A Comparison of Methods of MRSA Detection in Dutch Pig Stables

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Layman's summary:

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a *S. aureus* bacterium which is resistant to most β-lactam antibiotics. Antibiotics in this class make up a majority of the antibiotics prescribed to humans and include groups such as Penicillins and Cephalosporins. livestock associated MRSA (LA-MRSA) can be traced back to contact with animals and is genetically distinct from both hospital and community acquired MRSA. Pigs are an important reservoir of LA-MRSA, and close contact between pigs and humans on farms can lead to MRSA carriage by workers. This is a concern as MRSA carriage can lead to active MRSA infection in vulnerable populations, such as those who are hospitalized, possibly resulting in serious skin infections and even sepsis.

There is a growing body of research which seeks to understand the link between MRSA found in the environment on farms and its implication on human health. An important part of this is a thorough understanding of the occurrence of MRSA in that setting and the ability to measure it in a reproducible and robust way. This study aims to compare two different sampling methods for measuring airborne MRSA in the farm environment, passive dust sampling with electrostatic dust fall collectors (EDCs), and active sampling air pumps. The study described here is part of a larger study called Exclude MRSA whose objective is to reduce MRSA carriage in pigs by introducing benign bacteria into the noses of piglets which could competitively exclude MRSA. Additionally, the data collected may improve understanding of the ways that MRSA exists in the farm environment and make it easier to compare existing studies which used different sampling methods. Dust samples were taken from 7 Dutch pig farms, with measurements taken in multiple compartments housing pigs of varying ages. These samples were then analyzed for MRSA presence as well as total bacterial count. There was a strong positive relationship between the paired MRSA levels found in EDCs and those measured by pumps. This suggests that in the future EDCs can be used as the primary method for measuring MRSA presence in the pig farm setting.

Introduction:

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a *S. aureus* bacterium which is resistant to most β-lactam antibiotics, a group which includes Penicillins and Cephalosporins (1). Many people colonized with MRSA experience no ill health effects, however MRSA is an opportunistic pathogen and a study found that approximately 11% of patients who enter the hospital as carriers go on to develop and active MRSA infection during their stay (2). Moreover, research has shown that the risk for patients who are colonized with MRSA remains high even after their hospital stay, with one third of these patients developing an active MRSA infection in the year after discharge (3).MRSA is of increasing public health concern as it has properties which make it particularly dangerous if introduced into a healthcare setting, such as an ability to form biofilms and cause intravascular infections, in addition to the risk of serious skin infections and sepsis (4).

Prior to the 1990's MRSA infection was mainly found in hospitalized patients or outpatients who made frequent hospital visits (5). However, genetic studies have determined that the prevalence of MRSA infections in the European population has been increasing in people unconnected to hospitals (5). This is due to the rise in community associated MRSA (CA-

MRSA), some of which can be attributed to the spread of livestock-associated MRSA (LA-MRSA) strains (4,6). LA-MRSA was first described in 2004 when a 6-month-old girl was found to be MRSA positive during a pre-surgery screening, despite lacking the previously established risk factor of having been treated at a foreign hospital (7). The hospital could not decolonize the girl and it was found that her parents, who lived and worked on a pig farm, were also MRSA positive (7). This led researchers to explore livestock farming as a possible source of MRSA in the Dutch population. One such study done by Wulf et al. in 2006, investigated MRSA carriage in another group which has close livestock contact, veterinarians and veterinary students. They found contact with pigs conferred the highest relative risk of MRSA colonization when compared to contact with other types of animals (8). LA-MRSA shows no difference in clinal presentation from hospital-associated MRSA and CA-MRSA but is genetically distinct, with clonal complex 398 (CC398) being the most common clone in Europe (5,9). However, MRSA CC398, does show a reduced ability to spread from person to person, and a study done in a Dutch hospital showed that infections of CC398 were 72% less likely to create secondary cases, when compared with other MRSA strains (10). Nevertheless, MRSA CC398 is quickly evolving, and has demonstrated the ability to acquire foreign DNA, such as genes encoding various virulence factors (9). Studies have already found a sub-clade of CC398 which is presenting in patients without any history of livestock contact (11,12). This indicates that LA-MRSA, which started as a human pathogen and then adapted to colonize animals, is regaining genes which allow it to become more easily transmissible between humans making it a particularly worrying health threat (11,12).

Pigs are an important reservoir of LA-MRSA, and close contact between pigs and humans on farms can lead to MRSA colonization of the anterior nares, from which human to human spread can occur (13). This puts not only farm workers but also their families and close contacts at risk of contracting LA-MRSA. Research has found that the concentration of airborne MRSA in farms is highly correlated to the MRSA loads found in the noses of exposed people (14). Other important factors that influence MRSA transmission from animals to humans include, the prevalence of MRSA in the animals, the number of working hours spent in the stalls, as well as environmental exposure through contaminated dust (15). In the farm environment MRSA can be found in the air as clusters of cells or attached to other airborne particles such as fragments of skin cells or feed, which then fall as settled dust (13). MRSA can be cultured from settled dust for up to 30 days and can easily be re-aerosolized during routine farm activities such as high-pressure cleaning, making dust an important factor in the transmission of LA-MRSA to humans (16).

The parent study for this report, the EU Joint Programming Initiative on Antimicrobial Resistance (JPI-AMR) funded Exclude MRSA project (Preventing transmission of MRSA from livestock to humans through competitive exclusion), aims to reduce MRSA carriage in pigs by introducing benign bacteria into the noses of piglets which could competitively exclude MRSA. By occupying the bacterial niche normally inhabited by MRSA, the introduced bacteria would lower the burden of MRSA on the farm and therefore reduce risk of infection for the farm workers. Commercially available competitive exclusion products, such as Aviguard, have been successfully implemented in the poultry sector to combat Salmonella colonization (17). Over a three-year period, the efficacy of the intervention will be assessed in Ireland, Germany and The Netherlands. During this study the change in pig nasal and airborne MRSA abundance will be evaluated. To estimate the possible impact of this intervention on the prevalence of LA-MRSA carriage among farmers, it is important to have a complete understanding of the occupational

exposure and risk to become a MRSA carrier in farm workers. In Exclude-MRSA, airborne MRSA in the pig stables will be evaluated using settled dust collected using passive dust samplers, called electrostatic dust fall collectors (EDCs). The main aim of the study is to understand the relationship between MRSA as found by EDCs and that found by pumps. Additionally, if the EDCs are determined to be a good predictor of MRSA load in the environment, they could be used in place of active air sampling equipment in future studies. This could be an advantage as EDCs are easier to use and more cost effective than pumps and could therefore be implemented at a larger scale. Furthermore, an understanding of the relationship between pumps and EDCs could help to make studies more comparable in the future as EDC's simple construction means that it has few variables which can be changed form study to study, when compared to active pumps (18).

Previous studies have found EDCs to be useful and reproducible tools for measuring the microbial composition of dust found in farm environments (19). Additionally, EDCs are not size selective, they collect any size particle which is small enough to become airborne, providing a complete picture of the microbial environment (20). While the pumps used for active air sampling are size selective, selecting only particles small enough to be inhaled. The use of personal air samplers provided an estimate of what portion of the MRSA in the environment that workers are exposed, reflecting inhalation exposure during a workday. All this information will hopefully contribute to filling the knowledge gap of possible environmental reservoirs for MRSA in the pig farm environment, which currently poses a challenge in the creation of accurate risk modeling (18). If EDCs and pumps demonstrate to be equivalent methods of sampling then it will aid in growing the body of research which can be used to inform risk models such as the one proposed by Sørensen et al. in 2020, as currently the assumptions for the model are based on studies which used different sampling methods.

In the present study, we collected active (stationary and personal air sampling) and passive (EDCs) air samples on Dutch pig farms. MRSA loads were determined using qPCR targeting *femA*, *nuc* and *mecA* genes as a portion of the total bacterial load in the sample. The total number of all species of bacteria present was determined by 16S rRNA analysis. 16S rRNA is a gene that is highly conserved across bacterial species and can therefore be used to quantify the total number of bacteria found in a sample. For MRSA quantification, *nuc and femA* were selected as specific markers for all S. *aureus*, including antibiotic susceptible types, and *mecA* is responsible for MRSA's resistance to β-lactam antibiotics (21).The *mecA* gene encodes an altered penicillin-binding, methicillin resistant protein which is needed for bacterial wall synthesis which leads to the antibiotic resistance seen in MRSA (21,22). The presence of all three of these genes, *femA*, *nuc*, and *mecA*, strongly indicates the presence of MRSA in a sample.

Aims and Objectives

The aim of this study is to provide information on how MRSA measured in dust collected using a passive air sampling method (EDCs) compares to inhalable dust (active air sampling using stationary pumps) over a variety of pig farm environments. Understanding the relationship between these sampling methods could allow future research to use EDCs in place of pumps. EDCs have many advantages such as being cost effective and easy to use and could therefore be implemented at a larger scale. This information could also contribute to risk modeling by broadening the range of studies, both past and future, which can be used to inform the assumptions of risk modeling for MRSA in farms.

Methods:

Study population

Pig farmers were recruited from across the Netherlands. Farms could be of any size, and only farms using all organic practices were excluded, as they were unlikely to have MRSA present. A total of 7 farms were sampled. Every farmer was given a general questionnaire, asking about antimicrobial use and general biosecurity practices which could potentially influence the microbial diversity and abundance on the farm. On 6 of the farms, farm workers were recruited to wear personal air sampling devices during their shifts in the stables. The famer owners and every participant who wore a personal air sampler completed informed consent forms.

Questionnaire Data

A 26-item questionnaire was completed at each farm. The questions explored biosecurity practices at the farm as well as factors which may influence airborne dust such as the type of bedding used in the stables.

Sampling and storage

Three types of dust/air samples were collected at the farms: EDCs, stationary pump and personal pump air samples.

Electrostatic dust fall collectors (EDCs)

Electrostatic dust fall collectors (EDCs) were used to sample settled dust in the pig farm stables. EDCs contain two electrostatic dust cloths held by a plastic frame and placed onto a

Figure 1: Image of an EDC hanging in a pig stable

cardboard platform and hung (see Figure 1). EDCs were placed in an average of three different stalls, containing pigs of a range of age groups. At each farm we sampled the following compartments: a nursery compartment, a weaned piglets and a fattening pigs' compartment, except for 2 farms where only adult animals (fattening pigs) were sampled. At the farms where only adult animals were sampled, there was an effort made to place the EDCs and pumps in the compartments housing the oldest and youngest animals available to get a range of information.

The EDCs were hung at an average height of 1.5m in order to catch airborne matter and be out of reach of the animals. EDCs were then left in place for 7 days before being sent to the laboratory through the post by the farmer. An average of two EDCs were hung in each stall acting as duplicate measurements. Additionally, a blank EDC was taken to each farm but remained sealed and was sent back with the other samples and treated as a negative control. Once the EDCs arrived at the lab, both sides were sterilely removed from the plastic frame and placed in plastic zip top bags and stored at -20°C until analysis.

Active air sampling

Active air sampling was performed using Gilian Gilair 5 air sampling pumps along with Teflon filters (Filters: PE Drain Disc, Whatman: GE Healthcare, with Teflon 2.0μm 37mm: Pall Corporation) in GSP sampling heads. The pumps were used both for the stationary air measurements and the personal air sampling. Pumps were calibrated before use and the flow was checked after sampling to ensure consistency and to detect any malfunctioning pumps. The flow rate 3.5 liters per minute on average. At each farm a blank filter was transported to the farm but remained sealed and used as a negative control. An average of two pumps were placed in each stall and acted as duplicates. The pump sampling heads were clamped onto a metal rod which was 1.5m tall (see Figure 2). Any peculiarities, such as malfunctioning pumps or torn filters were recorded on the field forms. Personal air pumps were worn for approximately 6 hours while farm workers went about their daily tasks. A questionnaire was given to the participants wearing personal air samplers to gather further information on any activities which may have had an impact on the dust measurements. The personal air samplers were switched off during breaks when participants were in areas such as the canteen or office spaces and turned back on when they re-entered spaces containing animals. Stationary pumps were left in place for 6 hours and checked periodically. Once the sampling period was complete, the filters were sterile removed in the laboratory, placed into petri until analysis.

Figure 2: Image of field worker setting up stationary pump

Laboratory Methods

Once in the laboratory EDCs were thawed and placed into stomacher bags containing FE buffer and blended to suspend the material. The liquid was then transferred into in a 50ml tube and centrifuged at 50g for 1 minute, the top 2ml of the resulting suspension was either used immediately or frozen at -20°C until DNA extraction could proceed. In total, 300 μlmicroliters of the EDC liquid was added to a tube containing lysis buffer and zirconia beads.

Air filters were prepared for DNA extraction by being allowed to thaw and being placed into tubes containing zirconia beads. Next 600μl lysis buffer was added to the tubes. 500μl Phenol was added to both tubes containing the air filters and beads, as well as the tubes containing the EDC suspension and beads, and all tubes were bead beaten. The supernatant was then removed and used as the samples for DNA extraction and purification.

The elution volume was set to 67 μlmicroliters. Five microliters of DNA isolate was used in the following quantitative Real Time PCRs targeting, *femA, nuc*, *mecA*, and 16S rRNA, with all samples being run in duplicate. All targets were detected using the LightCyler480 and associated program. Each *femA/nuc/MecA* reaction consisted of 4 μl PCR grade water, 1 μl primer/probe mix and 10 μl LC480 probe mix. Each 16S rRNA reaction contained 7 μl PCR grade water, 1 μl primer 355F at 100uM, 1 μl primer 556R 10uM and 10 μl Cyber green master mix.

Statistical Analyses

Statistical analysis was performed using "R" software (version 4.1.0). The average Cp from the PCR replicates from the qPCR analysis was taken. Cp values over 40 were excluded, as they were considered outside of the reliable range of the PCR. Cell counts were calculated from formulas derived from the creation of a dilution curve made with a reference MRSA strain (B9 2-4662). The cell counts were then divided by their corresponding 16S measurements to give the relative abundance. Pump values were adjusted to account for flow rate and run time.

Next, as it is not possible to quantify MRSA presence with any one of the qPCR targets alone, MRSA abundance was estimated based on the presence of multiple targets. S. *aureus* count was estimated from the *nuc* or *FemA* count—whichever was highest—then the MRSA count was determined by taking the S. *aureus* count or *mecA* count whichever is lowest, as described in Bos et al in 2016. In cases where only *femA* or *nuc* values were present the S. *aureus* count was calculated from the available value. Pearson's correlation coefficients were calculated to indicate the correlation between targets. One sample was removed from the dataset due to a pump failure.

ANOVAs were performed to determine whether the MRSA levels found on the farms was significantly different, as well to determine if levels were significantly different in the compartments housing animals or different ages.

A Deming or orthogonal regression was performed on the paired EDC and pump data, as they are both continuous variables. The Deming regression was used as it accounts for error in both the x and y axis in place of the standard least squared regression which measures error only in the y axis, thus the Deming regression is often used in protocols which aim to compare measurement methods, such as in this study (23).

Results

Farm Characteristics

General farm characteristics are displayed in Table 1. Farms 2 and 7 had only adult animals present. All farms reported that no antibiotics were used in the week leading up the sampling in the compartments sampled.

Farm ID	Types of Animals Present	Number of EDCs	Number of Stationary Air Samples	Number of Personal air samples
1	Piglets and Sows, Weaned Piglets, Fattening Pigs	6	2	none
2	Piglets and Sows, Weaned Piglets, Fattening Pigs	9	6	2
3	Piglets and Sows, Weaned Piglets, Fattening Pigs	9	6	1
4	Fattening Pigs	6	4	1
5	Piglets and Sows, Weaned Piglets, Fattening Pigs	8	6	1
6	Piglets and Sows, Weaned Piglets, Sows	9	6	1
7	Fattening Pigs	6	4	1
Total	--	53	34	7

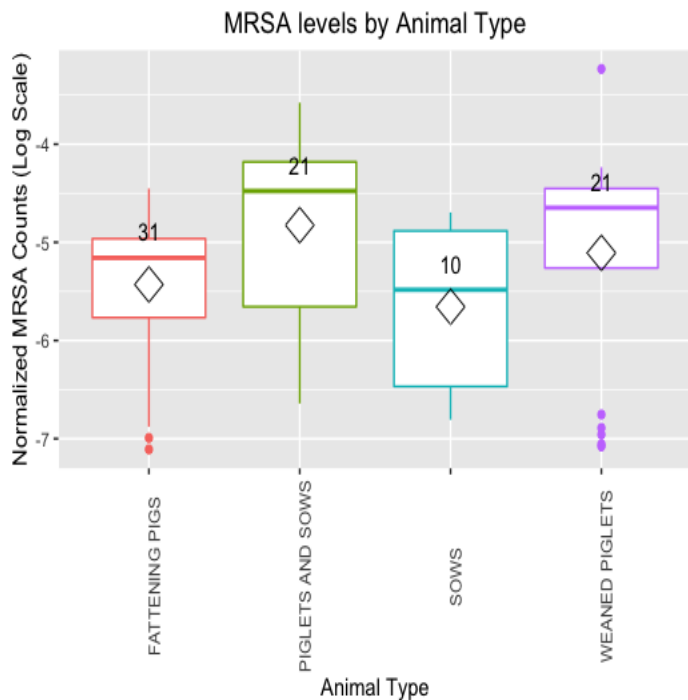*Table 1 Overview of samples collected*

MRSA levels by Animal Type

Figure 3:Boxplot of normalized MRSA level by animal category. The diamond icon indicates the mean MRSA measurement in that category, and the number over each box is the number of measurements taken in the category.

In all figures the MRSA load is expressed as the log transformed, relative (16S normalized) MRSA count.

None of the farms reported any antibiotic use in the week before sampling. None of the field blanks registered any S. aureus

MRSA could be measured in all but 3 of the 87 EDC and stationary pump samples, and was present on every farm sampled, one value was removed due to a pump failure. The MRSA loads found in the compartments housing young animals (sows and suckling piglets, and weaned piglets) were found by ANOVA to be statically higher different than those containing only adult animals (fattening pigs and sows) for both EDC and stationary pump measurement methods (Figure 3). The ANOVAs yielded p-values of less than 0.001. ANOVAs were also performed to test differences between MRSA levels on farms for both measurement methods. Farm 2 emerged as having significantly lower average MRSA counts than the others, using both measurement methods, the ANOVAs yielded a p-values less than 0.001.

Correlation between PCR Targets

There was a positive correlation found between all three gene targets (*mecA, femA,* and *nuc*). The highest Pearson correlation coefficient (PCC) was found between *femA* and *nuc* (PCC=0.98, p-value < 0.005). which indicates S. *aureus* presence in the sample. The correlation coefficients between *mecA* and *femA* (PCC=0.32, p-value < 0.005).), and *mecA* and *nuc* (PCC=0.34 p-value < 0.005) were similar. Figure 4 shows the normalized counts for all measurements from both methods plotted against each other, to illustrate the relationship between the relative amounts of each gene found in the samples.
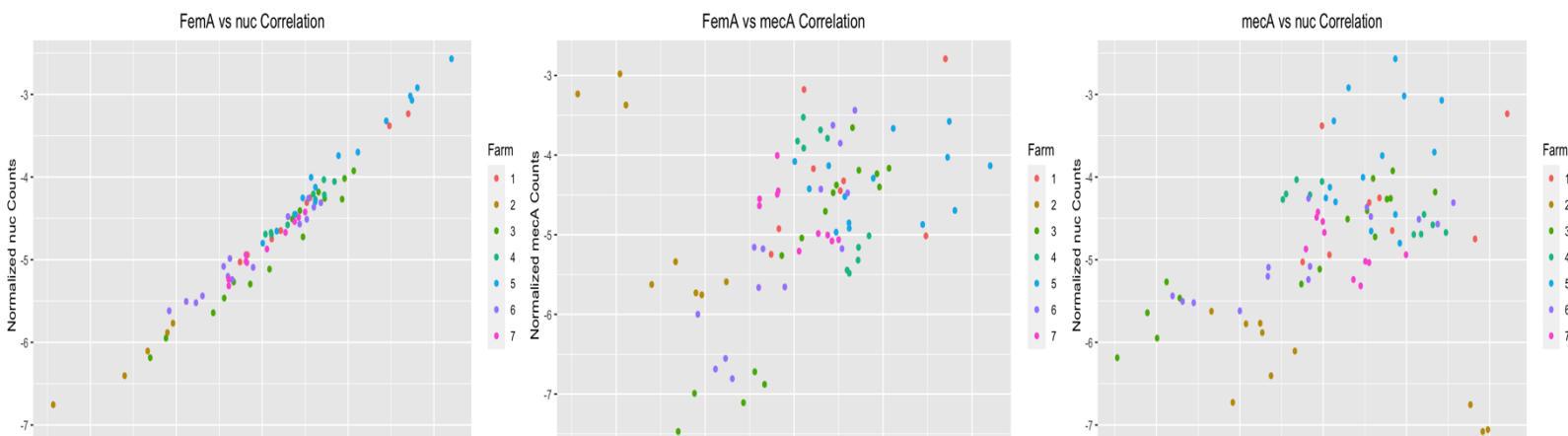

Figure 4: Correlation plots between normalized gene counts on a log scale

Comparison of EDC and Pump Measurements

A Deming orthogonal regression was performed on MRSA measured in inhalable dust samples by the pumps and the MRSA from the EDCs and found a positive relationship. There was an R-squared of 0.93, a slope of 1.02 indicating that a change in what was measured by the pumps had a closely corresponding change in what was measured by EDCs (Table 2). Figure 5 plots the median level of MRSA as measured by pumps against that measured by EDCs in the same compartment on a farm, along with the Deming regression line. In addition to the regression, a Pearson's correlation coefficient was also calculated and yielded a value of 0.90 (p-value < 0.005).
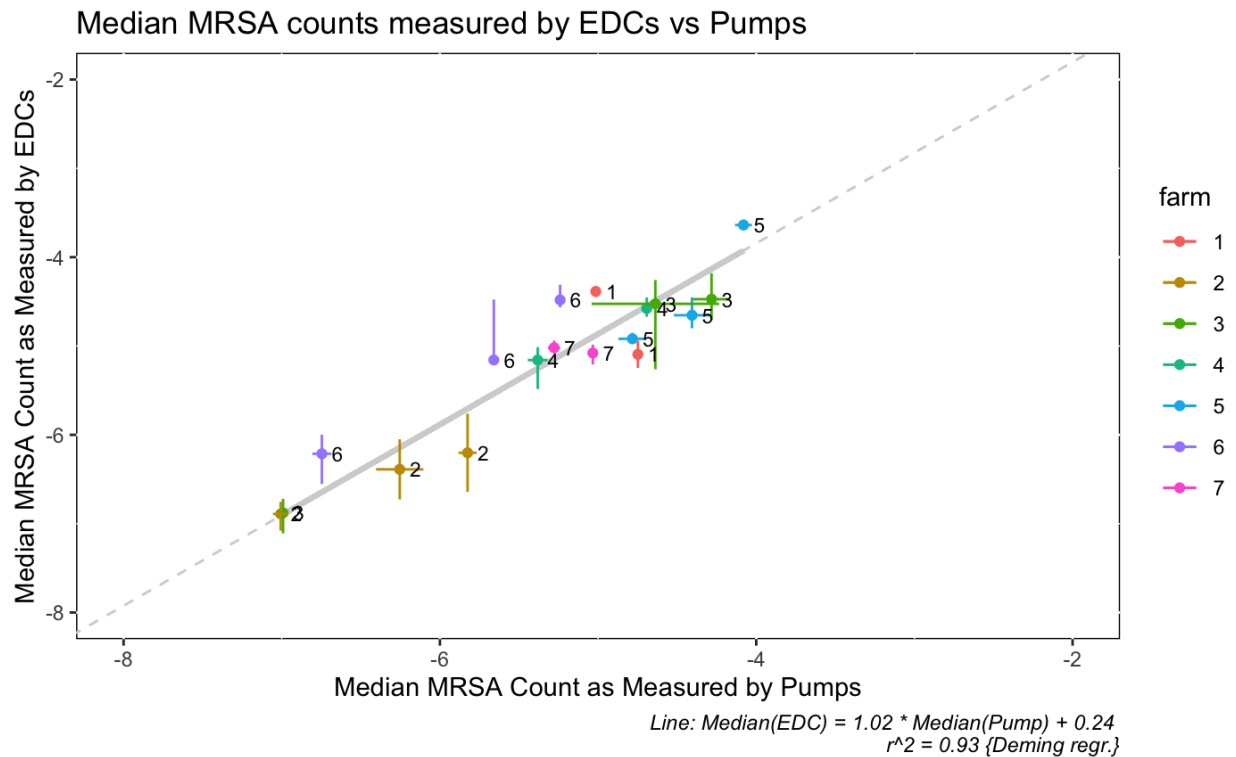

Figure 5: Median MRSA counts as measured by EDCs vs Pumps paired by compartment within each farm. The cross bars on each point indicate the maximum and minimum measurements taken in that compartment. The grey line indicates the Deming regression line.

Slope	Slope: 95% Confidence Interval	Intercept	Intercept: 95% Confidence Interval	R-squared
1.02	0.864 – 1.20	0.24	-0.663 – 1.23	0.93

Table 2: Deming regression summary statistics

MRSA levels by Sampling Location

Personal Pumps

There was at least one personal air sample taken on 6 of the 7 farms sampled. In total there were 7 personal samples all of which had measurable MRSA present in the filter. The Pearson correlation test between the average MRSA measured by the stationary pumps and the personal pumps yielded a non-significant result. Figure 6 illustrates the personal pumps relationship when compared to other locations sampled.
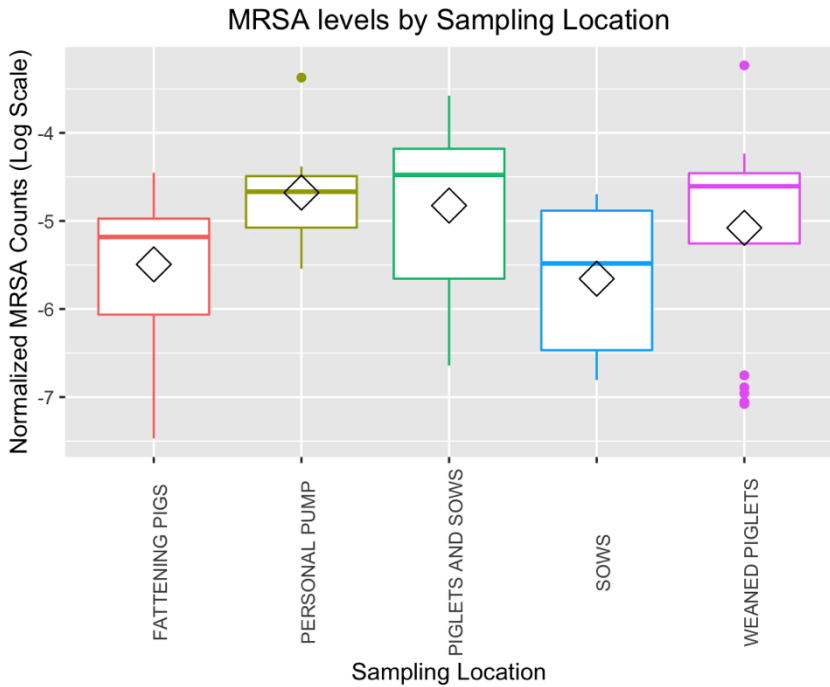
Figure 6: Boxplot indicating the distribution of MRSA by sampling area, the diamond symbol indicates the mean value in the category.

Questionnaire

The questionnaire data was gathered to help explain any differences between farms but due to the small number of farms included in the study no definite conclusions can be drawn from the information. The questions asked about practices such as cleaning procedures and biosecurity measures such as whether all materials used are placed along working lines (youngest to oldest animals). For example, one question asked about the types of cleaners used to clean the stables and Farm 2 is the only farm which cleaned with water alone, which could be one explanation for the overall lower MRSA level found at farm 2. Figure 7 is a Beeswarm plot, where the MRSA levels all the samples, EDC and stationary pump, are plotted by farm according to their answer to the question "What products are used to clean and disinfect the stables?".
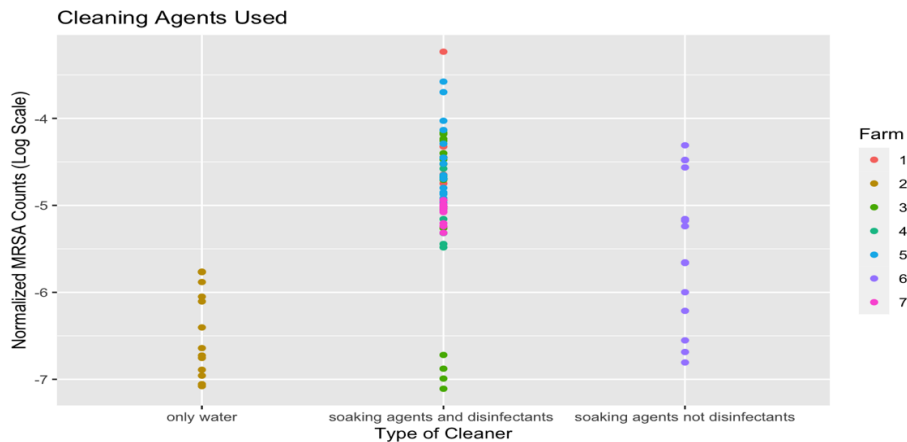

Figure 7: Beeswarm plot of normalized MRSA count by questionnaire responses on cleaning products used in stables

Normalization of Data

All the data in this report has been expressed as normalized values, meaning that for each sample the calculated amount of MRSA was then divided by the corresponding 16S value. As 16S is a gene element that is conserved across bacterial species, the 16S value can be used as a measure of total bacterial load in the sample. By dividing by the 16S value of a sample we are left with a relative abundance of MRSA. To confirm the results of this study, the Deming regression was also performed on the non-normalized data, and the strong positive relationship between MRSA as measured by the stationary pumps and that measured by the EDCs remained. Table 3 contains the Deming regression summary statistics for both the normalized and non-normalized values. In addition to the regression, a Pearson's correlation coefficient was also calculated with the non-normalized data and yielded a value of 0.84 (p-value < 0.005).

	Slope	Slope: 95% Confidence Interval	Intercept	Intercept: 95% Confidence Interval	R-squared
Normalized Data	1.02	0.858 – 1.19	0.24	-0.656 – 1.28	0.93
Non-Normalized Data	0.85	0.654 - 1.02	0.26	-0.28 - 0.955	0.89

Table 3: Comparison of Deming Regression summary statistics using normalized and non-normalized data.

Discussion

The information from this study adds to the existing body of work which demonstrates that EDCs are a viable way to quantify biological agents in air samples of various environments. In the present study, we showed that EDCs can be used to measure airborne MRSA levels in pig farms.

Benefits of EDCs

One of the major advantages of EDC use is their ability to be used for prolonged periods of time to potentially reflect cumulative exposure (19). Settled dust collected over a longer period is presumed to be an aggregate sample of what is airborne in the setting on average, in contrast with active air sampling which is highly subject to conditions at the time of sampling (20). For example, cleaning activities on the farm can stir up settled dust and re-aerosolize MRSA containing dust for a short period of time but would not be representative of the average amount of airborne dust (16). A longer sampling period for active air pumps is not viable if the sample is being used for a culture-based method, as the filters become saturated and the air flow dries out microorganisms, leading to a loss of diversity (19).

Additionally, while reproducibility was not directly measured in this study, EDCs have in the past, been found to produce reproducible results during repeated sampling campaigns in the same setting (19,20). In 2008, Noss et al. used EDCs to measure endotoxin levels in farms and in farm and non-farm homes. There they found that endotoxin levels measured in consecutive periods in the same home yielded a correlation value of 0.85 (20). demonstrating suitable reproducibility. Normand et al also found that fungal and bacterial diversity as measured by EDCs was reproducible when sampling in the same location. In addition, they found a 0.82 pairwise correlation value from EDCs left in the same locations for differing lengths of time (15 days and

1 month), indicating that EDC results could be comparable in studies which used differing sampling durations (19). A future study which confirms this capacity for reproducibility of EDCs for MRSA measurements would be beneficial.

Previous work has shown that in addition to measuring endotoxin and bacterial and fungal diversity, EDCs can be used to measure other types of environmental exposures such as fungal contamination, total particulate matter, bacterial bioburden, and viral RNA (24,25). This capacity could be utilized in future studies to employ EDCs on a large scale to get a complete picture of the airborne occupational exposures in indoor areas.

Personal air samples

Personal air samples were used in this study as they provide a measurement which is more representative of workers' actual exposure during the day. Workers move around different compartments and perform activities such as cleaning and handling animals which cause more dust to be released into the air, which means that stationary measurements could underestimate the actual occupational exposure (16). While there was no significant relationship between the MRSA measured personal pumps and the stationary pumps, there was a small number of personal samples in this study, and a more in-depth investigation into this relationship would be valuable in the future.

In a previous study, it was found that MRSA presence in the air is a strong indicator of MRSA carriage in the nose of workers (15). Personal air measurements could be an important part of future studies which aim to assess the human health impact of reducing antibiotic use in the livestock sector. As it was found that MRSA presence in the air is a strong indicator of MRSA carriage in the nose of workers (15). The number of hours worked in the farms and the level of antibiotic use in the animals also influences workers likelihood to be colonized with MRSA (26).

Differences between Compartments

In 2016, Bos et al. found the highest level of MRSA both in the air and nasal prevalence in workers who worked with the farrowing pig population. This result is mirrored in this study where the highest levels of MRSA were also seen in compartments containing the youngest animals. This may be due to the fact that younger animals generally receive larger amounts of antimicrobial drugs than older animals and undergo procedures such as teeth clipping which make them more susceptible to infection (26). While no antibiotic use was reported in the week before sampling in this study, we did not request access to information on past antimicrobial use or for a history of procedures done with the animals. Another possible explanation for higher MRSA carriage in younger animals is the possible re-use of the tools used in procedures such as teeth clipping between piglets, transferring bacteria between young animals in a way which is not seen in older animals (26).

Differences between Farms

There were significant differences between farms that could possibly be due to differences in biosecurity factors as examined in the questionnaire. Such as the significantly lower MRSA level found in Farm 2, and it was the only farm which indicated that they use only water to clean the compartments. Previous research has found that the use of certain common disinfectants (Quaternary Ammonium Compound based agents) is an important driver for

selection of MRSA in pig herds (27). Data on the types of disinfectants used was not gathered in this questionnaire but could be an interesting topic for further study.

Another factor which may have influenced the differences between farms is that they were sampled over a period of a few months, with the first farm sampled in the winter and the last in late spring. It has been found that in the winter months the bacterial load and numbers of airborne particles are higher in pig farms (28). At different times in the year, the levels of microorganisms and particles can vary due to changes in ventilation and animal density (19). In the winter animals are likely to be continuously present and ventilation may not be opened as often leading to more dust accumulation. However, as the data in this study was normalized to give relative amounts of MRSA present, the seasonal variation in overall bacterial load may not have a strong influence.

Normalization of Data

As has been previously mentioned the data in the is study was normalized to give the relative amounts of MRSA present in a sample. This was done in an effort to make samples taken with different sampling methods and at different times in the year more comparable. It is important to note however, that the results without normalization show the same strong positive relationship between MRSA measurement taken by the pumps and those measured by the EDCs, as the normalized data.

Drawbacks

One evident drawback of this study was the relatively small sample size of farms sampled. However, despite its small size this study sampled pigs at various ages and provides important information on the feasibility of large-scale EDC use, as well finding a significant correlation between the sampling methods employed.

Implications for Risk Modeling

In 2020, Sørensen et al., developed a model for LA-MRSA spread in pig farms, to be used as a tool for evaluating possible interventions. The model aims to aid in studying how different methods of reducing MRSA load in stables could limit the spread to humans, and even prevent spread to other farms. To do this they model how MRSA spreads within a pig herd through contaminated air, which can be used as a proxy for human risk (18).They also assess the potential impact of two types of interventions, where the MRSA shed by pigs is reduced, and where the MRSA level is reduced in the air without altering the prevalence in the animals (18).

Sørensen et al. note that they encountered a lack of previous studies with which to compare the models' predicted air concentrations of LA-MRSA. This was in part due to the fact that in the few studies that are available some used EDCs and others used active air sampling making them hard to compare to each other. The data presented in this study could help to make more previous studies results equivalent and thus fill the knowledge gap and help improve the assumptions made in the model.

The Continued Relevance of MRSA Monitoring

In an effort to curb the rise of antibiotic resistant bacteria, in 2009 the Dutch government introduced a strict set of rules for how antimicrobials can be used in livestock (29). Subsequently antibiotic use in the industry fell by 59% between 2009 and 2014 (29). Despite this, a study done

in the Netherlands in 2016 by Dierikx et al, found that by the time pigs arrived at slaughterhouses, all 56 of the batches of animals tested positive for MRSA. This may be due to animals from positive farms mixing with those from negative ones during transport, however this illustrates that MRSA presence in even a small number of farms is a risk despite a lower level of MRSA present on farms in the Netherlands in general (30). Additionally, it is not yet known what the "dose-response" effect of reducing antibiotic use will have on the presence of resistant bacteria, this in conjunction with the knowledge that LA-MRSA has shown to be highly adaptable means that continual long-term monitoring will be important for years to come (11,12,29).

Conclusion

This study demonstrates that EDCs are a viable candidate for a sampling method to be used in large testing campaigns looking to measure MRSA in pig farms, when compared to active air sampling. This study adds to the existing body of work which demonstrated that EDCs are a feasible, and easily standardized way to measure airborne dust. This data establishes that EDCs can be used accurately in a variety of pig stables areas with a range of airborne MRSA concentrations when compared to active air sampling.

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Appendix
'R' Code

```
---
title: "Exclude MRSA Final"
author: "Anne Rittscher"
date: "12/30/2021"
output: html_document
---

```{r setup, include=FALSE}
knitr::opts_chunk$set(echo = TRUE)
```
```

Load in Libraries

```{r}
library(ggplot2)
library(dplyr)
library(tidyr)
library(readxl)
library(e1071)
library(fitdistrplus)
library(beeswarm)
library(olsrr)
library(MethComp)
```

Load in data

```{r}
setwd("~/Desktop/Exclude MRSA/Final")
XMRSA<-read_xlsx("Copy of XMRSA PCR data
Master list_Nov29.xlsx", sheet="Sheet 1 -
XMRSA PCR data Master", range="A1:M995")
XMRSAPumpinfo<-read_xlsx("XMRSA PCR
data Master list_Nov09_with pumpinfo.xlsx",
sheet="Pump info", range="A1:S43")
```

Overview Plot

```{r}
ggplot(XMRSA, aes(x=Location.upper, y=Cp,
col=Target))+geom_boxplot()+theme(axis.text.x =
element_text(angle = 90))
```

Remove Everything that is NOT a replicate
(removing blanks and PCR controls), and add a
new column with original Cp values so they can be
refereed back to. NA Cp values were replaced by
50 to indicate that they is a very low amount or no
DNA in the sample and it was out of range of the
PCR.

```{r}
data1 <- XMRSA[XMRSA$Dupl %in% c("A",
"B"),]
data1$newCp=data1$Cp
```

```
data1$newCp[is.na(data1$newCp)] <- 50
head(data1)
data2<-data1[data1$newCp<41,]
```

Makes each target their own set where NTC are
excluded, certain columns are included, and the
table is pivoted. The absolute value is taken from
the duplicates and marked acceptable is the
difference is less than 1. Next columns are
renamed to refelct the target. Lastly the sets are
rejoined to make one large table with all the gene
targets, called "db".

```{r}
db.16S <- data2[data2$Target=="16S",] %>%
#select target
  filter(Sample.Type != "NTC") %>% #exclude
NTCs
  dplyr::select(Farm, Sample.ID, Sample.Type,
Location.upper, newCp, Dupl,Compartment) %>%
#include certain columns
  pivot_wider(names_from=Dupl,
values_from=newCp) %>% #flips rows and
columns
  mutate(Dupl.OK=ifelse(abs(A-B)>2, "*", ""))
%>% # takes absolute values of the duplicates, is
okay if diff <1
  rename("A.16S"="A",
"B.16S"="B","Dupl.OK.16S"="Dupl.OK")
#renaming columns
db.femA <- data2[data2$Target=="femA",] %>%
  filter(Sample.Type != "NTC") %>%
  dplyr::select( Sample.ID, newCp,Dupl) %>%
  pivot_wider(names_from=Dupl,
values_from=newCp) %>%
  mutate(Dupl.OK=ifelse(abs(A-B)>2, "*", ""))
%>%
  rename("A.femA"="A", "B.femA"= "B",
"Dupl.OK.femA"="Dupl.OK")
db.mecA<- data2[data2$Target=="mecA",] %>%
  filter(Sample.Type != "NTC") %>%
  dplyr::select(Sample.ID, newCp, Dupl) %>%
  pivot_wider(names_from=Dupl,
values_from=newCp) %>%
  mutate(Dupl.OK=ifelse(abs(A-B)>2, "*", ""))
%>%
  rename("A.mecA"="A", "B.mecA"= "B",
"Dupl.OK.mecA"="Dupl.OK")
```

```r
db.nuc <- data2[data2$Target=="nuc",] %>%
  filter(Sample.Type != "NTC") %>%
  dplyr::select(Sample.ID,newCp,Dupl) %>%
  pivot_wider(names_from=Dupl,
values_from=newCp) %>%
  mutate(Dupl.OK=ifelse(abs(A-B)>2, "*", "")) %>%
  rename("A.nuc"="A", "B.nuc"= "B",
"Dupl.OK.nuc"="Dupl.OK")

db <- left_join(db.16S, db.femA, by="Sample.ID")
db <- left_join(db, db.mecA, by="Sample.ID")
db <- left_join(db, db.nuc, by="Sample.ID")
rm(db.16S, db.femA, db.mecA, db.nuc)
```

```
Pump standardization, corrects for run time and
flow rate. data frame db is combined with pump
info to make dbpumpinfo
```
```r
PumpInfo<-
dplyr::select(XMRSAPumpinfo,c('Sample.ID','Flow Rate','Pump Run Time'))
PumpInfo<-na.omit(PumpInfo)
PumpInfo$RunTime<-
as.numeric(PumpInfo$`Pump Run Time`)
PumpInfo<-
dplyr::select(PumpInfo,c('Sample.ID','Flow Rate','RunTime'))
PumpInfo$Vol_Air_Sampled=(PumpInfo$`Flow Rate`*PumpInfo$RunTime)
colnames(PumpInfo)<-c("Sample.ID","AvgFlow Rate","RunTime","VolumeAirSampled")

PumpInfo$Sample.ID<-
as.character(PumpInfo$Sample.ID)
dbPumpInfo<-
left_join(db,PumpInfo,by="Sample.ID")
```
Next mean Cps per sample were calculated.The
new table containing cell counts is called db1.
```r
db1 <- dbPumpInfo %>%
  mutate(mean_Cp_16S=(A.16S+B.16S)/2) %>%
  mutate(mean_Cp_femA=(A.femA+B.femA)/2) %>%
  mutate(mean_Cp_mecA=(A.mecA+B.mecA)/2) %>%
  mutate(mean_Cp_nuc=(A.nuc+B.nuc)/2) %>%
  filter(!Location.upper %in% c("BANK EDC",
"BLANK", "BLANK EDC", "BLANK FILTER",
"PERSONAL PUMP"))
```

Next Cell counts were calculated. The log10 of
these values was then taken. The new table
containing normalized cell counts is called db2.
The formulas for the cell count calculation were
derived from calibration curves made by diluting
down know concentrations of DNA. If one of the
replicates was NA then the value from the
remaining replicate was taken.
```r
db2 <- db1 %>%
  mutate (OutcomeFemA=case_when(
  is.na(A.femA) & !is.na(B.femA) ~ B.femA,
  !is.na(A.femA) & is.na(B.femA) ~A.femA,
  !is.na(A.femA) & !is.na(B.femA) ~
mean_Cp_femA
  ))%>%
  mutate (Outcomenuc=case_when(
   is.na(A.nuc) & !is.na(B.nuc) ~ B.nuc,
   !is.na(A.nuc) & is.na(B.nuc) ~A.nuc,
   !is.na(A.nuc) & !is.na(B.nuc)~ mean_Cp_nuc
  ))%>%
  mutate (OutcomemecA=case_when(
   is.na(A.mecA) & !is.na(B.mecA) ~ B.mecA,
   !is.na(A.mecA) & is.na(B.mecA) ~A.mecA,
   !is.na(A.mecA) & !is.na(B.mecA)~
mean_Cp_mecA
  ))%>%
  mutate(cellcount.16S=10^((mean_Cp_16S-40.22)/-4.043)) %>%
  mutate(cellcount.femA=10^((OutcomeFemA-41.896)/-3.5474)) %>%
  mutate(cellcount.mecA=10^((OutcomemecA-39.716)/-3.2823)) %>%
  mutate(cellcount.nuc=10^((Outcomenuc-43.285)/-3.7116))
```
```
Adjusts cell count to cell count per meters squared
of air sampled by the pump. Lastly values were
normalized by dividing the cell count of FemA,
nuc, and MecA values by their corresponding 16S
value to get relative abundance values. Removes
one failed pump.
```
```r
db2b <- db2 %>%

mutate(cellcount.16S.corr=ifelse(Sample.Type=="PUMP", cellcount.16S/VolumeAirSampled*1000,
cellcount.16S))%>%

mutate(cellcount.femA.corr=ifelse(Sample.Type="PUMP",
cellcount.femA/VolumeAirSampled*1000,
cellcount.femA))%>%
```

```
mutate(cellcount.mecA.corr=ifelse(Sample.Type=
="PUMP",
cellcount.mecA/VolumeAirSampled*1000,
cellcount.mecA))%>%

mutate(cellcount.nuc.corr=ifelse(Sample.Type=="
PUMP", cellcount.nuc/VolumeAirSampled*1000,
cellcount.nuc))

db3 <- db2b %>%

mutate(normal.femA=log10(cellcount.femA.corr/c
ellcount.16S.corr)) %>%

mutate(normal.mecA=log10(cellcount.mecA.corr/
cellcount.16S.corr)) %>%

mutate(normal.nuc=log10(cellcount.nuc.corr/cellc
ount.16S.corr))

#Removes failed pump #303046 has no run time

db3<-db3[(db3$Sample.ID!=303046),]
```

Plotting normalized cell count values against animals type

```{r}
ggplot(db3, aes(x=Location.upper,
y=normal.femA))+geom_boxplot()+labs(title="Fe
mA")+theme(axis.text.x = element_text(angle =
90))
ggplot(db3, aes(x=Location.upper,
y=normal.mecA))+geom_boxplot()+labs(title="M
ecA")+theme(axis.text.x = element_text(angle =
90))
ggplot(db3, aes(x=Location.upper,
y=normal.nuc))+geom_boxplot()+labs(title="nuc")
+theme(axis.text.x = element_text(angle = 90))
```

Plot normalized cell counts against each other to determine relationship

```{r}
ggplot(db3,aes(x=normal.femA,y=normal.mecA))
+geom_point(aes(color=factor(Farm)))+ggtitle('Fe
mA vs mecA Correlation')+ylab('Normalized
mecA Counts')+xlab('Normalized FemA
Counts')+labs(color='Farm')+theme(plot.title =
element_text(hjust = 0.5))

ggplot(db3,aes(x=normal.femA,y=normal.nuc))+g
eom_point(aes(color=factor(Farm)))+ggtitle('Fem
A vs nuc Correlation')+ylab('Normalized nuc
Counts')+xlab('Normalized FemA
Counts')+labs(color='Farm')+theme(plot.title =
element_text(hjust = 0.5))

ggplot(db3,aes(x=normal.mecA,y=normal.nuc))+g
eom_point(aes(color=factor(Farm)))+ggtitle('mec
A vs nuc Correlation')+ylab('Normalized nuc
Counts')+xlab('Normalized mecA
Counts')+labs(color='Farm')+theme(plot.title =
element_text(hjust = 0.5))
```

Make a smaller table containing only the gene, value and animal type (location.upper)

```{r}
db4 <- db3 %>%
  dplyr::select(Location.upper, normal.femA,
normal.mecA,
normal.nuc,Sample.ID,Compartment,Farm,Sample
.Type)%>%
  pivot_longer(normal.femA:normal.nuc,
names_to="gene", values_to = "value")
```

Beeswarm plots of each gene (normalized cell counts) against the animals type (location.upper) clustered by farm.

```{r}
beeswarm(normal.femA  ~ Location.upper,
data=db3,pwcol =
as.numeric(Farm),col=sample(colors(), 27),
pch=19, method="swarm", cex=0.5)
        legend ("bottomright", legend=c("Farm 1",
"Farm 2","Farm 3","Farm 4","Farm 5","Farm
6","Farm7"),title = "Farm", pch = 16, col = 1:7)

beeswarm(normal.mecA  ~ Location.upper,
data=db3,pwcol =
as.numeric(Farm),col=sample(colors(), 27),
pch=19, method="swarm", cex=0.5)
beeswarm(normal.nuc  ~ Location.upper,
data=db3,pwcol =
as.numeric(Farm),col=sample(colors(), 27),
pch=19, method="swarm", cex=0.5)
```

Beeswarm plots of each gene by farm, these reveled that farm 2 is quite different from the others is normalized FemA and nuc values.

```{r}
beeswarm(normal.femA ~ Farm, data=db3,
method='swarm')
beeswarm(normal.mecA ~ Farm, data=db3,
method='swarm')
beeswarm(normal.nuc ~ Farm, data=db3,
method='swarm')
```

```
```

Normalized Cell counts by animals type all in one plot
```{r}
ggplot(db4,aes(x=Location.upper, y=value, col=gene))+geom_boxplot()+labs(title="Average Normalized Cell Counts",y="Gene Counts",x="Animal Type")+theme(axis.text.x = element_text(angle = 90))
```

Statistical Analysis of relationship between the cell counts of the different genes
```{r}
shapiro.test(db3$normal.femA)#p val=0.1560 NORMAL

shapiro.test(db3$normal.nuc)#p val= 0.1522 NORMAL

shapiro.test(db3$normal.mecA)#p val=0.1362 NORMAL
```

Statistical analysis of relationship between cell counts of the different genes,Pearson correlation because they are normally distributed
```{r}
cor.test(db3$normal.femA,db3$normal.nuc,method='pearson',use='complete.obs')#0.984
cor.test(db3$normal.femA,db3$normal.mecA,method='pearson',use='complete.obs')#0.319
cor.test(db3$normal.nuc,db3$normal.mecA,method='pearson',use='complete.obs')#0.338
```

Add in S.Aureus Counts and MRSA counts
S.Aureus counts are found by taking the highest value between FemA and nuc for a sample and MRSA count was taken between mecA and S.Aureus whichever is lower. For S.aureus counts if femA or nuc were missing a samle, the remaining value was used.
```{r}
#Get S.Aureus counts (highest b/w femA and nuc)

db3 <- db3 %>%
  mutate (S.AreusCounts=case_when(
    !is.na(db3$normal.femA) &
!is.na(db3$normal.nuc) ~
pmax(db3$normal.femA,db3$normal.nuc),
    !is.na(db3$normal.femA) &
is.na(db3$normal.nuc) ~ db3$normal.femA,
    is.na(db3$normal.femA) &
!is.na(db3$normal.nuc) ~ db3$normal.nuc))
```

```
#Get MRSA counts (lowest b/w mecA and S.aureus)
db3$MRSACounts<-pmin(db3$normal.mecA,db3$S.AreusCounts)

#Removes NA values
db3<-db3[!is.na(db3$MRSACounts),]
```

ANOVA to determine if significantly different by farm and animal type
```{r}
beeswarm(MRSACounts  ~ Farm, data=db3,pwcol = as.numeric(Farm),col=sample(colors(), 27), pch=19, method="swarm", cex=0.5)

db3a <- subset(db3, db3$Sample.Type=="EDC")
x <- aov(MRSACounts ~ as.factor(Farm)+ as.factor(Location.upper), data=db3a)
 summary(x)
TUKEY<-TukeyHSD(x)
plot(TUKEY)

db3b<-subset(db3, db3$Sample.Type=="PUMP")
x1 <- aov(MRSACounts ~ as.factor(Farm)+ as.factor(Location.upper), data=db3b)
 summary(x1)
aovMRSAfarm<-aov(MRSACounts ~ as.factor(Farm), data=db3)
summary(aovMRSAfarm)
```

Plotting Avg MRSA count per animal type.
```{r}
ggbox<- ggplot(db3,aes(x=Location.upper, y=MRSACounts,col=Location.upper))+
  geom_boxplot()+
  labs(title="MRSA levels by Animal Type",y="Normalized MRSA Counts (Log Scale)",x="Animal Type")+
  stat_summary(fun=mean, geom="point", shape=5, size=5, color="black", fill="black", position = position_dodge(width = 0))+
  theme(axis.text.x = element_text(angle = 90))+theme(plot.title = element_text(hjust = 0.5))
ggbox+
  annotate("text",
        x = 1:length(table(db3$Location.upper)),
        y = aggregate(MRSACounts ~ Location.upper, db3, median)[ , 2],
        label = table(db3$Location.upper),
        col = "black",
        vjust = - 1)
```

Questionnaire data

```{r}
Qs <-read.csv("Exclude MRSA Questionnaire
Answers.oct.csv", header=TRUE,
sep=",",row.names=1)
colnames(Qs)
colnames(Qs)<-
c('Question','1','2','3','4','5','6','7','Question.number')
rownames(Qs)
Qs1 <- as.data.frame(t(Qs))
colnames(Qs)
ncol(Qs1)
nrow(Qs1)

#delete extra column
dplyr::select(Qs1,-c(Question))
Qs<-subset(Qs1,select=c(-Question))
Qs<-
subset(Qs,rownames=c("Question","1","2","3","4"
,"5","6","7"))
#add on S.AUREUS & MRSA Counts
colnames(Qs) <- Qs[1,]
Qs <- Qs[2:nrow(Qs),]
Qs <- Qs[1:nrow(Qs)-1,]
Qs <- Qs %>%
dplyr::mutate(Farm2=seq.int(nrow(Qs)))

#Questionnaire data
colnames(Qs)<-
c('Q1','Q2','Q3','Q4','Q5','Q6','Q7','Q7A','Q8','Q9','
Q10A','Q10B','Q10C',

'Q11A','Q11B','Q11C','Q12','Q13','Q14','Q15','Q16'
,'Q17A','Q17B','Q17C',
          'Q17D','Q17E',
'Q18','Q19','Q20','Q21','Q22','Q23A','Q23B','Q23C'
,'Q23D',

'Q23E','Q23F','Q24','Q25','Q26','Q27','Q28','Farm')
```
Avg MRSA per farm and formatting of
questionnaire data
```{r}
#avg S.Aureus & MRSA per farm
Farm1Data<-subset(db3,Farm==1)
meanF1S.Areus<-
mean(Farm1Data$S.AreusCounts,na.rm=TRUE)
meanF1MRSA<-
mean(Farm1Data$MRSACounts,na.rm=TRUE)

Farm2Data<-subset(db3,Farm==2)
meanF2S.Areus<-
mean(Farm2Data$S.AreusCounts,na.rm=TRUE)
```

```
meanF2MRSA<-
mean(Farm2Data$MRSACounts,na.rm=TRUE)

Farm3Data<-subset(db3,Farm==3)
meanF3S.Areus<-
mean(Farm3Data$S.AreusCounts,na.rm=TRUE)
meanF3MRSA<-
mean(Farm3Data$MRSACounts,na.rm=TRUE)

Farm4Data<-subset(db3,Farm==4)
meanF4S.Areus<-
mean(Farm4Data$S.AreusCounts,na.rm=TRUE)
meanF4MRSA<-
mean(Farm4Data$MRSACounts,na.rm=TRUE)

Farm5Data<-subset(db3,Farm==5)
meanF5S.Areus<-
mean(Farm5Data$S.AreusCounts,na.rm=TRUE)
meanF5MRSA<-
mean(Farm5Data$MRSACounts,na.rm=TRUE)

Farm6Data<-subset(db3,Farm==6)
meanF6S.Areus<-
mean(Farm6Data$S.AreusCounts,na.rm=TRUE)
meanF6MRSA<-
mean(Farm6Data$MRSACounts,na.rm=TRUE)

Farm7Data<-subset(db3,Farm==7)
meanF7S.Areus<-
mean(Farm7Data$S.AreusCounts,na.rm=TRUE)
meanF7MRSA<-
mean(Farm7Data$MRSACounts,na.rm=TRUE)

#delete extra column
dplyr::select(Qs1,-c(Question))
Qs2<-subset(Qs1,select=c(-Question))
rownames(Qs2)
Qs3<-
subset(Qs2,rownames=c("Question","1","2","3","4"
,"5","6","7"))
```
#add in counts
```
AvgS.Aureus<-
c(0,meanF1S.Areus,meanF2S.Areus,meanF3S.Are
us,meanF4S.Areus,meanF5S.Areus,meanF6S.Areu
s,meanF7S.Areus,0)
AvgMRSA<-
c(0,meanF1MRSA,meanF2MRSA,meanF3MRSA,
meanF4MRSA,meanF5MRSA,meanF6MRSA,me
anF7MRSA,0)
Qs3$MRSAcount<-AvgMRSA
Qs3$S.Areus<-AvgS.Aureus
#add on S.AUREUS & MRSA Counts
Qs4<-Qs3
```

```
colnames(Qs4) <- Qs4[1,]
Qs4 <- Qs4[2:nrow(Qs4),]
Qs4 <- Qs4[1:nrow(Qs4)-1,]
colnames(Qs4)[43] <- "Avg S.Aureus"
colnames(Qs4)[44] <- "AvgMRSA"
Qs4 <- Qs4 %>%
dplyr::mutate(Farm2=seq.int(nrow(Qs4)))

colnames(Qs3) <- Qs3[1,]
Qs3 <- Qs3[2:nrow(Qs3),]
Qs3 <- Qs3[1:nrow(Qs3)-1,]
colnames(Qs3)[43] <- "Avg S.Aureus"
colnames(Qs3)[44] <- "AvgMRSA"
Qs3 <- Qs3 %>%
dplyr::mutate(Farm2=seq.int(nrow(Qs3)))
#Questionnaire data
colnames(Qs4)<-
c('Q1','Q2','Q3','Q4','Q5','Q6','Q7','Q7A','Q8','Q9','
Q10A','Q10B','Q10C',

'Q11A','Q11B','Q11C','Q12','Q13','Q14','Q15','Q16'
,'Q17A','Q17B','Q17C',
        'Q17D','Q17E',
'Q18','Q19','Q20','Q21','Q22','Q23A','Q23B','Q23C'
,'Q23D',

'Q23E','Q23F','Q24','Q25','Q26','Q27','Q28','AvgS.
Aureus','AvgMRSA','Farm')
```

Combine questionnaire and pcr data
```{r}
Qs5<- Qs4 %>%
  dplyr::select(Farm,Q18,Q22,Q20,Q7,Q25)

db4<-db3 %>%

dplyr::select(Farm,Sample.Type,S.AreusCounts,M
RSACounts,Compartment,Sample.ID,Location.up
per)

db5 <- left_join(db4, Qs5, by="Farm")
```

Plotting the effect of cleaning agents used against
MRSA Counts
```{r}
plotQ27 <- ggplot(db5, aes(x=`Q18`,
y=MRSACounts))+ggtitle('Cleaning Agents
Used')+xlab('Type of Cleaner')+ylab("Normalized
MRSA Counts (Log Scale)")+theme(axis.text.x =
element_text(angle =
0))+geom_point(aes(color=factor(Farm)))+labs(co
lor='Farm')
plotQ27
```

Plotting the effect of the placement of necessary
materials along working lines against MRSA
counts
```{r}
plotQ22 <- ggplot(db5, aes(x=`Q22`,
y=MRSACounts))+ggtitle('Are Nessasary
Materials Along Working Lines')+ylab('MRSA
Counts')+theme(axis.text.x = element_text(angle =
45))+geom_point(aes(color=factor(Farm)))+labs(c
olor='Farm')
plotQ22
```

~Linear Regression Analysis of EDC vs Pump data
Exploratory Plots

MRSA Counts,pumps vs EDCs clustered per farm
and S.Aureus counts pumps vs EDCs clustered per
farm
```{r}
PumpvEDCMRSA <- ggplot(db5,
aes(x=`Sample.Type`,
y=MRSACounts))+ggtitle('MRSA counts by
Sampling Method')+xlab('Sample
Type')+ylab('MRSA Counts')+theme(axis.text.x =
element_text(angle =
90))+geom_boxplot(aes(color=factor(Farm)))+labs
(color='Farm')
PumpvEDCMRSA

PumpvEDCS <- ggplot(db5, aes(x=`Sample.Type`,
y=S.AreusCounts))+ggtitle('S.Aureus counts by
Sampling Method')+xlab('Sample
Type')+ylab('S.Aureus Counts')+theme(axis.text.x
= element_text(angle =
90))+geom_boxplot(aes(color=factor(Farm)))+labs
(color='Farm')
PumpvEDCS
```

Subsetting EDCS and Pumps
```{r}
EDCs<-subset(db5,db5$Sample.Type=='EDC')
Pumps<-subset(db5,db5$Sample.Type=='PUMP')

EDCs1<-EDCs%>%

dplyr::select(Location.upper,S.AreusCounts,MRS
ACounts,Sample.ID,Compartment,Farm,Sample.T
ype) %>%
   mutate(FC=paste(Farm, Compartment))
colnames(EDCs1)
colnames(EDCs1)<-
c('Location.upper','EDC.S.AreusCounts',
```

```
'EDC.MRSACounts','Sample.ID','Compartment','F
arm','Sample.Type','FC')

Pumps1<-Pumps%>%

dplyr::select(Location.upper,S.AreusCounts,MRS
ACounts,Sample.ID,Compartment,Farm,Sample.T
ype) %>%
    mutate(FC=paste(Farm, Compartment))
colnames(Pumps1)
colnames(Pumps1)<-
c('Location.upper','Pump.S.AreusCounts',
'Pump.MRSACounts','Sample.ID','Compartment',
'Farm','Sample.Type','FC')

EDCsvPumps<-left_join(EDCs1,Pumps1,by='FC')

cor.test(EDCsvPumps$EDC.MRSACounts,EDCsv
Pumps$Pump.MRSACounts,method='pearson')
plot(EDCsvPumps$EDC.MRSACounts,EDCsvPu
mps$Pump.MRSACounts)

ggplot(EDCsvPumps,aes(x=EDC.MRSACounts,y
=Pump.MRSACounts,))+geom_point(aes(color=fa
ctor(Farm.y)))+ggtitle('Correlation of MRSA as
measured by Pumps vs EDCs')+ylab('MRSA
Count:Pumps')+xlab('MRSA
Count:EDCs')+labs(color='Farm')
```
```
Normality tests EDCs and Pumps, most sets are
not normal even with the removal of farm 2
```{r}
```
```
hist(EDCs$MRSACounts)
shapiro.test(EDCs$MRSACounts)#not normal

hist(EDCs$S.AreusCounts)
shapiro.test(EDCs$S.AreusCounts) # normal

hist(Pumps$MRSACounts)
shapiro.test(EDCs$MRSACounts)#not normal

hist(Pumps$S.AreusCounts)
shapiro.test(EDCs$S.AreusCounts) # normal
```
```
Average MRSA count per compartment EDC vs
Pump
```{r}
```
```
db6 <- db5 %>%

mutate(FCS=paste(Farm,Compartment,Sample.Ty
pe)) %>%
  mutate(FC=paste(Farm, Compartment)) %>%
  group_by(FCS) %>%
```
```
  summarize(mean(MRSACounts, na.rm=TRUE),
median(MRSACounts, na.rm=TRUE),
       min(MRSACounts,
na.rm=TRUE),max(MRSACounts, na.rm=TRUE),
n=n(),unique(FC)) %>%
  rename(MRSAmedian=`median(MRSACounts,
na.rm = TRUE)`) %>%
  rename(MRSAmean=`mean(MRSACounts,
na.rm = TRUE)`) %>%
  mutate(sample.type=ifelse(grepl("EDC",FCS),
"EDC", "PUMP")) %>%
  rename(FC=`unique(FC)`) %>%
  dplyr::select(-FCS)
```
```
Adding in Max and Min values so that they can be
plotted in the regression graph, calculated by
comaprtment
```{r}
```
```
db6a <- db6 %>% dplyr::select(FC, sample.type,
MRSAmedian) %>%
mutate(FCS=paste(FC,sample.type))
db6b <- db6 %>% dplyr::select(FC, sample.type,
`min(MRSACounts, na.rm = TRUE)`) %>%
mutate(FCS=paste(FC,sample.type))
db6c <- db6 %>% dplyr::select(FC, sample.type,
`max(MRSACounts, na.rm = TRUE)`) %>%
mutate(FCS=paste(FC,sample.type))
db6d <- db6 %>% dplyr::select(FC, sample.type,
MRSAmean) %>%
mutate(FCS=paste(FC,sample.type))
db6e <- db6 %>% dplyr::select(FC, sample.type,
n) %>% mutate(FCS=paste(FC,sample.type))
db7 <- left_join(db6a, db6b, by="FCS")
db7 <- left_join(db7, db6c, by="FCS")
db7 <- left_join(db7, db6d, by="FCS")
db7 <- left_join(db7, db6e, by="FCS")

db7a <- db7 %>% filter(sample.type.x=="EDC")
db7b <- db7 %>% filter(sample.type.x=="PUMP")
db8 <- left_join(db7a, db7b, by="FC")

rm(db6a, db6b, db6c, db6d, db6e,db7, db7a, db7b)
db8 <- db8 %>%
dplyr::select(FC,sample.type.x.x.x, MRSAmean.x,
MRSAmedian.x,`min(MRSACounts, na.rm =
TRUE).x`,`max(MRSACounts, na.rm = TRUE).x`,
n.x, sample.type.y.y.y, MRSAmean.y,
MRSAmedian.y,`min(MRSACounts, na.rm =
TRUE).y`,`max(MRSACounts, na.rm = TRUE).y`,
n.y)

colnames(db8)<-
c('FC','EDC','MeanMRSACountEDC','MedianMR
```

```r
SACountEDC','minMRSACountEDC',
'maxMRSACountEDC', 'n.EDC','Pump',
'MeanMRSACountPump','MedianMRSACountPu
mp','minMRSACountPump',
'maxMRSACountPump','n.Pump')

summary(Deming(db8$MedianMRSACountPump,
db8$MedianMRSACountEDC, vr=1))
```

Simple plot: avg MRSA pumps vs EDCs
```{r}
plot(db8$MeanMRSACountEDC,db8$MeanMRS
ACountPump)
```

Deming regression: Max & min values and final
graph Confidence intervals
```{r}

summary(Deming(db8$MedianMRSACountPump,
db8$MedianMRSACountEDC, vr=1))


Deming_line <- Deming
(db8$MedianMRSACountPump,
db8$MedianMRSACountEDC, vr=1)
DemCI<-Deming(db8$MedianMRSACountPump,
db8$MedianMRSACountEDC, vr=1,alpha= 0.05,
boot=1000,keep.boot=TRUE)

Slope<-Deming_line[2]
Intercept<-Deming_line[1]
db8a <- db8[complete.cases(db8),]
xmin <- min(db8a$MedianMRSACountPump)
ymin <- xmin*Deming_line[2]+Deming_line[1]
xmax <- max(db8a$MedianMRSACountPump)
ymax <- xmax*Deming_line[2]+Deming_line[1]

Corr <- cor(db8a$MedianMRSACountPump,
db8a$MedianMRSACountEDC,
method="pearson")
cor.test(db8a$MedianMRSACountPump,
db8a$MedianMRSACountEDC,
method="pearson")

p2 <-  ggplot(db8,
aes(x=MedianMRSACountPump,
y=MedianMRSACountEDC))+
  geom_abline(intercept=Intercept, slope=Slope,
col="lightgrey", linetype="dashed")+
  geom_segment(x=xmin, y=ymin, xend=xmax,
yend=ymax, col="lightgrey", size=1)+
  geom_point(aes(color=factor(substr(FC,1,1))))+
  geom_segment(aes(x=MedianMRSACountPump,
y=minMRSACountEDC,
           xend=MedianMRSACountPump,
yend=maxMRSACountEDC,
color=factor(substr(FC,1,1))),data=db8)+
  geom_segment(aes(x=minMRSACountPump,
y=MedianMRSACountEDC,
           xend=maxMRSACountPump,
yend=MedianMRSACountEDC,
color=factor(substr(FC,1,1))),data=db8)+
  #geom_smooth(method="lm", col="red",
se=TRUE)+
  ggtitle('Median MRSA counts measured by EDCs
vs Pumps')+
  xlab('Median MRSA Count as Measured by
Pumps')+
  ylab('Median MRSA Count as Measured by
EDCs')+
  labs(caption=paste("Line: Median(EDC)
=",round(Slope,2),"* Median(Pump) +",
           round(Intercept,2),"\n r^2
=",round(Corr,2), "{Deming regr.}"))+
  geom_text(aes(label=substr(FC,1,1)),
col="black", size=3, nudge_x=0.10, angle= 0)+
  theme(plot.caption = element_text(face="italic"),
      panel.background = element_rect(fill =
'white', color = 'black'),
      legend.key=element_rect(fill='white'))+
  coord_cartesian(xlim=c(-8,-2), ylim=c(-8,-2))+
  scale_colour_discrete("farm")

p2
```

Summary of Regression Statistics
```{r}
summary(lm(MeanMRSACountEDC~MeanMRS
ACountPump, data=db8))
```

_____Personal Pumps_____
Personal Pumps-Personal Pumps compared to
other "compartments"
```{r}
db <- dbPumpInfo %>%
  mutate(mean_Cp_16S=(A.16S+B.16S)/2) %>%
  mutate(mean_Cp_femA=(A.femA+B.femA)/2)
%>%
  mutate(mean_Cp_mecA=(A.mecA+B.mecA)/2)
%>%
  mutate(mean_Cp_nuc=(A.nuc+B.nuc)/2) %>%
  filter(!Location.upper %in% c("BANK EDC",
"BLANK", "BLANK EDC", "BLANK FILTER"))

PP1 <- db %>%
```

```r
mutate (OutcomefemA=case_when(
  is.na(A.femA) & !is.na(B.femA) ~ B.femA,
  !is.na(A.femA) & is.na(B.femA) ~A.femA,
  !is.na(A.femA) & !is.na(B.femA) ~
mean_Cp_femA
  ))%>%
 mutate (Outcomenuc=case_when(
   is.na(A.nuc) & !is.na(B.nuc) ~ B.nuc,
   !is.na(A.nuc) & is.na(B.nuc) ~A.nuc,
   !is.na(A.nuc) & !is.na(B.nuc)~ mean_Cp_nuc
  ))%>%
 mutate (OutcomemecA=case_when(
   is.na(A.mecA) & !is.na(B.mecA) ~ B.mecA,
   !is.na(A.mecA) & is.na(B.mecA) ~A.mecA,
   !is.na(A.mecA) & !is.na(B.mecA)~
mean_Cp_mecA
  ))%>%
  mutate(cellcount.16S=10^((mean_Cp_16S-
40.22)/-4.043)) %>%
  mutate(cellcount.femA=10^((OutcomefemA-
41.896)/-3.5474)) %>%
  mutate(cellcount.mecA=10^((OutcomemecA-
39.716)/-3.2823)) %>%
  mutate(cellcount.nuc=10^((Outcomenuc-
43.285)/-3.7116))

PP2 <- PP1 %>%

mutate(normal.femA=log10(cellcount.femA/cellco
unt.16S)) %>%

mutate(normal.mecA=log10(cellcount.mecA/cellc
ount.16S)) %>%

mutate(normal.nuc=log10(cellcount.nuc/cellcount.
16S))
#Get S.Aureus counts (highest b/w femA and nuc)
PP2 <- PP2 %>%
 mutate (S.AreusCounts=case_when(
   !is.na(normal.femA) & !is.na(normal.nuc) ~
pmax(normal.femA,normal.nuc),
   !is.na(normal.femA) & is.na(normal.nuc) ~
normal.femA,
   is.na(normal.femA) & !is.na(normal.nuc) ~
normal.nuc))

#Get MRSA counts (lowest b/w mecA and
S.aureus)
PP2$MRSACounts<-
pmin(PP2$normal.mecA,PP2$S.AreusCounts)

PP2<-PP2[!is.na(PP2$MRSACounts),]

ggplot(PP2,aes(x=Location.upper,
y=MRSACounts))+geom_boxplot()+labs(title="A
verage Normalized Cell Counts",y=" MRSA
Counts",x="Sample Location")+theme(axis.text.x
= element_text(angle = 90))

ggplot(PP2,aes(x=Location.upper,
y=MRSACounts,col=Location.upper))+
 geom_boxplot()+
 labs(title="MRSA levels by Sampling
Location",y="Normalized MRSA Counts (Log
Scale)",x="Sampling Location")+
 stat_summary(fun=mean, geom="point",
shape=5, size=5, color="black", fill="black",
position =          position_dodge(width = 0))+
 theme(axis.text.x = element_text(angle =
90))+theme(plot.title = element_text(hjust = 0.5))

PersonalPumps<-
subset(PP2,PP2$Location.upper=='PERSONAL
PUMP')

PersonalPumps1<-PersonalPumps%>%

dplyr::select(Location.upper,S.AreusCounts,MRS
ACounts,Sample.ID,Compartment,Farm,Sample.T
ype) %>%
   mutate(FC=paste(Farm, Compartment))
colnames(PersonalPumps1)<-
c('Location.upper','PP.S.AreusCounts',
'PP.MRSACounts','Sample.ID','Compartment','Far
m','Sample.Type','FC')

PersonalPumps2 <- PersonalPumps1 %>%

mutate(FCS=paste(Farm,Compartment,Sample.Ty
pe)) %>%
 mutate(FC=paste(Farm, Compartment)) %>%
 group_by(FCS)  %>%
 summarize(mean(PP.MRSACounts),unique(FC))
%>%
 dplyr::select(-FCS)
```
Average MRSA counts per farm (pumps)
```r
Farm2MRSApump<-subset(Pumps1,Farm==2)
mean(Farm2MRSApump$Pump.MRSACounts,na.
rm=TRUE)#-6.362029

Farm3MRSApump<-subset(Pumps1,Farm==3)
mean(Farm3MRSApump$Pump.MRSACounts,na.
rm=TRUE) #-4.966491
```

```
Farm4MRSApump<-subset(Pumps1,Farm==4)
mean(Farm4MRSApump$Pump.MRSACounts,na.
rm=TRUE) #-5.036958

Farm5MRSApump<-subset(Pumps1,Farm==5)
mean(Farm5MRSApump$Pump.MRSACounts,na.
rm=TRUE) #-4.423407

Farm6MRSApump<-subset(Pumps1,Farm==6)
mean(Farm6MRSApump$Pump.MRSACounts,na.
rm=TRUE) #-6.010262

Farm7MRSApump<-subset(Pumps1,Farm==7)
mean(Farm7MRSApump$Pump.MRSACounts,na.
rm=TRUE) # -5.155565
```
Average MRSA counts per farm (EDCs)
```{r}
#EDC avg vals
Farm2MRSAEDC<-subset(EDCs1,Farm==2)
mean(Farm2MRSAEDC$EDC.MRSACounts,na.r
m=TRUE) #-6.557605

Farm3MRSAEDC<-subset(EDCs1,Farm==3)
mean(Farm3MRSAEDC$EDC.MRSACounts,na.r
m=TRUE) #-5.345531

Farm4MRSAEDC<-subset(EDCs1,Farm==4)
mean(Farm4MRSAEDC$EDC.MRSACounts,na.r
m=TRUE) #-4.89248

Farm5MRSAEDC<-subset(EDCs1,Farm==5)
mean(Farm5MRSAEDC$EDC.MRSACounts,na.r
m=TRUE) #-4.489716

Farm6MRSAEDC<-subset(EDCs1,Farm==6)
mean(Farm6MRSAEDC$EDC.MRSACounts,na.r
m=TRUE) #-5.214306

Farm7MRSAEDC<-subset(EDCs1,Farm==7)
mean(Farm7MRSAEDC$EDC.MRSACounts,na.r
m=TRUE) # -5.043732
```
Personal Pumps vs Stationary Pumps. Farm
averages plotted against personal pump
measurements taken in the same farm.
```{r}
#add in counts
PersonalPumps2$AvgMRSAstationaryPumps<-c(-
6.362029,-4.966591,-5.036958,-4.423407,-
6.010262,-5.155565)
```

```
PersonalPumps2$AvgMRSAEDCs<-c(-6.557605,-
5.345531,-4.89248,-4.489716,-5.2124306,-
5.043732)
PersonalPumps2$Farm<-c(2,3,4,5,6,7)

PersonalPumps2<-PersonalPumps2[,-c(2)]

colnames(PersonalPumps2)<-c('Avg MRSA
Personal Pumps','Avg MRSA Stationary
Pumps','Avg MRSA EDCs','Farm')

cor.test(PersonalPumps2$`Avg MRSA Personal
Pumps`,PersonalPumps2$`Avg MRSA Stationary
Pumps`)
plot(PersonalPumps2$`Avg MRSA Personal
Pumps`,PersonalPumps2$`Avg MRSA Stationary
Pumps`)
```