products to limit the growth of spoilage bacteria.

Key words: Beer, brewing, spoilage, microbrewery, brewery, bacteria, contamination.

INTRODUCTION

Due to the microbial hurdles present, beer is generally recognized as a stable food product. Leistner (2000) defined microbial hurdles as a “combination of food preservation methods” which can be intrinsic with properties such as pH, water activity, redox potential, and competitive microbes (Leistner, 1992). Extrinsic hurdles include processing methods such a heating, cooling, and packaging. However, because of the fermentation environment, spoilage bacteria still survive and proliferate in beer despite the extrinsic and intrinsic properties that might inhibit their growth in other foods (Leistner, 1992, 2000).

Intrinsic properties of beer that present microbial hurdles include pH (typically pH 3.9-4.4), ethanol concentration (ranging from approximately 3.0 to 14% by volume), hop-derived compounds (iso-α acids typically in the range of 17-55 mg/L), CO_2 concentration (typically 0.5% w/w), and low oxygen concentration (<0.1 mg/L). Extrinsic microbial hurdles present in the brewing process include heating which is used during mashing, lautering, and boiling processes and clean-in-place (CIP) processes, which use a combination of temperature, turbulent flow, pressure, detergents, and sanitizing agents. The CIP processes are used to clean and

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sanitize beer contact surfaces between production runs in the brewery environment.

According to Ciont et al. (2022), beer may become contaminated during fermentation from the raw ingredients, poor sanitation, incorrect pasteurization, environment air pollution and inadequate ethanol production. Challenges to prevent bacterial spoilage in microbreweries include the presence of customers near the production/packaging area, the open-air environment around fermentation and maturation tanks, and the use of mobile pumps and modular hoses. Hygienic zones or the compartmentalization of the production environment into cleaner “areas” (zones) to protect food products from microbial cross-contamination is easier in closed food production facilities; however, microbreweries often allow open access to customers by virtue of operation. Furthermore, lactic acid bacteria are sometimes intentionally added to beer to produce sour ales in the same breweries producing non-sour products promoting cross-contamination. Beer spoilage bacteria typically include the genera *Lactobacillus, Pediococcus, Pectinatus,* and *Megasphaera* with various species spoiling the quality of beer. These bacteria have implicit factors that increase their ability to colonize beer despite the extrinsic, intrinsic, and processing hurdles in beer (Rodriguez et al., 2020; Vriesekoop et al., 2013).

Hops are derived from cone-shaped flowers of the hop plant *Humulus lupulus* and are not only added to beer to impart bitterness, flavour, and aroma, but also to inhibit bacteria via the release of hydronium ions which acts as a proton pump in the cell membrane to inhibit Gram-positive bacteria. This disrupts the cell membrane through a pH gradient shift resulting in cell death (Sakamoto and Konings, 2003; Simpson, 1993). However, some bacteria have mutated to develop implicit factors that increase their ability to colonize and subsequently spoil beer. Mutations to the *hor*A gene result in the removal of the hydronium ion protecting the Gram-positive spoilage bacteria cell membrane (Behr, 2006). Yeasts, such as *Brettanomyces* and *Saccharomyces cerevisiae* var *diastaticus* also can colonize beer, causing negative impacts to overall quality and stability (Suiker and Wösten, 2022). Spoilage yeasts were not targeted during this study to stay within the scope of bacterial contamination of beer.

The objective of the present study was to determine location and level of bacterial contamination in microbrewery unit operations, and to establish the relationship between surface and product contamination.

MATERIALS AND METHODS

The materials used includes HybriScan™ *D* Beer kits (Millipore Sigma, St. Louis, MO), Bacto Peptone (Beckton, Dickinson and Company, Sparks, MD), Sterile cotton tipped applicators (Puritan Medical Products Company LLC, Guilford, ME), peracetic acid (Envirotech, Modesto, CA) and Birko (Henderson, CO). Beer samples were collected from three microbreweries in South Carolina, USA.

Brewery descriptions

Three breweries of similar brewing system size, annual production level, floor plan, and location were chosen for the study.

(1) Brewery A: Two vessel (Mash Tun and Kettle) 7 brewery barrel (BBL, 1 BBL = 117 L) brewhouse with five 7 BBL fermentation tanks. Estimated annual output is 450 BBL (52,650 L).
(2) Brewery B: Two vessel (Mash Tun and Kettle) 5 BBL brewhouse with two 10 BBL, three 5 BBL, and one 2 BBL fermentation tanks. One 5 BBL tank was sampled for this study. Estimated annual output is 500 BBL (58,500 L).
(3) Brewery C: Two vessel (Mash Tun and Kettle) 10 BBL brewhouse with four 10 BBL fermentation tanks and 2 10 BBL horizontal lagering tanks. Estimated annual output is 600 BBL (70,200 L).

Sample location selection

After surveying and creating a flow chart of each facility, sampling locations were selected based on common unit operations within each brewery. Flowcharts and floorplans allowed the minimization of variability in sampling of the unit operations. Brewery floor plans permitted the identification of hygienic zones and the selection of eleven sampling sites (Figure 1). Sampling site section was based on the similarity between the three breweries and the relative chance of contamination.

Sampling sites (Figure 2) included the following locations (1) spray ball valve, (2) sample tap, (3) racking arm valve, (4) blow-off valve, (5) bottom valve, (6) carb stone valve, (7) yeast pitch, (8) cooled wort leaving heat exchanger, (9) early, (10) mid, and (11) late packaging runs (Figure 2). Sampling of the same fermentation tank was repeated 4 times to reduce variation between tanks.

Hygienic zones

Zone 1

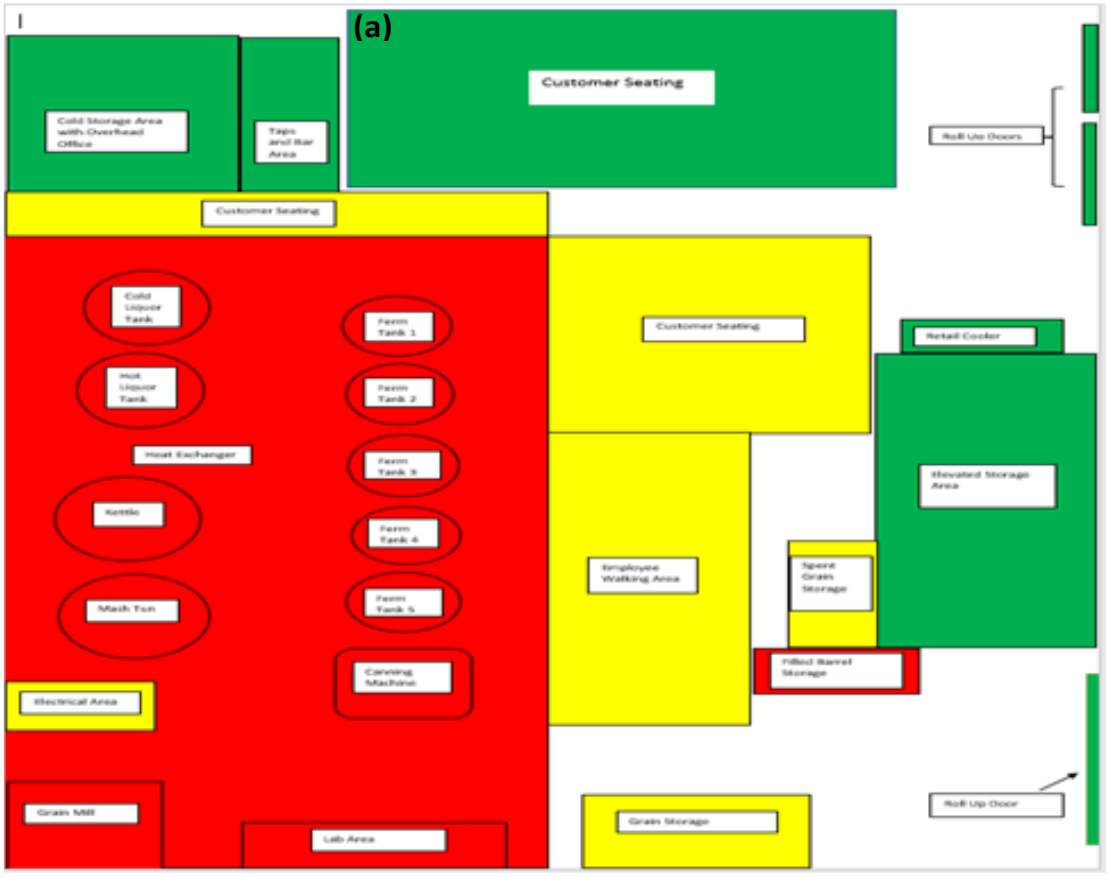
Colour coded red in Figure 1a, b, and c denoting zones that have direct contact with beer including the brew house, hot and cold liquor tanks, grain mill, heat exchanger, and fermentation tanks with a high risk of product contamination.

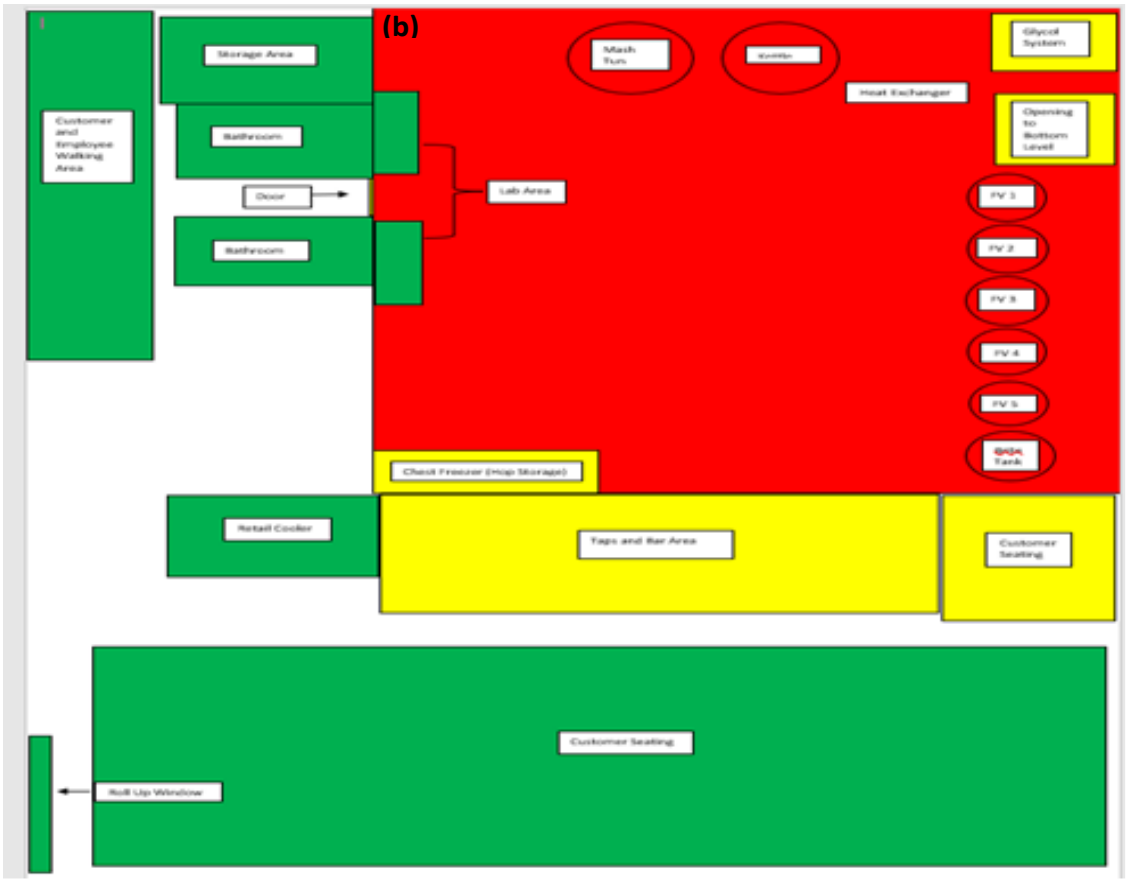
Zone 2

Colour coded yellow in Figure 1a, b, and c denoting zones that are adjacent to beer production having no physical barrier between the production areas. This area contains ingredient storage, employee walking areas, customer seating, tap/bar areas, or lab areas adjacent to production areas with no physical barriers with a medium risk of product contamination in these areas.

Zone 3

Colour coded green in Figure 1a, b, and c denoting zones completely removed from beer production areas including offices, retail space, finished product storage, taps and bar, customer seating that is not adjacent to production zones with a low risk of product contamination in these areas.


(a)
Customer Seating
Cold Storage Area with Overhead Office
Taps and Bar Area
Roll Up Doors
Customer Seating
Cold Liquor Tank
Hot Liquor Tank
Heat Exchanger
Kettle
Mash Tun
Ferm Tank 1
Ferm Tank 2
Ferm Tank 3
Ferm Tank 4
Ferm Tank 5
Canning Machine
Electrical Area
Grain Mill
Lab Area
Customer Seating
Employee Walking Area
Retail Cooler
Elevated Storage Area
Spent Grain Storage
Filled Barrel Storage
Roll Up Door
Grain Storage


(b)
Storage Area
Customer and Employee Walking Area
Bathroom
Door
Bathroom
Lab Area
Mash Tun
Kettle
Heat Exchanger
Glycol System
Opening to Bottom Level
FV 1
FV 2
FV 3
FV 4
FV 5
BBT Tank
Chest Freezer (Hop Storage)
Retail Cooler
Taps and Bar Area
Customer Seating
Customer Seating
Roll Up Window

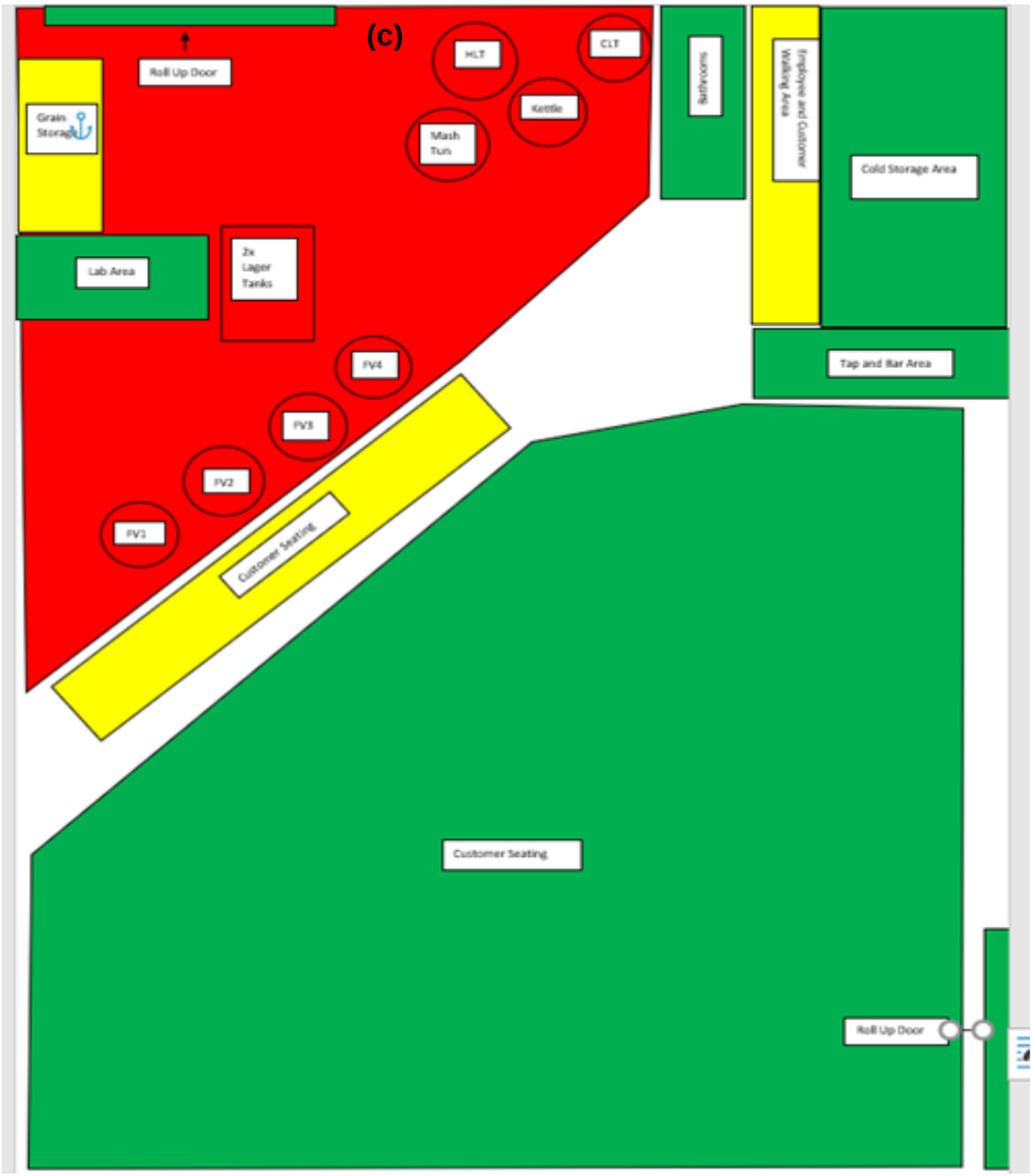


Figure 1. a. Brewery A floor plan; b. Brewery B floor plan; **c.** Brewery C floor plan.

Spray ball valve

A 1" to 1.5" in diameter butterfly valve made of 304 sanitary stainless steel positioned on the pipe coming from the CIP spray ball located
at the top of the fermentation tank, attached to the tank with a tri-clamp and gasket.

Sample tap

A 1" to 1.5" in diameter horizontal valve made of 304 sanitary stainless steel attached the fermentation tank just above the tank cone. This valve is used to collect samples of the beer during and after fermentation and attached to the tank by a tri-clamp and gasket.

Racking arm valve

A 1.5" to 2" in diameter butterfly valve made of 304 sanitary stainless steel that closes off the racking arm attached to the top cone of the fermentation tank, attached to the tank by a tri-clamp and gasket. The racking arm allows the transfer of beer from the

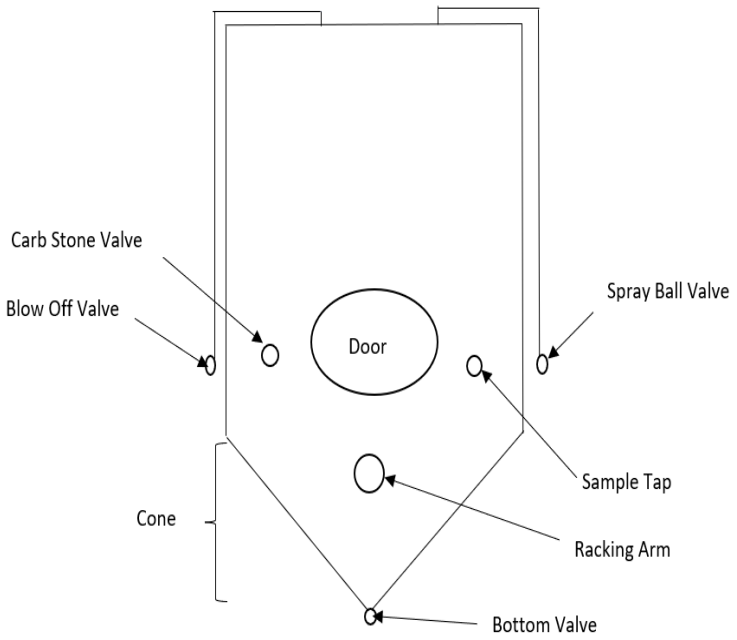


Figure 2. Surface swab sampling locations.

fermentation tank without agitating sediment collected in the tank cone.

Blow off valve

A 1" to 1.5" in diameter butterfly valve made of 304 sanitary stainless used to close the pipe running from the top of the fermentation tank allowing carbon dioxide escape during fermentation. This valve is attached to the tank by a tri-clamp and gasket and is left open during fermentation and closed upon completion of fermentation.

Bottom valve

A 1.5 to 2" in diameter butterfly valve made of 304 sanitary stainless steel used to close an opening at the bottom of the fermentation tank. This valve is attached to the tank by a tri-clamp and gasket and is located where cooled wort is pumped into the tank after leaving the heat exchanger.

Carb stone valve

A 1.5" to 2" in diameter ball valve made of 304 sanitary stainless steel that closes the opening of the carb stone assembly and attached to the tank by a tri-clamp and gasket. The carb stone assembly is attached to the fermentation vessel and aids in forcing carbon dioxide into solution during the carbonation process.

Yeast pitch

Yeast added to the fermentation tank containing cooled wort after being transferred to the tank. This can be a "fresh pitch" purchased from suppliers or a "harvested pitch" recovered from the tank cone after beer fermentation is complete.

Liquid sampling locations

Liquid sampling locations included the *Packaging Run Early*-beer collected from the keg filler head before filling the first keg of the production run; *Packaging Run Mid*-beer collected from the keg filler head halfway through the total volume of the production run; *Packaging Run Late*-beer collected from the keg filler head just before filling the final keg of the production run; and *Cooled Wort Exiting the Heat Exchanger*-cooled wort exiting the heat exchanger before entering the fermentation tank. Cooled wort samples reflect the overall bacterial counts of transfer lines connecting heat exchangers and fermentation tanks and the heat exchanger alone.

Open air sampling

Twenty-four-hour environmental air samples were collected from Brewery A as a preliminary study. Open tryptic soy agar plates were placed on the left and right side of the brew deck, above and below the fermentation vessel, in the grain storage area, in the barrel storage area, and on the heat exchanger. During the 24 h sampling period, both production and sales operations occurred with customers visiting the tap room. After sampling, the plates were collected, inverted, and incubated at 37°C for 24 h, after which colonies were aseptically selected then mixed in 2 mL of peptone then evaluated using the HybriScan assay.

Sample collection method

Surface swabs

Three milliliters of sterile 0.1% peptone water was used to collect samples in triplicate at the 11 sampling sites (Figure 3) from 15 mL test tubes. An 8.04 cm^2 area was sampled in a crosshatch pattern using sterile cotton tipped swabs (Figure 4) after which swabs were loaded back into the 15 mL test tubes. Yeast residue was swabbed

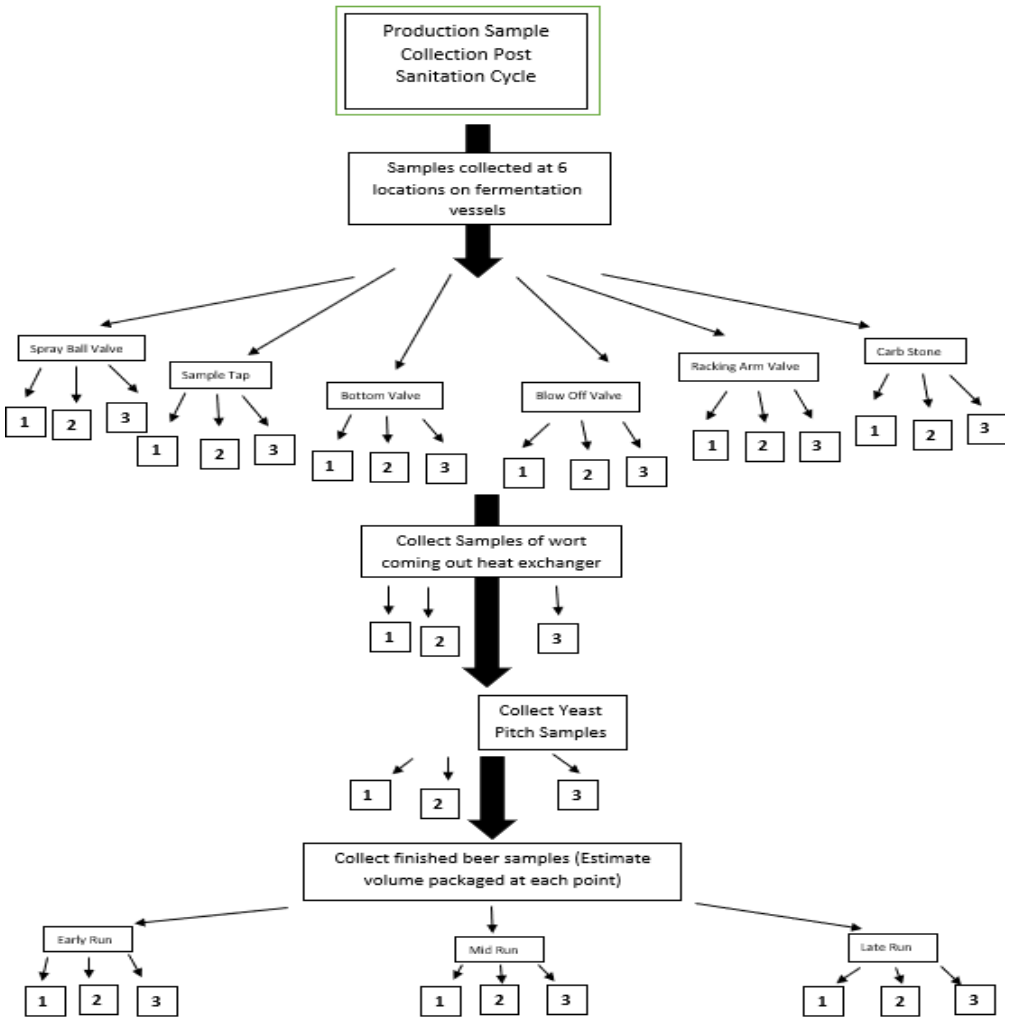


Figure 3. Sample collection diagram.

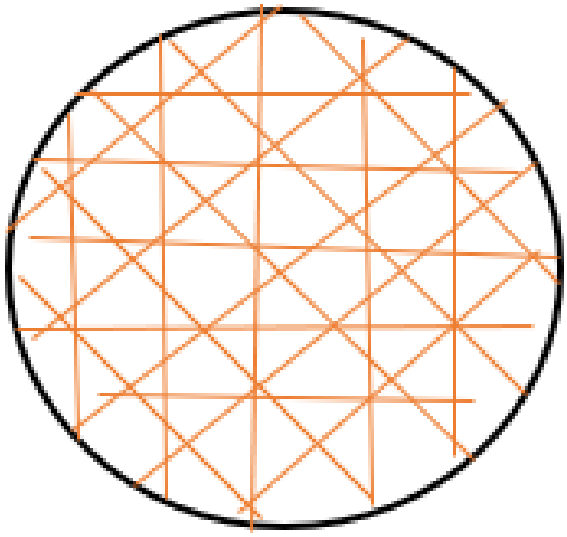


Figure 4. Sample surface swabbing pattern.

Table 1. Mean colony forming units (log cfu)/cm^2) for each brewery, across all surface swab sampling locations.

Brewery	log cfu/cm^2	Standard deviation (log cfu/cm^2)
A	4.7[a]	0.1
B	4.6[b]	0.3
C	4.8[a]	0.3

[a,b]Means with different superscripts are significantly different ($p<0.05$).

from inside fresh pitch packaging or inside the brink from harvested pitches. All samples were immediately stored under refrigeration post collection and analysed within 24 h.

Liquid samples

Beer samples were collected in triplicate in sterile 15 mL tubes and immediately refrigerated until analysis within 24 h.

Sample analysis method

Samples were analysed using Millipore Sigma HybriScan™ *D* Beer kits.

Bacterial species sensitivity of Millipore Sigma HybriScan™ D Beer kits

Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus brevisimilis, Lactobacillus buchneri, Lactobacillus casei, Lactobacillus collinoides, Lactobacillus coryniformis, Lactobacillus curvatus, Lactobacillus fermentum, Lactobacillus frucivorans, Lactobacillus linderi, Lactobacillus malefermentans, Lactobacillus parabuchneri (frigidus), Lactobacillus paracasei, Lactobacillus paraplantarum, Lactobacillus plantarum, Lactobacillus rhamnosus, Pediococcus acidilactici, Pediococcus claussenii, Pediococcus damnosus, Pediococcus inopinatus, Pediococcus parvulus, Pediococcus pentosaceus, Pectinatus cerevisiiphilus, Pectinatus frisigensis and *Megasphaera cerevisiae.*

Data analysis

Bacterial cell counts (cfu/10 µL) were calculated using a standard curve (Millipore Sigma HybriScan™ D). These values were expressed as cfu/mL then further converted to cfu/cm^2 for surface swab samples by dividing with 5.36 (brewery valves with diameter of 1.25" have a surface area of 8.04 cm^2 and 2 ml out of 3 ml peptone collected was sampled). The data were analyzed with statistical software of (SAS™) (Statistical Analysis System, SAS Institute, Cary NC) using a general linear model (GLM) and standard deviation for absorbance and cfu/mL were generated for sampling locations and breweries. Main effects for breweries and locations were significant ($p\leq0.05$) and means were separated at the $p\leq0.05$ level using the pdiff command of SAS™.

Validation of HybriScan™ detection threshold for samples

Validation of the detection threshold of beer samples using HybriScan™ technology was determined by comparing the standard curve provided from the manufacturer with each of the 5 HybriScan™ kit used. All samples tested were within the detection threshold of the HybriScan (<1.0 cfu/mL).

RESULTS AND DISCUSSION

Overall, analyses of the data demonstrated that there are a variety of potential locations within the microbrewery environment that may become sources of bacterial contamination. This is reflected in the 24 h environmental air samples which were all positive for the spoilage bacteria as detected by the HybriScan™ assay. In addition, number of microorganisms from surface swabs collected in Breweries A and C were greater than the number of microorganisms recovered from surfaces in Brewery B (Table 1) across all locations. As far as locations in the unit operations, the racking arm valve contained a higher bacterial count compared to other sampling locations (Table 2). This result was slightly skewed because Brewery C had an extremely high bacterial load found on the racking arm compared to Breweries A and B (Figure 5). In fact, the yeast pitch had the highest bacterial numbers for Brewery B while Brewery A had consistent bacterial loads found in all swab and liquid samples. There are reasons the racking arm may become a problem site for bacterial contamination.

One reason that the racking arm may harbour bacteria is the design which includes a threaded coupler and gasket that seals the connection on the 5-10 BBL fermentation tanks. This design creates niches for microorganisms that are difficult to remove during sanitation. Oftentimes, the racking arms are not completely disassembled during the CIP protocols in microbreweries. Furthermore, because of the design, the racking arms remain vertical during operation as they are lowered just above the trub (sedimentation) line in the fermentation tank allowing them to collect finished beer with minimal disruption of the trub. Trub deposits can become a source of bacterial contamination and support biofilm formation in an area that is already difficult to sanitize (Maifreni et al., 2015; Timke et al., 2008). In their study on biofilms, Ismail et al. (1999) reported that growth of aerobic and anaerobic bacteria in micropits (small pitting in stainless steel caused by abrasion) decreased the passive film of 304 stainless steel and cause increased rates of corrosion over time.

Table 2. Mean colony forming units (log cfu/cm^2) for each surface swab sampling location, across all three breweries.

Sampling location	Log values	Standard deviation (log cfu/cm^2)
Blow Off Valve (BOV)	4.8^{a,b}	0.5
Bottom Valve (BV)	4.7^{a,b}	0.3
Carb Stone Valve (CSV)	4.6^{b}	0.5
Racking Arm Valve (RAV)	4.9^{a}	0.6
Sample Tap (ST)	4.5^{b}	0.5
Spray Ball Valve (SBV)	4.6^{b}	0.5
Yeast Pitch (YP)	4.7^{a,b}	0.5

^{a,b}Means with different superscripts are significantly different (p≤0.05.) n = 36.

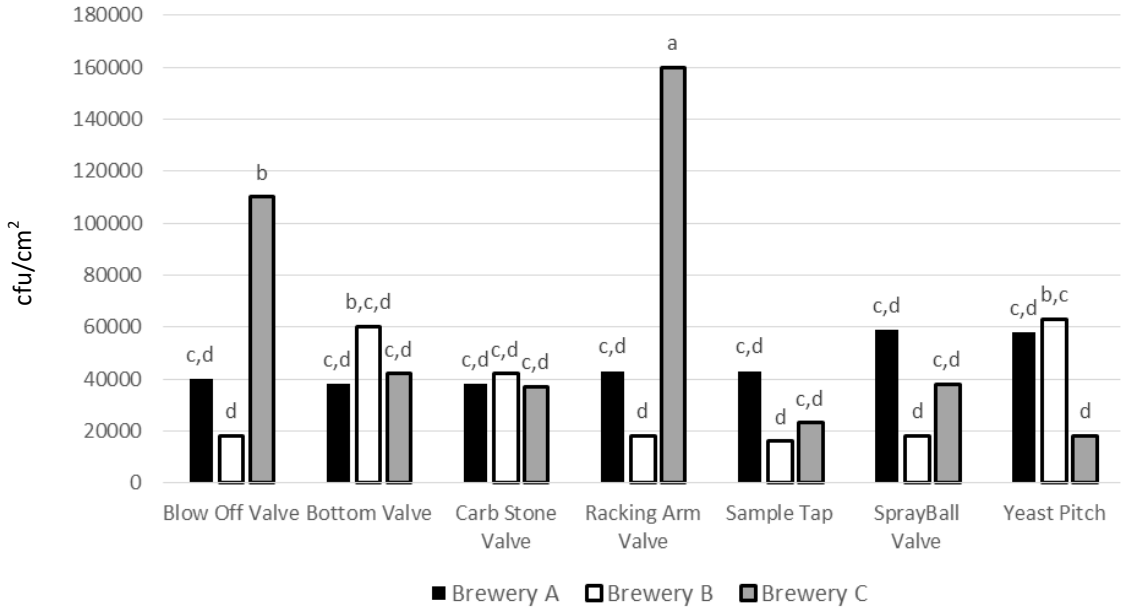


Figure 5. Mean colony forming units (cfu)/cm^2 at each surface swab sampling location across all breweries. Standard Error: 3960. ^{a,b,c,d}Means with different superscripts are significantly different (p<0.05); n=12.

Micropits favour bacterial adhesion on a surface. Since micropits are common on a frequently rotated apparatus like the racking arm, this phenomenon could be one of the reasons for the bacterial contaminations and potential biofilm formation centred around the racking arm, causing greater risk of contamination overtime as more micropits develop with use. Proper cleaning, sanitation, and maintenance of the racking arm can reduce the likelihood of biofilm formation and bacteria cross-contamination.

During peak fermentation (12-36 h after the introduction of yeast to the wort), active yeast will form krausen, which is a thick, brown/off-white foam that is often difficult to remove from the walls of the fermenter. Wang et al. (2021) evaluated the bitter-tasting components of krausen during beer fermentation and reported that these metabolites not only adhered to the surfaces of the fermentation tank, but also to the blow off piping, valve, and tubes. Krausen deposits, if not removed, could become a source of nutrients for contaminating strains of bacteria and could cause damage to the structural integrity of 304 stainless steel. These types of residues may be removed using hot water, caustic solutions, brushes, visual inspections and recleaning of soiled areas observed during a visual inspection as outlined in a proper sanitation standard operating procedure. A proper cleaning and sanitation protocol that accounts for the unique attributes of the racking arm and blow off apparats would protect the beer from bacterial contamination in these areas.

Although the highest levels of bacteria were recovered from the racking arm (4.9 log cfu/cm^2) and blowoff apparatus (4.8 log cfu/cm^2), other locations that were

Table 3. Mean colony forming units (log cfu/ml) for liquid samples taken at different points in the unit operations, across all three breweries.

Sampling location	Log (cfu/cm^2)	Standard deviation (log cfu/cm^2)
Packaging early run (PER)	5.3	0.4
Packaging mid run (PMR)	5.4	0.4
Packaging late run (PLR)	5.4	0.5
Wort out heat exchanger (WO)	5.3	0.4

Table 4. Mean colony forming units (log cfu/ml) for each brewery across all liquid samples.

Brewery	Log (cfu/ml)	Standard deviation (log cfu/ml)
A	5.1^b	0.1
B	5.1^b	0.1
C	6.0^a	0.4

^{a,b}Means with different superscripts are significantly different ($p<0.05$).

sampled also harboured bacteria that could serve as a source of product contamination. Bacteria were recovered from the bottom valve (4.7 log cfu/cm^2), carb stone valve (4.6 log cfu/cm^2), spray ball valve (4.6 log cfu/cm^2) and yeast pitch (4.7 log cfu/cm^2) in all three of the microbreweries tested (Table 2). During testing, there were no surfaces swabbed that were below the detection threshold of the assay. It is important for brewers at microbreweries to understand that spoilage bacteria are always present during the process.

There were no statistical differences in the four liquid samples taken at different times during the unit operations across all breweries and ranged from 5.3 to 5.4 log cfu/cm^2 (Table 3). However, liquid samples from each brewery were significantly different with Brewery C > Brewery A > Brewery B (Table 4).

Furthermore, when the data was separated by brewery, Brewery C had 4 to 6 times higher bacterial counts in all three of the Packaging Run samples compared to the Wort Out Heat Exchanger sample (Figure 6). Also, the Packaging Run early and late samples at Brewery C was higher than the other 2 liquid sample locations across the three breweries (Figure 6).

Differences in the type of kegging rigs, along with variations in the cleaning and sanitation protocols used at each brewery during packaging may have contributed to the variations in the bacterial counts in liquid samples.

Liquid samples collected from Brewery C at the packaging run early stage contained approximately 6.1 log cfu/ml bacteria compared to 5.0 log cfu/ml or less bacteria recovered from the same location in Breweries A and B (Figure 6). Similarly, liquid samples collected at the mid packaging and late packaging run stage contained approximately 5.9 log cfu/mL bacteria when collected from Brewery C compared to 5.3 log cfu/ml or less bacteria in the mid and late packaging run samples collected from Breweries A and B (Figure 6). This was most likely due to packaging equipment becoming contaminated during the packaging run as the early run samples bacterial contamination levels were lower. This equipment is most likely contaminated by the kegging rig touching the floor or some other unclean surface during packaging and transferring bacteria into the finished beer.

Conclusion

Difference in microbial counts recovered from surfaces in microbreweries may be caused by environmental contamination such as air, water, rodents/pest, food handlers, and customer proximity to production spaces. Main differences between the three breweries include floor plan, equipment layout, equipment quality and procedures, recipes, ingredient suppliers, employee training methods, and cleaning/sanitation protocols. Noting the floorplan of Brewery C with larger customer seating area adjacent to the production area compared to Breweries A and B, and that this Brewery C had higher bacterial counts leads to the possible cross contamination from the customer to the production area. “Open” concept brewery productions floors introduce bacterial contamination risks not commonly found in “closed” food production environment where strict hygiene practices can be enforced.

For this reason, it is important for brewers to closely follow their facilities’ cleaning and sanitation protocols. These include capping tank valves when not in use, monitor production surfaces for signs of wear, restricting access to areas with high contamination risk, and following good manufacturing processes for micro-

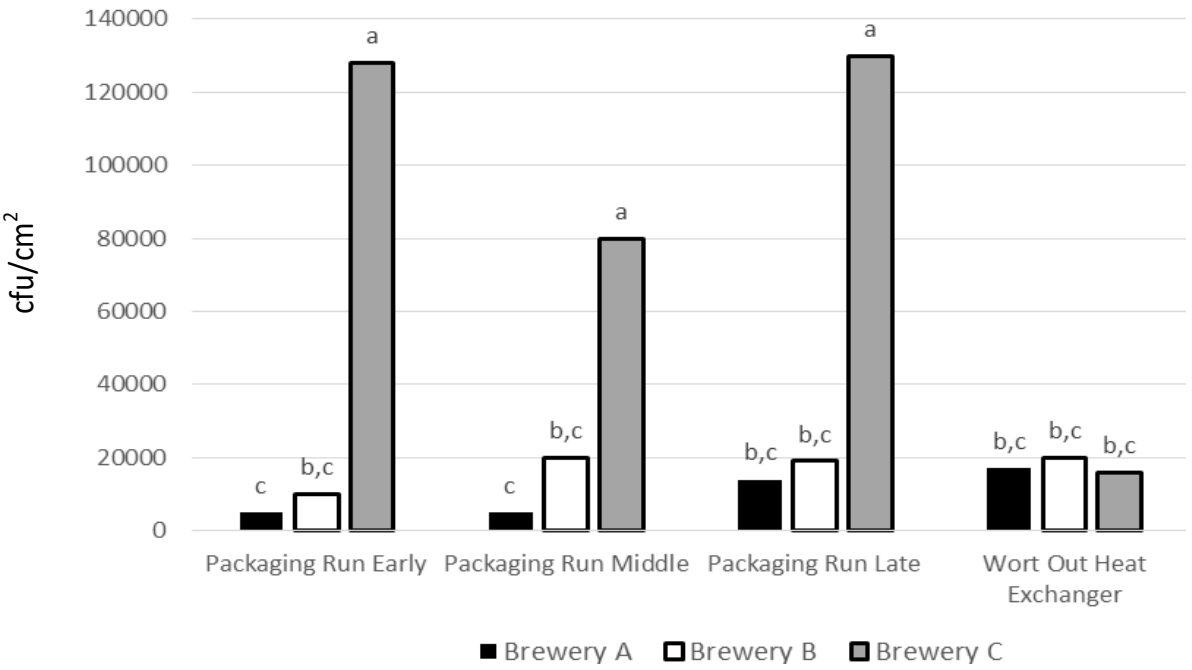


Figure 6. Mean colony forming units (cfu)/mL at each liquid sampling location across all breweries. [a,b,c]Means with different superscripts are significantly different ($p<0.05$) Standard Error: 22241; n=12.

breweries. Regular employee retraining is advised to ensure adherence to these protocols.

Overall, this study shows some vulnerabilities in the sanitary design of the fermentation equipment and standard cleaning protocols used by microbreweries and brewpubs. Future research should look for the impact of employee sanitation training on spoilage organisms counts, air flow controls for microbreweries, differentiate bacterial species on brewing surfaces, and assess yeast contamination through surface hygiene. Lastly, brewers and trade groups should work to increase consumer awareness of the importance of proper storage of microbrewery produced beer and that beer spoilage does not necessarily mean bad manufacturing practices by the brewer.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Technical Contribution No. 7135 of the Clemson University Experiment Station was appreciated.

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