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Amixicile Inhibits Anaerobic Bacteria within an Oral Microbiome Derived from Patients with Chronic Periodontitis

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

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Table of Contents

List of Tables

List of Figures

Abstract

AMIXICILE INHIBITIS ANAEROBIC BACTERIA WITHIN AN ORAL MICROBIOME DERIVED FROM PATIENTS WITH CHRONIC PERIODONTITIS

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Periodontitis is a chronic inflammatory disease caused by pathogenic bacteria residing in a complex biofilm within a susceptible host. Amixicile is a non-toxic, readily bioavailable novel antimicrobial that targets strict anaerobes through inhibition of the activity of Pyruvate Ferredoxin Oxidoreductase (PFOR), a major enzyme mediating oxidative decarboxylation of pyruvate. Our study aimed to evaluate the efficacy of amixicile, when compared to metronidazole, in inhibiting the growth of bacteria present in a microbiome harvested from patients with chronic periodontitis.

Plaque samples were harvested from patients with severe chronic periodontitis and cultured under anaerobic conditions. The microbiomes were grown in the presence of amixicile and metronidazole and the growth was compared to that of bacteria grown in the absence of the

antimicrobials. Following 24 hour growth the bacterial DNA was analyzed using quantitative PCR (qPCR) using primers specific for 12 bacterial species: *P. gingivalis (Pg), P. intermedia (Pi), F.nucleatum (Fn), S.gordonii (Sg), S. anginosus (Sa), V. atypical (Va), L. acidophilus (La), A.actinomycetemcomitans (Aa), T.denticola (Td), S.mutans (Sm),* and *S.sanguis (Ss).*

Both drug treatment groups yielded a statistical significant reduction for several anaerobic bacteria: Pi (P<.001), Fn (P<.001), Va (P<.001), and La (P<.001). Results indicated that amixicile and metronidazole had an effect on PFOR-containing bacteria and amixicile performed with similar efficacy to that of metronidazole. In conclusion, amixicile targets and reduces the quantities of anaerobic bacteria within an oral microbiome, and could be a potential new therapeutic antimicrobial in the treatment of periodontal disease.

Keywords: amixicile, metronidazole, micobiomes, periodontitis, q-PCR analysis

INTRODUCTION

Periodontitis is a chronic inflammatory disease caused by bacteria that colonize either at or below the gingival margin [1]. As plaque accumulates around teeth, it causes the inflammatory system to produce a wide array of cytokines, which are ultimately responsible for the destruction associated with disease. Unlike gingivitis, which is a reversible gingival inflammatory condition; periodontitis leads to the eventual breakdown of the junctional attachment apparatus to the tooth [1,2]. The types of clinical features seen in periodontitis are clinical attachment loss, radiographic bone loss, periodontal pockets, bleeding upon probing, suppuration upon probing, and varying degrees of tooth mobility [1]. The most recent data from the National Health and Nutrition Examination Survey (NHANES) suggest that the prevalence of chronic periodontitis exceeds 47% of U.S. adults [3].

Classic studies have demonstrated clear evidence to link dental plaque and calculus as major etiologic agents in the progression of periodontal disease [4,5]. Bacteria found in the oral cavity are extremely complex, with over 700+ species living amongst themselves in a layered ecosystem that enables them to be pathogenic [6]. Living in a biofilm enables the bacteria to receive nutrients, offers protection, and gene transfer. As the quantity of bacteria increase in the oral cavity, there is a shift in the types of microflora seen [6]. In health, the predominant bacterial species is aerobic Gram + cocci which includes the *Streptococcus* species [6]. However, in periodontitis the predominant species are anaerobic Gram – rods which include organisms such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Tannerella forsythia* [6].

Ultimately periodontal disease is multifactorial. It is the result of the interplay between specific pathogenic bacteria, host risk factors, and host-immune response. As microbiota dysbiosis occurs in subgingival plaque surrounding teeth, specific inflammatory pathways are activated in the host [4,5,6,7]. Once the microbiome is dominated by gram – anaerobic bacteria, the host immune system mounts a massive inflammatory response in an attempt to heal [56]. As a result, the destruction of the periodontium seen in periodontal disease is caused by the complex relationship between pathogenic bacteria that invade the oral biofilm and the types of immune responses generated within a susceptible host [56].

The first phase of treatment in periodontal disease is typically focused on mechanical therapy, which aims to reduce total numbers of all bacteria present at the site of infection. Clinicians will use a variety of hand instruments and ultrasonic devices to debride the teeth and soft tissues. Studies have confirmed that scaling and root planning accompanied with improved oral hygiene practices will allow for improvements in periodontal parameters [7]. Yet, the ability to effectively clean a diseased root surface is dependent upon multiple factors such as initial pocket depth, single versus multi-rooted teeth, and intrinsic anatomy of the tooth [8,9,10,11]. Generally speaking the deeper the probing depth the less likelihood of complete removal of etiologic agents of plaque and calculus [12]. Furthermore despite meticulous mechanical therapy, persistent bacteria can remain due their ability to invade host cells, survive and replicate, and then serve as a reservoir for future re-infections [13,14,15,16]. Some bacteria are also capable of altering the immune response of the host, thereby allowing them to be evasive [16].

Based on the infectious nature of periodontal disease, some clinicians have advocated for the use of antibiotics in their therapy as an adjunct method to control the bacterial load. The concept is based on the premise that there are specific types of bacteria associated with disease and therefore employing an antibiotic to target them would be beneficial [17,19,21,50-52]. Antibiotics target bacteria either by inhibiting bacterial growth (bacteriostatic) or directly killing bacteria (bactericidal). Antibiotics need to reach a minimum inhibitory concentration in order to be effective. There has been debate on how the concentration can be measured in the oral cavity and how much of the delivered systemic antibiotic will reach the gingival crevicular fluid in the periodontium [18].

In clinical practice, mainly broad-spectrum antibiotics are used as adjuncts in periodontal disease. The major problem with such antibiotics is inhibition of all bacteria present in infected sites. However, not all of the bacteria present in the periodontal infection are pathogenic. Use of specific antibiotics would spare commensal bacteria that are crucial for homeostasis and target solely pathogenic ones [17,19,21,50-52]. However, caveats with antibiotics include patient compliance, systemic side effects, and drug resistance. In a study published by Rams in 2014, which sought to measure the antibiotic resistance in human chronic periodontitis microbiota, researchers found that patients with chronic periodontitis frequently yielded sub gingival periodontal pathogen resistance to in vitro concentrations of antibiotics commonly used in clinical periodontal practice [20]. Systematic reviews analyzing the benefits of antibiotics in combination with mechanical therapy have determined that systemic antibiotics were uniformly beneficial in providing improvement in attachment loss when used as adjuncts to scaling and root

planing; although they were borderline significance when used as stand alone therapy [17,19,21,50-52].

Antibiotic use is highly varied amongst clinicians and to this day still remains subjective [17]. The Academy of Periodontology position paper on systemic antibiotic use set forth guidelines in 2004, which outline 3 main factors to consider when determining the use of antibiotics; the patient, the pathogenic microbiota and the drug. In summary it states that antibiotics should only be administered on the basis of clinical need for further treatment, the findings from microbiologic testing and the medical status and current medications of the patient [17]. It advocates for the conservative use of systemic antibiotics, and that antibiotics will offer the greatest benefit to patients who do not adequately respond to initial mechanical therapy [17]. Specific antibiotics have been indicated for the treatment of periodontal disease and include: amoxicillin, tetracyclines, metronidazole and combination drug therapy. Based on the current body of evidence, use of a systemic antimicrobial as an adjunctive therapy alters the microflora associated with periodontal disease [50-52]. However insufficient data for implementing optimal antibiotic regimens remain un-resolved in the periodontal literature [17, 50-52].

Certain antibiotics are ideal for periodontal infections based on their ability to target a specific type of bacteria, or ability to concentrate in the gingival crevicular fluid. Metronidazole is considered the gold standard for anaerobic infections and has been effective in reducing pathogens associated with periodontal disease and sparing indigenous bacteria [17,19,39,45-47]. By targeting specific anaerobic bacteria associated with disease and leaving commensal aerobic bacteria behind, this in theory allows for the biofilm to be modified and return to one that is no

longer pathogenic. When compared to a placebo, the administration of metronidazole in conjunction with mechanical therapy had a significant improvement in periodontal parameters, reduction of gram – bacteria and spirochetes, and reduced the surgical needs of patients treated with non-surgical therapy [46,47].

Amixicile is a promising novel antimicrobial that targets strict anaerobes by affecting a major metabolic pathway [22-25]. It selectively affects the disease promoting bacteria by affecting pyruvate:ferredoxin oxidoreductase (PFOR). PFOR catalyzes the conversion of pyruvate and Coenzyme A (CoA) to CO_2 and Acetyl-CoA, which is an important component of many metabolic pathways found in anaerobic bacteria and parasites. Bacteria such as *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum,* and *Tannerella forsythia,* utilize this pathway for energy (Appendices pp.52). This pathway is highly conserved, and therefore conceptually it has been proposed that this antimicrobial will not offer resistance [22-25]. In a mouse model, amixicile was shown to have an inhibitory effect on *Clostridium difficile* infection, less systemic side effects, and reduced number of resistant bacteria when compared to traditional drugs [25].

Since amixicile was shown to be effective on anaerobic bacteria, we believe that it will also have an effect on specific bacteria present in periodontal disease. Our study aimed to evaluate the efficacy of amixicile on a complex microbiome harvested from a periodontal pocket of patients with chronic periodontitis. Our hypothesis is that within the microbiome model, amixicile will selectively inhibit specific pathogens associated with periodontal disease and spare commensal

bacteria. We hypothesize that amixicile will selectively inhibit PFOR bacteria and have similar effects when compared to metronidazole.

MATERIALS AND METHODS

Study Population

The Virginia Commonwealth University Institutional Review Board (HM20005374) approved this study. All of the samples harvested in this study came from patients of record at VCU Graduate Periodontics Clinic. All participants of the study completed a comprehensive periodontal exam at the VCU Department of Periodontology, and received informed consent prior to plaque harvest.

Our inclusion criteria for all participants was as follows:

1. Adult patients (age 21+)
2. Non-diabetics
3. The patient cannot have taken antibiotics within the 6 months
4. Patient has not received periodontal therapy in the 6 months
5. Non-pregnant patients
6. Non-smokers
7. No patients who required premedication prophylaxis
8. No aggressive periodontitis

The diagnosis of disease severity was based on full mouth periodontal charting and clinical attachment levels, utilizing criteria outlines in Appendices pp. 53. Severe chronic periodontitis

was defined as inflammation of the periodontium with attachment loss of 5mm or more in conjunction with radiographic bone loss.

Biofilm Sample Collection

Bacterial samples were harvested from the pocket originating from the mesial of first molars. Local anesthesia was provided to all patients for comfort. All sites were air dried, and cotton roll isolation was used. Supra-gingival plaque was gently removed from the tooth, so that the free gingival margin was not disturbed. The sample was harvested sub-gingivally via a sterile curette and stored in 500 µl of SHI medium. The sample was immediately transported into an anaerobic chamber and another 500 µl of SHI medium[1] was added to lower the oxygen level of the sample. The sample was incubated overnight in an artificial atmosphere (composed of 80% N, 10% H, and 10% CO2) at 37 ^{o}C using a Coy anaerobic chamber (Ann Arbor, MI), and then aliquoted to 100 µl and stored in -80 ^{o}C with 10% of glycerol. Sample aliquots from ten patients were pooled together and aliquoted to 50 µl of each for the following study.

Antimicrobial Treatment

50 µl of pooled sample was added to 4 mL of BHI with 10% of filtered human serum (Valley Biomedical), then separated into four aliqouts. One aliquot was centrifuged and the pellet was kept at -20 ^{o}C for DNA isolation as baseline. The others were incubated at 37 ^{o}C in the anaerobic chamber with or without antimicrobial treatment. The concentrations of Amixicile and metronidazole (Sigma) used in this study are 25 µg/mL. Pellets from the overnight cultures were obtained for DNA preparation.

Propidium monoazide (PMA) Treatment

PMA dye (Biotium) was added to 1 mL of overnight culture to a final concentration of 50 µM. Samples were incubated in the dark for 5 minutes with occasional mixing, then exposed to light for 15 minutes with a 500 W halogen lamp. Pellets were collected for DNA isolation.

DNA isolation and qPCR

Cell pellets were re-suspended in 50 mM EDTA containing 10 mg/mL lysozyme and 100 U/mL mutanolysin (Sigma) and incubated at 37°C for 1 hr. DNA was isolated using the Wizard Genomic DNA purification kit (Promega) according to manufacturer's instructions. The DNA was then used to quantify the presence of bacterial species in the various samples using a 7500 Fast Real-time PCR machine (Thermo-Fisher). Purified DNA (1 µL) and species-specific primers were added to Fast SYBR Green Mastermix (Thermo-Fisher) and run using standard cycle conditions: 95°C for 20 sec (1 cycle); 95°C for 3 sec, 60°C for 30 sec (40 cycles). The species-specific 16S rDNA primer sequences used in this study are shown in below. The cycle threshold (Ct) data were collected and then converted to absolute fold change.

DNAseq library generation

1µg of purified gDNA was fragmented using a Covaris S2 ultrasonicator following the settings for Whole-genome Resequencing. ThruPLEX DNA-seq Kit (Rubicon Genomics) was used for library preparation according to manufacturer's instructions. Library samples were run on the Bioanalyzer to check the quantity and quality, then processed for next generation sequencing through Nucleic Acids Research Facilities in VCU.

16S rDNA primers

Porphyromonas gingivalis (Pg) Pg F: AGGCAGCTTGCCATACTGCG Pg R: ACTGTTAGCAACTACCGATGT	*Lactobacillus acidophilus* (La) La F: GGATAGAGGTAGTAACTGGCCTTTATT La R: CAGTTTCCGATGCAGTTCCTCG
Prevotella intermedia (Pi) Pi F: CCATCAGGTTATGCTGGGCA Pi R: GTTGCAGACCTCAGTCCGAA	*Aggregatibacter actinomycetemcomitans* (Aa) Aa F: AGTCGGACGGTAGCAGGTAA Aa R: GCTTGGTAGGCCTTTACCCC
Fusobacterium nucleatum (Fn) Fn F: CTGGCTCAGGATGAACGC Fn R: ATGGGACGCAAAGCTCTCTC	*Treponema denticola* (Td) Td F: AGCATGCAAGTCGAACGGTA Td R: AACTAGCTAATGGGACGCGG
Veillonella atypica (Va) Va F: CGGCTACTGATCATCGCCTT Va R: ATCTTAGTGGCGAACGGGTG	*Streptococcus mutans* (Sm) Sm F: GCACACCGTGTTTTCTTGAGTCG Sm R: CGGCTATGTATCGTCGCCTT
Streptococcus gordonii (Sg) Sg F: GCAATTGCACCACTACCAGA Sg R: TGCTCGGTCAGACTTTCGTC	*Streptococcus sanguinis* (Ss) Ss F: ACGCTGAAGAGAGGAGCTTG Ss R: GTGAGCCGTTACCTCACCAA
Streptococcus anginosus (Sa) Sa F: GAGTGCTAGGTGTTGGGTCC Sa R: TGTTCCGAAGAAACTTCCTATCTCT	16S universal F: AGAGTTTGATCCTGGCTCAG 16S universal R: GCTGCCTCCCGTAGGAGT

Statistical Analysis

Each run used three antimicrobials (control, Amixicile, and Metronidazole—each in duplicate) with 12 bacterial species (Pg, Pi, Fn, Sg, Sa, Va, La, Aa, Td, Sm, and Ss). The CT values were normalized by subtracting each 16s value difference with the non-controls. The corrected CT values were analyzed using a mixed-model ANOVA with the following factors: Antimicrobial treatment, bacterial species-a repeated, within-sample factor, and the Antimicrobial*Species interaction.

RESULTS

Plaque Harvest and Growth

All plaque samples contained enough bacteria to be grown under laboratory conditions. Every effort was made to eliminate supragingival plaque through use of a coronal scaling prior to subgingival plaque harvest. Due to the diversity within periodontal biofilms, samples were pooled together in order to generate a comprehensive diseased microbiome. The growth of bacteria under laboratory conditions was another variable that had to be controlled for. The growth of the bacteria within the microbiome was examined under various growth conditions including different types of media. All of which were tested to establish the optimal growth conditions. Once the methodology was consistent, qPCR was used to ensure that bacteria were growing under the established anaerobic conditions. Baseline samples were compared to a 24-hour culture. **Error! Reference source not found.** displays a comparison between (B) baseline bacterial sample PCR and (C) 24 hour incubation. The lower the CT value, the more bacteria are present in the sample. From **Error! Reference source not found.** there was an increase in all of the bacteria tested, which is indicated by a decrease in the CT value.

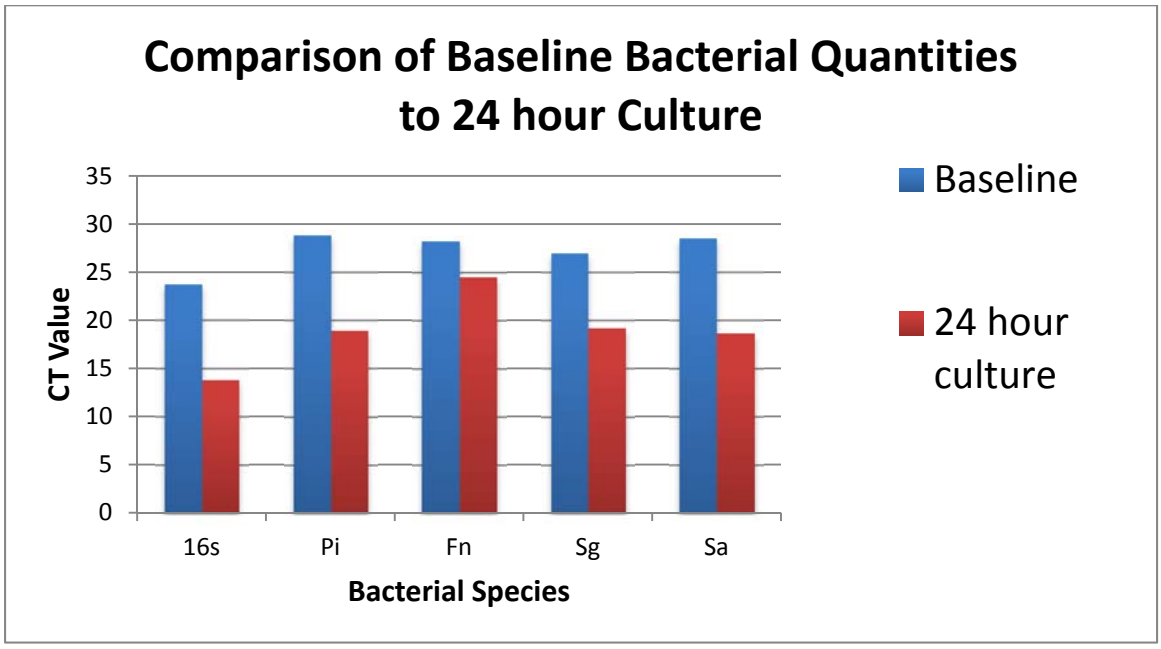

Figure 1. Comparison of Bacteria in Baseline Sample and 24 hour culture

Figure 1 displays the comparison of CT values between baseline plaque samples harvested and cultured for 24 hours under laboratory conditions. The decrease in CT value corresponds to a greater quantity of bacteria in the sample.

qPCR analysis was used to measure the relative quantities of bacteria within the samples. Some of the samples were treated with Propidium monoazide (PMA)which is a membrane-impermeant dye that selectively penetrates cells with compromised membranes, which can be considered dead. Once inside the cells, PMA intercalates into the DNA and can be covalently cross-linked to it, which strongly inhibits PCR amplification. We wanted to rule out any differences that might occur with our primers binding to total bacteria in sample versus live bacteria in sample.

Four separate biological replicate qPCR runs performed in duplicate under non-PMA conditions. All individual qPCR experiments were analyzed and can be found in the Appendices. The data was then compiled to represent an average cycle threshold (CT) values. The results of combining the four runs are summarized below. There were three separate biological replicate qPCR runs

performed in duplicate in PMA conditions. All individual non-PMA qPCR experiments were analyzed and can be found in the Appendices. The three PMA runs were combined and the data is summarized below. In the final portion of the results section, the non-PMA and PMA conditions are compared.

Total Bacteria in Sample (Non-PMA) Runs

The four previous individual runs were analyzed as one combined experiment. This was accomplished by adding an additional factor to the ANOVA model: Run ID. This permits each run to have a different mean level. Table 1 displays the corrected CT means compiled from the four individual qPCR runs. From Table 1 and Figure 2 there were differences in the relative abundance of the bacterial species tested in the control. High abundant species which is reflected by a low CT value were seen for: Pi, Fn, Sg, Sa, Va, and Ss. Whereas bacterial species Pg, La, Aa, Td, and Sm displayed a decreased abundance which is reflected by a higher CT value.

From Table 1 and Figure 2 there were statistical significant differences for Pi ($P<.001$), Fn ($P<.001$), Va ($P<.001$), La ($P<.001$) and Aa ($P<.001$). For the 3 treatment groups, there are 3 paired comparisons—2 with the control and 1 for amixicile vs metronidazole. A difference is declared if the p-value for the comparison is less than 0.05/3—a correction for multiple comparisons. In the table, if the active antimicrobial is significantly different from the control, then the active antimicrobial is labeled with a "-c" and if amixicile is different than metronidazole then each antimicrobial is labeled with "-x". From Table 1 and Figure 2 it demonstrates a difference from the control and amixicile in the following bacterial species: Pg, Pi, Fn, Sg, Va, La, Aa, and Td. A difference was seen from the control and metronidazole in the

following bacterial species: Pi, Fn, Sa, Va, La and Td. Lastly between amixicile and metronidazole, differences were observed for bacterial primers Pi and Va.

This information suggests that amixicile is affecting specific bacterial species within the microbiome, and is performing with similar efficacy to metronidazole in regards to targeting anaerobic bacteria. It also appears that when specific anaerobic bacteria were decreased after drug administration, specifically Pi, Fn, and Va, there was an increase in the abundance of aerobic species in the microbiome: La and Aa. A possible explanation for this trend could be that as selective bacteria species are eliminated from the microbiome is allows for growth of aerobic bacteria to obtain that niche. It could also be explained by the fact that all of the bacteria within the microbiome have varying growth rates, and therefore certain bacterial species appear to be in higher abundance versus other bacterial species.

The results for comparing each of the antimicrobials, separately within each bacterial species is shown in Appendix A Table 17 and these differences may be transformed into a fold-change by taking the differences with controls and exponentiating the difference. Exponentiating the 95% confidence intervals on the differences yields the 95% CI estimate for the fold estimate (and so, the CI's are not symmetric around the fold estimate). Table 2 and Figure 3 display the fold changes observed for all of the Non-PMA runs combined. Statistically significant reductions were seen for Pi (<.001), Fn (<.001), and Va (<.001). While a statistically significant increase was seen in La (<.001). The fold change decrease observed for both treatment groups on Pi, Fn and Va was similar and displays that both drugs treatment groups target select specific PFOR

containing bacteria. The fold change decrease seen in amixicile demonstrates that it is performing in the same manner as metronidazole.

Table 1. Corrected CT mean estimates for non-PMA Runs

Bacterial species	Antimicrobials	Corrected CT		
		Estimate	95% CI	
Pg (P=.015)	Control	31.27	30.59	31.95
	Amixicile-c	29.90	29.22	30.58
	Metronidazole	30.21	29.52	30.89
Pi (P<.001)	Control	17.51	16.83	18.20
	Amixicile-cx	24.33	23.65	25.01
	Metronidazole-cx	25.89	25.21	26.57
Fn (P<.001)	Control	23.38	22.69	24.06
	Amixicile-c	25.14	24.45	25.82
	Metronidazole-c	25.87	25.19	26.56
Sg (P=.005)	Control	18.23	17.55	18.91
	Amixicile-c	16.65	15.97	17.33
	Metronidazole	17.09	16.40	17.77
Sa (P=.006)	Control	16.65	15.97	17.34
	Amixicile	15.74	15.06	16.42
	Metronidazole-c	15.08	14.39	15.76
Va (P<.001)	Control	14.61	13.93	15.29
	Amixicile-cx	22.53	21.84	23.21
	Metronidazole-cx	24.27	23.59	24.95
La (P<.001)	Control	30.12	29.44	30.80
	Amixicile-c	27.26	26.58	27.94
	Metronidazole-c	28.05	27.36	28.73
Aa (P<.001)	Control	32.58	31.90	33.26
	Amixicile-c	30.73	30.05	31.42
	Metronidazole	31.51	30.83	32.19
Td (P=.001)	Control	28.61	27.92	29.29
	Amixicile-c	26.92	26.24	27.61
	Metronidazole-c	27.22	26.54	27.91
Sm (P=.848)	Control	30.82	30.14	31.50
	Amixicile	31.03	30.34	31.71
	Metronidazole	31.09	30.40	31.77
Ss (P=.032)	Control	15.14	14.46	15.82
	Amixicile	14.03	13.34	14.71
	Metronidazole	14.02	13.34	14.70

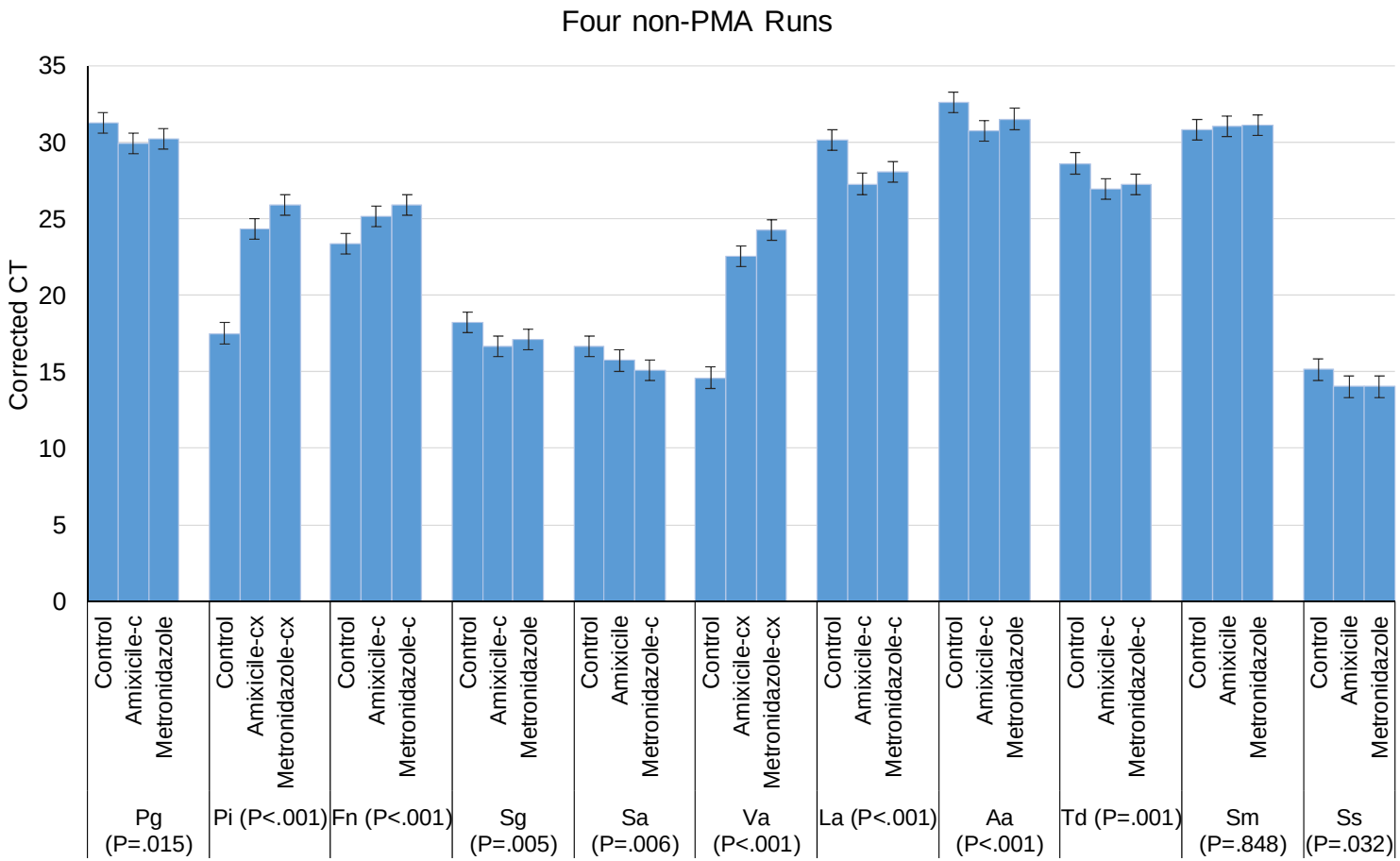

Figure 2. Corrected CT mean estimates for non-PMA Runs

Figure 2 includes the average CT values taken from samples from four biological replicates (microbiomes prepared on different days) each run in triplicate (n=12) under non-PMA conditions. ANOVA analysis was performed and applied to compare the control group to amixicile, control group to metronidazole and lastly compare amixicile and metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from amixicile and metronidazole.

Table 2. Fold change for non-PMA Runs

Bacterial species	Antimicrobials	Fold Estimate	95% CI	
Pg	Amixicile (P=.006)	2.580	1.322	5.035
	Metronidazole (P=.031)	2.087	1.069	4.073
Pi	Amixicile (P<.001)	0.009	0.005	0.017
	Metronidazole (P<.001)	0.003	0.002	0.006
Fn	Amixicile (P<.001)	0.295	0.151	0.576
	Metronidazole (P<.001)	0.177	0.091	0.346
Sg	Amixicile (P=.001)	2.982	1.528	5.821
	Metronidazole (P=.020)	2.209	1.132	4.311
Sa	Amixicile (P=.063)	1.887	0.967	3.684
	Metronidazole (P=.001)	2.988	1.531	5.832
Va	Amixicile (P<.001)	0.004	0.002	0.008
	Metronidazole (P<.001)	0.001	0.001	0.002
La	Amixicile (P<.001)	7.263	3.721	14.175
	Metronidazole (P<.001)	4.220	2.162	8.235
Aa	Amixicile (P<.001)	3.592	1.841	7.011
	Metronidazole (P=.030)	2.103	1.077	4.104
Td	Amixicile (P<.001)	3.209	1.644	6.263
	Metronidazole (P=.005)	2.604	1.334	5.081
Sm	Amixicile (P=.673)	0.866	0.444	1.691
	Metronidazole (P=.585)	0.831	0.426	1.621
Ss	Amixicile (P=.024)	2.168	1.111	4.232
	Metronidazole (P=.023)	2.178	1.116	4.251

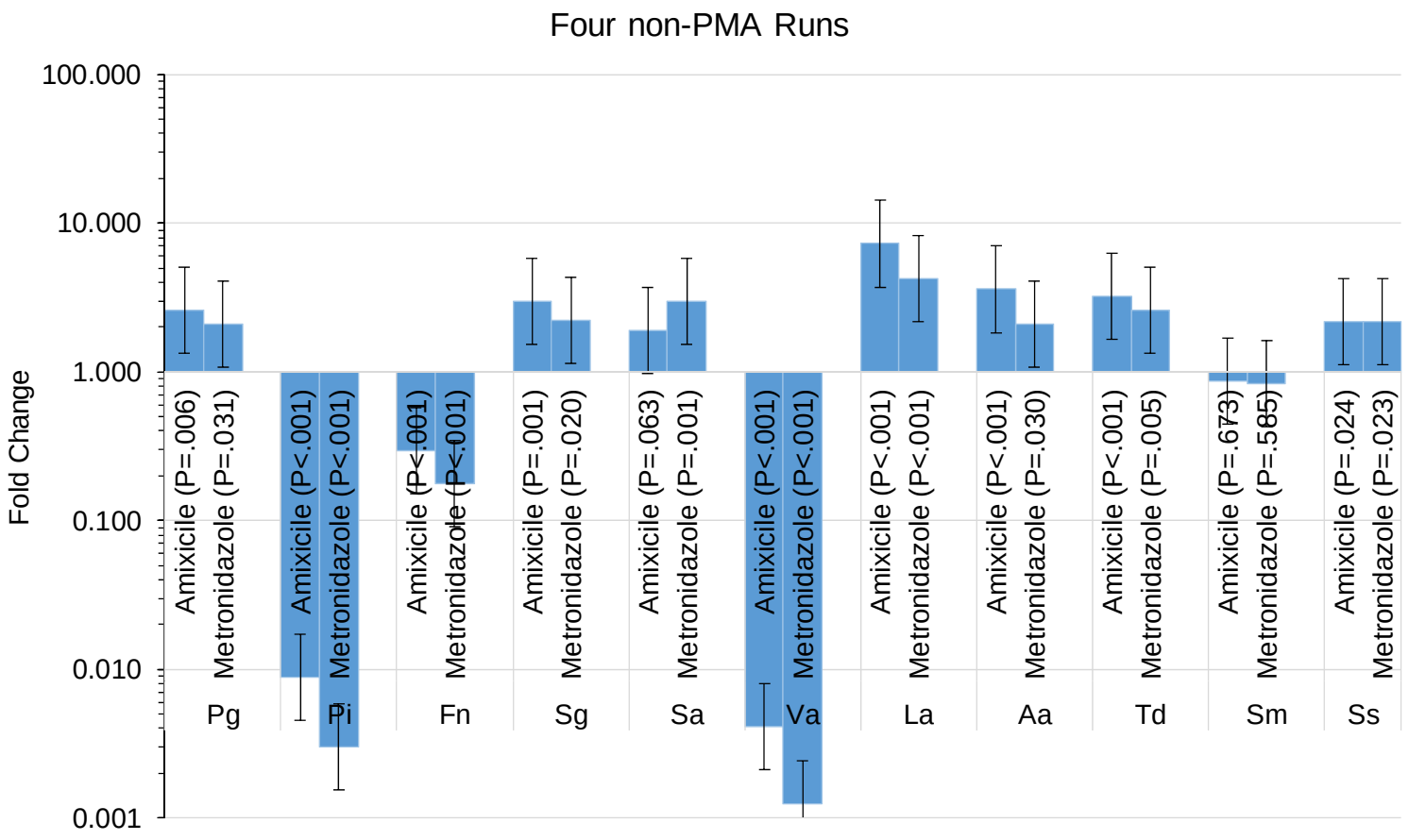

Figure 3. Fold change for non-PMA Runs (95% CI)

Figure 3 represents the fold change observed in bacterial species after treatment of either Amixicile or Metronidazole taken from samples from four biological replicates (microbiomes prepared on different days) each run in triplicate (n=12) under non-PMA conditions. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment.

Levels of Live Bacteria within Sample : (PMA) Runs

The three individual PMA runs were also analyzed as one combined experiment. The same data processing and analysis were performed on the aggregated non-PMA data was also performed on the aggregated PMA data. The average corrected CT estimates are shown in Table 3 and Figure 4. Similar trends were observed in regards to the abundance levels seen in the non-PMA data, and certain bacteria were present in high abundance relative to others. Higher abundant species represented by a low control CT value included Pi, Fn, Sg, Sa, Va, and Ss. Whereas a higher CT control value reflected lower abundant species and included Pg, La, Aa, Td and Sm.

Statistical significant differences were observed for Pi (P<.001), Fn (P<.001), Va (<.001) and La (P<.001). Within the three treatment groups, there are 3 paired comparisons—2 with the control and 1 for amixicile vs metronidazole. From Table 2 it demonstrates a difference from the control and amxicile in the following bacterial species: Pi, Fn, Sg, Va, La, and Td. A difference was seen from the control and metronidazole in the following bacterial species: Pi, Fn, Va, La and Sm. Lastly between amixicile and metronidazole, a difference was observed for Td species.

The results for comparing each of the antimicrobials, separately within each bacterial species is shown in Appendix A PMA Runs

Table 27. And these differences may be transformed into a fold-change by taking the differences with controls and exponentiating the difference. Exponentiating the 95% confidence intervals on the differences yields the 95% CI estimate for the fold estimate (and so, the CI's are not symmetric around the fold estimate). Table 4 and Figure 5 display the fold changes observed, and overall statistical significant reduction in both the amixicile and metronidazole treated groups in Pi, Fn and lastly Va. In the metronidazole group, a statistical significant increase in La

species was observed, however this was not significant for the amixicile treated group. Based on the similarity of the fold change decreases observed between the two treatment groups, both amixicile and metronidazole targeted specific PFOR bacteria within a microbiome and reduced their overall numbers following treatment.

Table 3. Corrected CT mean estimates for PMA Runs

Bacterial species	Antimicrobials	Corrected CT Estimate	95% CI	
Pg (P=.924)	Control	33.06	31.91	34.21
	Amixicile	33.42	32.02	34.83
	Metronidazole	33.15	31.89	34.41
Pi (P<.001)	Control	18.04	16.89	19.19
	Amixicile-c	25.67	24.52	26.82
	Metronidazole-c	26.94	25.79	28.09
Fn (P<.001)	Control	24.32	23.17	25.47
	Amixicile-c	28.01	26.86	29.16
	Metronidazole-c	27.81	26.66	28.96
Sg (P=.010)	Control	19.92	18.77	21.07
	Amixicile-c	17.39	16.24	18.54
	Metronidazole	18.85	17.71	20.00
Sa (P=.160)	Control	18.05	16.90	19.20
	Amixicile	16.84	15.69	17.99
	Metronidazole	16.57	15.42	17.72
Va (P<.001)	Control	15.41	14.27	16.56
	Amixicile-c	23.94	22.79	25.09
	Metronidazole-c	25.31	24.16	26.46
La (P<.001)	Control	31.31	30.16	32.46
	Amixicile-c	28.93	27.78	30.08
	Metronidazole-c	28.10	26.95	29.25
Aa (P=.268)	Control	32.49	31.34	33.64
	Amixicile	31.90	30.75	33.05
	Metronidazole	31.15	30.00	32.30
Td (P=.011)	Control	29.71	28.56	30.86
	Amixicile-cx	31.80	30.65	32.95
	Metronidazole-x	29.57	28.42	30.72
Sm (P=.011)	Control	34.30	33.15	35.45
	Amixicile	33.51	32.36	34.66
	Metronidazole-c	31.83	30.68	32.98
Ss (P=.045)	Control	16.87	15.72	18.02
	Amixicile	15.09	13.94	16.24
	Metronidazole	15.07	13.92	16.21

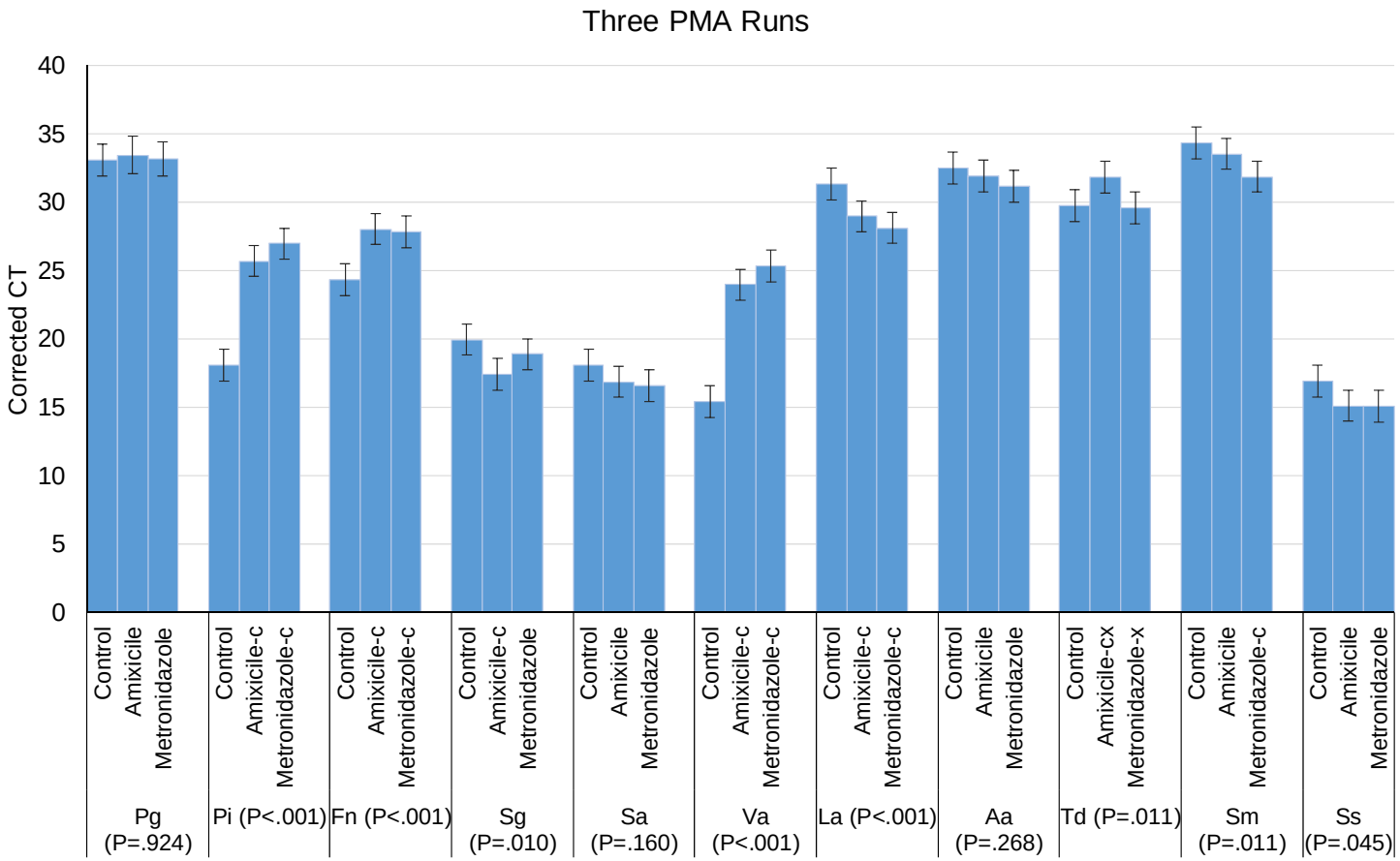

Figure 4. Corrected CT mean estimates for PMA Runs (95% CIs)

Figure 4 includes the average CT values taken from samples from three biological replicates (microbiomes prepared on different days) each run in triplicate (n=9) under PMA conditions. ANOVA analysis was performed and applied to compare the control group to amixicile, control group to metronidazole and lastly compare amixicile and metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from amixicile and metronidazole.

Table 4. Fold estimates for PMA Runs

Bacterial species	Antimicrobials	Fold Estimate	95% CI	
Pg	Amixicile (P=.696)	0.779	0.221	2.741
	Metronidazole (P=.918)	0.940	0.289	3.062
Pi	Amixicile (P<.001)	0.005	0.002	0.016
	Metronidazole (P<.001)	0.002	0.001	0.006
Fn	Amixicile (P<.001)	0.077	0.025	0.239
	Metronidazole (P<.001)	0.089	0.029	0.274
Sg	Amixicile (P=.002)	5.778	1.873	17.827
	Metronidazole (P=.197)	2.093	0.678	6.456
Sa	Amixicile (P=.141)	2.324	0.753	7.170
	Metronidazole (P=.073)	2.802	0.908	8.645
Va	Amixicile (P<.001)	0.003	0.001	0.008
	Metronidazole (P<.001)	0.001	0.000	0.003
La	Amixicile (P=.004)	5.196	1.684	16.029
	Metronidazole (P<.001)	9.267	3.004	28.589
Aa	Amixicile (P=.479)	1.499	0.486	4.624
	Metronidazole (P=.106)	2.527	0.819	7.796
Td	Amixicile (P=.012)	0.234	0.076	0.723
	Metronidazole (P=.869)	1.099	0.356	3.389
Sm	Amixicile (P=.338)	1.730	0.561	5.336
	Metronidazole (P=.003)	5.520	1.789	17.030
Ss	Amixicile (P=.032)	3.445	1.117	10.628
	Metronidazole (P=.030)	3.498	1.134	10.792

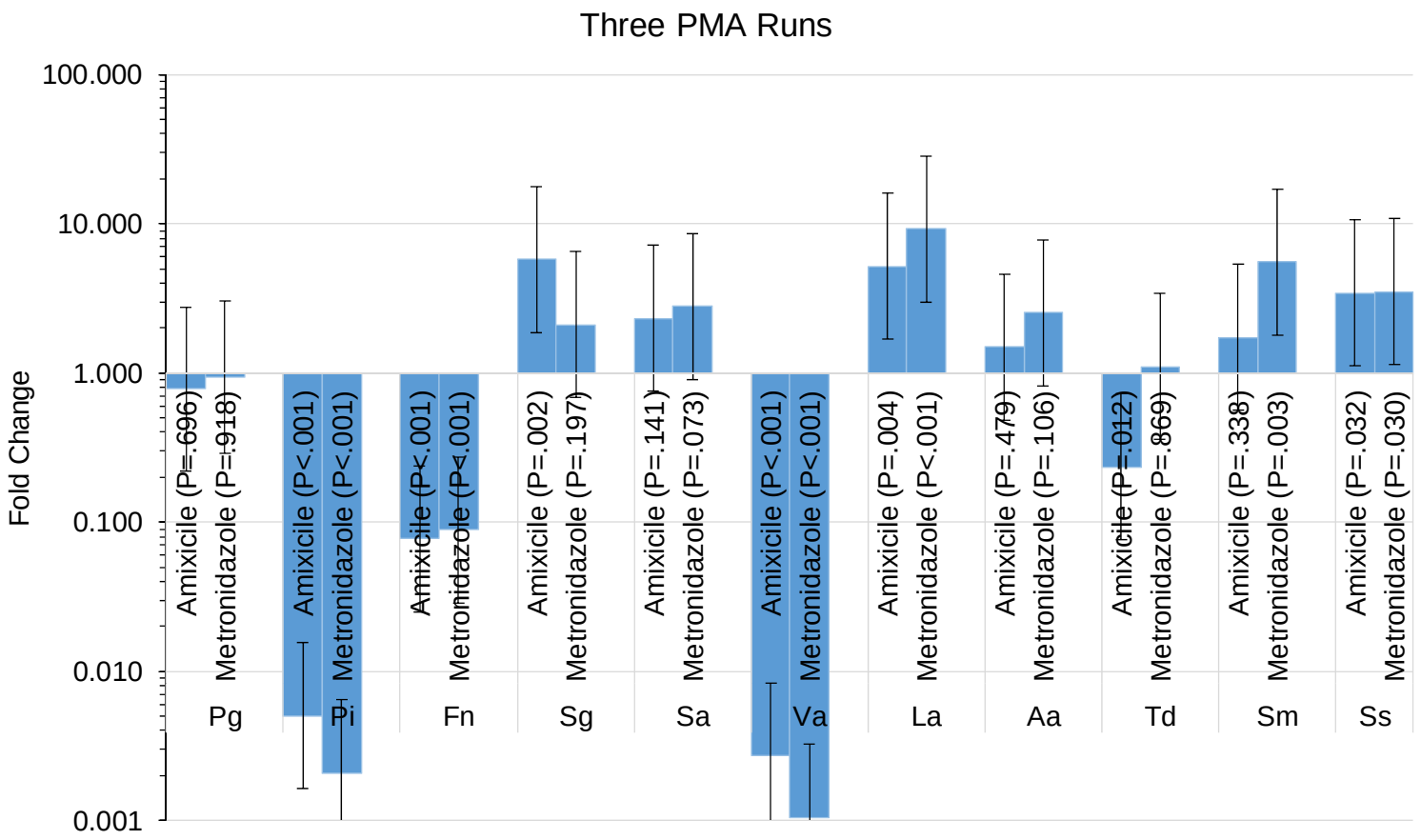

Figure 5. Fold estimates for PMA Runs (95% CIs)

Figure 5 represents the fold change observed in bacterial species after treatment of either Amixicile or Metronidazole taken from samples from three biological replicates (microbiomes prepared on different days) each run in triplicate (n=9) under PMA conditions. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment.

Comparison of levels of live and dead bacteria within microbiome

Our analysis involved examination of the extent of inhibition of bacterial growth by two antimicrobials: Amixicile and Metronidazole. Such inhibition could have been underestimated if the bacteria were metabolically inactive (and thus were dying) making the determination of the antimicrobial activity impossible. Thus we also determined the viability of the bacteria within the microbiomes by comparing samples analyzed in the presence and absence of propidium monoazide (PMA). PMA is a membrane-impermeant dye that selectively penetrates cells with compromised membranes, which can be considered dead. PMA was used prior to qPCR to rule out any outliers in the data. When PMA is used it will bind to any non-viable cells, and thereby prevent binding the DNA primers used. This allows for the analysis to only reflect the total living bacteria within the microbiome after antimicrobial treatment.

Based off the data, it appeared that the application of PMA to the DNA eliminated statistical changes that were observed in bacterial species La, Aa, and Td in the non-treated PMA qPCR. The reason for increased number of statistical significant differences seen in the non-PMA qPCR runs could be the result of the primers binding to dead bacteria in the microbiome. Addition of the PMA reagent eliminates this possible error by allowing the primers to only bind to live bacteria present in the microbiome, which allows for the data to more accurately reflect the live state of the microbiome and how the drugs affects bacteria. Both Non-PMA and PMA treated groups shared statistically significant reductions in the following bacterial species: Pi ($P<.001$), Fn ($P<.001$), and Va ($<.001$). All of these bacteria are PFOR containing bacteria. Therefore

within our experiment, it appeared that the application of the PMA aided in determination of the effect amixicile and metronidazole had on the oral microbiomes.

DISCUSSION

Periodontitis is a complex poly microbial infection that has been associated with gram negative bacteria such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia* and *Fusobacterium nucleatum* [6, 27]. Unlike acute infections that are typically caused by exogenous bacterial agents, periodontitis is a complex chronic infection characterized by endogenous oral microorganisms [16, 27]. Oral micro-biofilms enable bacteria to live in a layered ecosystem that involves adherence to a solid surface (the tooth) and is surrounded by a microbial polysaccharide and protein matrix [16, 27, 43]. This complex eco-system provides numerous protective advantages to the bacteria including: nutrient availability and uptake, removal of potentially harmful metabolic products, and the development of an appropriate chemical ecosystem necessary for the bacteria to survive [27]. It has been shown that there are specific associations among bacteria living in dental biofilms [6]. Socransky identified six groups of oral bacterial species and grouped them according their spatial relationships which include; yellow, green, purple, orange and red complexes [6]. These complexes represent a group of distinct bacterial species that tend to aggregate together and contribute to the collective survival of the complex within the micro-biofilm. Complexes green and purple act as early colonizers, and have the ability to attach directly to the tooth. Orange and red complexes tend to be associated with pathogenic bacteria that cause periodontal destruction [6].

As a result, biofilms are often difficult therapeutic targets because they are dynamic communities. The structure of the biofilm allows bacterial species to be more resistant to antibiotics and the immune system than planktonic bacteria [27, 43]. Furthermore, there is a difference in the types of bacteria that are found in supragingival and subgingival plaque.

Supragingival plaque is characterized by Gram + cocci, whereas subgingival plaque is characterized by a zone of Gram – spirochetes [27, 48]. Traditionally, periodontitis is first managed with mechanical therapy aimed at reducing the overall quantity of bacteria and implementing better oral hygiene practices to the patient [7]. Numerous studies have confirmed the benefits of mechanical therapy in the treatment of periodontal disease such as decrease in probing depths, detoxification of root surfaces and clinical attachment gain following implementation of mechanical therapy [7, 29-32]. Despite its effectiveness, mechanical therapy is unable to remove all pathogens associated with disease. The trend observed in clinical practice is that as disease severity increases the odds of effective removal decrease [8-10]. Additionally another factor to consider is the ability for bacterial re-contamination following debridement [14,31]. Research indicates that is takes as little as 42 days for the sub-gingival microflora to re-establish [14,31]. Therefore strict maintenance schedules are required for all patients presenting with periodontal disease [33-35].

Since periodontal diseases are chronic polymicrobial infections, the use of antibiotics has been advocated to aid in the reduction of disease causing bacteria. Antibiotics target specific microbes and kill them through either a bactericidal or bacteriostatic mechanism. In order for an antibiotic to be effective it must be able to reach and penetrate the pathogens [37-39]. When observing in vivo micro-biofilms, the available amount of the antibiotic that is able to reach and have effects on the bacteria is reduced due to the complex structure of the biofilm [37-39]. Research has demonstrated a 100 to 1,000 fold increase in antimicrobial tolerance in biofilms compared to planktonic cells [37]. Several mechanisms have been proposed to explain the drug resistance of a biofilm and can be classified into 3 groups: intrinsic, mutational and acquired [37,43]. Rams

published a study in 2014 to determine the occurrence of in vitro antibiotic resistance among selected periodontal patients cultured from patients with chronic periodontitis [20]. Researchers found that of all the antibiotics investigated which included; amoxicillin, metronidazole, doxycycline, clindamycin, and combination drug therapy; no single antibiotic or combination of antibiotics evaluated demonstrated in vitro inhibition of all the assessed periodontal pathogens [20].

Taking resistance into consideration, the American Academy of Periodontology wrote a position paper advocating for microbiological testing prior to administration of antibiotic therapy [17]. Microbiological testing has been confirmed to aid in the diagnosis of periodontal disease activity and severity [53, 54]. In clinical practice, microbiological testing is seldom implemented, as the majority of periodontal diseases respond positively to mechanical therapy. However, new developments in microbiological testing have made it simple, and cost effective to utilize in clinical practice [53]. Advancements in microbiological testing enable clinicians to be able to use whole saliva in order to determine bacterial species present [53]. A group of researchers found that that whole saliva is superior to pooled periodontal pocket samples to detect *P. gingivalis, P. intermedia, P. nigrescens, and T. denticola* in the oral cavity [54]. From our data, we can see that specific periodontal pathogens, specifically; *P. intermedia, T. denticola* and *Veillonella atypica;* were present in sub-gingival plaque samples harvested from patient with severe chronic periodontitis. Determining the types of bacterial species present in the biofilm is critical as it enables the clinician to determine the appropriate antimicrobial to use so that specific pathogens can be targeted.

At this point in time there is insufficient evidence to advocate for the sole use of antibiotics as mono-therapies in the treatment of periodontal diseases [50-52]. Another branch of research concerning periodontal disease and antibiotics is the use of antibiotics as adjunctive therapy to mechanical therapy. Systematic reviews have displayed conflicting evidence in regards to observed additional benefits when using antibiotics as an adjunctive therapy [50-52]. It appears that the administration of systemic antibiotics has certain effects on the sub-gingival microbiota, but usually does not completely eliminate all of the disease causing bacteria [17,50-52]. In 2002 Herrera et al published findings to support the use of antimicrobials in specific clinical situations such as patients with deep pockets, patients with active disease, or patients with specific microbiologic profiles [50]. Whereas in another systematic review published in 2003, Haffajee et al concluded that while it appears a benefit exists when antibiotics are added as an adjunctive therapy, there is insufficient data to define an optimal antibiotic protocol [51]. From these systematic reviews, the American Academy of Periodontology outlined their recommendations for antibiotic use that included: aggressive cases of periodontal disease, refractory periodontitis and immune compromised patients [17, 40, 41]. Furthermore, the evidence seems to support that the quality of mechanical debridement and the time of the prescription of the drug may influence the clinical outcome [52]. The greatest benefit seen with antibiotics is post thorough meticulous mechanical debridement. Additionally the debridement should be completed within a short window (preferable <1 week) under antibiotic prophylaxis in order for the greatest clinical benefit to be achieved [52].

Metronidazole, an antibiotic compound, has been used and studied extensively in the treatment of various anaerobic infections [45]. It is considered the gold standard, and has been shown to be

effective in reducing the periodontal pathogens associated with disease [39, 46, 47]. It is considered to be a pro-drug as it requires metabolic activation by sensitive organisms, and ultimately interferes with bacterial nucleic acid synthesis [39]. Metronidazole has been used as an adjunct to scaling and root planning. When compared to a placebo, the administration of metronidazole in conjunction with mechanical therapy had a significant improvement in periodontal parameters [46, 47]. Researchers observed that after 6.4 years of follow up, the surgical needs were reduced when metronidazole was dispensed in conjunction with mechanical debridement after the first and second annual examinations [46, 47]. Despite its ability to target strict anaerobes associated with disease, metronidazole undoubtedly has unwelcome side effects that include: nausea, gastrointestinal disturbances, disulfram reaction, and neuropathies [39]. Due to these unwanted side effects this can lead to issues regarding patient compliance. Therefore the decision to utilize an antibiotic must be thoroughly and carefully considered for each patient.

Amixicile is a novel antimicrobial and like metronidazole, it targets specific anaerobic bacteria. However amixicile targets and affects the main metabolic pathway strict anaerobes use for energy [22-25]. It selectively affects the pyruvate:ferredoxin oxidoreductase (PFOR) pathway. PFOR catalyzes the conversion of pyruvate and Coenzyme A (CoA) to CO_2 and Acetyl-CoA and is an important component of many metabolic pathways found in anaerobic bacteria and parasites [22-25]. Animal research models have evaluated the effects when administering systemic amixicile in the treatment of a *Clostriudum difficile* infection and compared it to traditional Vancomycin. Researchers found amixicile was efficacious in eradicating the disease, but also displayed low toxicity, excellent drug metabolism, and an absence of mutation-based

drug resistance [25]. They concluded that amixicile could be a potential new drug to be used in infections caused by PFOR-expressing bacteria [22-25].

Our study aimed to evaluate how an oral microbiome cultured from patients with periodontal disease would respond to amixicile. To our knowledge this is the first study to investigate the effects of amixicile on an oral in vitro microbiome. Researching a microbiome cultured from patient's disease is lab intensive however it is more clinically relevant than solely looking at single species cultures. Our methodology confirmed that a microbiome could be grown successfully under anaerobic conditions, and that the microbiomes contained both Gram + and Gram – bacteria, which are both present in periodontal disease.

Our hypothesis was that amixicile would selectively target anaerobic bacteria, and reduce their prevalence in the microbiome derived from patients with chronic periodontitis. Secondly, we hypothesized that when compared to metronidazole, amixicile would act with similar efficacy in reducing the quantities of anaerobic bacteria. Based on the data, it appears that an effect was seen when amixicile was applied to a cultured oral microbiome. A statistically significant ($P<.001$) reduction was seen in selective quantities of bacterial species, which included: *P. intermedia, F. nucleatum* and *V. atypica*. All of these bacterial species utilize the PFOR pathway. When the data was evaluated to determine fold changes that occurred in the given bacterial species, both amixicile and metronidazole displayed a statistically significant ($P<.001$) decrease in the relative quantities of *P. intermedia, F. nucleatum* and *V. atypica*.

The data supports the notion that amixicile targets specific anaerobic bacteria within an oral microbiome and performs with a similar degree of efficacy to metronidazole. All of the species that were affected have been implicated in the development and progression of periodontal disease [6]. *Fusobacterium nucleatum* is a microbe associated with initiation of the microbial shift from a primarily gram + to gram – biofilm [16]. This microbial shift is crucial in the development of periodontal disease, and the clinical attachment loss that follows. In vitro analysis has confirmed that *F. nucleatum* coaggregates with all of the following bacteria: *P. gingivalis, Treponema denticola, A. actinomycetemcomitans, P. intermedia, Eubacterium* species, *Selenomonas* species and *Actinomyces* species [44]. In theory, if *F.nucleatum* could be targeted at an earlier stage, it could prevent the transition for a gram + to gram – micro-biofilm. This could potentially reduce the harmful effects the micro-biofilm causes in periodontal disease.

The results from this study advocate for further research to be performed regarding the use of amixicile as a potential new antimicrobial in the treatment of periodontal disease. While this study is only in vitro, it demonstrates that amixicile targets strict anaerobes and reduces their quantity. While antibiotics have forever changed the practice of medicine, the issues with increasing drug resistance cannot be ignored. Within oral biofilms, resistance to amoxicillin, tetracyclines, and metronidazole has been reported [16-18, 20,21,28,38]. Due to the effect that amixicile targets a highly conserved pathway within anaerobes, conceptually it supports the idea that it will lend to increased drug resistance in the bacteria.

The potential use of amixicile as an adjunct to mechanical therapy is very exciting. When patient present with severe periodontal disease, managing deep periodontal pockets and attachment loss

is difficult. Furthermore, clinicians anticipate that mechanical therapy will not remove all pathogens within the periodontal pocket. As the Academy of Periodontology outlines, antibiotic therapy needs to be considered for patients presenting with severe disease. Ideally, thorough mechanical debridement should be performed and subsequent re-evaluation should occur. If little improvements are seen in control of inflammation with mechanical therapy, then microbiological testing should be performed on the patient to determine the types of bacteria present. When sites display bleeding and deep probing depths, they have been associated with specific periodontal pathogens including *P.gingivalis, A. actinomycetemcomitans*, and *Fusobacterium* species [44]. As most bacteria associated with severe periodontal disease belong to anaerobic phyla, amixicile could provide additional benefits to patients and possibly reduce the need for surgical therapy in the future.

Limitations to this research include a lack of effect seen with *P.gingivalis*. *P.gingivalis* did not respond to either amixicile or metronidazole treatment. *P.gingivalis* has been regarded as a "keystone pathogen" and its presence has been linked with active disease in periodontal pockets [55]. Ideally amixicile and metronidazole should both have an effect on *P.gingivalis* because *P.gingivalis* is a gram – anaerobe. However little change was observed from the control and the antimicrobial treatment groups. Multiple factors could explain this finding. First, *P.gingivalis* is a sensitive anaerobe to grow in laboratory conditions. Its overall quantity in a biofilm is typically smaller when compared to other bacterial species and which was reflected by the higher CT values. The high CT value would indicate a lower overall quantity of DNA present in the microbiome. Furthermore issues with the primers and their ability to bind to the wild type *P.gingivalis* cultured could also explain the low CT values.

Another limitation is the difficulty culturing and growing live complex microbiomes under laboratory conditions. As a result, the effect of the host environment was not investigated in this study. Furthermore only 12 bacterial species primers were tested in this in vitro microbiome. Obviously it is impossible to culture all of the bacteria under laboratory conditions, as over 700 species exist. Culturing oral plaque is technique sensitive, which is why samples were pooled from multiple patients, and multiple qPCR runs were performed and grouped together.

Future research involving Amixicile should focus on the effects it would have on induced periodontal disease, and other anaerobic infections in animal models. The systemic side effects, optimal dosing, and overall effect on periodontal disease remain to be determined with future research. Ultimately randomized clinical trials in human subjects would be needed in order to allow Amixicile to be FDA approved in the treatment of periodontal disease, and possibly other diseases that are the result of anaerobic dominated infections.

CONCLUSIONS

Amixicile is a promising new antimicrobial in the treatment of anaerobic bacterial infections. The effect of amixicile and metronidazole was dependent on the bacteria being analyzed. Amixicile and metronidazole had an effect on PFOR-containing bacteria, specifically changes were seen for *P. intermedia, F. nucleatum* and *V. atypical*. When comparing amixicile to metronidazole, amixicile performed with similar efficacy with the largest effect seen for PFOR bacteria. The data supports the notion that amixicile targets specific anaerobic bacteria within an oral microbiome cultured from patients with chronic periodontitis and performs with a similar degree of efficacy to metronidazole. Such a specific, non-toxic and bioavailable antimicrobial would be highly desirable for the treatment of periodontal disease.

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Appendices

Amixicile targets specific anaerobic bacteria that utilize the pyruvate-ferredoxin oxidoreductase pathway (PFOR)

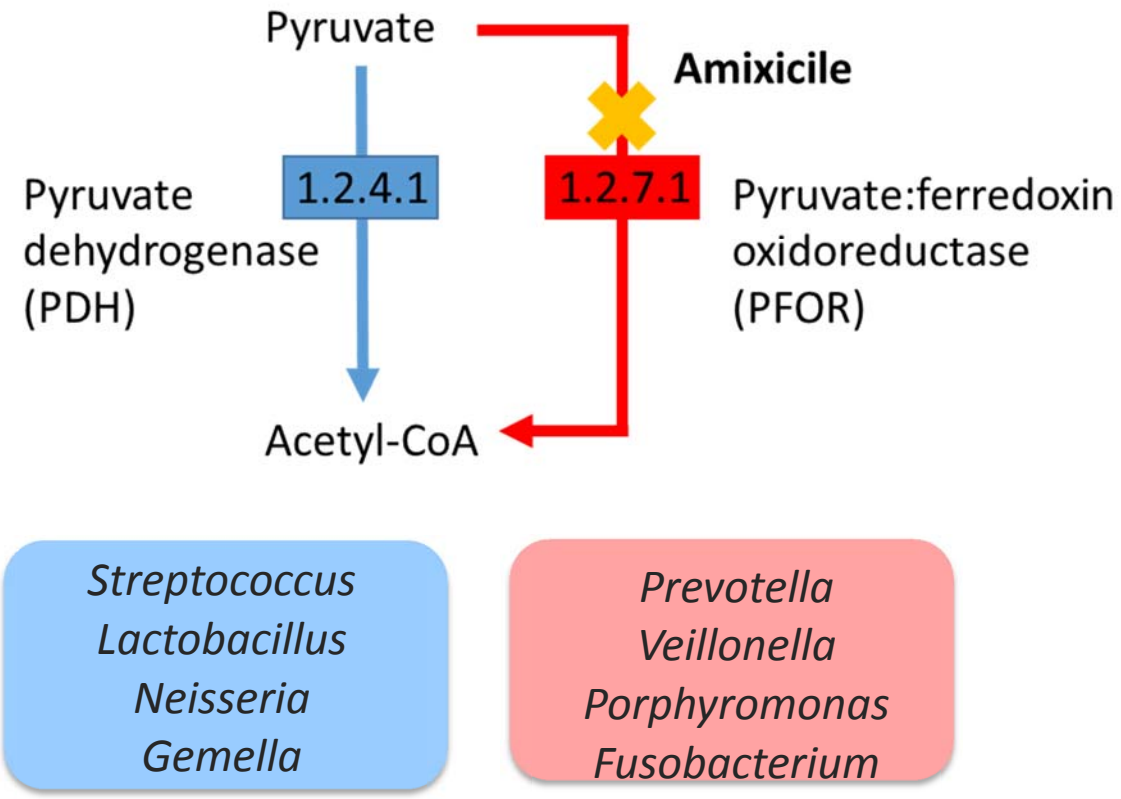

Figure 6. This figure displays how amixicile targets specific anaerobic bacteria by blocking the PFOR metabolic pathway.

Guidelines for the diagnosis of periodontitis according to the American Academy of Periodontology 2015

Guidelines for Determining Severity of Periodontitis

	Slight (Mild)	Moderate	Severe (Advanced)
Probing depths	>3 & <5 mm	≥5 & <7 mm	≥7 mm
Bleeding on probing	Yes	Yes	Yes
Radiographic bone loss	Up to 15% of root length or ≥2 mm & ≤3 mm	16% to 30% or >3 mm & ≤5 mm	>30% or >5 mm
Clinical attachment loss[1]	1 to 2 mm	3 to 4 mm	≥5 mm

Figure 7. Classification of Periodontal Disease Severity

Run 2017-02-28_144220

Table 5. Corrected CT mean estimates for Run 2017-02-28_144220

Bacterial Species	Antimicrobial	Corrected CT Estimate	95% CI	
Pg (P=.065)	Control	32.88	31.98	33.78
	Amixicile	31.49	30.59	32.39
	Metronidazole	31.66	30.76	32.55
Pi (P<.001)	Control	18.95	18.05	19.84
	Amixicile-c	26.80	25.90	27.69
	Metronidazole-c	28.31	27.41	29.20
Fn (P=.003)	Control	24.48	23.58	25.37
	Amixicile	25.86	24.96	26.76
	Metronidazole-c	26.81	25.91	27.71
Sg (P<.001)	Control	20.01	19.11	20.90
	Amixicile-c	17.76	16.86	18.66
	Metronidazole-c	17.48	16.58	18.37
Sa (P=.026)	Control	18.41	17.52	19.31
	Amixicile	17.24	16.34	18.13
	Metronidazole-c	16.66	15.76	17.55
Va (P<.001)	Control	16.52	15.63	17.42
	Amixicile-cx	24.52	23.62	25.41
	Metronidazole-cx	26.41	25.51	27.30
La (P<.001)	Control	32.41	31.52	33.31
	Amixicile-c	29.84	28.94	30.73
	Metronidazole-c	30.43	29.54	31.33
Aa (P=.002)	Control	34.94	34.04	35.83
	Amixicile-c	32.69	31.80	33.59
	Metronidazole-c	32.87	31.97	33.77
Td (P<.001)	Control	32.48	31.59	33.38
	Amixicile-c	28.94	28.04	29.84
	Metronidazole-c	28.96	28.07	29.86
Sm (P=.252)	Control	32.60	31.71	33.50
	Amixicile	32.86	31.97	33.76
	Metronidazole	31.85	30.95	32.75
Ss (P=.283)	Control	16.55	15.65	17.44
	Amixicile	15.89	14.99	16.79
	Metronidazole	15.56	14.66	16.45

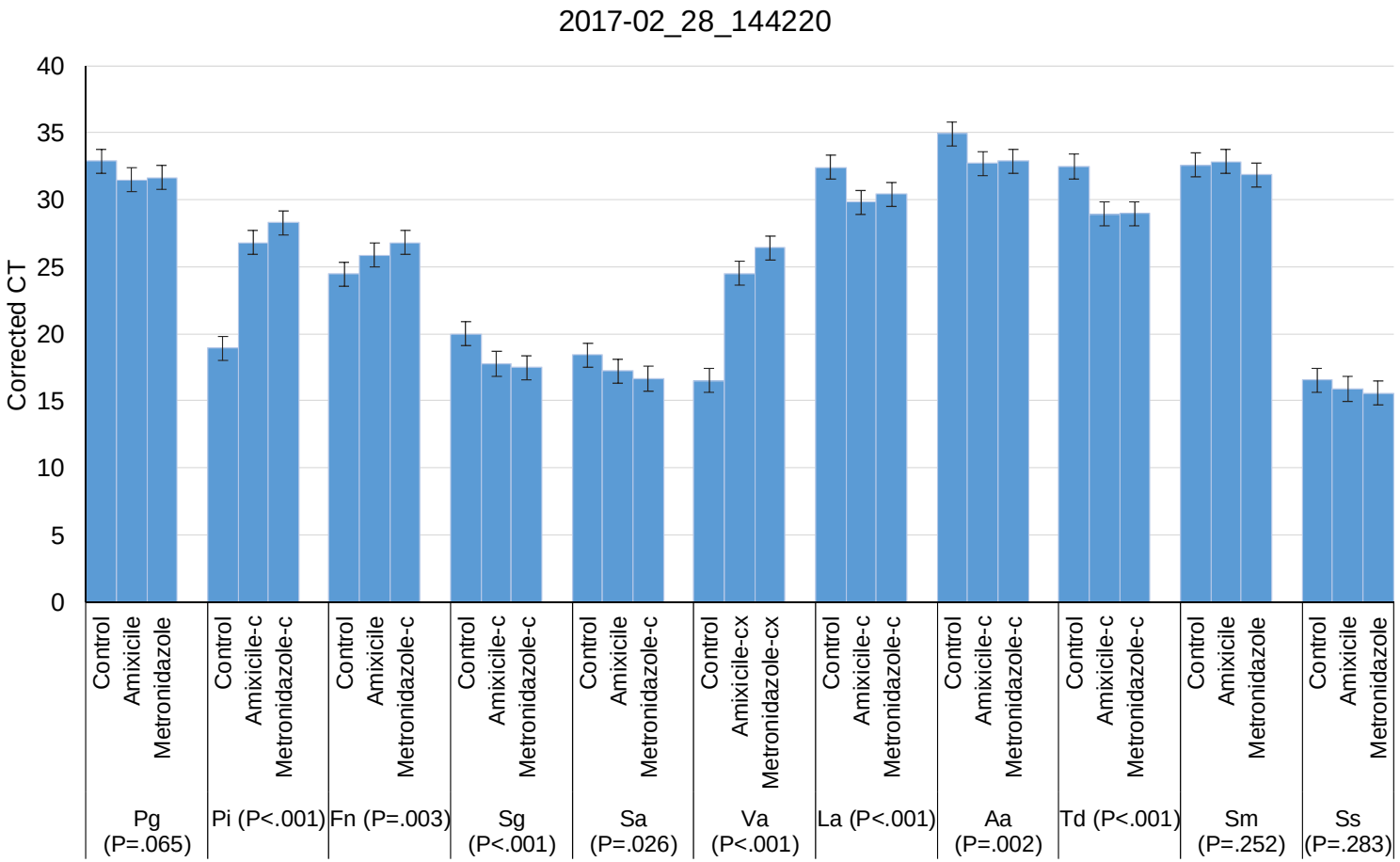

Figure 8. Corrected CT mean estimates for Run 2017-02-28_144220 (95% CIs)

Figure 6 represents the average CT values taken of Run 2017-02-28_144220. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statisitically significant difference from Amxicile and Metronidazole

Table 6. Differences in the Corrected CT mean estimates for Run 2017-02_28_144220

Bacterial Species	Compare	Corrected CT Estimate	95% CI	
Pg	CvA (P=.033)	1.391	0.124	2.658
	CvM (P=.058)	1.223	-0.044	2.491
	AvM (P=.789)	-0.168	-1.435	1.100
Pi	CvA (P<.001)	-7.850	-9.118	-6.583
	CvM (P<.001)	-9.359	-10.626	-8.091
	AvM (P=.021)	-1.509	-2.776	-0.241
Fn	CvA (P=.034)	-1.383	-2.650	-0.115
	CvM (P<.001)	-2.332	-3.599	-1.064
	AvM (P=.137)	-0.949	-2.216	0.318
Sg	CvA (P=.001)	2.248	0.981	3.516
	CvM (P<.001)	2.530	1.263	3.798
	AvM (P=.653)	0.282	-0.985	1.549
Sa	CvA (P=.068)	1.175	-0.092	2.442
	CvM (P=.008)	1.756	0.488	3.023
	AvM (P=.357)	0.581	-0.687	1.848
Va	CvA (P<.001)	-7.992	-9.259	-6.724
	CvM (P<.001)	-9.882	-11.149	-8.614
	AvM (P=.005)	-1.890	-3.158	-0.623
La	CvA (P<.001)	2.574	1.307	3.842
	CvM (P=.003)	1.980	0.713	3.247
	AvM (P=.346)	-0.594	-1.862	0.673
Aa	CvA (P=.001)	2.242	0.974	3.509
	CvM (P=.002)	2.066	0.799	3.334
	AvM (P=.779)	-0.176	-1.443	1.092
Td	CvA (P<.001)	3.542	2.274	4.809
	CvM (P<.001)	3.519	2.252	4.787
	AvM (P=.971)	-0.023	-1.290	1.245
Sm	CvA (P=.676)	-0.262	-1.529	1.005
	CvM (P=.234)	0.753	-0.514	2.020
	AvM (P=.112)	1.015	-0.252	2.282
Ss	CvA (P=.298)	0.658	-0.610	1.925
	CvM (P=.122)	0.989	-0.279	2.256
	AvM (P=.598)	0.331	-0.936	1.598

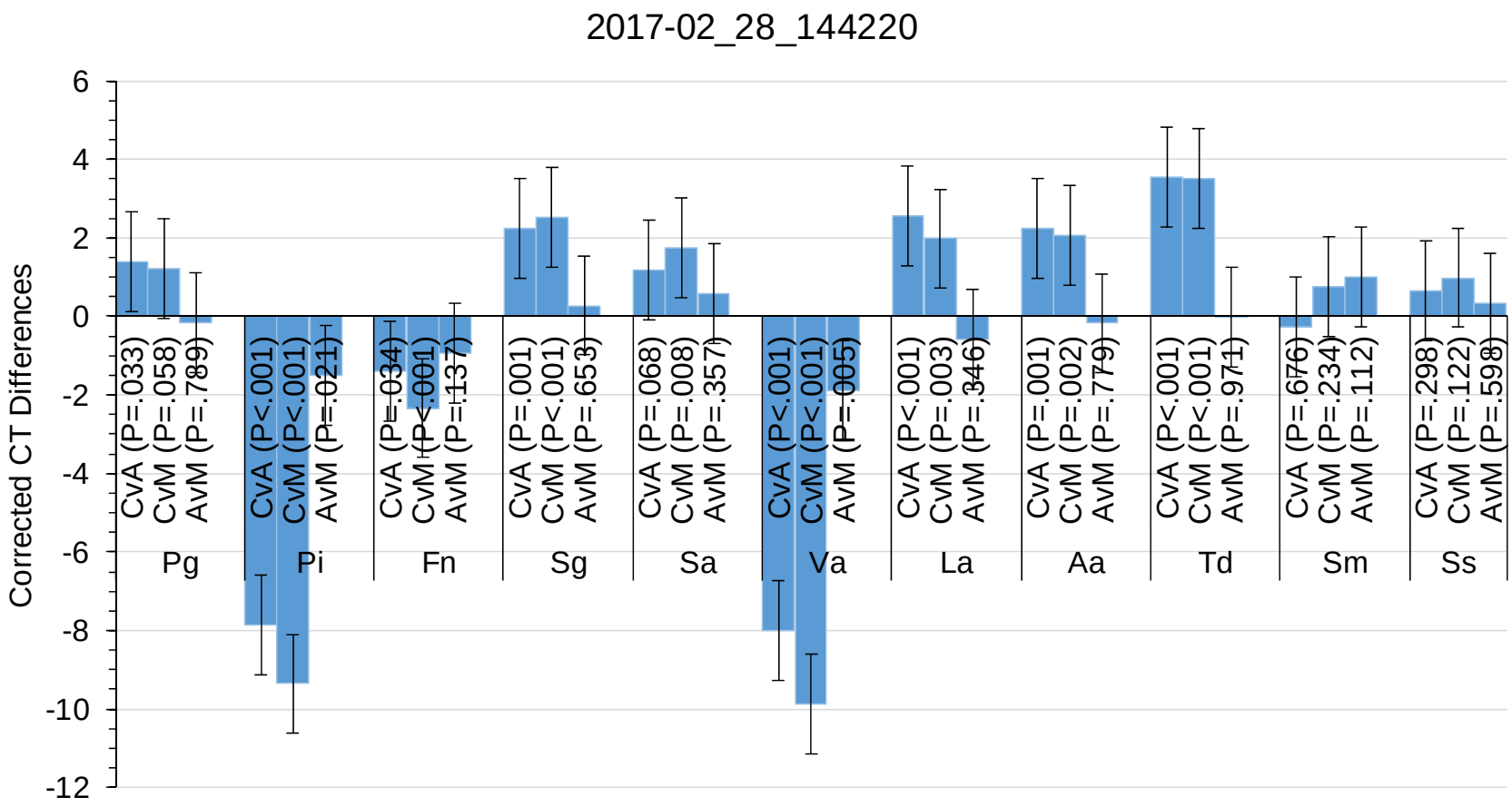

Figure 9. Differences in the Corrected CT mean estimates for Run 2017-02_28_144220 (95% CIs)

Figure 7 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 7. Fold estimates for Run 2017-02-28_144220

Bacterial Species	Antimicrobial	Fold Estimate	95% CI	
Pg	Amixicile (P=.033)	2.623	1.090	6.314
	Metronidazole (P=.058)	2.335	0.970	5.621
Pi	Amixicile (P<.001)	0.004	0.002	0.010
	Metronidazole (P<.001)	0.002	0.001	0.004
Fn	Amixicile (P=.034)	0.384	0.159	0.923
	Metronidazole (P<.001)	0.199	0.083	0.478
Sg	Amixicile (P=.001)	4.751	1.974	11.437
	Metronidazole (P<.001)	5.777	2.400	13.906
Sa	Amixicile (P=.068)	2.258	0.938	5.435
	Metronidazole (P=.008)	3.377	1.403	8.129
Va	Amixicile (P<.001)	0.004	0.002	0.009
	Metronidazole (P<.001)	0.001	0.000	0.003
La	Amixicile (P<.001)	5.955	2.474	14.336
	Metronidazole (P=.003)	3.945	1.639	9.496
Aa	Amixicile (P=.001)	4.730	1.965	11.386
	Metronidazole (P=.002)	4.188	1.740	10.081
Td	Amixicile (P<.001)	11.646	4.838	28.035
	Metronidazole (P<.001)	11.466	4.763	27.600
Sm	Amixicile (P=.676)	0.834	0.346	2.008
	Metronidazole (P=.234)	1.685	0.700	4.057
Ss	Amixicile (P=.298)	1.578	0.655	3.798
	Metronidazole (P=.122)	1.985	0.824	4.777

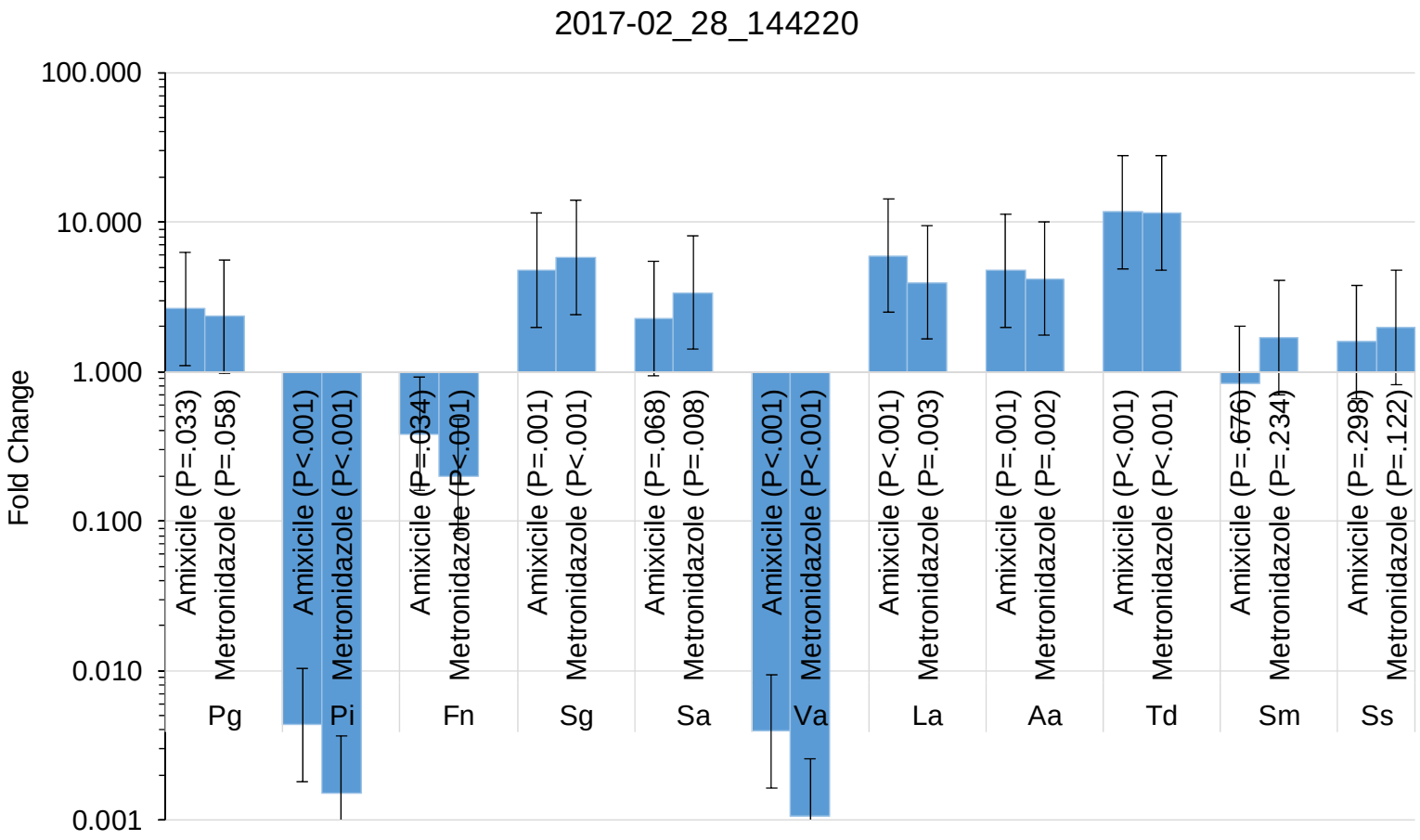

Figure 10. Fold estimates for Run 2017-02_28_144220 (95% CIs)

Figure 8 represents the fold change observed for Run 2017-2_28_144220 n bacterial species after treatment of either Amixicile or Metronidazole. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment

Run 2017-03-07_1

Table 8. Corrected CT mean estimates for Run 2017-03-07_1

		Corrected CT		
Bacterial species	Antimicrobials	Estimate	95% CI	
Pg (P=.004)	Control	32.36	31.93	32.79
	Amixicile-c	31.30	30.87	31.73
	Metronidazole-c	31.59	31.16	32.02
Pi (P<.001)	Control	18.29	17.86	18.72
	Amixicile-cx	25.64	25.21	26.07
	Metronidazole-cx	27.19	26.76	27.62
Fn (P<.001)	Control	24.70	24.27	25.13
	Amixicile-c	30.15	29.73	30.58
	Metronidazole-c	29.70	29.27	30.13
Sg (P<.001)	Control	21.37	20.94	21.80
	Amixicile-cx	20.48	20.05	20.91
	Metronidazole-x	21.92	21.49	22.35
Sa (P<.001)	Control	17.99	17.56	18.42
	Amixicile-x	17.28	16.85	17.71
	Metronidazole-cx	16.41	15.98	16.84
Va (P<.001)	Control	16.15	15.73	16.58
	Amixicile-cx	24.56	24.13	24.99
	Metronidazole-cx	25.53	25.10	25.96
La (P<.001)	Control	30.86	30.43	31.29
	Amixicile-c	29.16	28.74	29.59
	Metronidazole-c	29.33	28.90	29.76
Aa (P=.002)	Control	32.78	32.35	33.21
	Amixicile-c	33.95	33.53	34.38
	Metronidazole	33.24	32.81	33.67
Td (P=.235)	Control	28.70	28.27	29.13
	Amixicile	28.31	27.88	28.74
	Metronidazole	28.21	27.78	28.64
Sm (P<.001)	Control	30.64	30.21	31.07
	Amixicile-cx	33.15	32.72	33.58
	Metronidazole-x	31.31	30.88	31.74
Ss (P<.001)	Control	16.90	16.47	17.33
	Amixicile-c	15.77	15.34	16.20
	Metronidazole-c	15.63	15.20	16.06

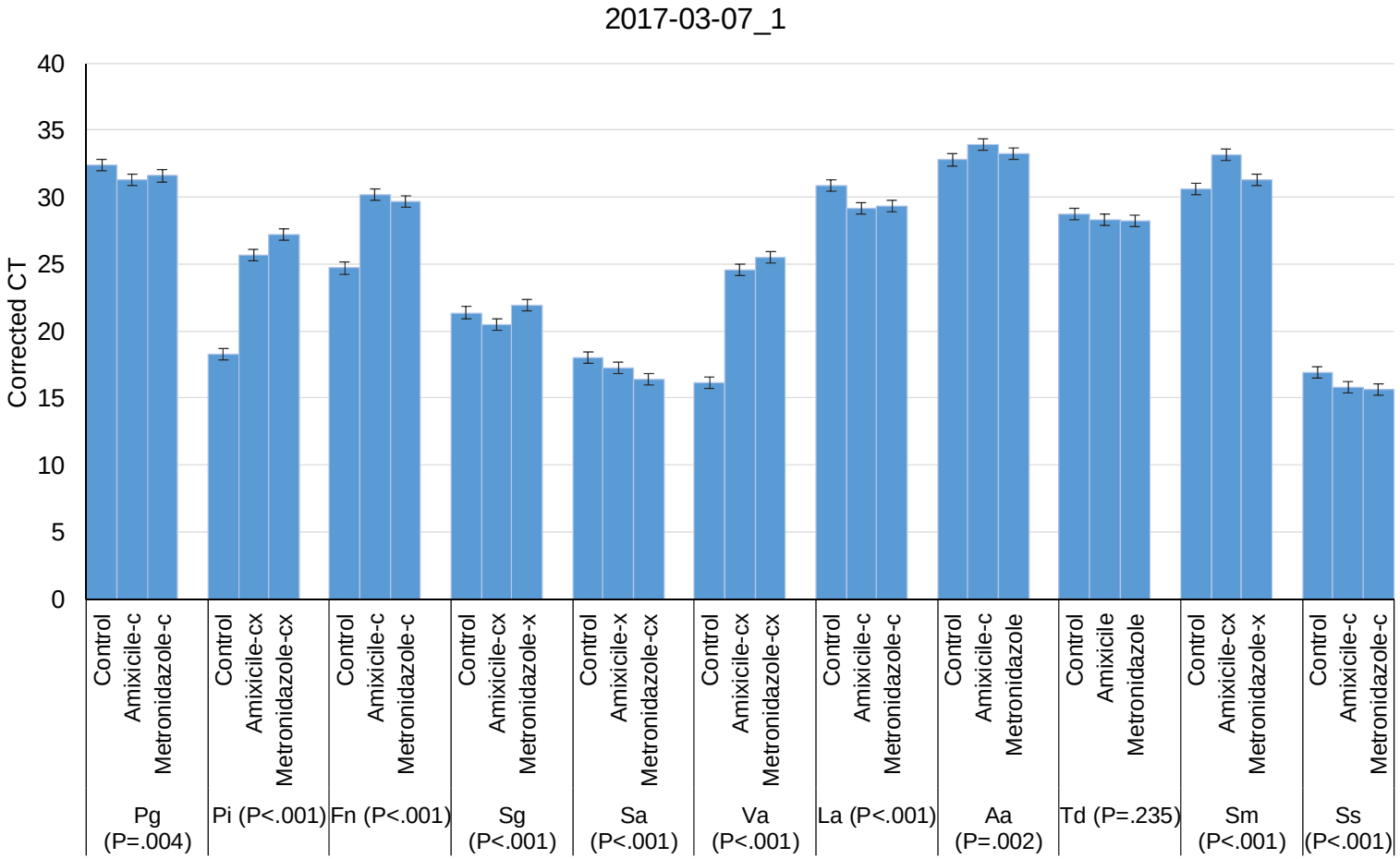

Figure 11. Corrected CT mean estimates for Run 2017-03-07_1 (96% CIs)

Figure 9 represents the average corrected CT values taken of Run 2017-03-07_1. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statisitically significant difference from Amxicile and Metronidazole

Table 9. Differences in the Corrected CT mean estimates for Run 2017-03-07_1

		Corrected CT		
Bacterial species	Compare	Estimate	95% CI	
Pg	CvA (P=.001)	1.057	0.450	1.664
	CvM (P=.015)	0.768	0.161	1.375
	AvM (P=.339)	-0.289	-0.896	0.318
Pi	CvA (P<.001)	-7.349	-7.956	-6.741
	CvM (P<.001)	-8.902	-9.509	-8.295
	AvM (P<.001)	-1.554	-2.161	-0.946
Fn	CvA (P<.001)	-5.457	-6.065	-4.850
	CvM (P<.001)	-4.999	-5.606	-4.391
	AvM (P=.133)	0.459	-0.148	1.066
Sg	CvA (P=.005)	0.894	0.287	1.501
	CvM (P=.078)	-0.542	-1.150	0.065
	AvM (P<.001)	-1.436	-2.044	-0.829
Sa	CvA (P=.025)	0.701	0.094	1.309
	CvM (P<.001)	1.576	0.969	2.183
	AvM (P=.006)	0.874	0.267	1.482
Va	CvA (P<.001)	-8.405	-9.012	-7.797
	CvM (P<.001)	-9.377	-9.984	-8.769
	AvM (P=.003)	-0.972	-1.579	-0.365
La	CvA (P<.001)	1.696	1.088	2.303
	CvM (P<.001)	1.533	0.925	2.140
	AvM (P=.588)	-0.163	-0.770	0.444
Aa	CvA (P<.001)	-1.171	-1.779	-0.564
	CvM (P=.135)	-0.457	-1.065	0.150
	AvM (P=.023)	0.714	0.107	1.321
Td	CvA (P=.199)	0.390	-0.217	0.998
	CvM (P=.110)	0.490	-0.117	1.097
	AvM (P=.740)	0.100	-0.508	0.707
Sm	CvA (P<.001)	-2.516	-3.124	-1.909
	CvM (P=.032)	-0.670	-1.277	-0.063
	AvM (P<.001)	1.846	1.239	2.454
Ss	CvA (P<.001)	1.127	0.520	1.734
	CvM (P<.001)	1.267	0.660	1.874
	AvM (P=.641)	0.140	-0.467	0.747

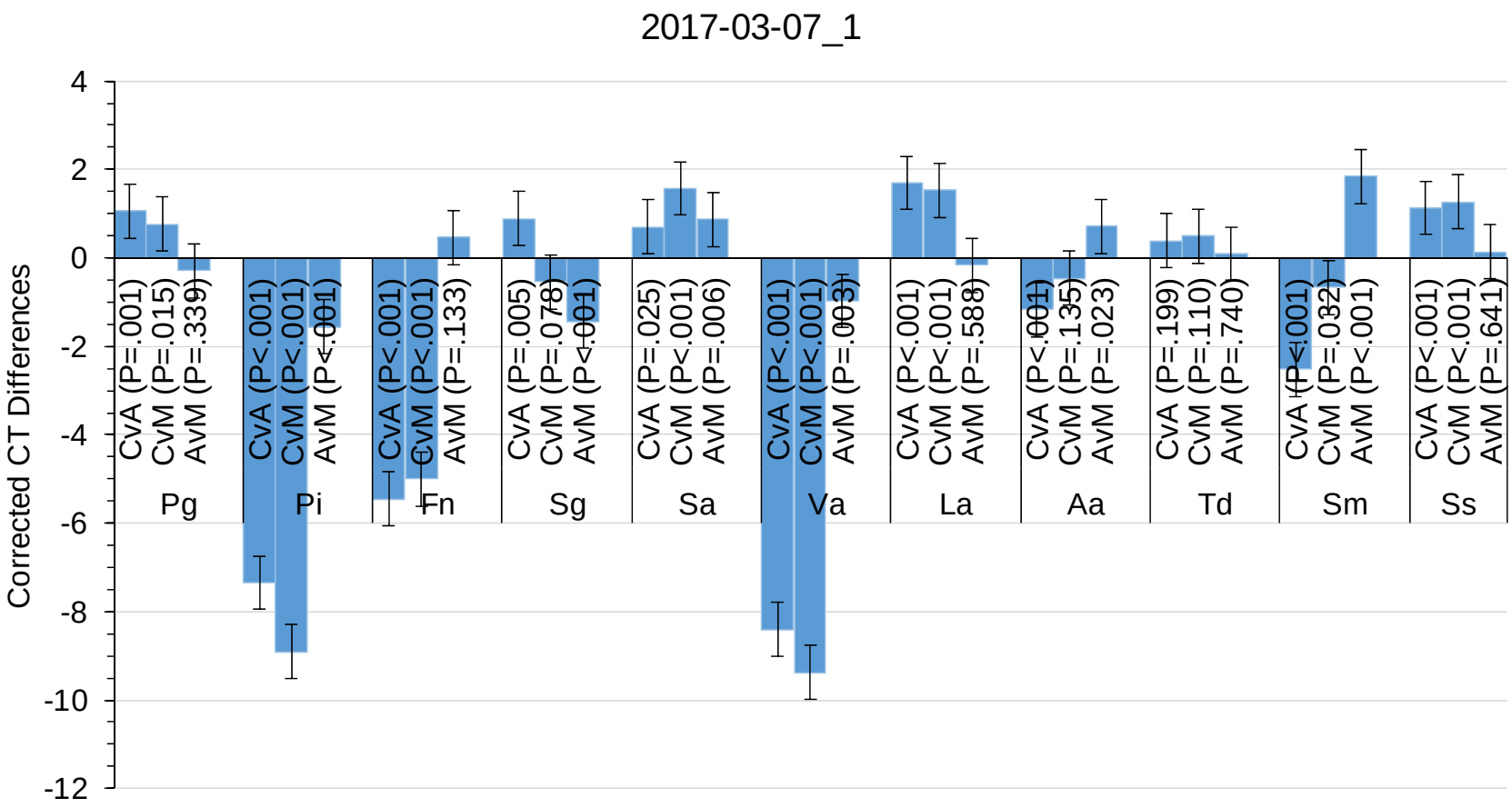

Figure 12. Differences in the Corrected CT mean estimates for Run 2017-03-07_1 (95% CI)

Figure 10 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 10. Fold change for Run 2017-03-07_1

Bacterial species	Antimicrobials	Fold		
		Estimate	95% CI	
Pg	Amixicile (P=.001)	2.081	1.366	3.170
	Metronidazole (P=.015)	1.703	1.118	2.595
Pi	Amixicile (P<.001)	0.006	0.004	0.009
	Metronidazole (P<.001)	0.002	0.001	0.003
Fn	Amixicile (P<.001)	0.023	0.015	0.035
	Metronidazole (P<.001)	0.031	0.021	0.048
Sg	Amixicile (P=.005)	1.858	1.220	2.831
	Metronidazole (P=.078)	0.687	0.451	1.046
Sa	Amixicile (P=.025)	1.626	1.067	2.477
	Metronidazole (P<.001)	2.981	1.957	4.541
Va	Amixicile (P<.001)	0.003	0.002	0.004
	Metronidazole (P<.001)	0.002	0.001	0.002
La	Amixicile (P<.001)	3.239	2.126	4.934
	Metronidazole (P<.001)	2.893	1.899	4.407
Aa	Amixicile (P<.001)	0.444	0.291	0.676
	Metronidazole (P=.135)	0.728	0.478	1.110
Td	Amixicile (P=.199)	1.311	0.860	1.997
	Metronidazole (P=.110)	1.405	0.922	2.140
Sm	Amixicile (P<.001)	0.175	0.115	0.266
	Metronidazole (P=.032)	0.629	0.413	0.958
Ss	Amixicile (P<.001)	2.184	1.434	3.327
	Metronidazole (P<.001)	2.406	1.580	3.666

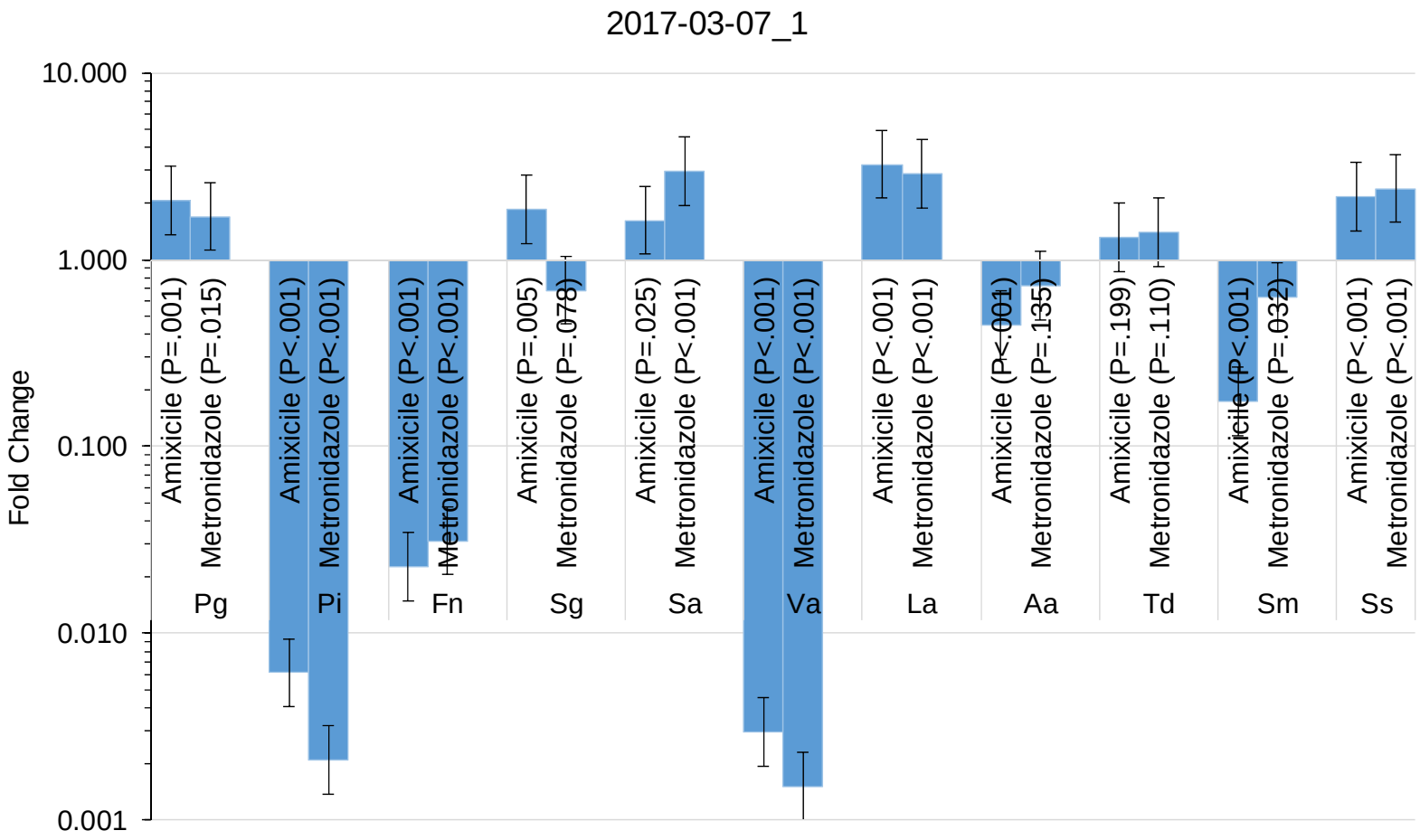

Figure 13. Fold change for Run 2017-03-07_1 (95% CIs)

Figure 11 represents the fold change observed for RUN 2017-03-07_1 in bacterial species after treatment of either Amixicile or Metronidazole. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment

Run 2017-03-21_162558

Table 11. Corrected CT mean estimates for Run 2017-03-21_162558

Bacterial species	Antimicrobials	Corrected CT		
		Estimate	95% CI	
Pg (P<.001)	Control	31.11	30.72	31.51
	Amixicile-cx	28.59	28.19	28.98
	Metronidazole-cx	29.94	29.54	30.33
Pi (P<.001)	Control	17.34	16.95	17.74
	Amixicile-cx	23.67	23.27	24.06
	Metronidazole-cx	25.89	25.50	26.29
Fn (P<.001)	Control	23.29	22.89	23.68
	Amixicile-x	23.00	22.60	23.39
	Metronidazole-cx	24.92	24.52	25.31
Sg (P<.001)	Control	17.11	16.71	17.50
	Amixicile-cx	14.59	14.20	14.99
	Metronidazole-cx	15.71	15.32	16.11
Sa (P<.001)	Control	16.56	16.16	16.96
	Amixicile-c	15.64	15.24	16.03
	Metronidazole-c	15.28	14.88	15.67
Va (P<.001)	Control	14.82	14.42	15.21
	Amixicile-cx	21.54	21.15	21.94
	Metronidazole-cx	23.99	23.60	24.39
La (P<.001)	Control	29.59	29.19	29.99
	Amixicile-cx	27.10	26.71	27.50
	Metronidazole-cx	27.82	27.42	28.21
Aa (P<.001)	Control	32.47	32.08	32.87
	Amixicile-cx	29.58	29.18	29.97
	Metronidazole-cx	31.36	30.97	31.76
Td (P<.001)	Control	27.87	27.48	28.27
	Amixicile-cx	26.39	25.99	26.78
	Metronidazole-x	27.69	27.30	28.09
Sm (P<.001)	Control	29.68	29.28	30.07
	Amixicile-cx	30.43	30.03	30.82
	Metronidazole-cx	32.01	31.62	32.41
Ss (P<.001)	Control	14.90	14.50	15.29
	Amixicile-c	13.33	12.93	13.72
	Metronidazole-c	13.99	13.60	14.39

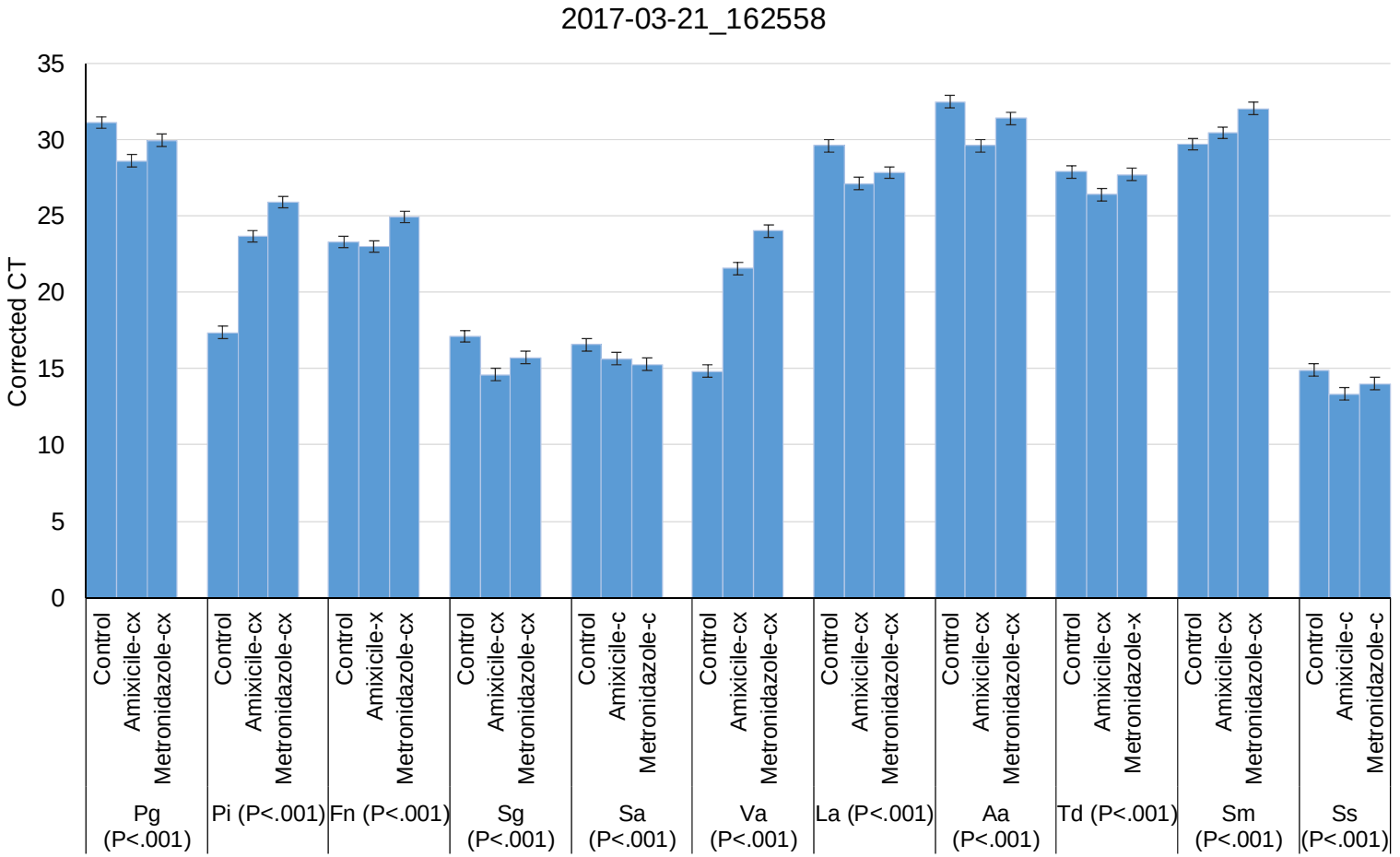

Figure 14. Corrected CT mean estimates for Run 2017-03-21_162558 (95% CI)

Figure 12 represents the average corrected CT values taken of Run 2017-03-21_162558. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 12. Differences in the Corrected CT mean estimates for Run 2017-03-21_162558

Bacterial species	Compare	Corrected CT Estimate	95% CI	
Pg	CvA (P<.001)	2.527	1.968	3.087
	CvM (P<.001)	1.176	0.616	1.735
	AvM (P<.001)	-1.352	-1.911	-0.792
Pi	CvA (P<.001)	-6.324	-6.884	-5.765
	CvM (P<.001)	-8.549	-9.109	-7.990
	AvM (P<.001)	-2.225	-2.785	-1.666
Fn	CvA (P=.303)	0.287	-0.273	0.846
	CvM (P<.001)	-1.634	-2.193	-1.074
	AvM (P<.001)	-1.921	-2.480	-1.361
Sg	CvA (P<.001)	2.515	1.955	3.074
	CvM (P<.001)	1.395	0.835	1.954
	AvM (P<.001)	-1.120	-1.679	-0.560
Sa	CvA (P=.002)	0.923	0.363	1.482
	CvM (P<.001)	1.282	0.722	1.841
	AvM (P=.200)	0.359	-0.201	0.918
Va	CvA (P<.001)	-6.722	-7.281	-6.163
	CvM (P<.001)	-9.175	-9.735	-8.616
	AvM (P<.001)	-2.453	-3.013	-1.894
La	CvA (P<.001)	2.485	1.926	3.045
	CvM (P<.001)	1.773	1.214	2.333
	AvM (P=.014)	-0.712	-1.271	-0.152
Aa	CvA (P<.001)	2.893	2.334	3.452
	CvM (P<.001)	1.107	0.547	1.666
	AvM (P<.001)	-1.786	-2.346	-1.227
Td	CvA (P<.001)	1.487	0.928	2.046
	CvM (P=.519)	0.179	-0.381	0.738
	AvM (P<.001)	-1.308	-1.868	-0.749
Sm	CvA (P=.010)	-0.751	-1.311	-0.192
	CvM (P<.001)	-2.338	-2.898	-1.779
	AvM (P<.001)	-1.587	-2.146	-1.027
Ss	CvA (P<.001)	1.570	1.010	2.129
	CvM (P=.002)	0.904	0.345	1.464
	AvM (P=.021)	-0.666	-1.225	-0.106

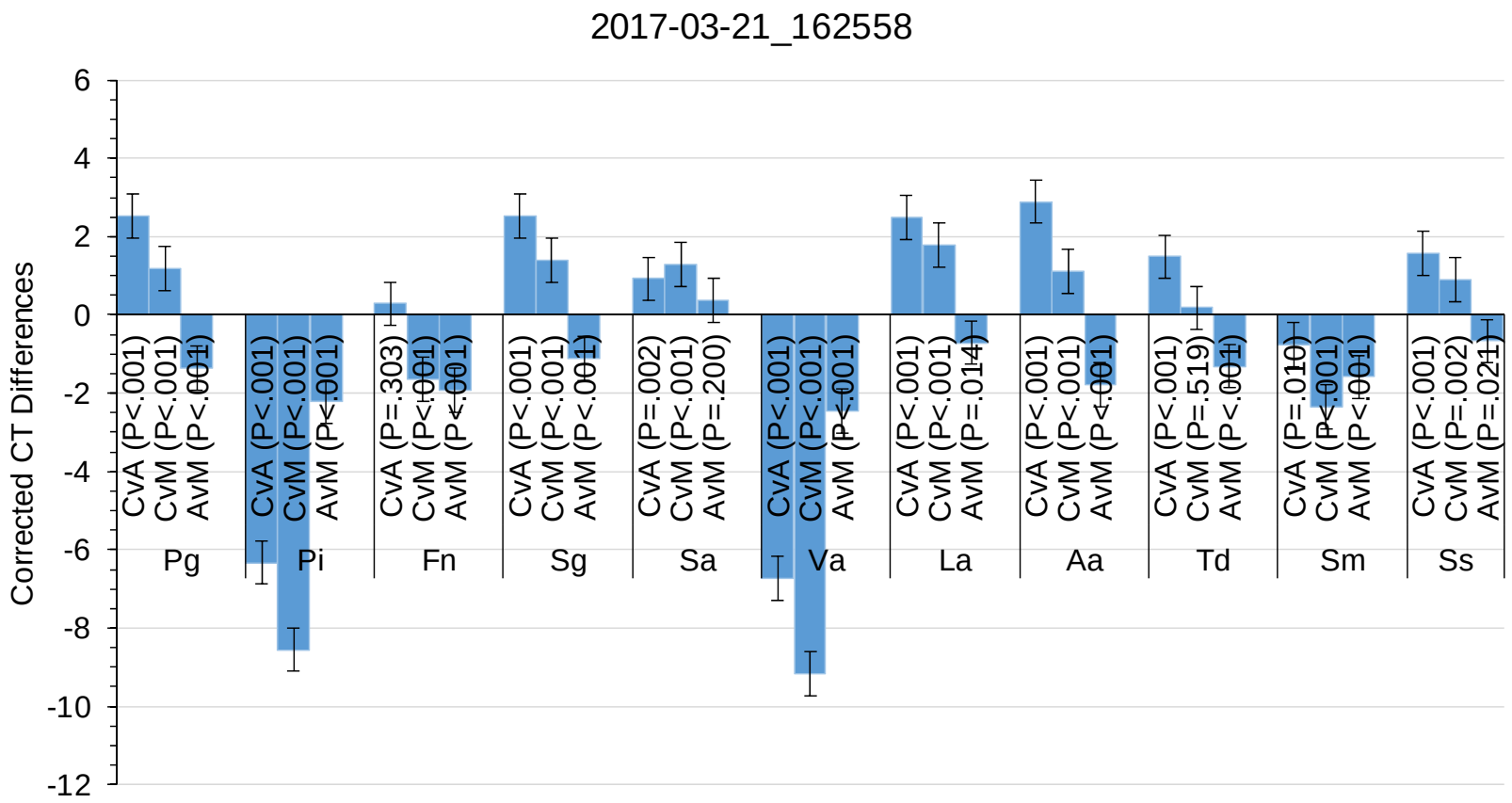

Figure 15. Differences in the Corrected CT mean estimates for Run 2017-03-21_162558

Figure 13 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 13. Fold change for Run 2017-03-21_162558

Bacterial species	Antimicrobials	Fold Estimate	95% CI	
Pg	Amixicile (P<.001)	5.765	3.912	8.496
	Metronidazole (P<.001)	2.259	1.533	3.329
Pi	Amixicile (P<.001)	0.012	0.008	0.018
	Metronidazole (P<.001)	0.003	0.002	0.004
Fn	Amixicile (P=.303)	1.220	0.828	1.798
	Metronidazole (P<.001)	0.322	0.219	0.475
Sg	Amixicile (P<.001)	5.715	3.878	8.422
	Metronidazole (P<.001)	2.629	1.784	3.875
Sa	Amixicile (P=.002)	1.895	1.286	2.793
	Metronidazole (P<.001)	2.431	1.650	3.583
Va	Amixicile (P<.001)	0.009	0.006	0.014
	Metronidazole (P<.001)	0.002	0.001	0.003
La	Amixicile (P<.001)	5.600	3.800	8.253
	Metronidazole (P<.001)	3.419	2.320	5.038
Aa	Amixicile (P<.001)	7.428	5.040	10.947
	Metronidazole (P<.001)	2.154	1.461	3.174
Td	Amixicile (P<.001)	2.803	1.902	4.131
	Metronidazole (P=.519)	1.132	0.768	1.668
Sm	Amixicile (P=.010)	0.594	0.403	0.875
	Metronidazole (P<.001)	0.198	0.134	0.291
Ss	Amixicile (P<.001)	2.968	2.014	4.375
	Metronidazole (P=.002)	1.871	1.270	2.758

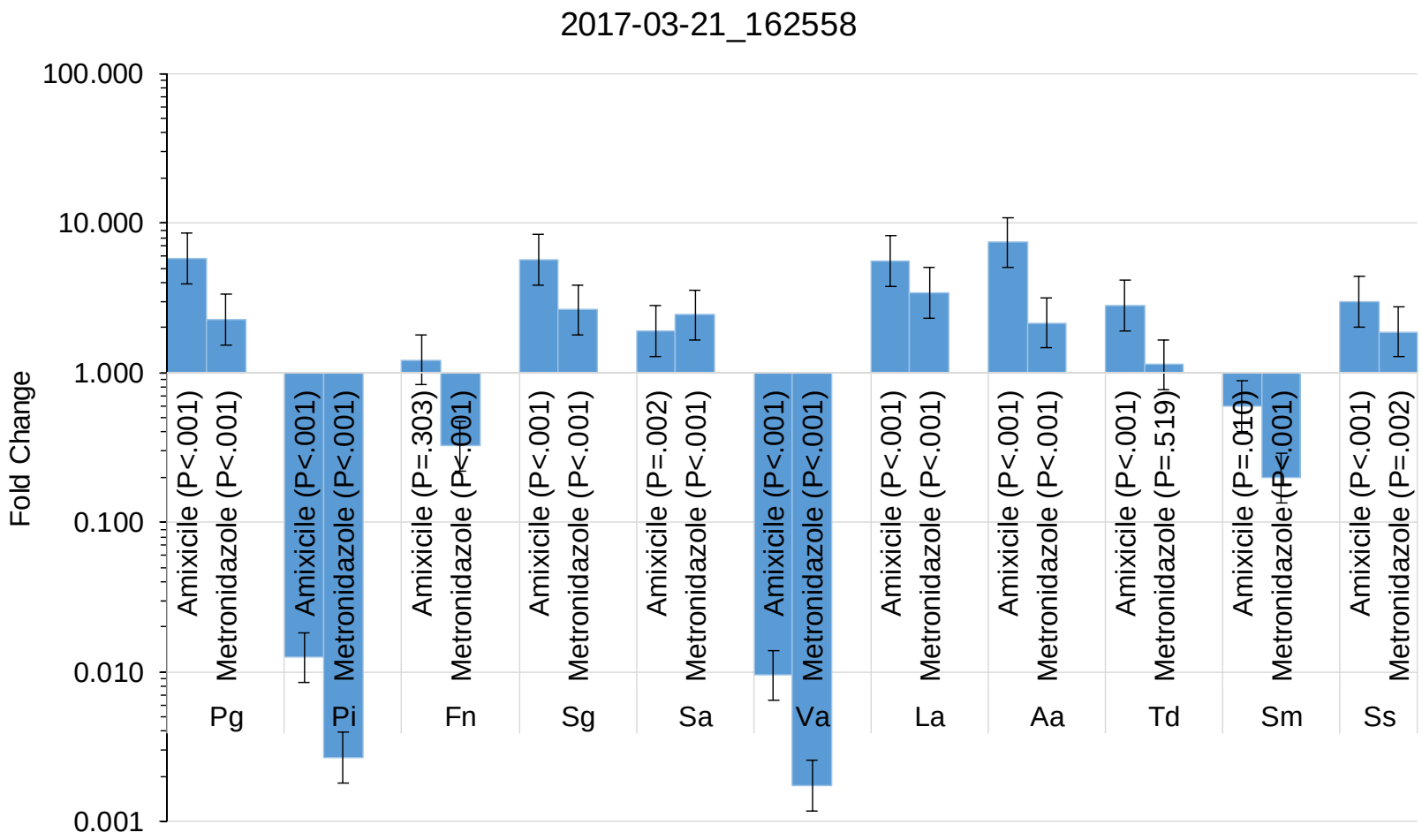

Figure 16. Fold change for Run 2017-03-21_162558 (95% CI)

Figure 11 represents the fold change observed for RUN 2017-03-07_1 in bacterial species after treatment of either Amixicile or Metronidazole. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment

Run 2017-04-13_175325

Table 14. Corrected CT mean estimates for Run 2017-04-13_175325

Bacterial species	Antimicrobials	Corrected CT		
		Estimate	95% CI	
Pg (P=.079)	Control	28.72	28.06	29.38
	Amixicile	28.23	27.56	28.89
	Metronidazole	27.64	26.98	28.30
Pi (P<.001)	Control	15.47	14.81	16.14
	Amixicile-c	21.22	20.55	21.88
	Metronidazole-c	22.17	21.51	22.83
Fn (P=.098)	Control	21.04	20.38	21.70
	Amixicile	21.53	20.86	22.19
	Metronidazole	22.07	21.41	22.73
Sg (P=.048)	Control	14.43	13.76	15.09
	Amixicile	13.78	13.12	14.44
	Metronidazole-c	13.24	12.57	13.90
Sa (P=.003)	Control	13.66	13.00	14.32
	Amixicile	12.79	12.13	13.46
	Metronidazole-c	11.96	11.29	12.62
Va (P<.001)	Control	10.93	10.27	11.60
	Amixicile-cx	19.49	18.83	20.15
	Metronidazole-cx	21.14	20.48	21.80
La (P<.001)	Control	27.63	26.96	28.29
	Amixicile-cx	22.94	22.28	23.60
	Metronidazole-cx	24.60	23.94	25.27
Aa (P<.001)	Control	30.13	29.47	30.79
	Amixicile-cx	26.71	26.05	27.37
	Metronidazole-cx	28.55	27.89	29.22
Td (P=.009)	Control	25.37	24.70	26.03
	Amixicile-c	24.06	23.39	24.72
	Metronidazole-c	24.03	23.37	24.69
Sm (P<.001)	Control	30.36	29.70	31.02
	Amixicile-cx	27.66	27.00	28.32
	Metronidazole-cx	29.18	28.51	29.84
Ss (P=.015)	Control	12.23	11.57	12.89
	Amixicile	11.12	10.45	11.78
	Metronidazole-c	10.90	10.23	11.56

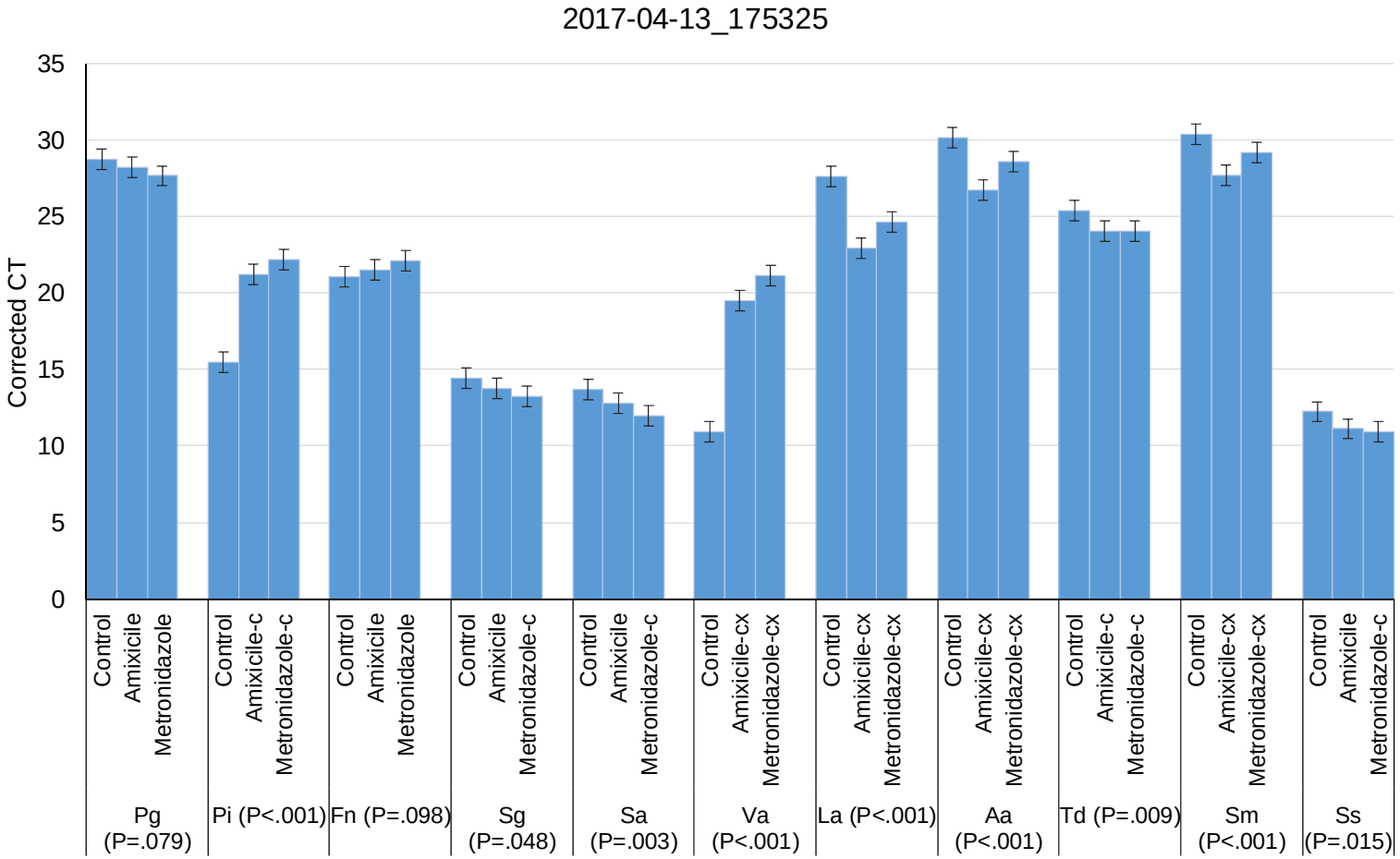

Figure 17. Corrected CT mean estimates for Run 2017-04-13_175325 (95% CIs)

Figure 15 represents the average corrected CT values taken of Run 2017-04-13_175325. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 15. Differences in the Corrected CT mean estimates for Run 2017-04-13_175325

Bacterial species	Compare	Corrected CT Estimate	95% CI	
Pg	CvA (P=.291)	0.493	-0.444	1.430
	CvM (P=.026)	1.079	0.141	2.016
	AvM (P=.212)	0.586	-0.352	1.523
Pi	CvA (P<.001)	-5.744	-6.681	-4.807
	CvM (P<.001)	-6.696	-7.634	-5.759
	AvM (P=.047)	-0.952	-1.890	-0.015
Fn	CvA (P=.299)	-0.485	-1.422	0.452
	CvM (P=.033)	-1.027	-1.964	-0.090
	AvM (P=.247)	-0.542	-1.479	0.395
Sg	CvA (P=.168)	0.649	-0.288	1.586
	CvM (P=.015)	1.190	0.253	2.127
	AvM (P=.247)	0.542	-0.396	1.479
Sa	CvA (P=.069)	0.867	-0.071	1.804
	CvM (P<.001)	1.704	0.767	2.641
	AvM (P=.078)	0.837	-0.100	1.774
Va	CvA (P<.001)	-8.554	-9.492	-7.617
	CvM (P<.001)	-10.206	-11.143	-9.269
	AvM (P=.001)	-1.651	-2.589	-0.714
La	CvA (P<.001)	4.687	3.750	5.624
	CvM (P<.001)	3.022	2.085	3.959
	AvM (P=.001)	-1.665	-2.602	-0.728
Aa	CvA (P<.001)	3.416	2.479	4.354
	CvM (P=.002)	1.574	0.637	2.511
	AvM (P<.001)	-1.843	-2.780	-0.905
Td	CvA (P=.008)	1.309	0.372	2.246
	CvM (P=.007)	1.334	0.397	2.271
	AvM (P=.957)	0.025	-0.912	0.962
Sm	CvA (P<.001)	2.702	1.765	3.639
	CvM (P=.015)	1.184	0.247	2.121
	AvM (P=.002)	-1.518	-2.455	-0.581
Ss	CvA (P=.022)	1.112	0.174	2.049
	CvM (P=.007)	1.333	0.396	2.270
	AvM (P=.633)	0.221	-0.716	1.158

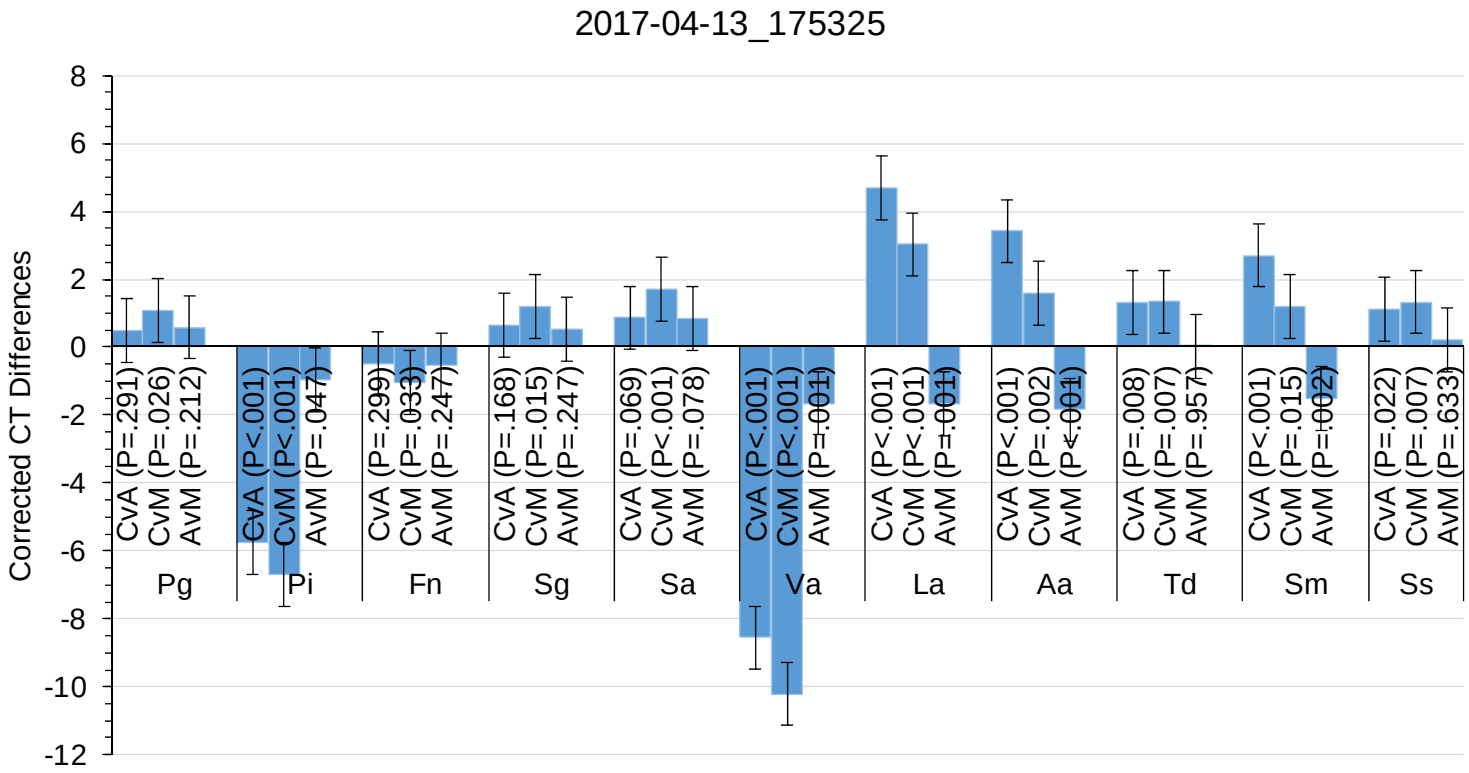

Figure 18. Differences in the Corrected CT mean estimates for Run 2017-04-13_175325

Figure 16 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 16. Fold estimates for Run 2017-04-13_175325

Bacterial species	Antimicrobials	Fold Estimate	95% CI	
Pg	Amixicile (P=.291)	1.407	0.735	2.695
	Metronidazole (P=.026)	2.112	1.103	4.044
Pi	Amixicile (P<.001)	0.019	0.010	0.036
	Metronidazole (P<.001)	0.010	0.005	0.018
Fn	Amixicile (P=.299)	0.715	0.373	1.368
	Metronidazole (P=.033)	0.491	0.256	0.940
Sg	Amixicile (P=.168)	1.568	0.819	3.002
	Metronidazole (P=.015)	2.282	1.192	4.369
Sa	Amixicile (P=.069)	1.823	0.952	3.491
	Metronidazole (P<.001)	3.258	1.701	6.237
Va	Amixicile (P<.001)	0.003	0.001	0.005
	Metronidazole (P<.001)	0.001	0.000	0.002
La	Amixicile (P<.001)	25.759	13.453	49.321
	Metronidazole (P<.001)	8.125	4.243	15.557
Aa	Amixicile (P<.001)	10.677	5.577	20.444
	Metronidazole (P=.002)	2.977	1.555	5.701
Td	Amixicile (P=.008)	2.477	1.294	4.743
	Metronidazole (P=.007)	2.521	1.317	4.826
Sm	Amixicile (P<.001)	6.508	3.399	12.461
	Metronidazole (P=.015)	2.272	1.187	4.350
Ss	Amixicile (P=.022)	2.161	1.129	4.137
	Metronidazole (P=.007)	2.519	1.315	4.823

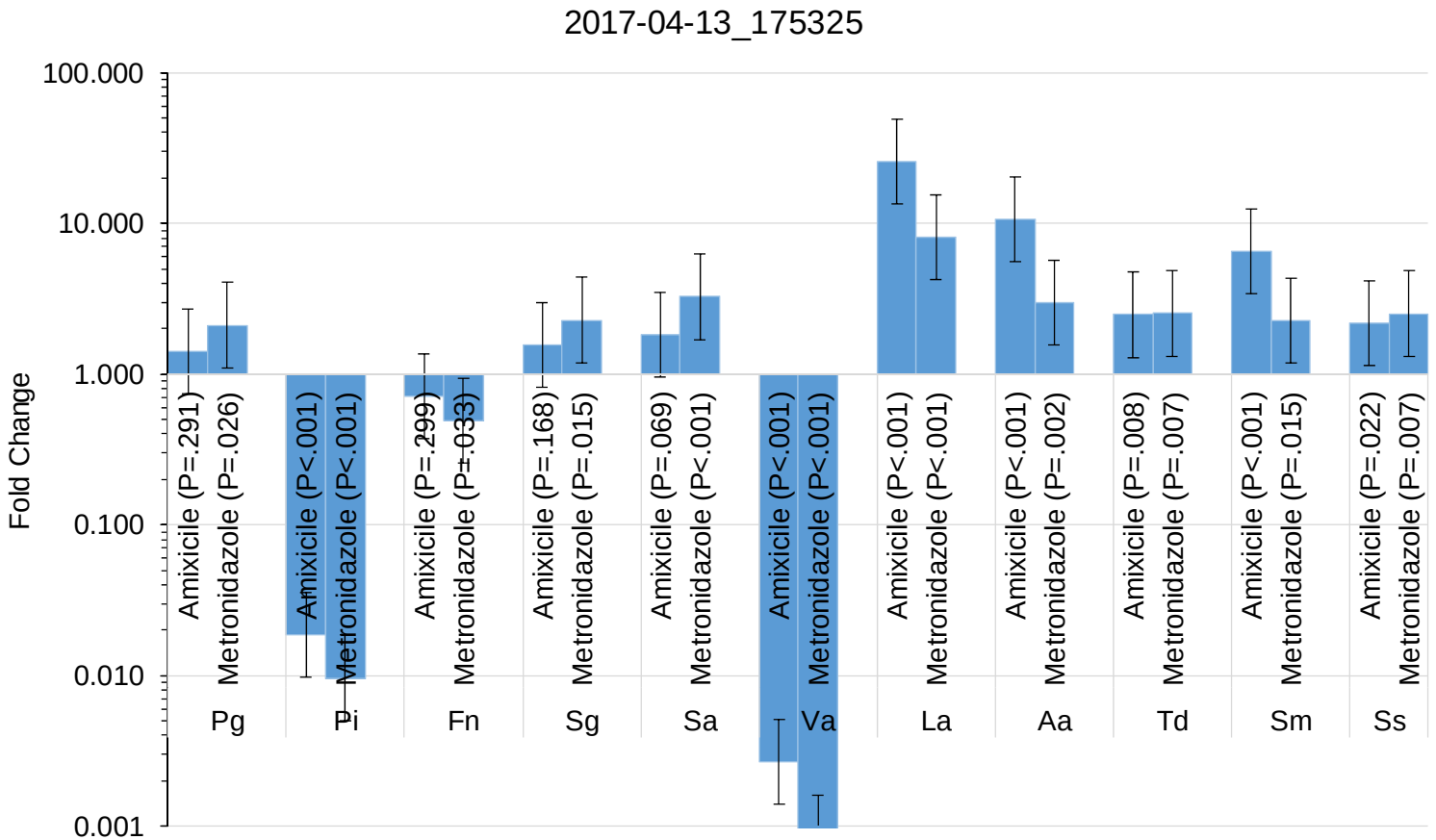

Figure 19. Fold estimates for Run 2017-04-13_175325 (95% CIs)

Figure 17 represents the fold change observed for Run 2017-04-13_175325 in bacterial species after treatment of either Amixicile or Metronidazole. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment

Non-PMA Runs

Table 17. Differences in the Corrected CT mean estimates for non-PMA Runs

Bacterial species	Compare	Corrected CT Estimate	95% CI	
Pg	CvA (P=.006)	1.367	0.402	2.332
	CvM (P=.031)	1.061	0.097	2.026
	AvM (P=.533)	-0.306	-1.270	0.659
Pi	CvA (P<.001)	-6.817	-7.781	-5.852
	CvM (P<.001)	-8.377	-9.341	-7.412
	AvM (P=.002)	-1.560	-2.525	-0.595
Fn	CvA (P<.001)	-1.759	-2.724	-0.795
	CvM (P<.001)	-2.498	-3.462	-1.533
	AvM (P=.133)	-0.738	-1.703	0.226
Sg	CvA (P=.001)	1.576	0.612	2.541
	CvM (P=.020)	1.143	0.178	2.108
	AvM (P=.377)	-0.433	-1.398	0.532
Sa	CvA (P=.063)	0.916	-0.048	1.881
	CvM (P=.001)	1.579	0.614	2.544
	AvM (P=.177)	0.663	-0.302	1.628
Va	CvA (P<.001)	-7.918	-8.883	-6.953
	CvM (P<.001)	-9.660	-10.625	-8.695
	AvM (P<.001)	-1.742	-2.706	-0.777
La	CvA (P<.001)	2.861	1.896	3.825
	CvM (P<.001)	2.077	1.112	3.042
	AvM (P=.111)	-0.783	-1.748	0.181
Aa	CvA (P<.001)	1.845	0.880	2.810
	CvM (P=.030)	1.072	0.108	2.037
	AvM (P=.116)	-0.773	-1.737	0.192
Td	CvA (P<.001)	1.682	0.717	2.647
	CvM (P=.005)	1.380	0.416	2.345
	AvM (P=.538)	-0.302	-1.266	0.663
Sm	CvA (P=.673)	-0.207	-1.172	0.758
	CvM (P=.585)	-0.268	-1.232	0.697
	AvM (P=.901)	-0.061	-1.026	0.904
Ss	CvA (P=.024)	1.117	0.152	2.081
	CvM (P=.023)	1.123	0.158	2.088
	AvM (P=.989)	0.007	-0.958	0.971

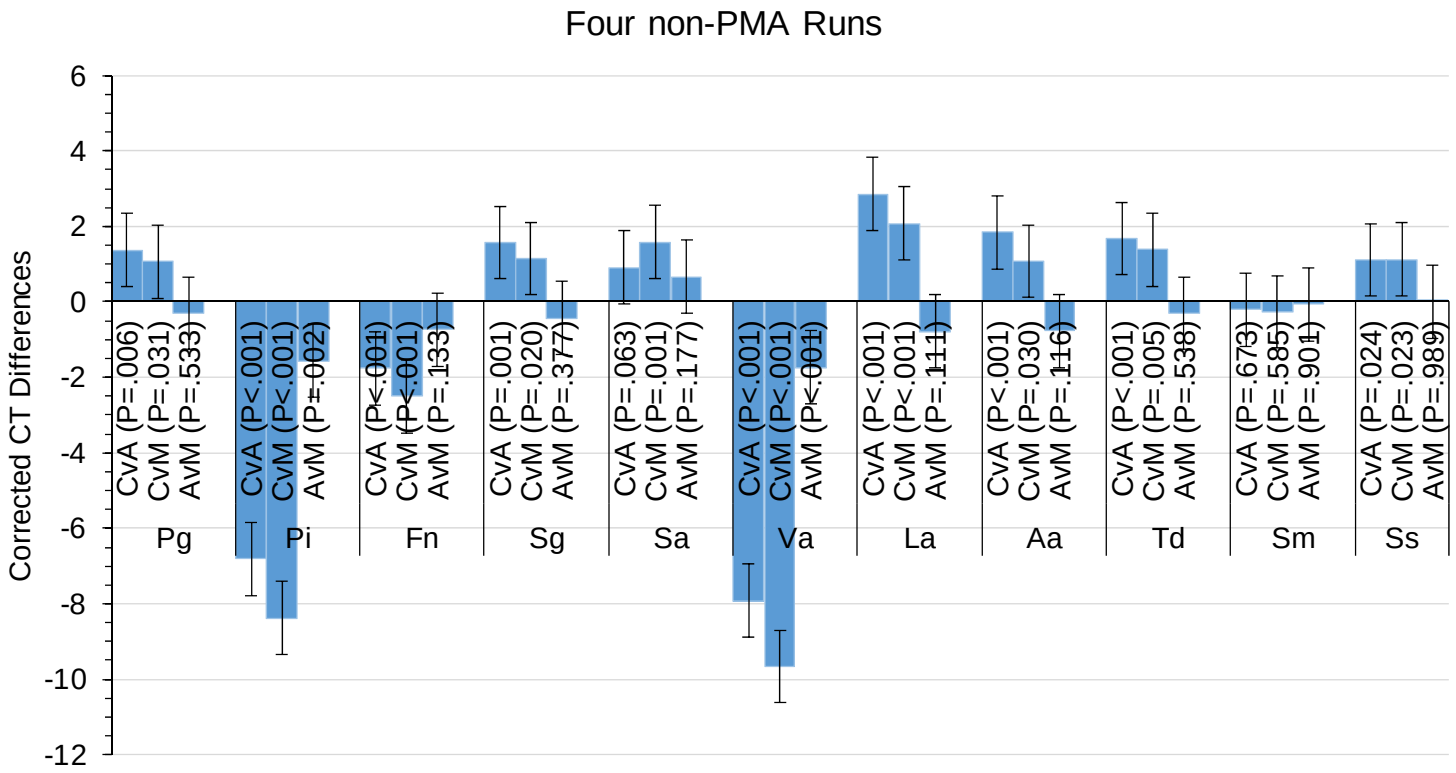

Figure 20. Differences in the Corrected CT mean estimates for non-PMA Runs

Figure 18 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Run 2017-02-28_pma

Table 18. Corrected CT mean estimates for Run 2017-02-28_pma

Bacterial species	Antimicrobials	Corrected CT		
		Estimate	95% CI	
Pg (P=.793)	Control	33.23	31.83	34.62
	Amixicile	33.86	31.89	35.82
	Metronidazole	33.81	32.42	35.21
Pi (P<.001)	Control	18.41	17.01	19.80
	Amixicile-c	25.90	24.51	27.30
	Metronidazole-c	27.20	25.80	28.59
Fn (P=.010)	Control	23.15	21.76	24.55
	Amixicile	25.19	23.80	26.59
	Metronidazole-c	26.29	24.90	27.69
Sg (P=.020)	Control	20.00	18.60	21.39
	Amixicile-c	17.11	15.71	18.50
	Metronidazole	18.69	17.30	20.09
Sa (P=.127)	Control	17.54	16.14	18.93
	Amixicile	16.26	14.87	17.66
	Metronidazole	15.53	14.14	16.93
Va (P<.001)	Control	15.93	14.53	17.32
	Amixicile-c	23.19	21.80	24.59
	Metronidazole-c	24.85	23.45	26.24
La (P=.003)	Control	31.85	30.45	33.24
	Amixicile-c	28.95	27.55	30.34
	Metronidazole-c	28.52	27.12	29.91
Aa (P=.790)	Control	31.83	30.44	33.23
	Amixicile	32.29	30.90	33.69
	Metronidazole	31.64	30.25	33.04
Td (P<.001)	Control	30.26	28.87	31.66
	Amixicile-cx	33.49	32.10	34.89
	Metronidazole-x	29.64	28.24	31.03
Sm (P=.670)	Control	34.64	33.25	36.04
	Amixicile	33.78	32.38	35.17
	Metronidazole	34.17	32.78	35.57
Ss (P=.078)	Control	16.64	15.25	18.04
	Amixicile	14.59	13.20	15.99
	Metronidazole	14.75	13.35	16.14

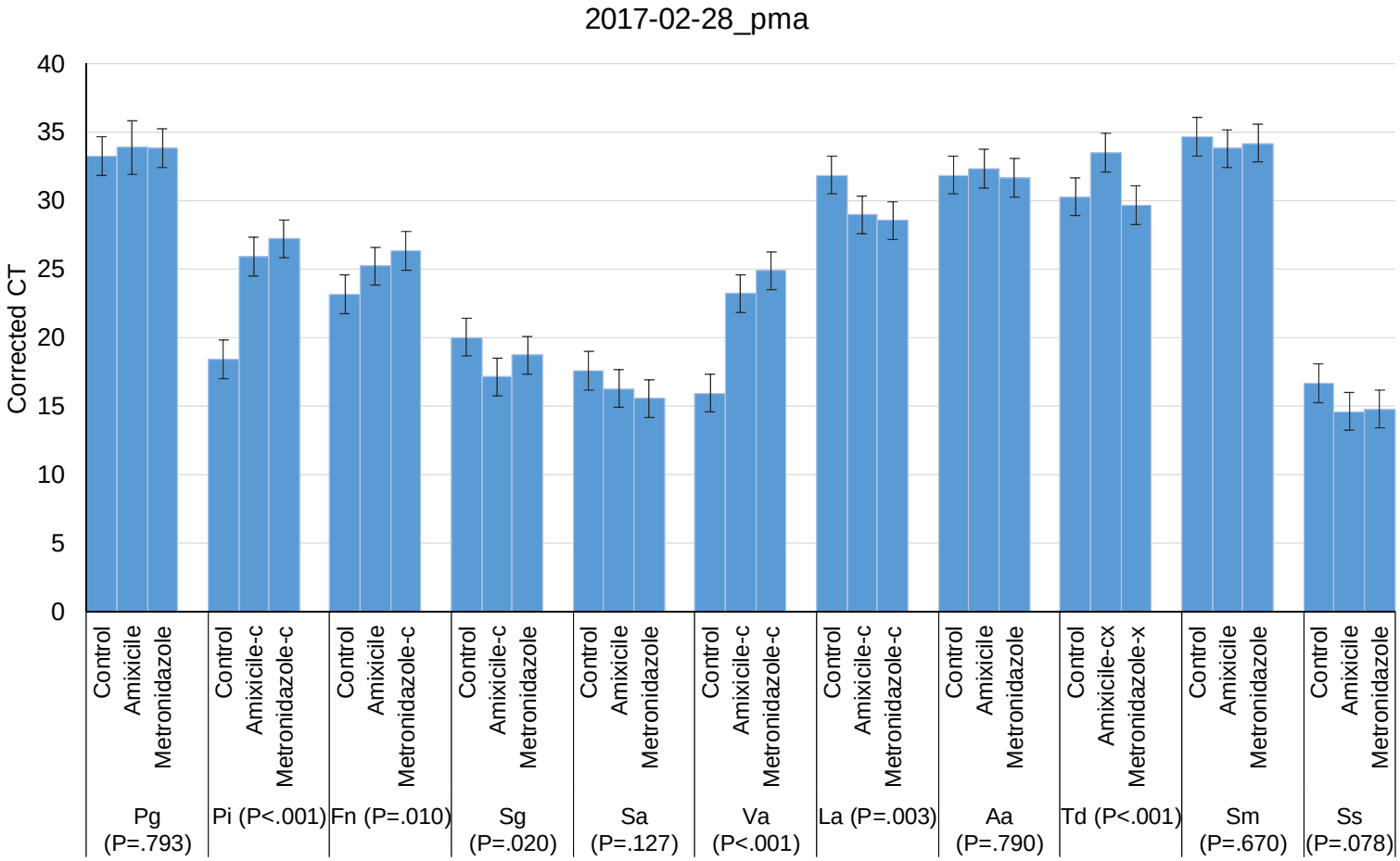

Figure 21. Corrected CT mean estimates for Run 2017-02-28_pma (95% CIs)

Figure 19 represents the average corrected CT values taken of Run 2017-02-28_pma. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 19. Differences in the Corrected CT mean estimates for Run 2017-02-28_pma

Bacterial species	Compare	Corrected CT Estimate	95% CI	
Pg	CvA (P=.598)	-0.628	-3.038	1.783
	CvM (P=.548)	-0.586	-2.559	1.386
	AvM (P=.972)	0.041	-2.369	2.452
Pi	CvA (P<.001)	-7.493	-9.466	-5.520
	CvM (P<.001)	-8.787	-10.760	-6.814
	AvM (P=.190)	-1.294	-3.267	0.679
Fn	CvA (P=.043)	-2.039	-4.012	-0.066
	CvM (P=.003)	-3.140	-5.113	-1.167
	AvM (P=.263)	-1.100	-3.073	0.873
Sg	CvA (P=.006)	2.891	0.919	4.864
	CvM (P=.186)	1.306	-0.667	3.279
	AvM (P=.111)	-1.585	-3.558	0.387
Sa	CvA (P=.196)	1.276	-0.697	3.249
	CvM (P=.046)	2.006	0.033	3.979
	AvM (P=.455)	0.730	-1.242	2.703
Va	CvA (P<.001)	-7.265	-9.238	-5.292
	CvM (P<.001)	-8.920	-10.893	-6.947
	AvM (P=.097)	-1.655	-3.628	0.318
La	CvA (P=.005)	2.900	0.927	4.873
	CvM (P=.002)	3.331	1.358	5.303
	AvM (P=.659)	0.430	-1.542	2.403
Aa	CvA (P=.638)	-0.458	-2.431	1.515
	CvM (P=.846)	0.188	-1.784	2.161
	AvM (P=.508)	0.647	-1.326	2.620
Td	CvA (P=.002)	-3.230	-5.203	-1.257
	CvM (P=.523)	0.623	-1.350	2.596
	AvM (P<.001)	3.853	1.880	5.826
Sm	CvA (P=.376)	0.868	-1.105	2.841
	CvM (P=.628)	0.473	-1.500	2.446
	AvM (P=.685)	-0.395	-2.368	1.578
Ss	CvA (P=.043)	2.046	0.073	4.018
	CvM (P=.059)	1.894	-0.079	3.867
	AvM (P=.876)	-0.152	-2.124	1.821

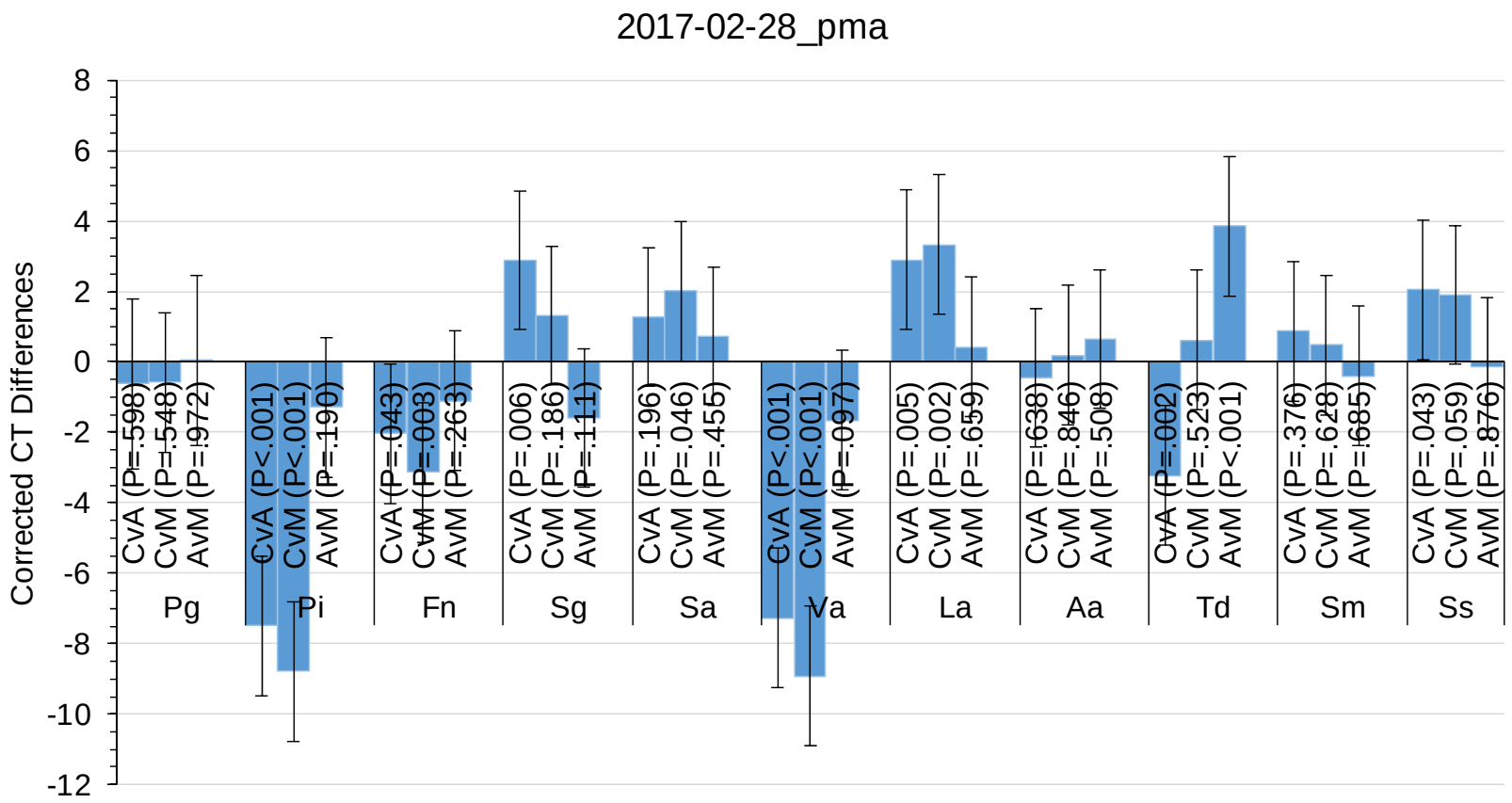

Figure 22. Differences in the Corrected CT mean estimates for Run 2017-02-28_pma

Figure 20 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 20. Fold estimates for Run 2017-02-28_pma

Bacterial species	Antimicrobials	Fold Estimate	95% CI	
Pg	Amixicile (P=.598)	0.647	0.122	3.441
	Metronidazole (P=.548)	0.666	0.170	2.614
Pi	Amixicile (P<.001)	0.006	0.001	0.022
	Metronidazole (P<.001)	0.002	0.001	0.009
Fn	Amixicile (P=.043)	0.243	0.062	0.955
	Metronidazole (P=.003)	0.113	0.029	0.445
Sg	Amixicile (P=.006)	7.420	1.890	29.126
	Metronidazole (P=.186)	2.473	0.630	9.706
Sa	Amixicile (P=.196)	2.421	0.617	9.505
	Metronidazole (P=.046)	4.017	1.023	15.769
Va	Amixicile (P<.001)	0.007	0.002	0.026
	Metronidazole (P<.001)	0.002	0.001	0.008
La	Amixicile (P=.005)	7.465	1.902	29.302
	Metronidazole (P=.002)	10.060	2.563	39.488
Aa	Amixicile (P=.638)	0.728	0.185	2.857
	Metronidazole (P=.846)	1.140	0.290	4.473
Td	Amixicile (P=.002)	0.107	0.027	0.418
	Metronidazole (P=.523)	1.540	0.392	6.046
Sm	Amixicile (P=.376)	1.825	0.465	7.163
	Metronidazole (P=.628)	1.388	0.354	5.448
Ss	Amixicile (P=.043)	4.129	1.052	16.206
	Metronidazole (P=.059)	3.717	0.947	14.591

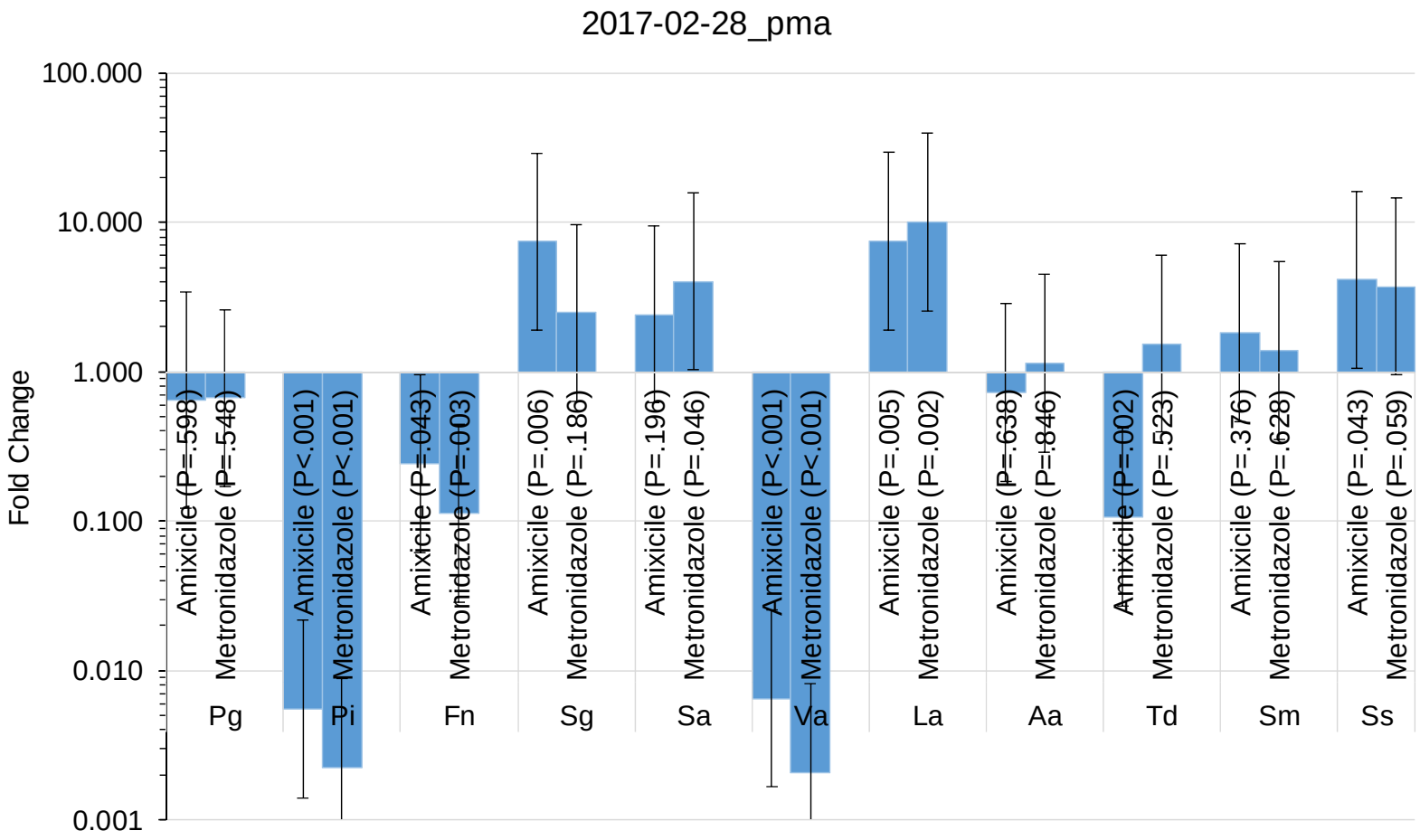

Figure 23. Fold estimates for Run 2017-02-28_pma (95% CIs)

Figure 21 represents the fold change observed for Run 2017-02-28_pma in bacterial species after treatment of either Amixicile or Metronidazole. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment

Run 2017-03-07_pma

Table 21. Corrected CT mean estimates for Run 2017-03-07_pma

Bacterial species	Antimicrobials	Corrected CT		
		Estimate	95% CI	
Pg (P=.006)	Control	32.82	32.18	33.46
	Amixicile-x	32.82	32.18	33.45
	Metronidazole-cx	31.48	30.84	32.12
Pi (P<.001)	Control	18.38	17.74	19.01
	Amixicile-c	26.85	26.21	27.49
	Metronidazole-c	27.63	26.99	28.27
Fn (P<.001)	Control	25.15	24.51	25.79
	Amixicile-cx	33.06	32.42	33.69
	Metronidazole-cx	29.39	28.76	30.03
Sg (P<.001)	Control	21.74	21.10	22.38
	Amixicile-cx	19.30	18.66	19.94
	Metronidazole-x	21.58	20.94	22.22
Sa (P=.003)	Control	20.26	19.63	20.90
	Amixicile	19.16	18.52	19.80
	Metronidazole-c	18.63	17.99	19.27
Va (P<.001)	Control	16.30	15.66	16.94
	Amixicile-c	26.01	25.37	26.64
	Metronidazole-c	25.65	25.01	26.29
La (P<.001)	Control	31.03	30.39	31.67
	Amixicile-x	30.56	29.92	31.19
	Metronidazole-cx	27.72	27.09	28.36
Aa (P<.001)	Control	32.25	31.61	32.88
	Amixicile-x	31.28	30.64	31.92
	Metronidazole-cx	29.12	28.48	29.76
Td (P<.001)	Control	29.77	29.13	30.41
	Amixicile-cx	31.97	31.33	32.61
	Metronidazole-cx	28.33	27.69	28.97
Sm (P<.001)	Control	35.68	35.05	36.32
	Amixicile-cx	34.11	33.47	34.74
	Metronidazole-cx	29.01	28.37	29.64
Ss (P<.001)	Control	18.16	17.52	18.80
	Amixicile-c	17.00	16.36	17.64
	Metronidazole-c	16.16	15.53	16.80

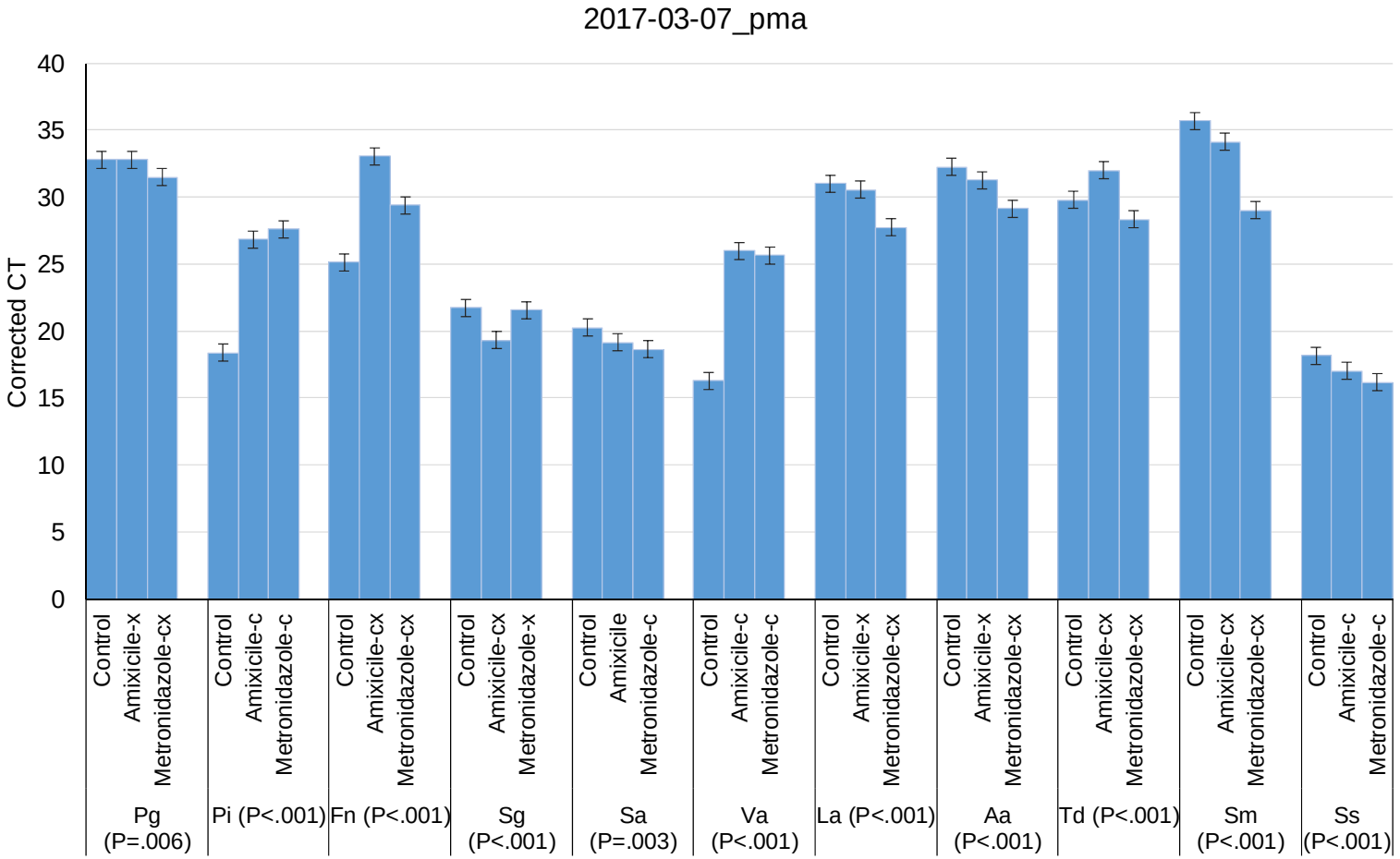

Figure 24. Corrected CT mean estimates for Run 2017-03-07_pma (95% CIs)

Figure 22 represents the average corrected CT values taken of Run 2017-03-07_pma. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 22. Differences in the Corrected CT mean estimates for Run 2017-03-07_pma

Bacterial species	Compare	Corrected CT Estimate	95% CI	
Pg	CvA (P=.996)	0.002	-0.901	0.905
	CvM (P=.005)	1.337	0.435	2.240
	AvM (P=.005)	1.335	0.433	2.238
Pi	CvA (P<.001)	-8.475	-9.378	-7.573
	CvM (P<.001)	-9.251	-10.154	-8.349
	AvM (P=.089)	-0.776	-1.679	0.127
Fn	CvA (P<.001)	-7.904	-8.807	-7.002
	CvM (P<.001)	-4.244	-5.146	-3.341
	AvM (P<.001)	3.661	2.758	4.563
Sg	CvA (P<.001)	2.441	1.539	3.344
	CvM (P=.720)	0.160	-0.742	1.063
	AvM (P<.001)	-2.281	-3.184	-1.378
Sa	CvA (P=.018)	1.107	0.205	2.010
	CvM (P<.001)	1.632	0.729	2.535
	AvM (P=.245)	0.525	-0.378	1.427
Va	CvA (P<.001)	-9.706	-10.609	-8.804
	CvM (P<.001)	-9.352	-10.255	-8.450
	AvM (P=.430)	0.354	-0.549	1.256
La	CvA (P=.289)	0.477	-0.426	1.380
	CvM (P<.001)	3.308	2.405	4.210
	AvM (P<.001)	2.831	1.928	3.734
Aa	CvA (P=.036)	0.969	0.067	1.872
	CvM (P<.001)	3.124	2.222	4.027
	AvM (P<.001)	2.155	1.252	3.058
Td	CvA (P<.001)	-2.200	-3.103	-1.297
	CvM (P=.003)	1.442	0.539	2.344
	AvM (P<.001)	3.642	2.739	4.544
Sm	CvA (P=.001)	1.578	0.675	2.481
	CvM (P<.001)	6.679	5.776	7.581
	AvM (P<.001)	5.101	4.198	6.003
Ss	CvA (P=.013)	1.164	0.261	2.066
	CvM (P<.001)	1.999	1.097	2.902
	AvM (P=.068)	0.836	-0.067	1.738

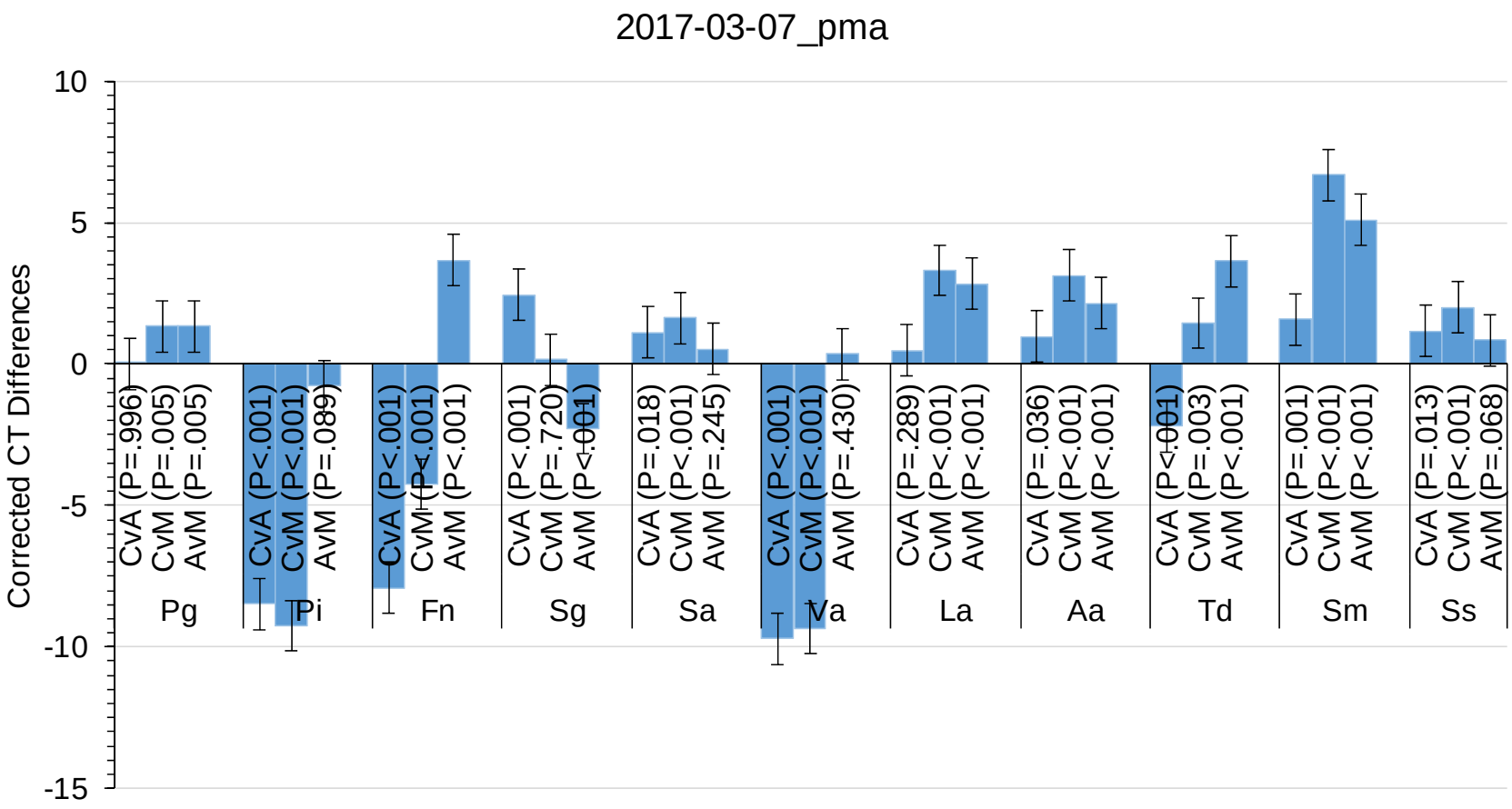

Figure 25 Differences in the Corrected CT mean estimates for Run 2017-03-07_pma

Figure 23 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 23. Fold estimates for Run 2017-03-07_pma

Bacterial species	Antimicrobials	Fold Estimate	95% CI	
Pg	Amixicile (P=.996)	1.001	0.536	1.872
	Metronidazole (P=.005)	2.527	1.352	4.724
Pi	Amixicile (P<.001)	0.003	0.002	0.005
	Metronidazole (P<.001)	0.002	0.001	0.003
Fn	Amixicile (P<.001)	0.004	0.002	0.008
	Metronidazole (P<.001)	0.053	0.028	0.099
Sg	Amixicile (P<.001)	5.431	2.905	10.154
	Metronidazole (P=.720)	1.117	0.598	2.089
Sa	Amixicile (P=.018)	2.155	1.152	4.028
	Metronidazole (P<.001)	3.099	1.658	5.794
Va	Amixicile (P<.001)	0.001	0.001	0.002
	Metronidazole (P<.001)	0.002	0.001	0.003
La	Amixicile (P=.289)	1.392	0.744	2.602
	Metronidazole (P<.001)	9.902	5.297	18.512
Aa	Amixicile (P=.036)	1.958	1.047	3.660
	Metronidazole (P<.001)	8.719	4.664	16.301
Td	Amixicile (P<.001)	0.218	0.116	0.407
	Metronidazole (P=.003)	2.716	1.453	5.078
Sm	Amixicile (P=.001)	2.986	1.597	5.582
	Metronidazole (P<.001)	102.454	54.803	191.538
Ss	Amixicile (P=.013)	2.240	1.198	4.188
	Metronidazole (P<.001)	3.998	2.138	7.474

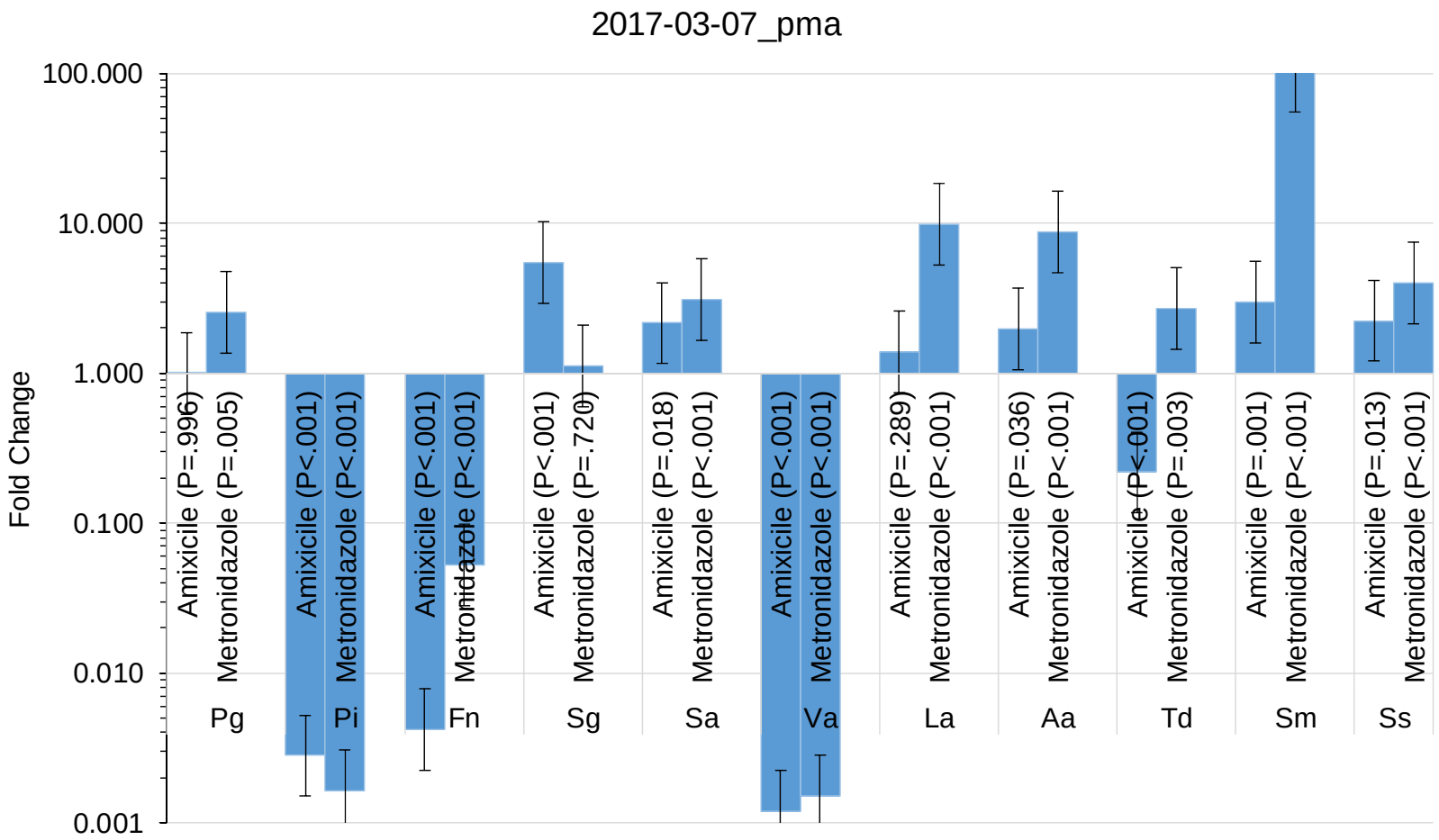

Figure 26 Fold estimates for Run 2017-03-07_pma (95% CIs)

Figure 24 represents the fold change observed for Run 2017-03-07_pma in bacterial species after treatment of either Amixicile or Metronidazole. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment

Run 2017-04-13_184418PMA

Table 24. Corrected CT mean estimates for Run 2017-04-13_184418PMA

Bacterial species	Antimicrobials	Corrected CT		
		Estimate	95% CI	
Pg (P<.001)	Control	33.14	32.80	33.47
	Amixicile-c	35.07	34.59	35.54
	Metronidazole-c	35.73	35.26	36.20
Pi (P<.001)	Control	17.33	16.99	17.67
	Amixicile-cx	24.25	23.92	24.59
	Metronidazole-cx	25.99	25.65	26.33
Fn (P<.001)	Control	24.65	24.31	24.98
	Amixicile-cx	25.78	25.44	26.11
	Metronidazole-cx	27.74	27.41	28.08
Sg (P<.001)	Control	18.02	17.69	18.36
	Amixicile-c	15.76	15.43	16.10
	Metronidazole-c	16.29	15.96	16.63
Sa (P<.001)	Control	16.36	16.02	16.69
	Amixicile-c	15.09	14.75	15.43
	Metronidazole-c	15.53	15.20	15.87
Va (P<.001)	Control	14.02	13.68	14.35
	Amixicile-cx	22.63	22.30	22.97
	Metronidazole-cx	25.44	25.10	25.78
La (P<.001)	Control	31.05	30.71	31.39
	Amixicile-cx	27.29	26.96	27.63
	Metronidazole-cx	28.05	27.72	28.39
Aa (P<.001)	Control	33.38	33.04	33.72
	Amixicile-c	32.14	31.80	32.48
	Metronidazole-c	32.68	32.34	33.02
Td (P<.001)	Control	29.08	28.75	29.42
	Amixicile-cx	29.94	29.60	30.27
	Metronidazole-cx	30.74	30.41	31.08
Sm (P=.376)	Control	32.57	32.23	32.90
	Amixicile	32.64	32.30	32.98
	Metronidazole	32.32	31.99	32.66
Ss (P<.001)	Control	15.81	15.48	16.15
	Amixicile-cx	13.67	13.33	14.01
	Metronidazole-cx	14.29	13.95	14.62

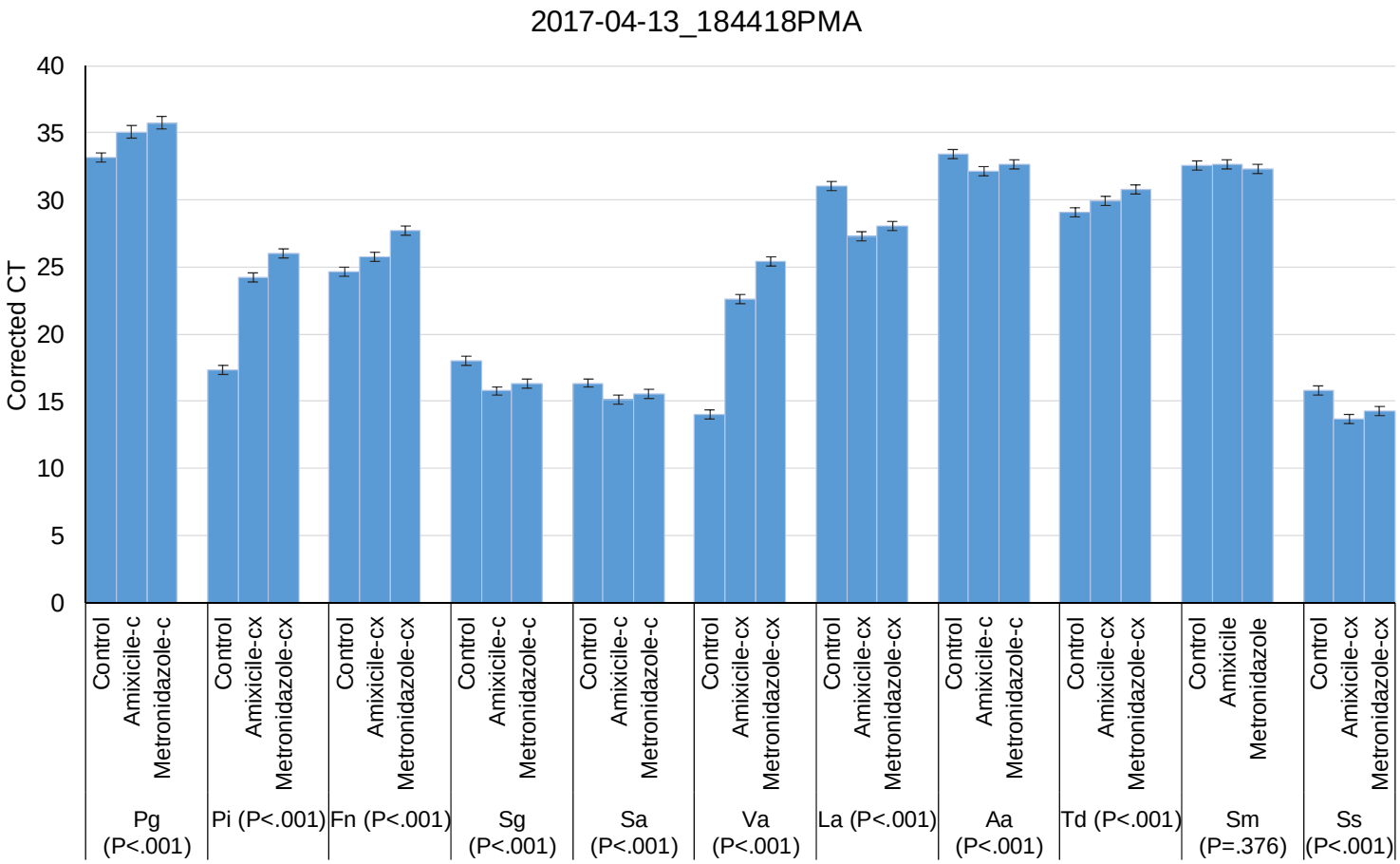

Figure 27. Corrected CT mean estimates for Run 2017-04-13_184418PMA (95% CIs)

Figure 25 represents the average corrected CT values taken of Run 2017-04-13_1184418PMA. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 25. Differences in the Corrected CT mean estimates for Run 2017-04-13_184418PMA

Bacterial species	Compare	Corrected CT Estimate	95% CI	
Pg	CvA (P<.001)	-1.933	-2.515	-1.351
	CvM (P<.001)	-2.594	-3.176	-2.012
	AvM (P=.053)	-0.661	-1.332	0.010
Pi	CvA (P<.001)	-6.925	-7.402	-6.449
	CvM (P<.001)	-8.662	-9.138	-8.185
	AvM (P<.001)	-1.736	-2.213	-1.260
Fn	CvA (P<.001)	-1.129	-1.605	-0.653
	CvM (P<.001)	-3.097	-3.573	-2.621
	AvM (P<.001)	-1.968	-2.444	-1.492
Sg	CvA (P<.001)	2.259	1.783	2.736
	CvM (P<.001)	1.730	1.254	2.206
	AvM (P=.031)	-0.529	-1.005	-0.053
Sa	CvA (P<.001)	1.267	0.791	1.743
	CvM (P=.001)	0.822	0.345	1.298
	AvM (P=.066)	-0.445	-0.922	0.031
Va	CvA (P<.001)	-8.617	-9.093	-8.141
	CvM (P<.001)	-11.423	-11.899	-10.946
	AvM (P<.001)	-2.805	-3.282	-2.329
La	CvA (P<.001)	3.755	3.279	4.231
	CvM (P<.001)	2.998	2.522	3.474
	AvM (P=.003)	-0.757	-1.233	-0.281
Aa	CvA (P<.001)	1.240	0.764	1.716
	CvM (P=.005)	0.700	0.223	1.176
	AvM (P=.028)	-0.541	-1.017	-0.064
Td	CvA (P=.001)	-0.851	-1.328	-0.375
	CvM (P<.001)	-1.658	-2.134	-1.182
	AvM (P=.002)	-0.807	-1.283	-0.330
Sm	CvA (P=.752)	-0.074	-0.551	0.402
	CvM (P=.306)	0.242	-0.234	0.719
	AvM (P=.184)	0.317	-0.160	0.793
Ss	CvA (P<.001)	2.144	1.668	2.621
	CvM (P<.001)	1.526	1.050	2.003
	AvM (P=.013)	-0.618	-1.094	-0.142

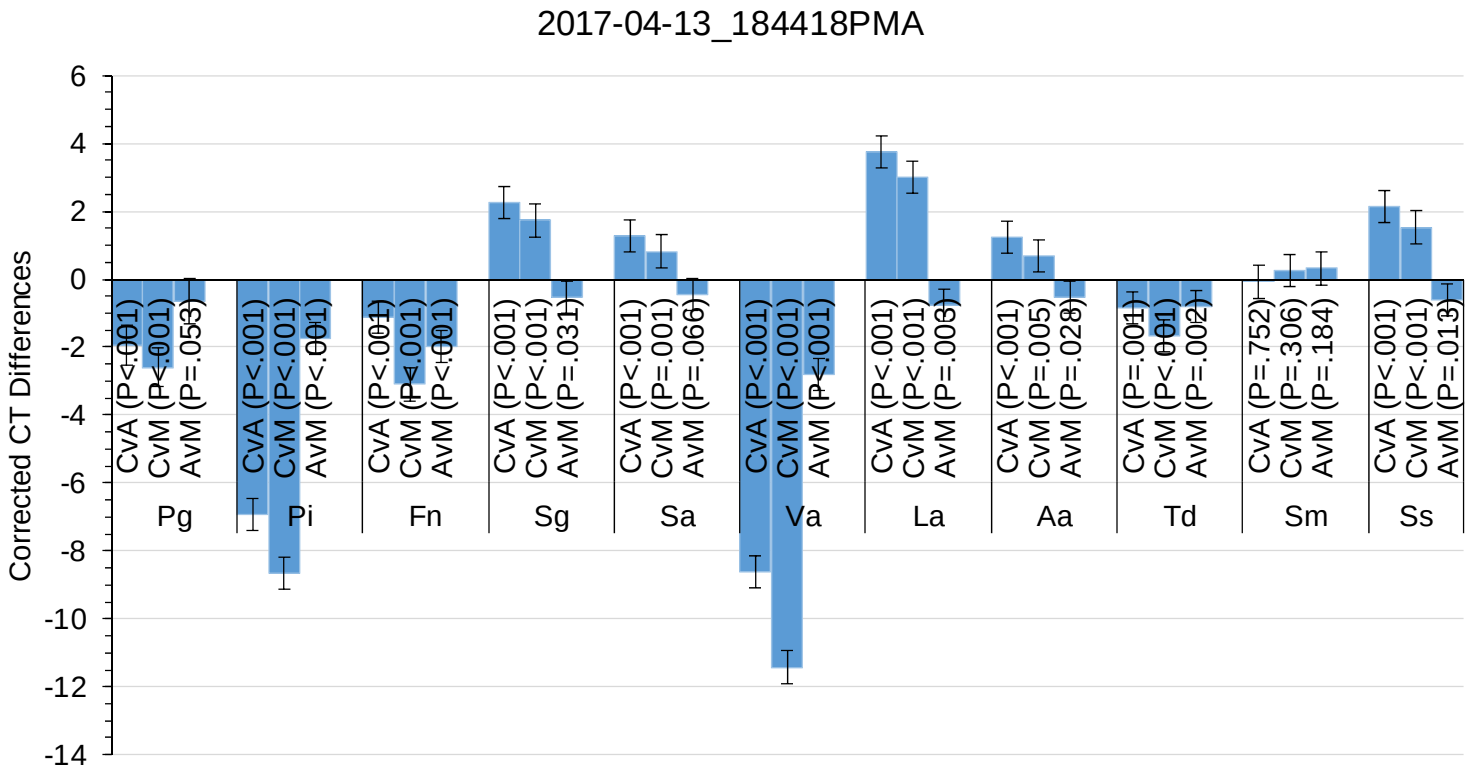

Figure 28. Differences in the Corrected CT mean estimates for Run 2017-04-13_184418PMA

Figure 26 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole

Table 26. Fold estimates for Run 2017-04-13_184418PMA

Bacterial species	Antimicrobials	Fold Estimate	95% CI	
Pg	Amixicile (P<.001)	0.262	0.175	0.392
	Metronidazole (P<.001)	0.166	0.111	0.248
Pi	Amixicile (P<.001)	0.008	0.006	0.011
	Metronidazole (P<.001)	0.002	0.002	0.003
Fn	Amixicile (P<.001)	0.457	0.329	0.636
	Metronidazole (P<.001)	0.117	0.084	0.163
Sg	Amixicile (P<.001)	4.788	3.442	6.661
	Metronidazole (P<.001)	3.318	2.385	4.615
Sa	Amixicile (P<.001)	2.407	1.730	3.348
	Metronidazole (P=.001)	1.767	1.271	2.459
Va	Amixicile (P<.001)	0.003	0.002	0.004
	Metronidazole (P<.001)	0.0004	0.0003	0.0005
La	Amixicile (P<.001)	13.501	9.705	18.782
	Metronidazole (P<.001)	7.989	5.743	11.114
Aa	Amixicile (P<.001)	2.362	1.698	3.286
	Metronidazole (P=.005)	1.624	1.167	2.259
Td	Amixicile (P=.001)	0.554	0.398	0.771
	Metronidazole (P<.001)	0.317	0.228	0.441
Sm	Amixicile (P=.752)	0.950	0.683	1.321
	Metronidazole (P=.306)	1.183	0.850	1.646
Ss	Amixicile (P<.001)	4.421	3.178	6.151
	Metronidazole (P<.001)	2.881	2.071	4.008

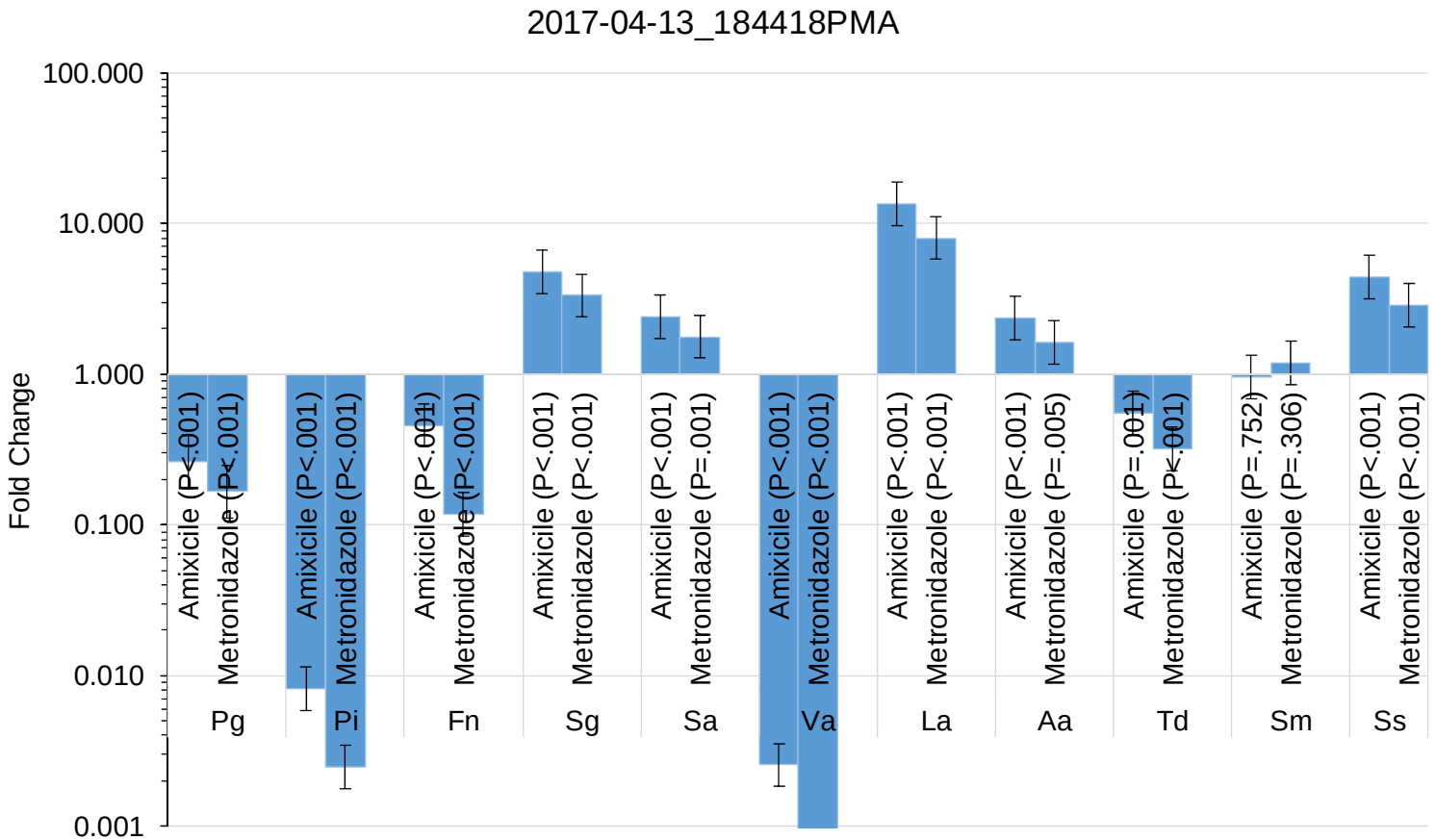

Figure 29. Fold estimates for Run 2017-04-13_184418PMA (95% CIs)

Figure 27 represents the fold change observed for Run 2017-04-13_184418pma in bacterial species after treatment of either Amixicile or Metronidazole. A P Value <.001 represented a statistical significant change in the numbers of bacteria from the control and antimicrobial treatment

PMA Runs

Table 27. Differences in the Corrected CT mean estimates for PMA Runs

Bacterial species	Compare	Corrected CT Estimate	95% CI	
Pg	CvA (P=.696)	-0.360	-2.175	1.455
	CvM (P=.918)	-0.089	-1.793	1.615
	AvM (P=.777)	0.271	-1.614	2.156
Pi	CvA (P<.001)	-7.631	-9.257	-6.006
	CvM (P<.001)	-8.900	-10.525	-7.275
	AvM (P=.125)	-1.269	-2.894	0.357
Fn	CvA (P<.001)	-3.691	-5.316	-2.066
	CvM (P<.001)	-3.493	-5.119	-1.868
	AvM (P=.811)	0.197	-1.428	1.823
Sg	CvA (P=.002)	2.531	0.905	4.156
	CvM (P=.197)	1.065	-0.560	2.691
	AvM (P=.077)	-1.465	-3.091	0.160
Sa	CvA (P=.141)	1.217	-0.409	2.842
	CvM (P=.073)	1.487	-0.139	3.112
	AvM (P=.743)	0.270	-1.355	1.895
Va	CvA (P<.001)	-8.529	-10.155	-6.904
	CvM (P<.001)	-9.898	-11.524	-8.273
	AvM (P=.098)	-1.369	-2.994	0.256
La	CvA (P=.004)	2.377	0.752	4.003
	CvM (P<.001)	3.212	1.587	4.837
	AvM (P=.312)	0.835	-0.790	2.460
Aa	CvA (P=.479)	0.584	-1.042	2.209
	CvM (P=.106)	1.337	-0.288	2.963
	AvM (P=.361)	0.754	-0.872	2.379
Td	CvA (P=.012)	-2.094	-3.719	-0.468
	CvM (P=.869)	0.136	-1.490	1.761
	AvM (P=.008)	2.229	0.604	3.855
Sm	CvA (P=.338)	0.791	-0.835	2.416
	CvM (P=.003)	2.465	0.839	4.090
	AvM (P=.044)	1.674	0.049	3.299
Ss	CvA (P=.032)	1.785	0.159	3.410
	CvM (P=.030)	1.807	0.181	3.432
	AvM (P=.979)	0.022	-1.603	1.647

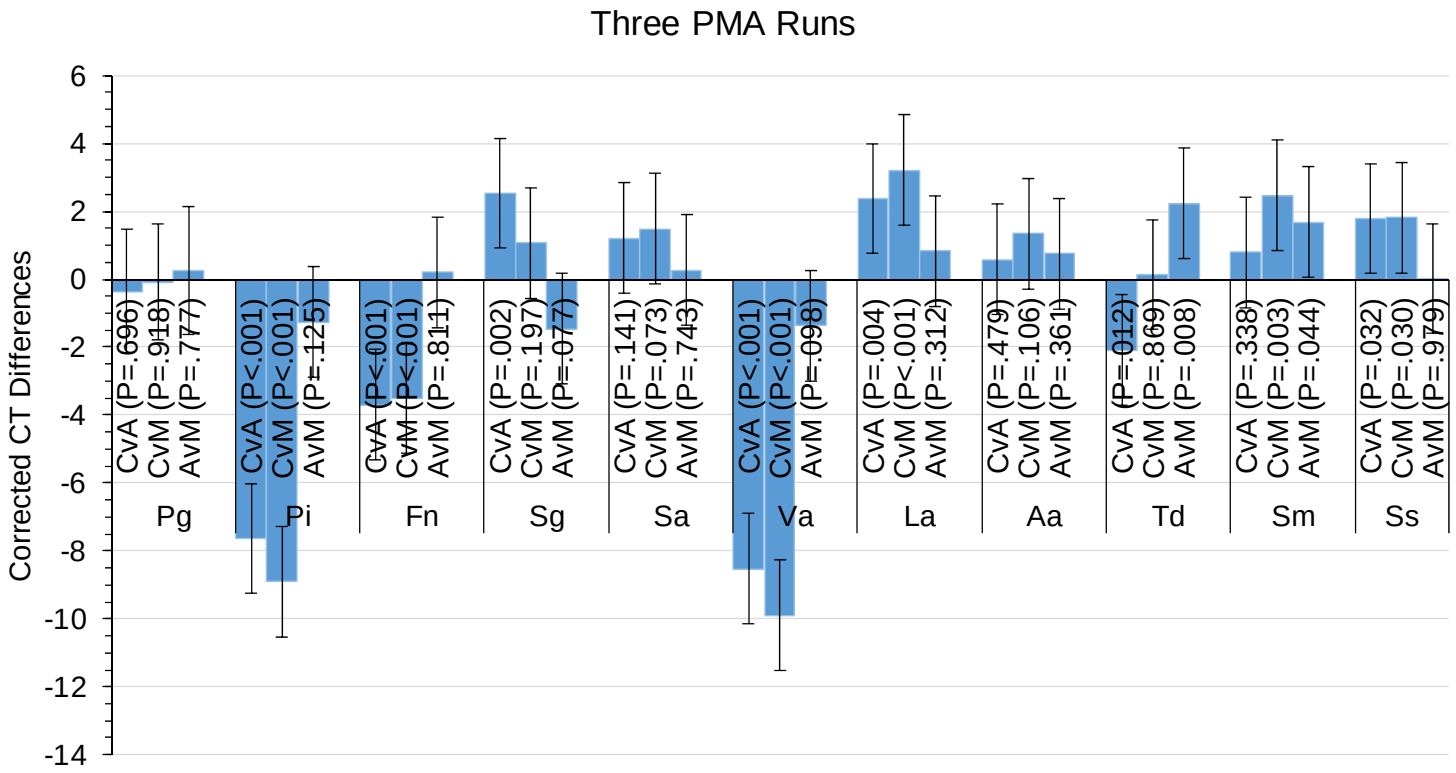

Figure 30. Differences in the Corrected CT mean estimates for PMA Run

Figure 29 represents the differences in corrected CT mean estimates from the original CT values prior to standardization with 16s primer. ANOVA analysis was performed and applied to compare the control group to Amixicile, control group to Metronidazole and lastly compare Amixicile and Metronidazole. A “c” represents a statistically significant difference from control and antimicrobial. An “x” represents a statistically significant difference from Amixicile and Metronidazole