Markers Of Antimicrobial Resistance in the Bovine Gut with Relevance to Human and Veterinary Medicine

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Contents

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Abstract:

Antimicrobial Resistance poses a large threat to both human and animal healthcare. Antibiotics used on farm are the same or very similar to those used in human healthcare, which leads to concerns over the development of resistant organisms. The aim of this study was to create resistance profiles of isolates from bovine gut and environment on farms, from organisms of medical and veterinary importance.

Three dairy farms were enrolled for the study. Samples were taken that represented the bovine gut and environment. Environmental samples were taken from similar locations across all three farms. The antibiograms were produced using the SIRscan 2000 and MICs were calculated to begin identifying values for antibiotics that do not have clinical breakpoints in veterinary medicine. Amongst the three farms enrolled, 807 strains were isolated, with the most prevalent resistance being seen against Tetracycline. Many of the organisms were multi-class resistant. *Escherichia coli* had high MIC values against Tetracyclines that were greater than or equal to 64mg/l, correlating with the high levels of resistance seen.

All farms showed resistance to multiple antibiotics in different classes, the profiles varied between the bovine gut and environment on farm and amongst all three farms. This study showed a snapshot of the resistance profiles that can be found. Longitudinal studies would provide data as to how resistance changes over time and as exposure to antibiotics changes also. By providing information as to what resistance is routinely seen on farm and how resistance evolved over time. A better understanding will be gained with greater detail and a clearer view of the effects of antibiotic use in the cattle industry.

List of Abbreviations:

Abbreviation	Definition
AMR	Antimicrobial Resistance
BG	Bovine Gut
CIA	Critically Important Antimicrobial list
DCT	Dry Cow Therapy
ECOFF	Epidemiological cut-off value for resistance
EMA	European Medical Agency
ENV	Environment
ESBL	Extended Spectrum Beta Lactamases
EUCAST	European Society for Clinical Microbiology and Infectious Disease
FDA	USA Food and Drug Administration
MIC	Minimum Inhibitory Concentration
RUMA	Responsible use of Medicines in Agriculture Alliance
SDCT	Selective Dry Cow Therapy
WHO	World Health Organisation
ZD	Zone Diameter

1.0 Introduction:

Antimicrobials are one of the most important and successful form of chemotherapy in modern medicine (Aminov, 2010). It is established that they have significantly contributed to the control of infectious disease, allowing certain surgical procedures to progress successfully and increase the life expectancy of humans and animals worldwide. However, with the overuse of antimicrobials, consequences have arisen. Antimicrobial resistance is now a common underlying problem, with multi-drug resistant bacteria appearing at an increasing rate imposing significant burdens on healthcare (Hadley and Hancock, 2010). Alongside this, development of antibiotics by pharmaceutical companies has decreased dramatically, only contributing to around 0.2% of new drug development (Spellburg *et al*., 2010). Novel antibiotic discovery is unlikely to help the issue of antimicrobial resistance. Limited antimicrobial drug development will occur compared to other areas of healthcare, due to the likelihood that pathogens will evolve resistance to novel antibiotics as seen with existing antibiotics (Palmer and Kishony, 2013). For example, Macrolides were discovered in 1948, and resistance was first observed in 1955. Aminoglycosides were discovered in 1963 and resistance was already being observed in 1964 (Lewis, 2013). It is evident that producing new antibiotics will not stop the development of resistant organisms.

1.1 Resistance Emergence:

Antimicrobial resistance can emerge through transmission of genetic information from one bacterium to another (Smilie *et al*., 2010). This is done in a number of different ways, for example, through plasmids and mobile DNA elements. Virtually any gene in intestinal bacteria

has the ability to be mobilised and transferred via mobile genetic elements (Smilie *et al*., 2010). Resistance genes can be transferred between bacteria that are in the same species but also across different genera (Huddleston, 2014). Under selective pressure, bacteria that carry an appropriate resistance gene can become clonally dominant This can be known as Colonisation Resistance (CR) (Butaye *et al*., 2014) and is a mechanism whereby the gut microflora protects itself against the arrival of new and often harmful organisms. The use of antibiotics and exposure of food animals to antimicrobial agents may increase the number of pathogens in animals through reduced CR. This could potentially lead to more pathogens in the food supply (Barza, 2010). The risk of foodborne infections could be due to resistant bacteria being able to colonize animals or persist in the environment better than susceptible ones (Tollesfon and Karp,2004). The widespread use of antibiotics in human and animal medicine is likely to induce substantial changes in the gut environment (Sommer *et al*., 2009). Genes that carry resistance, which persist as a result of the antibiotics used could potentially become more abundant than others in the human gut microbiome (Butaye *et al.,* 2014). The evidence supporting the movement of resistance genes from in the farm environment is shown when looking at the flora of farm workers and animals. When looking at farms that used antimicrobial growth promoters, it was seen that there were more resistant bacteria in the intestinal microflora of the farm workers and farm animals that those who did not employ this practice on farm. Furthermore, a study by Alexander *et al*. (2010), showed drug resistant *Escherichia coli (E. coli)* was present in or on beef carcasses after evisceration, after 24 hours in the chiller and in ground beef for 1-8 days thereafter. It is evident that people on farm and those more distantly related to farms practice can become susceptive to resistance found on farm (Marshall and Levy, 2011).

Under the appropriate selective pressures, bacteria that carry the resistance genes can grow and expand at the expense of bacteria that are inhibited (Butaye *et al.,* 2014). Livestock, such as cattle, are considered the primary reservoir for bacteria such as non-typhoidal *Salmonella* sp*., E. coli* and *Campylobacter* sp. which may cause enteric infections in humans after moving through the food chain via improper handling and cooking (Carrattoli, 2008). Extended Spectrum Beta Lactamases (ESBLs) are enzymes that have the ability to degrade common Penicillins and Cephalosporins. ESBL producing bacteria have been seen in human medicine for a long time with new appearances also seen in animal medicine. ESBL producing bacteria have frequently been reported in animals but only in the last few years have the same isolates been seen in both humans and animals (Carrattoli, 2008). The increase in antimicrobial resistance seen in bacteria of animal origin resembles the process in humans seen before (Ewers *et al.,* 2012). Evidence like this could suggest that there is an overlap between the ESBL positive bacteria found in animals and humans, and therefore there is a possible relationship between the two. Within the last decade, the number of publications reporting ESBL producing- *E. coli* isolated from food producing animals has increased (Ewers *et al*., 2012). Noticeably most ESBL enzymes identified in livestock are likewise present in bacteria from humans (Smet *et al*., 2010). However, the enzyme identified in the different strains of ESBL producers does not show a zoonotic link between the two, leaving the question open (Ewers *et al.,* 2012). If the same strain was identified, this could be potentially used as evidence to suggest a zoonotic link between the two. A better understanding is needed as to what factors contribute to the dissemination of resistant microbes among and between humans and animals. This includes an evaluation of the role of faecal flora in the dissemination of antimicrobial resistance as well as on the mechanisms of linkage and transmissibility of resistance determinants in the natural environments (Carrattoli, 2008).

In recent studies, including one conducted by Guerra *et al* (2014), residual resistance was found, with bacteria showing to be co resistant to most of the antibiotics used in the study. An additional study by Poirel *et al* (2012), sampled 50 isolates from cattle within a herd, where a high prevalence of resistance was seen, particularly to β-lactams as well as Tetracyclines. Fischer *et al*., (2013) took samples from pig farms in Germany and found resistance in *Salmonella* sp. isolates that were studied on farm. The bacteria were shown to be susceptible to a broad range of β-lactams and resistant to Chloramphenicol, Streptomycin, Sulphonamides and Trimethoprim. It is difficult to follow resistant organisms when no recorded documentation is found to follow resistance trends. With different methodologies being used, including samples taken at different times in the year and in different geographical areas, the flow of ESBL producing *E.coli* could not be traced. Detailed knowledge of antimicrobial use should be recorded in human, livestock and companion animal medicine (Ewers *et al.,* 2012). This could provide a retrospective view on how and when resistance emerged and potentially move from livestock to animals.

1.2 Antimicrobial resistance in the gut:

The human intestinal tract is a reservoir for more antibiotic resistance genes than any other studied natural environment such as soil or marine and lake environments (Huddleston, 2014). Although it is understood that this potential reservoir can hold resistant organisms, little is known about the diversity of the microbes within the BG (Callaway *et al.,* 2014). The microbial community can be classed according to its taxonomy, of which the three most dominant phyla observed are *Bacteroidetes* (such as *Bacteroides fragilis*), *Firmicutes* (e.g. *Bacillus* sp.) and *Proteobacteria* (such as *E. coli*) (Jamie & Mizrahi, 2012). *Bacteroidetes* are often the most abundant phyla found in the microbial community in the Bovine Gut (BG), and the ratio of *Firmicutes* to *Bacteroidetes* is shown to affect energy harvesting and body fat in

humans and mice. This is mirrored in cattle, where a decreased presence of *Bacteroidetes* in the microbiota correlates with increased fat in the blood and the tissue. (Turnbaugh *et al*., 2006). Although it is possible for the microbiota to have an effect on the animal's health and wellbeing, it does not mean that diet or the environment can alter it. Cows fed the same diets have substantial differences in their bacterial communities (Welkie *et al*., 2010). This suggests that microbiota in the bovine gut cannot be presumed. Research may eventually lead to a full understanding of the diversity of the gastrointestinal (GI) flora and how it can be modified. For example, a particular microbiome profile in food producing animals could be able to increase production efficacy, product quality and/or food safety. This could then be progressed further by preventing colonisation of the gut by pathogens, including zoonotic pathogens (Dowd *et al*., 2008). Intestinal *E. coli* can be readily disseminated in different ecosystems through water and are used as indicators of faecal contamination, therefore could also be used to track the evolution of antimicrobial resistance within and into different ecosystems (Kummerer., 2009).

Within the phyla of *Bacteroidetes*, most of the bacteria are anaerobic. The rumen is inhabited by a high density of resident microbiota, consisting of bacteria, protozoa, archaea and fungi to degrade the plant material (Arumugam *et al.,* 2011). IT acts as a pregastric anaerobic chamber of which 95% of its contents is bacteria. (Bruc *et al.,* 2009). While there are mostly anaerobic bacteria in the gut, it is the aerobic bacteria that are of concern; zoonotic pathogens need to be at least facultative aerobic in order to be a threat to humans. Zoonotic pathogenic bacteria such as *Salmonella* Enterica and *E. coli* O157 can live in the lower gut of cattle and cause human illness through carcass and/or food contamination (Shelton *et al*., 2006). Anaerobic bacteria cannot survive outside the gut, and therefore would not be found in faecal samples. Of the aerobic bacteria found in faecal samples, *E. coli* is most commonly detected but represents less than 1% of intestinal bacterial populations (Dowd *et al.,* 2008).

Gram negative bacteria, like *E. coli*, asymptomatically colonise in the guts of birds and mammals and the intestinal population differs between animals of the same species. Therefore, knowledge regarding *E. coli* in these intestinal populations is limited to single papers and does not represent all species (Guenther *et al.,* 2011). *E. coli* can grow relatively easily in the laboratory unlike the phyla *Bacteroidetes*, which are fastidious and require specialised anaerobic growth conditions. Culture based methods of identification can be time consuming and show only 1% of the bacteria present that can be cultured. This leads results to be potentially biased in their evaluation of microbial diversity, tending to overestimate the importance of bacterial species such as *E. coli* that easily grow on the agar surface (Dowd *et al.,* 2008). Conducting anaerobic test would help to identify bacteria that are found within the intestinal bacterial populations and so would help to stop the over representation of aerobic bacteria in studies.

Studies have shown that the environment can also host these pathogens. *E. coli* identified as ESBL producers were seen in the environment decades before ESBL producing *E. coli* outbreaks occurred in human clinical settings (Guenther *et al.,* 2011). The movement of antibiotic residues from farm into the environment can lead to the percentage of resistant bacteria increasing in the environment. Once administered as much as 30-90% of veterinary antibiotics, depending on the class, are excreted into manure (Sarmah *et al.,* 2006). The exposure of these antibiotics in the environment causes unnatural pressures on the surrounding bacteria leading resistant organisms forming (Sarmah *et* al., 2006). The transfer of animal manure in agricultural soils and spill over from manure lagoons can be found to be a predominant source of resistance at farm level (Heuer *et al*., 2011). When comparing pristine soils against agricultural soils, antibiotic resistance differs qualitatively and quantitatively. For example, in soil samples taken from the Rocky Mountain National Park in USA, Tetracycline

resistance genes were not detected by real time PCR whilst they were abundant in soil samples subject to agriculture activities in the same region (Yang *et al.,* 2010). Originally the frequency of bacteria carrying antimicrobial resistant genes in soil was explained by the presence of antibiotic producers that harbour these genes to protect themselves from secondary metabolites. Antibiotics at low environmental concentrations may have functions other than antibiotics including signalling and metabolic purposes (Heuer *et al.,* 2011). Antibiotic-producing bacteria occur naturally throughout the environment, colonising plants, soil and detritus in aquatic environment, aquatic plants and animals. The large-scale mixing of these environmental bacteria with exogenous bacteria from anthropogenic sources such as farm drainage and waste processing provides the ideal selective and ecological conditions for new resistant strains to arise: thus, soil water and other nutrient-enriched habitats can act as hotspots for horizontal gene transfer. The reservoir of resistance genes in the environment is due to a mix of naturally occurring resistance and those present in animal and human waste and the selective effects of pollutants, which can co-select for mobile genetic elements carrying multiple resistance genes (Wellington *et al.,* 2013).

1.3 Antimicrobial Use On Farm:

Antimicrobials used in animal healthcare are the same or very similar to those given to humans (Tollefson and Karp,2004). Antibiotics are mostly used to treat humans after diagnosis, whilst in agriculture antimicrobials were also used as a preventative measure in feed, until being against the law in the European Union (EU) in 2006, and in treating a whole herd when only a few animals showed symptoms (Landers *et al.,* 2012).). Individual animal treatment was seen as impractical in large herds so group treatment was preferred when the first and possibly only animal showed symptoms (Schwarz *et al.,* 2001). This method of antimicrobial use in animal husbandry had been in place since the early 1950s to improve growth and feed efficiency (Angulo *et al*., 2004). There was also the presumption that all antimicrobial growth promoters

used developed no cross-resistance with antimicrobials used for human medicine (Schwarz *et al.,* 2001). In 1999, using antimicrobials at sub-therapeutic levels for promotion of growth came under scrutiny and the World Health Organisation (WHO) recommended discounting use if the same antimicrobial classed was used in humans (WHO, 1999). Before the ban was proposed, the amount of antibiotics was around 13,288 tons in the EU and Switzerland, of which 29% was used in veterinary medicine, 6% used as growth promoters and 65% used in human medicine (FEDESA, 2001). In the USA, 70% of the 16,200 tons of antibiotics used was in livestock farming, in the same time. This was eight times the amount that was used in human medicine (Kummerer, 2003). To address the issue of inappropriate used of antimicrobial agents. Reduction should be facilitated as demonstrated in Denmark and Sweden. The two countries showed that dramatically reducing the use of antimicrobial growth promotors with human analogues reduced the incidence of antimicrobial resistance and public health risks (Angula *et al.,* 2004). Sweden was one of the first countries to discontinue the use of growth promoters in 1986 (Cogliani *et al*., 2011). There was widespread agreement and disagreement with this proposed ban: on the one hand, there was the theoretical hazard to human health which had arisen from use of growth-promoting antibiotics, however, when examined independently without political or commercial influence the risk was seen as very small or even zero in many cases (Phillips et al., 2004). Even though the relationship between antibiotic growth promoters and spread of antimicrobial resistance remained unknown, similar resistance genes have been isolated from human pathogens, bacteria of animal origin and environmental bacteria(Kemper,2008).

The most widely used groups of antibiotics in animal husbandry in the EU are Tetracyclines, Macrolides, Penicillins, Aminoglycosides and Sulphonamides (Haller *et al*., 2002). Discontinuing the use of these antibiotics as growth promoters was decided in the

European Union in 2006, because using antimicrobials in this way potentially stimulates selection and dissemination of resistance genes in bacteria (Angula *et al.,* 2009). Resistant bacteria can be transmitted to humans through the consumption or handling of foods of animal origin or via direct contact with the animals themselves (Tollesfon and Karp, 2004). Discontinuation of antimicrobial growth promoters was seen to lead to a decrease in resistant bacteria found in agriculture, food products and humans (Aarestrup *et al*., 2001). This supports a relationship between the two.

Since the EU ban in January 2006, attitudes towards how antibiotics are used on farm have changed. Additional pressures from the general public and research has enforced the decrease and appropriate use of antimicrobials. More recently the movement from Blanket Dry Cow Therapy (DCT) to Selective Dry Cow Therapy (SDCT) has been proposed as an additional way to reduce antibiotic use on farm. Antibiotic treatments at the end of lactation, DCT, are used to eliminate intramammary infections and prevent new infections during the dry period, where the cattle are not milked (Berry and Hillerton, 2002). In many countries DCT is a standard way to dry off cows but due to concerns about antibiotic resistance SDCT was proposed as an alternative (Huijps and Hogeveen, 2007). This was supported by public pressure and consumer concerns about antibiotic residues in milk and antimicrobial resistance levels shown through research (Robert *et al*., 2008).

One of the reasons SDCT was not well received was due to the issue of mastitis control as it was believed that antibiotic therapy at the end of lactation was the only and most effective way of eliminating infections and preventing new ones (Eberhart, 1986). A study by Berry and Hillerton (2002) showed that cows left untreated saw significantly more cases of clinical mastitis. Evidence like this suggests that farmers' attitudes to moving away from

antibiotic use would not be positive. Later studies disprove this, for example, a study in Denmark (Bennedsgaard *et al*., 2010) found that their farms participating were able to reduce the use of antimicrobials in their herd during the study and after, without apparent negative effects on production and udder and herd health.

A list of critically important antibiotics was introduced in an attempt to control the rise of resistant organisms. WHO (2012) created a document for public and animal health authorities involved in managing antimicrobial resistance to ensure that the critically or highly important antimicrobials listed were used prudently in both human and animal healthcare. Among this list were 3rd or 4th Cephalosporins and Fluoroquinolones were mentioned. Fluoroquinolones and 3rd generation Cephalosporin are drugs of choice for invasive *Salmonella* sp. infections in humans. Resistant *Salmonella* sp. resulted from use of antimicrobial agents in food animals which can travel to human through the food supply (Angulo *et al*.,2009). This is a possible example to show that certain antibiotic should not be used in animal healthcare, so that it can increase the potential to treat effectively in human healthcare. Due to these health risks, there is the need to emphasize more prudent treatment with antimicrobials to minimize the dissemination of resistant *Salmonella* sp. (Angulo *et al*., 2009).

Cefquinome and Marbofloxacin are part of the critically important antibiotics list (CIA). According the World Health Organisation (WHO, 2017). This document was created to ensure that the antibiotics listed were to be used prudently to help tackle the issue of resistance (WHO, 2011). The extended list also includes Tyrosine, Ceftazidime, Tigecycline, Ampicillin, Amoxicillin and Gentamicin. This list corresponds to both human and veterinary use. Third and fourth generation Cephalosporins which include Cefquinome, Cefotaxime, Ceftazidime and Cefepime are some of the antibiotics of highest priority on the CIA list.

Table 1 shows which class the antibiotics belong to and which generation the Cephalosporins are.

Table 1: A table of the antibiotics used according to their class. In the cephalosporin table, the generation of the antibiotic is put in brackets.

Antibiotic Class	Antibiotics
Cephalosporins	Cefoxitin (2nd) Cefuroxime (2nd) Cefotaxime(3rd) Ceftazidime(3rd) Cefepime(4th) Cefquinome(4th)
β-Lactams	Ampicillin Amoxicillin Amoxicillin-Clavulanic acid Piperacillin Piperacillin-Tazobactam
Tetracyclines	Oxytetracycline Tigecycline
Fluoroquinolones	Ciprofloxacin Marbofloxacin
Aminoglycosides	Gentamicin
Carbapenems	Ertapenem Meropenem Imipenem

The development of resistance in both human and veterinary medicine became the main topic of discussion, for example WHO conferences in Berlin (1997) and Geneva (1998). The outcome of these discussions was that the use of antimicrobial drugs in humans and animals is interrelated and that monitoring systems should be established to focus on resistance in pathogenic and commensal bacteria of animal origin (Caprioli *et al*., 2000). Defined daily dose has been considered as the most accurate method in assessing use of antimicrobials. The defined daily does refers to the average maintenance dose per day for a drug (WHO,2017). Such systems are already seen with supervision of prescribing, dispensing and administration in hospital treatment of humans (Hillerton *et al*., 2016). The European Medicine Agency (EMA) has a method for standardizing antimicrobial sales and annual figures in each country,

based on the number of animals and their theoretical weight, within each species (Grave *et al*., 2012). With this form of regulation, various countries have seen a decrease in antimicrobial use in general. New Zealand is this third lowest user of antimicrobials in animal production and used much less than in human medicine (Hillerton *et al*., 2016). In 2010, China was the largest antimicrobial consumer for livestock and it is estimated that the industry will use 30% of the global antimicrobial production by 2030 (Boeckel *et al*., 2015). Great lengths are taken to decrease the use of antimicrobials, especially in the EU. However, it is projected that antimicrobial consumption will rise by 67% by 2030, and nearly double in Brazil, Russia, India, China and South Africa. This rise is likely to be due to growth in consumer demand for livestock products in middle income countries and a shift to large-scale farms where antimicrobials are used routinely (Boeckel *et al*., 2015). Evidence like this can show the need for an international partnership to monitor and control the rise of resistance, as opposed to individual countries tackling the issue in numerous different ways.

1.4 Minimum Inhibitory Concentrations (MIC)

Susceptibility testing is not commonly seen in veterinary medicine as opposed to in human medicine. The methods used to distinguish antimicrobial resistance are disc diffusion method and/or MIC calculation. MICs are defined as the lowest concentration of an antimicrobial that will inhibit growth on an organism (Andrews, 2001). This can be determined by visible growth of an organism, after overnight incubations determined by eye. MICs can be used to give a definitive answer when a borderline result is obtained by other methods or when disc diffusion methods are not appropriate (Andrews, 2006). An example of this is where common veterinary antibiotics, Cefquinome and Oxytetracycline, do not have clinical breakpoints published. The European Union Committee for Antimicrobial Susceptibility Testing (EUCAST) are one of the main providers for clinical breakpoints to assess the level of

resistance within an organism. EUCAST employs certain criteria to determine breakpoints, these being: MIC frequency distribution analysis, assessment of MIC values in the context of the presence or absence of known mechanisms of resistance, evaluation of MICs based on drug levels in patients receiving antibiotic therapy and the response rates in patients with infection compared to the drug MICs associated with their infecting pathogens (Doern, 2011).

Marbofloxacin (a Fluroquinolone), Cefquinome (a Cephalosporin) and Oxytetracycline (a Tetracycline) are antibiotics used in veterinary practice, but some of these antibiotics are commonly used in practice. However, little is known about what resistance is found against these antibiotics. The lack of clinical breakpoints determined by EUCAST hinders the opportunity to create resistance profiles and hence have a better understanding as what effects antibiotic use on farm brings. An example of where MIC values are known is Ampicillin. Ampicillin is a well-known β-lactam that is commonly used due to its ability destroys both Gram-positive and Gram-negative bacteria (Rozas *et al,* 2010). The MIC and breakpoint values, according to EUCAST, for Ampicillin are already well established. The breakpoint value between resistance and susceptibility is 14mm for *Enterobacteriacae* (EUCAST, 2016) in the disc diffusion method.

Evidence has shown that there is a rise in antibiotic resistance due to the overuse of antimicrobials in treatment for both human and animal disease. However, attitudes towards the way these antimicrobials are being used as changed, as seen in the 2006 ban for them to be used as a preventative measure in feed in agriculture. There is still little research to show what types of resistance profiles can be shown on the farms. This study will bring to look at what resistance can be seen in farm level and discuss whether trends and patterns can be determined.

This will help being to bring further understanding as to the effects of antimicrobial use in agriculture.

1.5 Aims and Objectives

The overall aim of the study is to determine the levels of resistance on farms and to establish the MIC values of antibiotics without clinical breakpoints to determine the resistance level of those antibiotics. This will be done by achieving the following:

- Isolate bacteria from the BG and Environment (ENV) across three dairy farms enrolled in the study
- Identify the organisms isolate through a series of biochemical tests
- Conduct antibiograms against different antibiotics to test resistance
- Compare resistance profiles between the farm and the environment within the farm and also against the two other farms involved
- Calculate MIC values for *E.coli* isolated on Farm 1
- Use MIC values to calculate ECOFFs and compare the values to identify any resistant organisms for the antibiotics Marbofloxacin, Cefquinome an Oxytetracycline.

2.0 Materials and Methods:

2.1 Farm Enrolment:

Three dairy farms all located in South Central England were enrolled in the study. Contact with and enrolment of these farms were made through WestPoint Farm Vets. The farms were kept anonymous throughout the study and labelled Farm 1, Farm 2 and Farm 3. The sizes of the herds were 300, 200 and 200 respectively and the breed across all three farms was Holstein Friesian. The calving status amongst the farms varied: Farm 1 undergoes block calving, Farm 2 calves all year round and Farm 3 also undergoes block calving but over a longer period of time than Farm 1. Block calving involves is where all cows within the herd are calved at approximately the same time in the year. All herds on the farms were on a silage based diet with mixed rations, a common diet to encourage maximum performance and health, and had access to grazing at some point throughout the year. Samples were taken that represented 30% of the herd. Only 15% of Farm 1 could be represented due to the farm leaving the practice half way through the study, due to the farm moving practice. All three farms were solely used for milk production, with no other livestock present. Samples were collected over a series of visits from October 2015 to March 2016. Freeze tag numbers and names of environmental locations were used to identify samples. This also allowed logging of repeat sample collections from the same cow. Ethical approval was not required in this study, due to the cattle not directly being approached for sole purpose of the research. The samples were taken during veterinary visits already scheduled the inspection gloves taken to isolate samples.

2.2 Sample Isolation and Identification

Bacterial strains from 160 animals present across the three farms were isolated and identified. Faecal samples were taken by swabbing of veterinarian examination gloves

following rectal examinations. The samples were individually bagged at the location to avoid cross contamination of samples. Blue shoe covers were worn to collect samples from the holding yard, scraper tractor tread, crush, feed passage and cubicle shed. The samples were then taken from the blue shoe covers with swabs to transfer them to the lab. Organisms targeted for isolation were *E. coli*, *Salmonella* sp., *Pseudomonas* sp., *Klebsiella* sp. *Proteus* sp., and *Streptococcus* sp. These organisms were targeted due to their status in healthcare, with common diseases caused by these species. In order to facilitate targeting of these organisms, samples were grown on six different agars. The six agars used were: Nutrient agar (OXOID), MacConkey agar (OXOID), Brilliant Green agar (OXOID), Modified Edwards agar (OXOID), Xylose Lysine Deoxychocolate (XLD) agar (OXOID) and Sorbitol MacConkey agar (OXOID). Nutrient agar was used to help growth of the organisms, MacConkey agar was chosen to help distinguish bacteria that ferment lactose from those that do not. Brilliant Green agar was used to help identify the potential presences of *Salmonella* Sp. Modified Edwards agar is a selective medium for isolation of *Streptococcus* sp. and XLD agar was used as a selective growth medical for differentiation *Salmonella* Sp. Sorbitol MacConkey agar is a variant of MacConkey agar and used for the detection of *E. coli* O157:H7. Results from each agar allowed the different targeted organisms to be isolated after overnight growth at 37°C. Isolated samples were then grown on Nutrient and MacConkey Agar, which was used as the first step of identification. Gram staining, oxidase, catalase and indoles tests were performed. The results from these biochemical tests allowed identification of the organisms at a species level.

2.3 Microorganism and culture methods

Once all samples were isolated, overnight culture on nutrient agar were transfer to Micro bank vials (Pro-lab diagnostics, UK), for storage at -80°C. Isolated were revived by

taking from storage and transfer using a sterile swab on Brain Heart Infusion agar and incubating aerobically at 37°C for 24 hours before being used. All media used was from Oxoid Ltd (Basingstoke, Hampshire UK). Isolates were then stored on microbank beads frozen at -80°C.

2.4 Antibiotics used in this study:

Twenty antibiotics were chosen for the study. All antibiotics were sourced from one veterinary practice. The antibiotics were chosen to cover a range of classes, generations and be used in both human and veterinary medicine. For the antimicrobial profiles, the antibiotics were sub-grouped in groups of 5-6 antibiotics per plates (Table 2). Vet A and Vet B were not both used at the same time: Vet A was used for gram negative bacteria and Vet B was used for gram positive bacteria. Tylosin can only be used against gram positive bacteria, being used in the treatment of *Streptococcus* and *Staphylococcus* Sp. Infection (Entorf *et al.* 2014). ESBLA A, ESBLA B and Human plates were used against all samples isolated.

Table 2: A table of the antibiotics used separated according to their panels. The concentrations described are per disc.

Panel Name:	**Vet A**	**Vet B**	**ESBL A**	**ESBL B**	**Human**
Antibiotics	30µg Cefquinome 25µg Amoxicillin 10 µg Ampicillin 30 µg Oxytetracycline 5 µg Marbofloxacin	30µg Cefquinome 25µg Amoxicillin 10 µg Ampicillin 30 µg Oxytetracycline 5 µg Marbofloxacin 30 µg Tylosin	5 µg Cefotaxime 10/20 µg Amox/Clav 30 µg Piperacillin 30/6 µg Piper/Tazo 30 µg Cefuroxime 30 µg Cefoxitin	10 µg Ceftazidime 30 µg Cefepime 10 µg Ertapenem 10 µg Meropenem 10 µg Imipenem	30 µg Ceftazidime 5 µg Ciprofloxacin 10 µg Gentamicin 15 µg Tigecycline

2.5 SIRscan 2000

To establish the resistance profiles of the isolated bacteria, the disc diffusion method was used (Kindly provided by Pro Lab Diagnostics and i2a). This disc diffusion method was developed in 1940 and is still the official method used in clinical microbiology for routine susceptibility testing (Balouiri *et al*., 2015). It provides qualitative results by using the diameter to categorize the reading as susceptible, intermediate or resistant (Jorgensen & Ferraro, 2009). The disc diffusion method was done according the EUCAST guidelines version 6 (EUCAST,2016) and standard antibiotic discs were used (OXOID) (i2a) (MAST). The SIRscan 2000 is an automatic plate reader equipped with the SirWeb programme that enables complete management of results (i2a). The plate is put into the SIRscan and a photo of the plate is taken which then appears on the screen. Pre-logged light settings and set up of antibiotic panels allows the SIRscan to instantaneously read all the zones and interpret them. This can then be modified by the user to create readings that are correct for the plate. The SIRscan was calibrated at the start of the study. The results are then automatically loaded onto a database which can be accessed later. The SIRscan 2000(i2a) is able to automatically determine the results of the zones of inhibition through colour co-ordination of circumferences (resistant – red, intermediate – yellow, susceptible – green). This is done through taking a photo of the plate showing the diameters with an image on the computer. The data saved from each plate can be broken down into the qualitative results or 'raw' diameter readings to allow evaluation of results as desired. All resistance profiles were recorded once.

2.5 MIC calculation

All *E. coli* strains that were taken from Farm 2 were included in this pilot study. *E. coli* was chosen as it was the most common organism isolated throughout the study. A total of 85 organisms were used, 39 from visit 1, 32 from visit 2 and 19 from visit 3 (Table 3).

The MICs were determined through the method of broth micro dilution. This method is susceptibility testing technique, using sterile 96 well micro-dilution plates (VWR), inoculated with the organisms, Mueller Hinton broth, and antibiotic at set concentrations (Rodriguez-Tudela, 2008). All wells contained 100µl of four times strength Mueller Hinton Broth. All antibiotics were diluted in water from powder form (OXOID). 100µl aliquots of the antibiotics were then added from well 1-10 through two-fold dilution with the concentration starting in well one at 64mg/l the highest concentration to 0.00125mg/l. Bacteria suspended in Ringer's solution at 0.5 McFarland Standard was added at 100µl also to wells 1-11 and 100µl sterile ringers solutions were added to well 12. Well 11 acted as a positive buffer, containing bacteria only and well 12 acted as a negative bacteria containing no bacteria. The plates were kept in a shaking incubator at 80-100rpm at 37°C for 18 hours. Each MIC calculation was replicate three times. Turbidity was seen by eye the following day by two people and recorded using Microsoft Excel 2016. If turbidity was seen across all wells then the MIC was noted as greater than or equal to 64mg/l. However, it is understood that the MIC may be higher.

2.6 Statistical analysis

In conducting statistical analysis, T tests were performed to analyse the variation between groups. Parametric assumptions have been met and t tests were deemed the most appropriate method to determine statistical signifigance. Any value under 0.05 is classed as significant. Only assumpoions and associations can be made through the trends in the data when the results of the t test value does not fall under 0.05. T test were calculated on results for organisms showing resistance in relation to species and in relation to antibiotics used.

3.0 Results

3.1 Organisms isolated

The first set of results show the isolation and identification of the isolates taken from all three farms, as described in 2.2. The organisms isolated were *E.coli, Klebsiella* sp., *Pseudomonas sp., Proteus* sp. And *Streptococcus* sp. Other organisms were also isolated throughout the study. These isolates included *Corynebacterium* sp., *Streptobacillus* sp, *Bacillus* sp., *Enterococcus* sp. And *Staphylococcus* sp. These organisms were put into the Other category as part of the results. A breakdown of the isolates taken from farm 1 can be shown in table . In total 338 organisms were isolated across the two visits in the study. 69% of the isolates were represented in the BG and 31% were represented in the environment. *E.coli* was the most prevalent isolate (72.7% in the BG and 63.1% in the ENV). Samples taken from the environmental locations were of a much smaller size than in the BG. These observations were seen in Farm 2 and Farm 3, shown in tables 4 and 5 respectively. On Farm 2, 280 organisms were isolated (table 4). It was visited three times throughout the study. The most common organisms isolated on Farm 2 were *E.coli* (31.2%) and *Proteus* sp. (32.8%). *E.coli* was the most prevalent in the BG (33%) and *Proteus* sp. Was the most prevalent in the ENV (34.8%). On Farm 3, visited four times throughout the study (table 5), 270 organisms were isolated overall. No species from the *Other* category were isolated. Similar to Farm 1 and 2, *E.coli* was isolated the most in the BG (33%), however, *Proteus* sp. Was isolated the most in the ENV (34.8%).

Table 3: Table showing the percentage and number of organisms isolated from Farm 1, separated into organisms isolated per visit and then overall and sub grouped into those isolated from the bovine gut, environment and combined.

Species	**Visit 1 October 2015**			**Visit 2 November 2015**			**Total Number (both visits)**		
	Location			**Location**			**Location**		
	Overall	**Bovine Gut**	**Environment**	**Overall**	**Bovine Gut**	**Environment**	**Overall**	**Bovine Gut**	**Environment**
E. coli	92 (80%)	71 (86%)	21 (66%)	151 (67.7%0	107 (70%)	44 (62%)	243 (71.8%)	178 (75.7%)	65 (63.1%)
Pseudomonas sp.	7 (6.1%)	5 (6%)	2 (6%)	12 (5.4%)	8 (5.5%)	4 (6%)	19 (5.6%)	13 (5.5%)	6 (5.8%)
Proteus sp.	10 (8.6%)	2 (2%)	8 (25%)	32 (14.3%)	16 (10%)	16 (21%)	42 (12.4%)	18 (7.6%)	24 (23.3%)
Streptococcus sp.	2 (1.7%)	1(1%)	1 (3%)	22 (9.8%)	16 (10%)	6 (9.5%)	24 (7.1%)	17 (7.2%)	7 (6.7%)
Klebsiella sp.	1 (0.8%)	1(1%)	-	3 (1.3%)	2 (1%)	1 (1.5)	4 (1.1%)	3 (1.2%)	1 (0.9%)
Staphylococcus sp.	1 (0.8%)	1 (1%)	-	1 (0.4%)	1 (0.5%)	-	2 (0.6%)	2 (0.8%)	-
Corynebacterium sp.	2 (1.7%)	2 (2%)	-	2 (0.8%0	2 (1%)	-	4 (1.1%)	4 (1.6%)	-

Table 4: Table showing the percentage and number of organisms isolate from Farm 2, separated into organisms isolated per visit and then overall and sub grouped into those isolated from the bovine gut, environment and combined.

Species	Visit 1 November 2015			Visit 2 January 2016			Visit 3 February 2016			Total Number (all visits)		
	Source			Source			Source			Source		
	Overall	BG	ENV	Overall	BG	ENV	Overall	BG	ENV	Overall	BG	ENV
E. coli	38 (40%)	31 (41%)	7 (37%)	32 (39%)	26 (44%)	6 (26%)	19 (18.4%)	14 (18%)	5 (22%)	89 (31.2%)	71 (33%)	18(27%)
Pseudomonas sp.	5 (5.2%)	2 (3%)	3 (18%)	5 (6%)	3 (5%)	2 (9%)	5 (4.8%)	5 (6%)	-	15 (5.3%)	10 (4.6%)	5 (7.5%)
Proteus sp.	30 (31.6%)	24 (32%)	6 (32%)	17 (20.7%)	10 (17%)	7 (30%)	45 (43.6%)	35 (44%)	10 (44%)	92 (32.8%)	69 (32.2%)	23 (34.8%)
Streptococcus sp.	16 (16.8%)	13 (17%)	3 (16%)	23 (28%)	18 (31%)	5 (22%)	24 (23.3%)	18 (22%)	6 (26%)	63 (22.5%)	49 (22.8%)	14 (21%)
Klebsiella sp.	6 (6.3%)	5 (7%)	1 (6%)	5 (6.1%)	2 (3%)	3 (13%)	3 (2.9%)	3 (3%)	-	14 (5%)	10 (4.6%)	4 (6%)
Staphylococcus sp.	-	-	-	-	-	-	2 (1.9%)	1 (1%)	1 (4%)	2 (0.7%)	1 (0.4%)	1 (1.5%)
Enterococcus sp.	-	-	-	-	-	-	3 (2.9%)	3 (3%)	-	3 (1.1%)	3 (!.2%)	- (0%)
Corynebacterium sp.	-	-	-	-	-	-	2 (1.9%)	1 (1%)	1 (4%)	2 (0.7%)	1 (0.4%)	1 (1.5%)

Table 5: Table showing the percentage and number of organisms isolate from Farm 3, separated into organisms isolated per visit and then overall and sub grouped into those isolated from the bovine gut, environment and combined.

Species	**Visit 1 January 2016**			**Visit 2 February 2016**			**Visit 3 March 2016**			**Visit 4 March 2016**			**Total Number (all visits)**		
	Source			**Source**			**Source**			**Source**			**Source**		
	Overall	**BG**	**ENV**	**Overall**	**BG**	**ENV**	**Overall**	**BG**	**ENV**	**Overall**	**BG**	**ENV**	**Overall**	**BG**	**ENV**
E. coli	32 (55%)	19 (57%)	13 (52%)	25 (37.8)	19 (38%)	9 (47%)	37 (47%)	32 (59%)	5 (22%)	30 (44.7%)	24 (48%)	6 (35%)	127 (47%)	94 (50%)	33 (39%)
Pseudomonas sp.	4 (6.8%)	-	4 (16%)	14 (21.2%)	9 (18%)	5 (26%)	9 (11.5%)	-	9 (39%)	3 (4.4%)	-	3 (18%)	30 (11%)	9 (4.8%)	21 (25%)
Proteus sp.	6 (10.3%)	5 (15%)	1 (4%)	13 (19.6%)	10 (20%)	3 (16%)	22 (28%)	14 (26%)	6 (26%)	17 (25%)	13 (26%)	4 (23.5%)	56 (21%)	42 (22.4%)	14 (16.8%)
Streptococcus sp.	11 (18.9%)	7 (21%)	4 (16%)	10 (15%)	9 (18%)	1 (5%)	7 (8.9%)	6 (11%)	1 (4%)	17 (25%)	13 (26%)	4 (23.5%)	45 (9.2%)	35 (18.7%)	10 (12%)
Klebsiella sp.	5 (8.6%)	2 (6%)	3 (12%)	4 (6%)	3 (6%)	1 (5%)	3 (3.8%)	2 (4%)	1 (4%)	-	-	-	12 (4.4%)	7 (3.7%)	5 (6%)
Staphylococcus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Corynebacterium sp.	-		-	-	-	-	-	-	-	-	-	-	-	-	-

3.2 Antibiotic resistance in relation to species

Once the organisms were isolated and identified, resistance profiles were created according to the method described in 2.3 and 2.4. Manual and SIRscan readings were used to determine which organism were resistant to antibiotics. This was then analysed to note which organisms showed resistance to certain classes of antibiotics and whether the organism showed resistance to more than one class. The locations were separated to BG and ENV only, where all the separate environmental locations represented one environmental sample.

Farm 1 showed a higher prevalence of organisms that were resistant to two or more classes of antibiotic (table 6). This is especially true for *Klebsiella* sp. And specie from the "Other" category. Multi-class resistance is more common in the samples from the ENV across all farms.

Table 6: Table showing the number and percentage of targeted bacterial organisms that show resistance within the bovine gut, environment and overall on Farm 1

		E.coli			Proteus			Pseudomonas			Streptococcus			Klebsiella			Other		
Bovine Gut		**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**
	No Resistance	5 (11%)	21 (27%)	26 (22%)	1 (7%)	-	1 (6%)	-	1 (20%)	1 (16%)	-	-	-	1 (100%)	-	1 (50%)	-	-	-
	Resistance to one class	11 (26%)	29 (38%)	40 (33.4%)	3 (21%)	2 (100%)	5 (30%)	-	2 (40%)	2 (32%)	4 (40%)	-	4 (26%)	-	-	-	-	-	-
	Resistance to 2+ classes	26 (62%)	26 (34%)	52 (44%)	9 (69%)	-	9 (64%)	2 (100%)	2 (40%)	4 (64%)	6 (60%)	5 (100%)	11 (74%)	-	1 (100%)	1 (50%)	-	1 (100%)	1 (100%)
Environment		**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**
	No Resistance	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Resistance to one class	1 (7%)	1 (4.7%)	2 (5.5%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Resistance to 2+ classes	13 (93%)	20 (95.3%)	33 (94.5%)	8 (100%)	8 (100%)	16 (100%)	3 (100%)	-	3 (100%)	-	1 (100%)	1 (100%)	1 (100%)	-	1 (100%)	1 (100%)	-	1 (100%)
Overall		**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**	**Visit 1**	**Visit 2**	**Overall**
	No Resistance	5 (8.9%)	21 (22%)	26 (16.9%)	1 (4.7%)	-	1 (3.2%)	-	1 (20%)	1 (10%)	-	-	-	1 (50%)	-	1 (33%)	-	-	-
	Resistance to one class	12 (21.4%)	30 (31%)	42 (27.4%)	3 (14.2%)	2 (20%)	5 (16%)	-	2 (40%)	2 (20%)	4 (40%)	-	4 (25%)	-	-	-	-	-	-
	Resistance to 2+ classes	39 (69%)	46 (47%)	85 (55.5%)	17 (81%)	8 (80%)	25 (81%)	5 (100%)	2 (40%)	7 (70%)	6 (60%)	6 (100%)	12 (72%)	1 (50%)	1 (100%)	2 (66.6%)	1 (100%)	1 (100%)	2 (50%)

Table 7: Table showing the number and percentage of *E.coli, Proteus* sp. and *Pseudomonas* sp. that show resistance within the bovine gut, environment and overall on Farm 2

	E.coli					***Proteus* sp.**					***Pseudomonas* sp.**		
Bovine Gut		**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**
	No Resistance	43%	69%	42&	26%	9%	75%	9%	31%	0%	33%	0%	33%
	Resistance to one class	29%	23%	29%	19%	40%	12.5%	40%	31%	0%	33%	0%	33%
	Resistance to 2+ classes	29%	8%	29%	55%	51%	12.5%	51%	38%	0%	33%	0%	33%
Environment		**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**
	No Resistance	22.33%	67%	0%	0%	40%	100%	20%	53%	0%	0%	0%	0%
	Resistance to one class	53.1%	16.5%	100%	43%	19%	0%	40%	16.3%	16%	50%	50%	39%
	Resistance to 2+ classes	24.5%	16.5%	0%	57%	41%	0%	40%	26.7%	67%	50%	50%	61%
		Visit 1	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**
Overall	**No Resistance**	40.67%	69%	32%	13%	32%	71%	11%	38%	7%	20%	0%	9%
	Resistance to one class	31%	22%	47%	31%	31%	18%	49%	33%	52%	40%	50%	47%
	Resistance to 2+ classes	28.33%	9%	21%	56%	27%	12%	40%	29%	41%	40%	50%	44%

Table 8: Table showing the percentage of *Streptococcus* sp., *Klebsiella* sp. and species from the *Other* category that show resistance within the bovine gut, environment and overall on Farm 2.

		***Streptococcus* sp.**				***Klebsiella* sp.**				**Other**			
Bovine Gut		**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**
	No Resistance	0%	39%	83%	41%	85%	0%	0	28%	0%	0%	0%	0%
	Resistance to one class	50%	17%	17%	28%	17%	0%	50%	22%	0%	0%	0%	0%
	Resistance to 2+ classes	50%	44%	0%	31%	0%	0%	50%	50%	100%	0%	100%	100%
Environment		**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**
	No Resistance	0%	60%	80%	47%	83%	0%	0%	28%	0%	0%	0%	0%
	Resistance to one class	50%	20%	10%	27%	17%	0%	0%	6%	0%	0%	100%	50%
	Resistance to 2+ classes	50%	20%	10%	26%	0%	100%	0%	66%	100%	0%	0%	50%
Overall		**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Overall**	**Visit 1**	**Visit 2**	**Visit 2**	**Overall**
	No Resistance	44%	43%	77%	55%	31%	0%	33%	21%	0%	0%	0%	0%
	Resistance to one class	31%	17%	18%	22%	33%	0%	33%	22%	0%	0%	50%	25%
	Resistance to 2+ classes	33%	40%	5%	23%	35%	100%	33%	57%	100%	0%	50%	75%

Table 9: Table showing the number and percentage of *E.coli, Proteus* sp. and *Pseudomonas* sp. that show resistance within the bovine gut, environment and overall on Farm 3

		E.coli					***Proteus* sp.**					***Pseudomonas* sp.**				
Bovine Gut		**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**
	No Resistance	(11.25%)	0%	3%	4%	4.5%	7.25%	0%	29%	0%	9%	0%	0%	33%	0%	8.25%
	Resistance to one class	(33.5%)	32%	38%	33%	34%	21.25%	0%	14%	38%	18%	0%	0%	17%	0%	4.25%
	Resistance to 2+ classes	(55.25%)	68%	59%	63%	61.5%	71.25%	100%	57%	62%	73%	0%	100%	50%	0%	87.5%
Environment		**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**
	No Resistance	(7.75%)	0%	0%	0%	1.9%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Resistance to one class	(46.5%)	33%	80%	25%	46.1%	50%	75%	0%	25%	37.5%	32.75%	20%	11%	25%	22%
	Resistance to 2+ classes	(48%)	66%	20%	75%	52%	50%	25%	100%	75%	62.5%	67.25%	80%	89%	75%	78%
Overall		**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**
	No Resistance	11.75%	0%	(3%)	2%	4.25%	13.25%	0%	20%	0%	3%	0%	0%	16%	0%	4%
	Resistance to one class	34.75%	32%	43%	26%	34%	17%	33%	10%	26%	22%	31.25%	10%	14%	25%	20%
	Resistance to 2+ classes	53.5%	68%	54%	72%	61.75%	67%	66%	70%	64%	75%	70.75%	90%	70%	75%	76%

Table 10: Table showing the percentage of *Streptococcus* sp., *Klebsiella* sp. and species from the *Other* category that show resistance within the bovine gut, environment and overall on Farm 3

		***Streptococcus* sp.**					***Klebsiella* sp.**					**Other**				
Bovine Gut		**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**
	No Resistance	19.75%	0%	33.5%	46%	24%	12.5%	0%	50%	0%	3%	0%	0%	0%	0%	0%
	Resistance to one class	18%	12.5%	16.5%	23%	17.5%	29%	66%	0%	0%	23.75%	25%	0%	0%	100%	100%
	Resistance to 2+ classes	62.25%	87.5%	50%	31%	57%	33.25%	33%	50%	0%	29.25%	0%	0%	0%	0%	0%
Environment		**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visti 4**	**Overall**
	No Resistance	37.5%	0%	100%	50%	47%	0%	0%	50%	0%	12.5%	0%	0%	0%	0%	0%
	Resistance to one class	0.5%	0%	0%	0%	0.1%	43.75%	0%	0%	0%	11%	0%	0%	0%	100%	100%
	Resistance to 2+ classes	37.75%	100%	0%	50%	52.9%	56.25%	100%	50%	0%	76.5%	0%	0%	0%	0%	0%
Overall		**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Overall**	**Visit 1**	**Visit 2**	**Visit 3**		**Overall**
	No Resistance	29.25%	0%	66%	48%	35.8%	6.25%	0%	50%	0%	14%	50%	0%	0%	0%	0%
	Resistance to one class	17.5%	6%	8%	11%	10.6%	33.75%	33%	0%	0%	16%	50%	0%	0%	100%	100%
	Resistance to 2+ classes	53.25%	93%	26%	41%	53.6%	35%	66%	50%	0%	70%	0%	0%	0%	0%	0%

Separating the data between the BG and ENV showed where the resistance can be found. Across all three farms, resistance was seen in organisms across the BG and ENV. However, the profiles of resistance was different across all three farms.

All cows included in the study were deemed “healthy” with no cases on ongoing treatment and all the farm locations were cleaned on a regular basis. However, isolates from each farm show a different resistance profile, which could reflect farm antimicrobial use as well as exposure to movement of resistant organisms and lack of biosecurity. Biosecurity refers to the measure designed to reduce the risk of transmission of organisms. An example of this in this instance would be wildlife, like birds that fly on and of the farm and neighbouring fields.

Resistant organisms can more from the BG and ENV and visa versa. The difference between the resistance found in the BG and ENV means that one cannot be representative of another or used to be representative of the overall resistance pattern. Further work is needed to determine for the resistance can more from the BG to the ENV or the other way around. Resistance profiles were then measured according to resistance against each antimicrobial tested.

3.3 Class Resistance

Comparing the resistance profiles on each farm, in the BG and ENC allowed a more direct comparison between the levels of resistance seen. When focusing on Farm 1, the profiles vary greatly between the BG and ENV. For example, the resistance profile for *E.coli* showed higher resistance levels to Cephalosporins (46%), β-lactams (51%) and Tetracyclines (88.5%) in the ENV (table 7) than in BG (8%, 14% and 72% respectively). For all species in both the BG and ENV, the most frequently observed resistance was against the Tetracycline class, although the percentages vary slightly 100% of the *Klebsiella* sp. In the ENV were resistant to tetracycline, whereas 50% of resistance against tetracycline was seen in the BG. This pattern of higher resistance in the ENV compared to the BG was true for all species on Farm 1.

Table 11: Table showing the percentage of bacteria that is resistant to classes of antibiotics in the BG, ENV and overall for Farm 1

		E.coli (%)	*Proteus* sp. (%)	*Pseudomonas* sp.(%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)
Overall	Cephalosporin	18	26	17	0	0
	Beta Lactams	22	26	33	9	33
	Aminoglycoside	26	29	33	32	33
	Tetracyclines	75	91	83	59	66
	Carbapenems	2	12	8	0	0
	Fluoroquinolone	4	12	0	18	0
	Macrolide	0	0	0	23	0
		E.coll (%)	*Proteus* sp.(%)	*Pseudomonas* sp. (%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)
Bovine Gut	Cephalosporin	8	19	12	0	0
	Beta Lactams	14	12	12	6	0
	Aminoglycoside	28	31	38	25	50
	Tetracyclines	72	93	75	50	50
	Carbapenems	3	63	0	0	0
	Fluoroquinolone	0	0	0	19	0
	Macrolide	0	0	0	6	0
		E.coli (%)	*Proteus* sp. (%)	*Pseudomonas* sp. (%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)
Environment	Cephalosporin	46	38	25	0	0
	Beta Lactams	51	31	75	17	100
	Aminoglycoside	20	25	25	50	0
	Tetracyclines	86	88	100	83	100
	Carbapenems	0	19	25	0	0
	Fluoroquinolone	11	12	0	17	0
	Macrolide	0	0	0	50	0

The most prevalent resistance towards an antibiotic class differs on Farm 2 (table 12) compared to Farm 1. Like Farm 1, *E.coli, Pseudomonas* sp., *Klebsiella* sp., and species from the Other category showed most resistance to Tetracycline (43%, 37%, 33% and 75% respectively). *Proteus* sp. Overall showed more resistance to Cephalosporin (27%) but *Proteus* sp. isolates from the ENV saw higher resistance to Tetracycline. *Pseudomonas* sp. isolated from the BG showed a higher resistance prevalence to Tetracycline. *Klebsiella* sp. showed a similar pattern, higher resistance was observed against β-lactams (40%) in the BG rather than Cephalosporin.

Species from the "Other" category were not isolated in Farm 3 (table 13). On Farm 3, across the BG and ENV, the highest level of resistance was observed against Tetracyclines, as seen on the other two farms. On the other two farms, differences were seen in the resistance profiles between the BG and ENV, whereas on Farm 3 more similarities can be seen. Unlike Farm 1 and Farm 2, a trend of high prevalence of resistance against the antibiotics in the ENV compared to the BG was not shown in the results for Farm 3.

Table 12: Table showing the percentage of bacteria that is resistant to classes of antibiotics in the BG, ENV and overall for Farm 2

		E.coli (%)	*Proteus* sp. (%)	*Pseudomonas* sp. (%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)	Other (%)
Overall	Cephalosporin	24	27	18	2	22	0
	Beta Lactams	17	23	26	9	27	25
	Aminoglycoside	3	9	0	25	0	0
	Tetracyclines	43	18	37	22	33	75
	Carbapenems	11	18	18	0	16	0
	Fluoroquinolone	9	4	0	18	0	0
	Macrolide	0	0	0	24	0	0
		E.coli (%)	*Proteus* sp. (%)	*Pseudomonas* sp. (%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)	Other (%)
Bovine Gut	Cephalosporin	27	18	33	19	10	0
	Beta Lactams	17	16	13	5	40	50
	Aminoglycoside	3	4	0	24	0	0
	Tetracyclines	47	41	40	14	40	50
	Carbapenems	7	14	13	0	10	0
	Fluoroquinolone	0	5	0	16	0	0
	Macrolide	0	0	0	21	0	0
		E.coli (%)	*Proteus* sp. (%)	*Pseudomonas* sp. (%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)	Other (%)
Environment	Cephalosporin	26	21	15	0	38	0
	Beta Lactams	19	16	36	16	12	0
	Aminoglycoside	6	14	0	0	0	0
	Tetracyclines	33	33	27	67	25	100
	Carbapenems	10	14	22	0	25	0
	Fluoroquinolone	3	3	0	16	0	0

Table 13: Table showing the percentage of bacteria that is resistant to classes of antibiotics in the BG, ENV and overall for Farm 3

		E.coli (%)	*Proteus* sp. (%)	*Pseudomonas* sp. (%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)
Overall	Cephalosporin	27	25	10	0	12
	Beta Lactams	21	19	30	13	48
	Aminoglycoside	4	3	0	20	0
	Tetracyclines	35	35	30	20	24
	Carbapenems	11	13	30	0	12
	Fluoroquinolone	1	6	0	10	0
	Macrolide	0	0	0	37	0
		E.coli (%)	*Proteus* sp. (%)	*Pseudomonas* sp. (%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)
Bovine Gut	Cephalosporin	28	25	0	0	0
	Beta Lactams	22	22	0	11	50
	Aminoglycoside	2	1	0	22	0
	Tetracyclines	40	27	100	19	33
	Carbapenems	8	16	0	0	17
	Fluoroquinolone	0	7	0	11	0
	Macrolide	0	0	0	37	0
		E.coli (%)	*Proteus* sp. (%)	*Pseudomonas* sp. (%)	*Streptococci* sp. (%)	*Klebsiella* sp. (%)
Environment	Cephalosporin	24	24	12	0	50
	Beta Lactams	24	24	36	33	50
	Aminoglycoside	9	12	0	0	0
	Tetracyclines	22.5	24	12	33	0
	Carbapenems	14	12	36	0	0
	Fluoroquinolones	4.5	6	0	0	0
	Macrolide	0	0	0	33	0

Across all three farms the percentage of tetracycline resistance exceeded all other classes. More variation in the farm's resistance profiles was seen when comparing the class that had the second most prevalent level of resistance. In Farm 1, resistance was shown to be present against Aminoglycosides. This is true for all species (*E. coli* 28%, *Pseudomonas* sp. 38%, *Streptococcus* sp. 25% and *Klebsiella* sp. 50%) except species from the Other category. This differs to the profile seen in Farm 2, where different results were seen for the second most prevalent. Overall, *Proteus sp.* (28%), *Pseudomonas sp.* (47%), *Klebsiella sp.* (36%) and species from the Other category (25%) showed the class to be β-Lactams. *E. coli* had the result for the Cephalosporin class at 29% and *Streptococcus sp.* showed it for Macrolides (24%). While on Farm 1 the same pattern was seen in the BG and ENV, the trend in the BG of Farm 2 differs to the profile in the ENV. Within the BG, *E. coli* (44%), *Proteus sp.* (32%) and *Pseudomonas sp.* (33%) had Cephalosporin as the second most resistant class. *Klebsiella* sp. and species from the Other category saw it for Aminoglycosides and *Streptococcus sp.* (29%) saw it from Macrolides again. In the ENV, again *E. coli* (44%) and *Proteus sp.* (33%) has this result for Cephalosporins, but this time Tetracyclines were the second most prevalent for *Pseudomonas sp.* (57%)and *Klebsiella sp. (*50%).

In Farm 3, *E. coli* showed the class with the second highest resistance to be Aminoglycosides, in the BG (56%) and in the ENV (64%). β-Lactam resistance against *Proteus* sp. in the ENV (50%), which was the second most prevalent resistance in the ENV, differing to the BG, where the class was Aminoglycosides. Within the *Pseudomonas* sp. isolated on Farm 3, resistance against Cephalosporins and β-Lactams was at 46%. In the ENV the second most resistant class was against *Streptococcus* sp. (30%). The resistance profile on Farm 3 had the most similarity to the profile seen in Farm 1. However, there were still notable differences between all three farms. The differences in the resistance profiles mean that no conclusions can be made to draw relationships between the three farms.

Within the Gram negative species, the least prevalent resistant was seen against Carbapenems and Fluoroquinolones as seen in Farm 1 (table 7). This was also the case for Farm 3 (table 9). Within Farm 2, more variability was seen across the species. All farms show the least resistance against the Fluoroquinolone class within the Gram negatives. In Farm 3, low levels of resistance were also seen against Aminoglycosides (4% for *E. coli* and 11% for *Proteus* sp.). Higher resistance were seen against Aminoglycosides in *Pseudomonas* sp. The second lowest percentage of resistance was seen against Carbapenems and Cephalosporins, with the result of 33%. The classes Carbapenems and Cephalosporin had the lowest percentages of resistance in Farm 3. However, the result of 33% resistance against classes in Farm 1 and 2 coincided with the highest levels of resistance. The proportional difference of percentage across all three farms suggest the different AMR profiles present. Again, these differences in the percentage of resistance and the ration of different resistance percentages show a different profile in the three farms. At this point no relationships can be drawn between the resistance found in the BG or ENV and the three farms enrolled showed little similarity.

3.4 Statistical comparison of organisms in relation to organisms and anitbiotics

Tables 14-16 shows the value for the t tests performed when comparing the percentage of resistance values in the bovine gut versus the environment, and between farms.

Table 14: T test values for the bovine gut results versus the environmental results on Farm 1,2 and 3.

Organism	Farm 1	Farm 2	Farm 3
E.coli	0.060101725	0.408737345	0.465913814
Proteus sp.	0.466915818	0.422189883	0.386183692
Pseudomonas sp.	0.068838902	0.489566677	0.486156737
Streptococcus sp.	0.030996228	0.384084824	0.490176826
Klebsiella sp.	0.228525824	0.5	0.5

Values that fall below 0.05 are statistically significant. This is only true for *Streptococuss sp.* On Farm 1. The remaing values fall above this are not stastically significant and therefore only assumptions of the data cannot be made. For Table 15,no values fell below 0.05 and for Table 16 only *E.coli* and *Proteus sp.* Showed significant signifigance.

Table 15: T Test results for the BG from farm to farm comparisons

Organism	Farm 1 v Farm 2	Farm 1 v Farm 3	Farm 2 v Farm 3
E.coli	0.296690835	0.315746846	0.459668934
Proteus sp.	0.061162349	0.093969783	0.5
Pseudomonas sp.	0.274562826	0.244516364	0.495002422
Streptococcus sp.	0.443131179	0.452776233	0.486675404
Klebsiella sp.	0.5	0.5	0.5

Table 16: T test results for ENV for farm to farm comparisons

Organism	Farm 1 v Farm 2	Farm 1 v Farm 3	Farm 2 v Farm 3
E.coli	0.038490983	0.06368379	0.473157762
Proteus sp.	0.027314799	0.050913731	0.473512955
Pseudomonas sp.	0.038981693	0.067176568	0.433362068
Streptococcus sp.	0.111267863	0.064283016	0.433362068
Klebsiella sp.	0.235908297	0.228525824	0.276363088

T test were also performed data showing the number of organisms that were resistance to none, one of two or more antibiotics. Tables 17 – 20 showed comparisons between farms. No values fell below 0.05 which means that only assumptions of the data can be made and values did not show to be significantly significant.

Table 17: T test results for bacteria showing resistance for Farm 1 against Farm 2

	E.coli	*Proteus sp.*	*Pseudomonas sp.*	*Streptococci sp.*	*Klebsiella sp.*
No Resistance	0.4841807	0.5	0.393897877	0.5	0.5
Resistant to 1	0.5	0.484382014	0.5	0.5	0.5
Resistant to 2+	0.444049149	0.5	0.5	0.5	0.489158578

Table 18: T test results for bacteria showing resistance on Farm 2 against Farm 3

	E.coli	*Proteus sp.*	*Pseudomonas sp.*	*Streptococci sp.*	*Klebsiella sp.*
No Resistance	0.5	0.5	0.495692375	0.486794772	0.11169752
Resistant to 1	0.232192631	0.478693096	0.5	0.499242583	0.5
Resistant to 2+	0.5	0.468408799	0.5	0.5	0.5

Table 19: T test results for bacteria showing resistnance on Farm 1 against Farm 3

	E.coli	*Proteus sp.*	*Pseudomonas sp.*	*Streptococci sp.*	*Klebsiella sp.*
No Resistance	0.488345273	0.5	0.408201491	0.485855978	0.276370099
Resistant to 1	0.116060216	0.5	0.5	0.499565694	0.5
Resistant to 2+	0.480471759	0.5	0.5	0.5	0.488526193

The results of the t tests in overall show few results are statistically significant which meant only assumptions of the results could be made.

3.4 Determining ECOFFS

Epidermiological cut-off values (ECOFFs) were used to identify the upper limit of wild type (WT) population. For Marbofloxacin (Figure 14) the value was 8mg/l; for Oxytetracycline (Figure 15) the value was 16mgl. For Ampicillin the ECOFF was 16mg/l and Cefquinome (Figre 17) had the value of 8mg/l.

The ECOFFs were determined from MICs of all *E.coli* on Farm 1 in the study. With a smaller sample size. When looking at the graphs, estimates can be determined. Ampicillin has the same ECOFF value as Oxytetracycline, with Cefquinome and Marbofloxacin having lower ECOFF values at 8mg/l. The next stage was to compare the Zone Diamter (ZD) values with the MICs to determine any correlation between the two value.

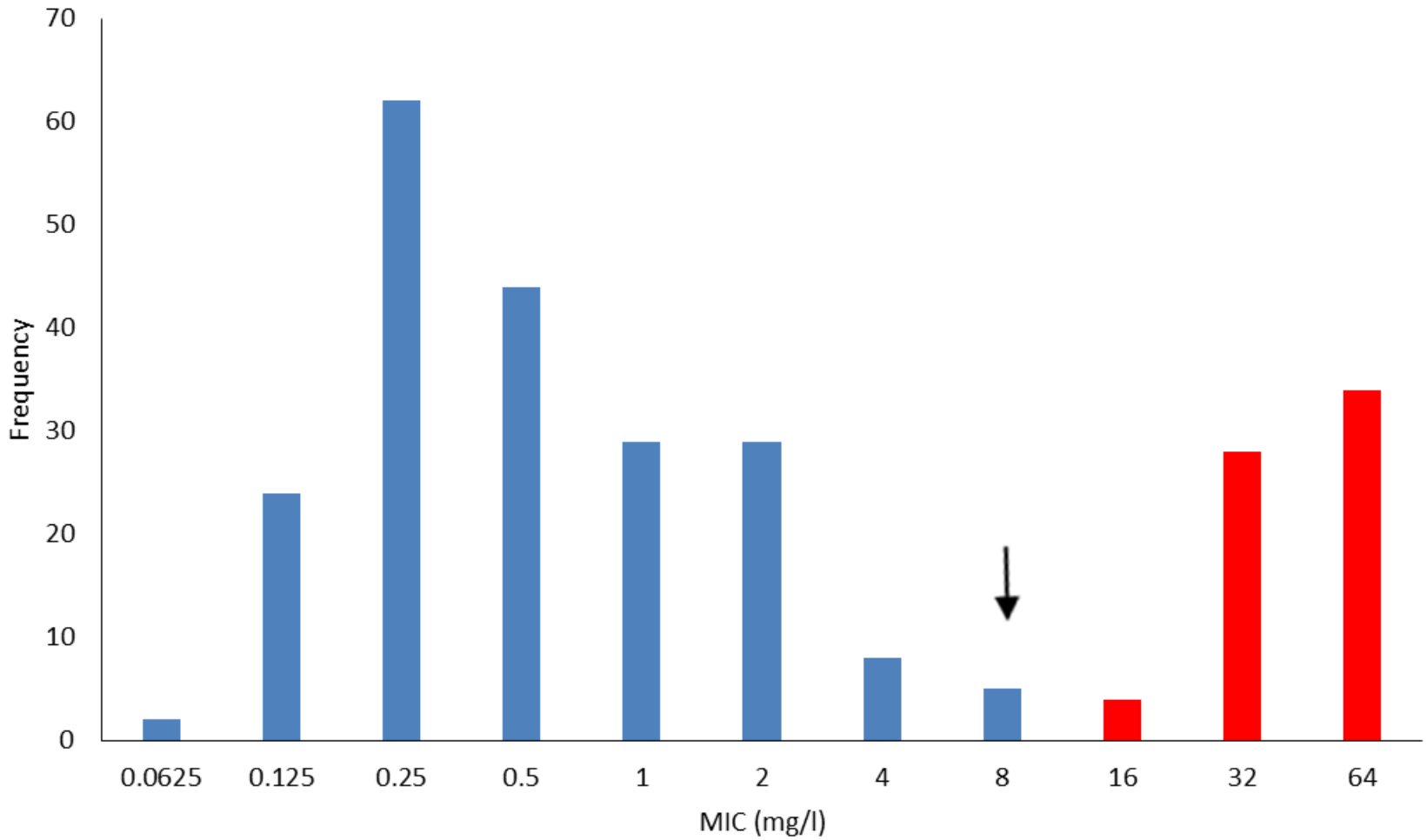

Figure 1: Bar chart showing distribution analysis of MIC values for Marbofloxacin. The black arrow represents with the ECOFF cut off value is (8mg/l). The red bars the fall to the right of the ECOFF value are suggestive of resistant isolates. The blue bars indicate the WT isolates. Any data plotted at x=64mg/l represents organisms that hold an MIC that is greater than or equal to 64mg/l.

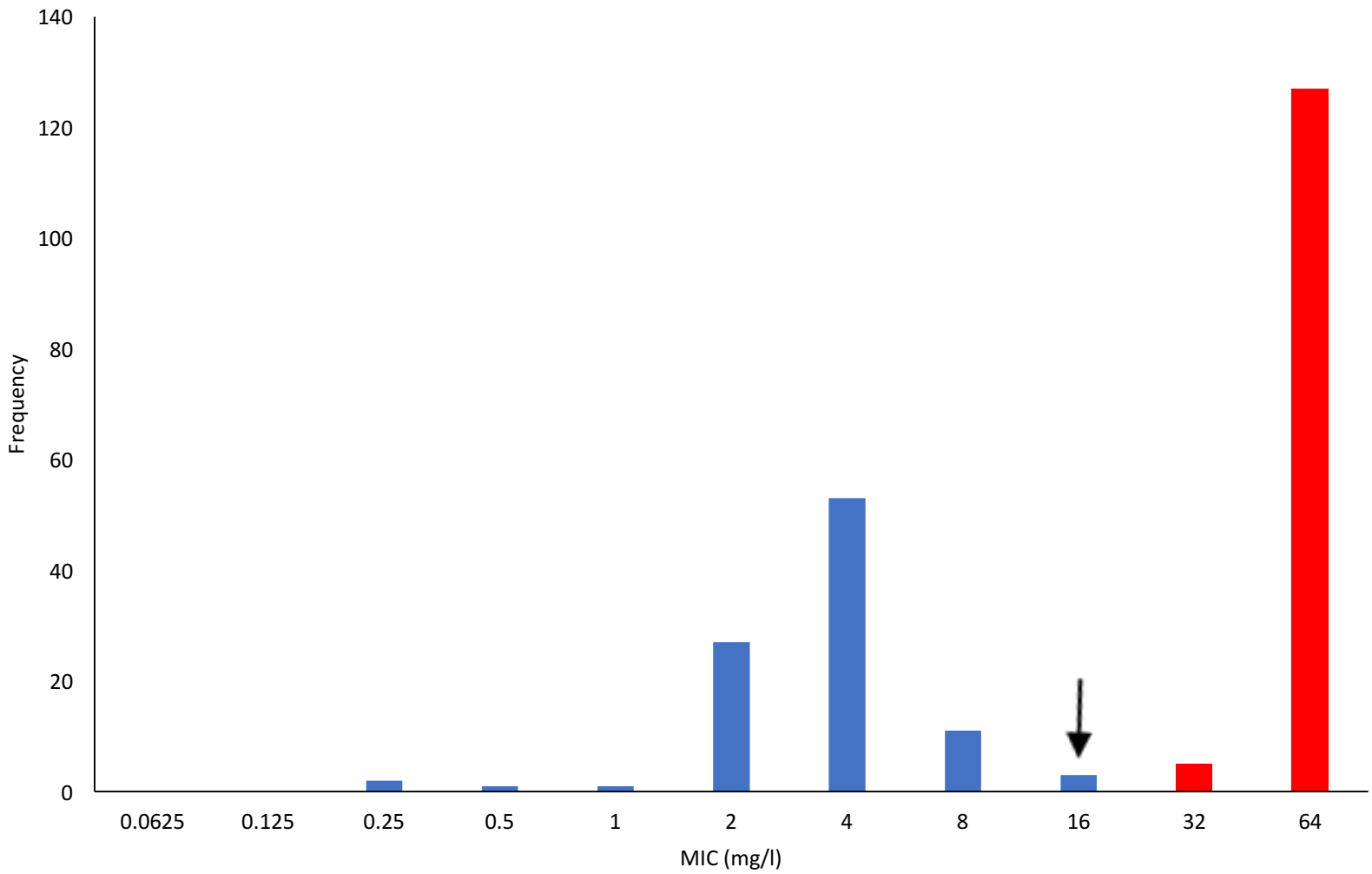

Figure 2: Bar chart showing distribution analysis of MIC values for Oxytetracycline. The black arrow represents with the ECOFF cut off value is (16mg/l). The red bars the fall to the right of the ECOFF value are suggestive of resistant isolates. The blue bars indicate the WT isolates. Any data plotted at x=64mg/l represents organisms that hold an MIC that is greater than or equal to 64mg/l.

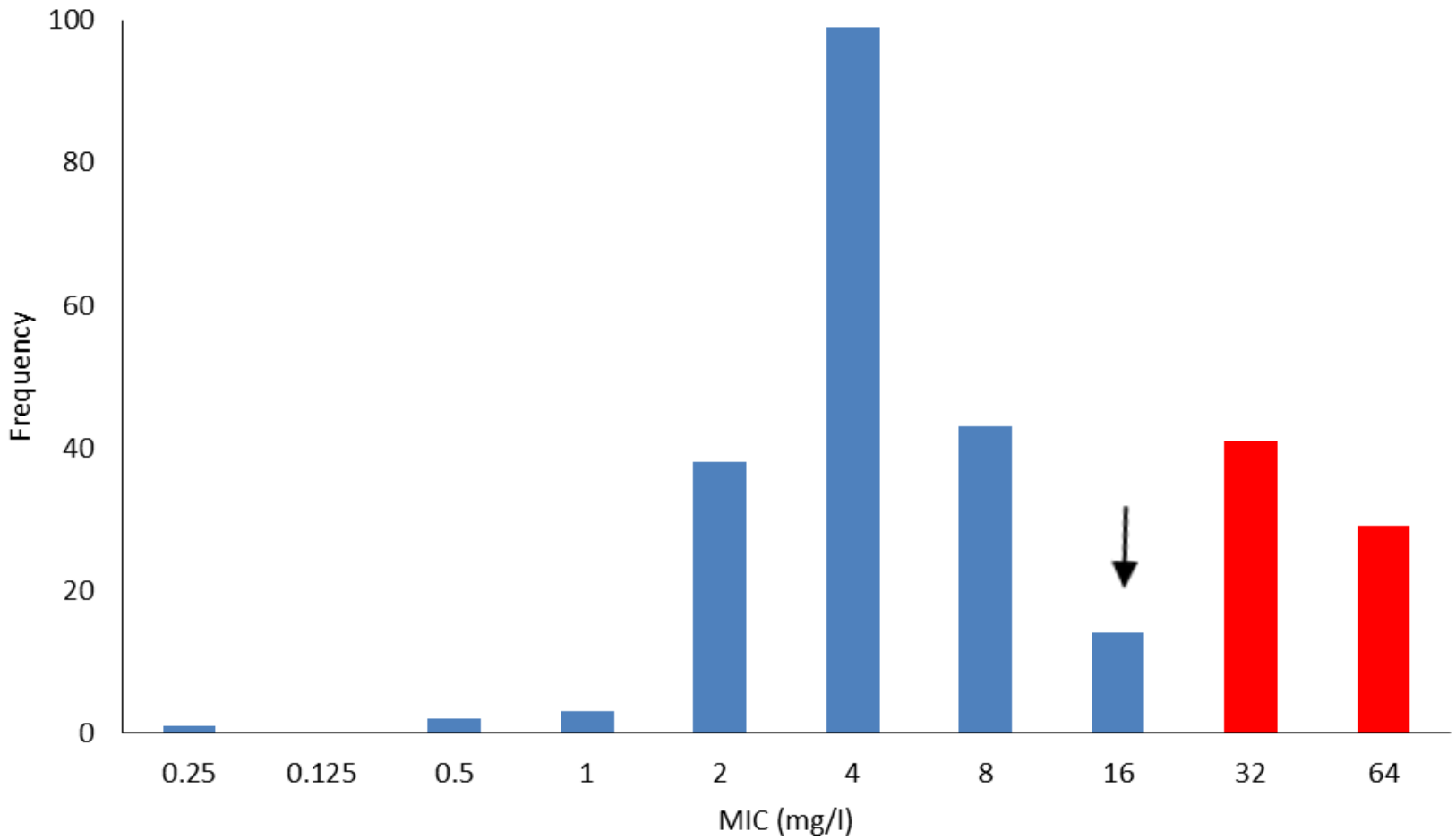

Figure 3: Bar chart showing distribution analysis of MIC values for Cefquinome. The black arrow represents with the ECOFF cut off value is (8mg/l). The red bars the fall to the right of the ECOFF value are suggestive of resistant isolates. The blue bars indicate the WT isolates. Any data plotted at x=64mg/l represents organisms that hold an MIC that is greater than or equal to 64mg/l.

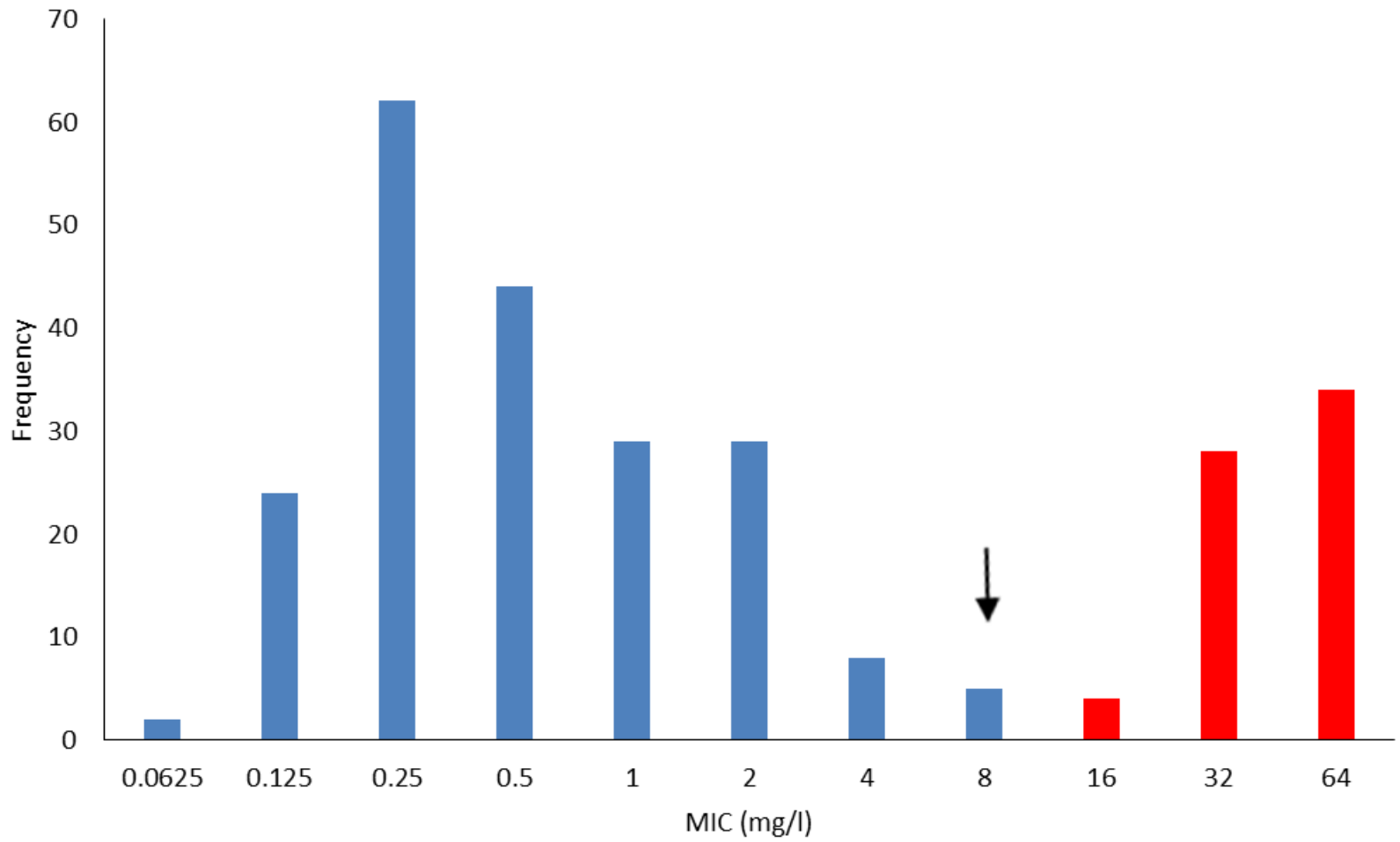

Figure 4: Bar chart showing distribution analysis of MIC values for Ampicillin. The black arrow represents with the ECOFF cut off value is (8mg/l). The red bars the fall to the right of the ECOFF value are suggestive of resistant isolates. The blue bars indicate the WT isolates. Any data plotted at x=64mg/l represents organisms that hold an MIC that is greater than or equal to 64mg/l.

3.6 MIC v ZD calculations for *E.coli* on Farm 2

The MIC concentrations for *E. coli* on Farm 2 were calculated according to the method described in 2.5. *E. coli* on Farm 2 were chosen to follow on from previous calculations for MIC on Farm 1. The MIC for Marbofloxacin, Cefquinome and Oxytetracycline were calculated and three technical replicates were performed. These MIC results were then used to determine levels of potential resistance against their antibiotics. The MIC value was then compared to the ZD value for each isolates shown in Figures 5 to 10.

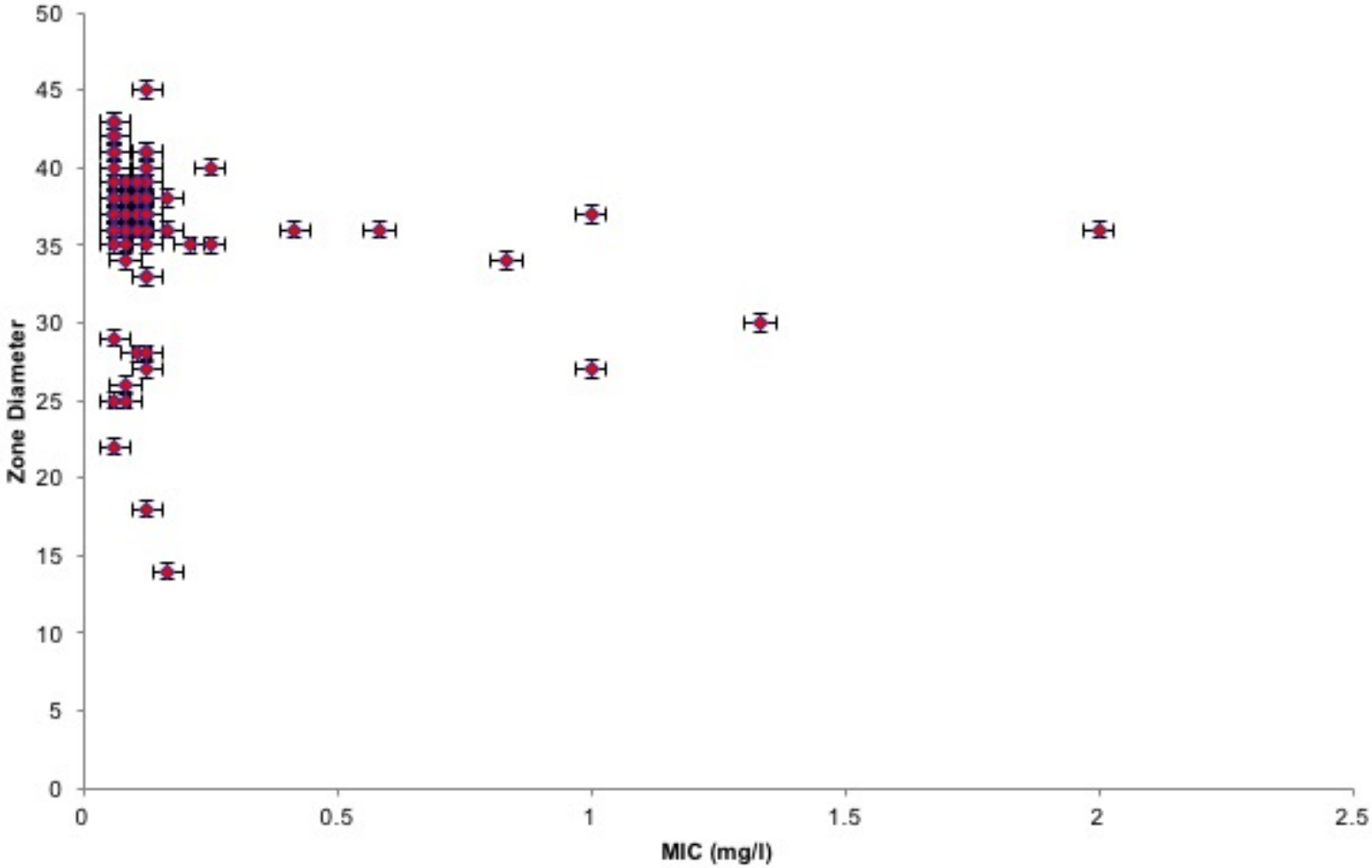

Figure 5: Scatter graph showing the average of the MIC replicates against ZD for Marbofloxacin for *E. coli* on Farm 2, showing standard deviations (blue squares represent the environmental samples; red diamonds represent samples taken from the bovine gut).

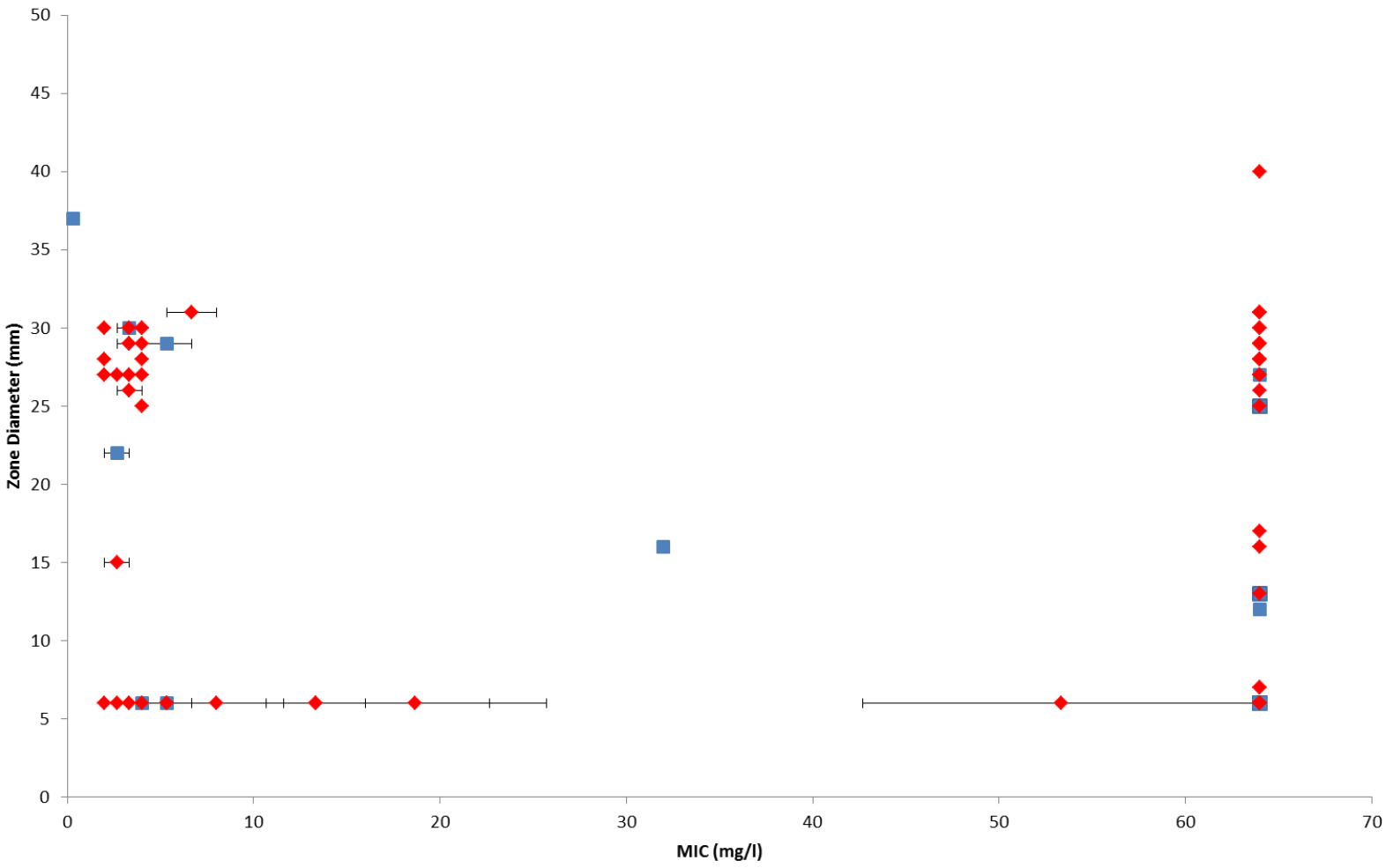

Figure 6: Scatter graph showing the average of the MIC replicates against ZD for Oxytetracycline for *E. coli* on Farm 2, showing standard deviations (blue squares represent the environmental samples; red diamonds represent samples taken from the bovine gut). Any data plotted at x=64mg/l represents organisms that hold an MIC that is greater than or equal to 64mg/l

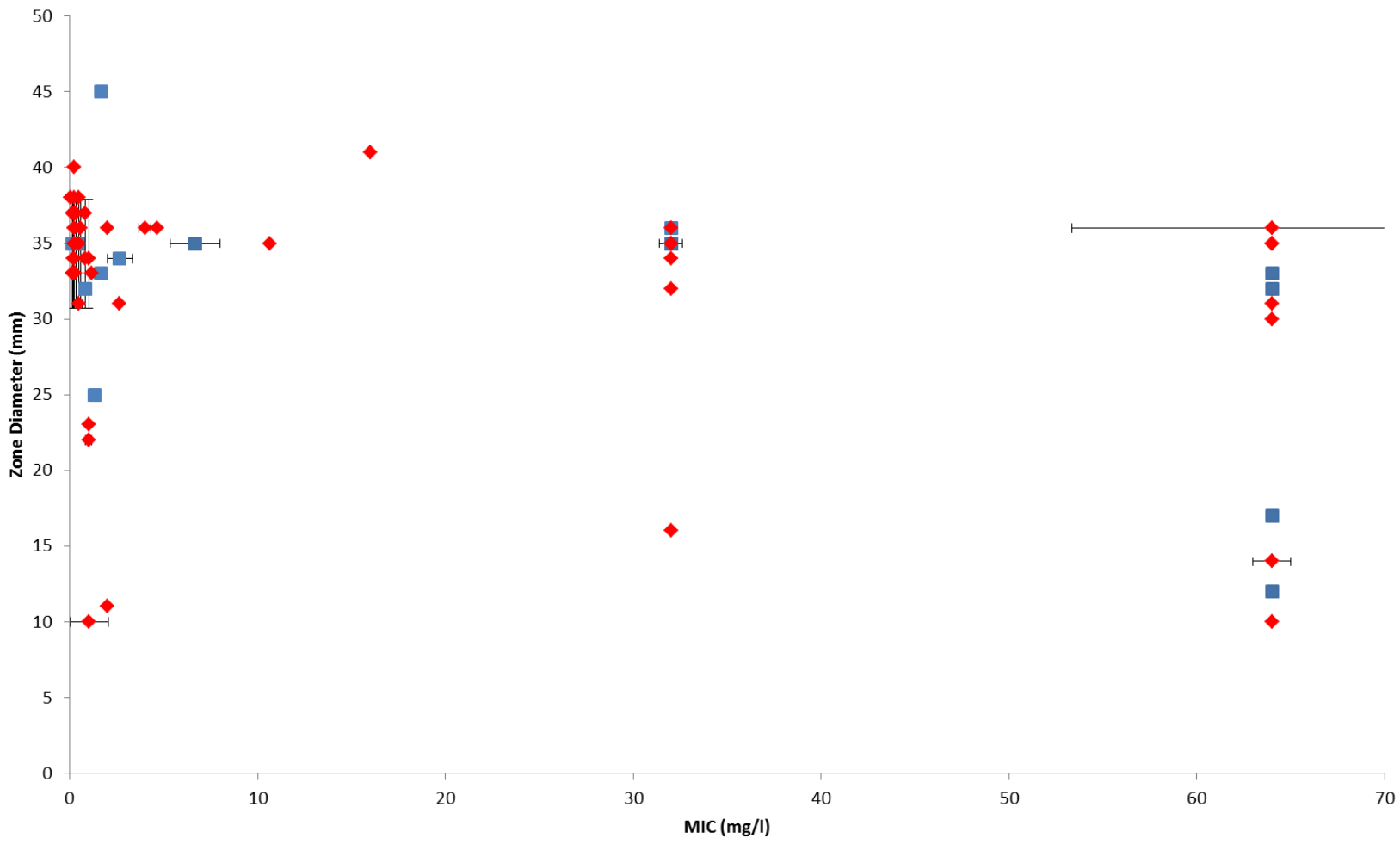

Figure 7: Scatter graph showing the average of the MIC replicates against ZD for Cefquinome for *E. coli* on Farm 2, showing standard deviations (blue squares represent the environmental samples; red diamonds represent samples taken from the bovine gut). Any data plotted at x=64mg/l represents organisms that hold an MIC that is greater than or equal to 64mg/l.

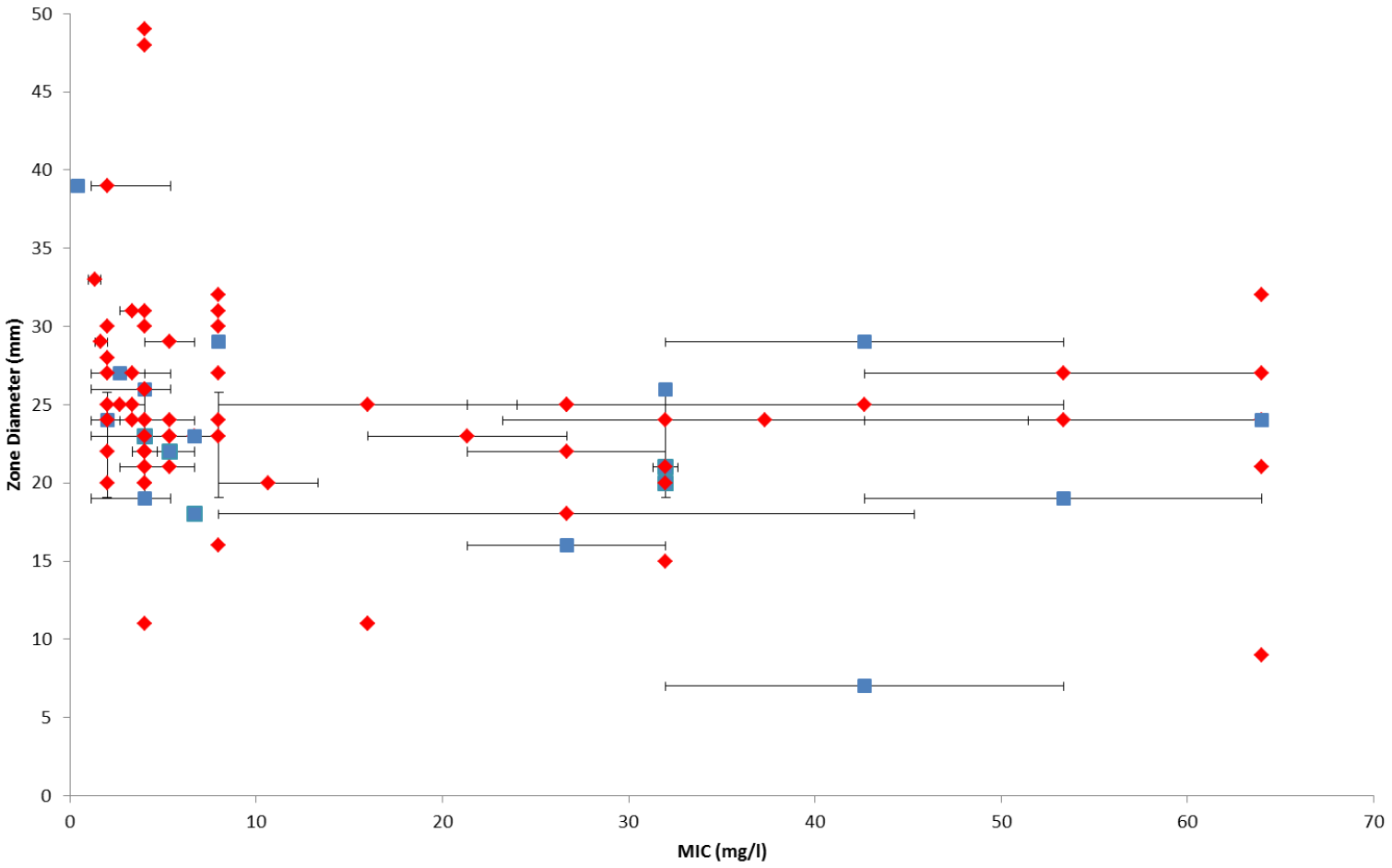

Figure 8: Scatter graph showing the average of the MIC replicated against ZD for Ampicillin for *E. coli* on Farm 2, showing standard deviations (blue square represent environmental samples; red diamonds represent samples taken from the bovine gut). Any data plotted at x=64mg/l represents organisms that hold an MIC that is greater than or equal to 64mg/l.

The results for Marbofloxacin (Figure 18) showed a cluster towards the far left of the graph. The highest MIC concentration was 1mg/l. All Zone Diameter (ZD) values were above 20mm. A ZD of 20mm was commonly deemed susceptible across most antibiotics according to EUCAST (2016) for *Enterobacteriacae*. This suggests that the majority *E. coli* in this study were susceptible to Marbofloxacin. The results for Marbofloxacin showed only one distinct cluster, unlike the other three antibiotics.

Oxytetracycline showed four distinct clusters (Figure 19). The four clusters can be seen at high MIC concentration values and at low MIC concentration values and at high and low ZD values. The largest cluster was seen at 64mg/l and 6mm. This is the opposite relationship than Marbofloxacin, which showed a cluster at lower MIC concentrations that were below 0.5mg/l and high ZD values. The other clusters also showed that some of the *E. coli* organisms were also susceptible to Oxytetracycline. Cefquinome also did not show one distinct cluster, however, the majority of results were towards the left of the scatter graph (Figure 20) where the organisms had low MIC values and a ZD value above 20mm. A second cluster is shown around 32mg/l for the MIC value and a ZD value above 20mm. The final small cluster formed at >32mg/l and low ZD values and this was also the cluster seen for Oxytetracycline. These clusters suggest the *E. coli* were both susceptible and resistant to Cefquinome and more strain variance was seen than with Oxytetracycline and Marbofloxacin. Ampicillin (Figure 21) showed three clusters, around 0-10mg/l, around 32mg/l and at 64mg/l. The majority like in the cluster towards the lower MIC concentrations. As with Cefquinome, *E. coli* show variance within the isolates where some have lower ZD values and higher MIC concentration suggesting these isolates have resistance and those organisms that show high ZD values and low MIC concentrations to show susceptibility. The variances found in these results are similar to that stated by EUCAST (2016), where the

majority or the MIC concentrations ranged from 1-8mg/l being the most common and higher MICs also stated. Organisms that have a higher ZD and low MIC represent little or no resistance. Likewise, lower ZD values and high MIC represent organisms that have higher resistance towards an antibiotic. For an organism to have either a high ZD and a high MIC, shows a potentially contradictory conclusion. Further research is needed to conclude what the relationship between ZD and MIC represents in this case

Within the data, organisms from the BG (red) and ENV (blue) were represented. No difference was seen between the two groups. This was not the case for the class resistance data or resistance that represented the species. This method could be used as an epidemiological tool for determining MICs and prevalence of resistance, environmental samples to represent the whole farm, with the herd included.

4.10 Single v Dual Target

Antibiotics that work as a dual target contain more than one antibiotic class. In this instance. Amoxicillin was combined with Clavalunic acid and Piperacillin was combined with Tazobactam. Table 20 shows the percentage of resistance found in the single target and dual target antibiotics.

Table 20: Table showing the percentage of bacteria showing resistance to these set antibiotics.

	Farm 1	**Farm 2**	**Farm 3**
Amoxicillin	84%	43%	66%
Amoxicillin-Clavulanic acid	20%	46%	39%
Piperacillin	8%	20%	15%
Piperacillin-Tazobactam	4%	34%	5%

The results do not show a direct relationship of resistance seen between single and dual target antibiotics, as the difference in percentage between the two varies in each example. Further studies are required to understand how dual target antibiotics can lower resistance, and if this is directly proportional to resistance seen against single target antibiotics.

4.0 Discussion

4.1 Antibiotic use and animal welfare

For cattle, the predominant use of antibiotics is for the control of mastitis in dry cows, where antibiotic treatment may be giving for clinical of sub clinical mastitis (Briyne *et al.,* 2016). Dry cow therapy has traditionally used intramammary antibiotic therapy immediately after the last lactation. It is possibly that the extent of the use of antimicrobials used to control mastitis could be reduced if there was a wider awareness and application of best practice guidelines, such as the responsible use of medicines in agriculture (RUMA, 2005). The use of antibiotics during lactation to control mastitis and to reduce pain and inflammation is recognised as essential on welfare grounds. This should be accompanied with a suitable prescribing practice and regular bacterial isolation ad sensitivity testing. Having good animal husbandry, biosecurity and milking hygiene will help minimise mastitis without potential overuse of antibiotics (Briyne *et al.,* 2016). The risks seen with the development of resistant bacteria can potentially be traced to intensive animal production, as well as mismanagement seen in nutrition, chemotherapy and housing conditions (Trevis *et al.,* 2006). Good animal welfare is not only achieved through eliminating pain and disease but also by proper management of animal welfare (Loor *et al.,* 2003). Poor animal welfare has commonly been used as the reason for antimicrobial resistance being found, however the farms involved in this study showed good animal welfare procedures ad yet high level of resistance were found.

This could potentially show that resistance may not come from mismanagement alone. Therefore further research is required to determine why high levels of resistance can be found, examples of which could be unknown. Further work is needed to determine what factors would case resistant organisms to survive in this environment.

4.2 Antibiotic classes used on farm

Section 3.2 showed the different antibiotics used on farm. There are only a few 1st and 2nd generation Cephalosporin approved worldwide for the treatment of mastitis infection, whilst Ceftiofur, a 3rd generation cephalosporin, and Cefquinome, a 4th generation cephalosporin, have been developed specifically for veterinary use (EMA, 1998). Cefquinome has been approved in several countries for the treatment of respiratory disease in cattle and swine, foot rot in cattle as well as mastitis in dairy herds (Hornish & Katarski, 2002). Ceftiofur and Cefquinome were used across all farms in this study. In 2012, the Food and Drug Administration's centre for veterinary medicine issued an order prohibiting certain used of cephalosporins in food producing animals. This was because cephalosporins are commonly used in treating human disease (FDA, 2014); cephalosporin was include in the CIA for the same reason. Although these restrictions are set, all generations of cephalosporin are still being used across farms and resistance towards them is seen. All farms used cephalosporins in this study and resistance was seen (Table 7,8 and 9).

Tetracyclines are commonly used in animal husbandry; in some food producing animal species they are the first line of therapy (EMA, 2013). Tetracyclines are the most commonly sold antimicrobial in veterinary medicine: in the UK 46% of the antibiotics used for food producing animals were tetracycline in 2010 (ESVAC, 2012). Because Tetracycline are

commonly used, they were one of the first antibiotic classes to show resistance in initial studies (Schwarz & Dancla, 2001). Several different mechanisms of tetracycline resistance were noted, with active efflux pumps and ribosome protection being the most prevalent (Schwarz & Dancla, 2001). Tigecycline is a form of Glycylcycline, which is a new antibiotic derived from tetracyclines, designed to overcome common mechanisms of tetracycline resistance. The two mechanisms are resistance mediated by acquired efflux pumps and/or ribosomal protection. Tigecycline is authorised only for human use, treating complicated skin and soft tissue infections (EMA, 2013). It is also authorised by the FDA (2006) for community-acquired pneumonia. Currently Tigecycline resistance has been mostly seen in human medicine and has not been recognised in veterinary medicine (EMA, 2013). A study by Ruzauskas (2009) showed only 2% Tigecycline resistance amongst isolates taken from cattle, swine and poultry. This study showed higher levels of resistance against Tigecycline, even though the antibiotic as not used. Further studies would determine whether this is an example of cross over resistance or class resistance, due to the large use of tetracycline across the three farms.

Carbapenems are not a commonly used antibiotic class for veterinary medicine in large animals (Dowling, 2013), which may be why little resistance was seen compared to other classes in this study. Ciprofloxacin was the only fluroquinolone being tested in this study. Fluroquinolones use in human medicine is commonly used and so agricultural use of this class is low to prevent the selection pressure for pathogens to develop resistance (McEwan & Fedoraka, 2002). Pencillins, specifically Amoxicillin is one of the older antibiotics available and sued in both human and veterinary medicine. This is unlike Gentamicin which is rarely used in cattle and more commonly used in human medicine. A study by Schroder *et al.,* (2002) showed that gentamicin had a lower prevalence of resistance in *E.coli* at 18.6% in

cattle; ampicillin was also low at 12%. The aim of investigating a wide range of antibiotics was to begin developing a clear understanding as to what possible resistance can be seen on farm.

4.3 Resistance in the BG and ENV

All three farms showed different resistance profiles in the isolates taken in the BG and ENV. Likewise different resistant profiles for different species isolatd were also seen across the three farms. All three farms were exposed to similar classes of antimicrobials and this was seen in the resistance profiels between Farm 1 and Farm 2 but could not be noted in Farm 3. Fluroquinolones were not prescribed on Farm 3 and resistance against the class was still seen (table 9). The reason for this could not be determined, and further studies into farm biosecurity and its potential effect are required to determine how this is possible.

The different resistant profiles from the isolates in the ENV could be due to the antibiotics being directly excreted through animal faeces and resistance in the bacteria themselves being excreted. The isolates in the ENV come from the different cattle who are being exposed to different antibiotics. After administration of an antibiotic, a significant fraction is excreted in either the parent form or their active metabolite)Zhou *et al.,* 2013). The percentage that this would occur at differs among different antibiotics depending on their stability. Antibiotic residues have been reported in the surrounding environment on farms, where the concentration can range from 26-83µg/g in soils (Li *et al.,* 2011). When antibiotics are introduced in this way, they could potentially cause development of antibiotic resistance through exerting a selective pressure on the microbial community (Pruden *et al.,* 2006). This supports the different profiles of resistance found in the ENV in this study, and where the

percentage of resistance against certain antibiotic classes of antibiotic were higher in the ENV than in the BG samples. *Pseudomonas* sp. On Farm 3 (table 9) is an example of higher resistance seen in the ENV than in the BG. It is possible that as the antibiotic is excreted from the cow into the environment, selective pressures cause the surrounding bacteria in the ENV to become resistant in order to survive against the introduction of new antibiotics into its surroundings. As most antibiotics are water soluble, many that are used in the cattle industry are poorly absorbed in the bovine gut resulting in up to 90% of the compound being excreted before it can be metabolised (Zhao *et al.,* 2010). Antimicrobials are designed to overcome this factor by having physiochemical properties that allow absorption, distribution and bioavailability within the bovine gut (Dowling, 2013). However, excretion of the compound can still be observed. Different antibiotics were excreted into the same environment and potentially allowing the bacteria to pick up or develop multiple resistance mechanisms from the surroundings. This is observed in section 3.3, where bacteria with resistance against multiple classes, were more widely seen in the ENV samples than in the BG samples. It was seen that an organism can have resistance to more than one class of antibiotic.

It is known that the addition of antimicrobials into the gut of any species will eliminate antibiotic sensitive bacteria and allow the populations density of the resistant bacteria to increase (Campagnolo *et al.,* 2002). Therefore, when samples were taken from faecal matter representing the BG, it is likely that multiple resistant organisms can be present, as shown in section 3.3. Both the ENV and BG provide the opportunity for organisms carrying resistance to survive over antibiotic sensitive bacteria. However with more multi resistance bacteria being found in the ENV, it is possible that the bacteria in the environment are able to better hold resistance to multiple classes of antibiotic that in the BG. This may be that the properties

of the surroundings in the ENV favour this more than in the BG and further studies are required to determine the reason for this. There are different physiological and chemical properties within the two different environments of the BG and END. The bacteria in the BG are not exposed to weather conditions and likewise the bacteria in the ENV are not exposed to the conditions found in the BG: different levels of acidity as well as an immune system found in the gut eco system. This makes it difficult to compare the two sets of data. This is because both are facing different selective pressures and so make them directly incomparable. The two environments hold differences in the selective pressures and exposure; yet still interact with one another causing a potential movement of organisms between the two. Animal faeces can be found in the environment and cattle can pick up resistant organisms from contact within the environment. These factors could suggest that the movement of organisms overcome the factors influencing the individual environment.

4.4 Determining ECOFFs

EUCAST defines the Epidemiological cut-off value (ECOFF) as an MIC value that identifies the upper limit of the wild type (WT) population. The ECOFF value can be determined by visual inspection or through statistical calculation (Turnbridge *et al.,* 2006). An example of a visual inspection is the commonly used “eyeball method” to determine cut offs. While this method can be applied as "estimation”, it can lack reproductively (Valsesia *et al*., 2015). Nonetheless, the method was successfully used by Keller *et al*. (2015) and showed ECOFFS can be determined through finding the frequency of MIC values. This method was used for determining the ECOFFs in the isolates from this study.

The distribution analysis shows variation between the antibiotics. Marbofloxacin (figure 5 showed a trend of low MIC concentrations associated with high ZD values (Appendix D), the ECOFF was low at 0.5mg/l. Likewise for Oxytetracycline (figure 6), the

ECOFF was 16mg/l and the cluster where there were high MICs and low ZDs are represented in the orange bars for suggestive resistant isolate. Where there was also a cluster towards the other end of the values, part of a normal distribution cure was seen and so the ECOFF was determined. Ampicillin (figure 8) has the same ECOFF value as Oxytetracycline, however the prevalence of suggestive resistant strains is much smaller and a whole normal distribution curve can be seen. The ECOFF for Ampicillin according to EUCAST (2016) is 8mg/l. It is possible that the ECOFF is different due to the fact that EUCAST works with human samples and the strains studied here have come from cattle. Oxytetracycline shows a similar profile to that of Ampicillin. Cefquinome's ECOFF value of 8mg/l falls in the middle of the ECOFFs found in this study (Figure 7. Like Ampicillin, a whole normal distribution curve can be noted, although it is weighted to the left, similar to Marbofloxacin. Cefquinome and Ampicillin hold similarities, but the distribution for Cefquinome is shifted far to the left where there are lower MIC concentrations, makes its less comparable that Oxytetracycline.

4.5 Human and veterinary antibiotics

Antibiotics designed for human use may not have the same attributes that are required for effective dosing and treatment in animal healthcare. Different antibiotics in one class can hold different chemical elements changing their efficacy. Different generations seen in Cephalosporin and the addition of Clavulanic acid to Amoxicillin are examples of where there are differences seen within the group of antibiotics in one class. Cephalosporin is generally stable against the plasmid mediated β-lactamases produced by both Gram positive and Gram negative bacteria. Several types of β-lactamases produced may be mediated by either plasmid of chromosomally and may hydrolyse either/both penicillin and cephalosporin, causing cross resistance. 2nd and 3rd generation Cephalosporin have a greater reaction against

Gram negative β-lactamases compared to 1st generation (Boothe, 2015). The addition of clavulanic acid to Amoxicillin acts as a β-lactamase inhibitor to improve its treatment against resistant bacteria. The disadvantage of developing antibiotics this way to improve efficacy is the limit of additions to its analogue which can be a single chemical core or ones which can counteract resistance mechanisms (Coates et al., 2011). Furthermore, antibiotic concentrations can be very different in different tissues and organs, which complicate the determination of therapeutic thresholds. Likewise, antibiotics used in humans and cattle cannot be deemed to have the same effects due to different tissues, size and organs and pharmacokinetics (Rodloff et al., 2008).

Intermediate values, according to EUCAST (2016) were commonly seen against the human antibiotics tested in this study. Antibiotics with this intermediate value were Gentamicin and Tigecycline. The use of intermediate values could be used as a buffer to prevent the fluctuating interpretation of the results that are susceptible at some points and resistant at others merely due to minor, random variation in test conditions (Rodloff *et al*., 2008). The classification of "intermediate" also represents antibiotics that are effective against one organism in one target site, for example the urinary tract, but is not as effective in another target site. This is due to the pharmacodynamics and pharmacokinetics of the antibiotic (Rodloff *et al.,* 2008).

4.6 Cross resistance

All antibiotics used in veterinary medicine are the same or closely related to antimicrobials used in human medicine which will induce cross resistance (Ungemach *et al.,* 2006). When looking more closely, molecular mechanisms could provide insight as to how resistance can carry over across species and antibiotic classes. Molecular analysis of antibiotic resistant genes, plasmids and transposons has demonstrated identical elements found in humans and

animals. The mobile genetic elements can move from animals to humans through the environment (Ungemach *et al*., 2006). For example, bacteria are released into the environment through animal faeces, carrying resistance with it. Specific food items, water and direct contact can spread the bacteria from animal's microflora to human microflora (Tueber, 2001).

It is also possible that cross resistance is seen through the different classes having the same resistant mechanisms. Most antibiotics are targeted at intracellular processes, and must be able to penetrate the bacterial cell envelope. In particular, the outer membrane of Gram – negative bacteria provide a formidable barrier that must be overcome. There are essentially two pathways that antibiotics can take through the outer membrane: a lipid-mediated pathway for hydrophobic antibiotics and general diffusion pores for hydrophilic antibiotics (Delcour, 2009). Aminoglycosides, older Tetracyclines (including Oxytetracycline) and β-lactams are hydrophilic and so can use simple diffusion to enter the cell. Modification in the outer membrane, like changing the lipid and/or porin composition, will influence the antibiotic's ability to enter the cell (Delcour, 2008). This means that the organism is able to be resistant to different classes of antibiotic with only one mechanism.

Efflux pumps alongside low permeability can cause synergy and multidrug resistance, especially with *Pseudomonas* sp. (Schweizer et al., 2003). A documented mechanism to reduce outer membrane permeability was to lower porin expression through environmental factors or mutations (Delcour, 2009). For example, the uptake of tetracycline by *E. coli* cells were shown to be reduced in a mutant lacking OmpF expression confirming that tetracycline use the pathway of diffusions through porins based on increase resistance in mutants with decreased OmpF expression (Cohen et al., 1988). The upregulation of the marA gene leads to increased levels of sRNA micF which inhibits translation of OmpF RNA and this decreased expression leads to increased resistance (Delcour, 2009). This is an example of where gene

mediated antibiotic resistance is used and so in this case cells become insensitive to a variety of hydrophilic antibiotics as listed above.

4.7 Single target versus dual target antibiotics

Dual target antibiotics were created to improve the efficacy of the treatment. Amoxicillin is a first choice narrow-spectrum antibiotic; its combination with Clavulanic Acid is suggested to the treatment of patients with suspected Gram negative infections caused by β-lactamases-producing organisms. Clavalunate is able to inactivate β-lactamases, which prevents penicillin degradation and consequently antimicrobial resistance (Salvo *et al*., 2007). In this study Amoxicillin, Piperacillin, Amoxicillin-clavulanic acid, Piperacillin-Tazobactam was used as examples to see the difference between single and dual target antibiotics. Below show the percentage of bacteria resistant to these antibiotics.

The combination of clavulanic acid should make for a better antimicrobial that can work against strains which would be resistant to amoxicillin alone. Tazobactam would have a similar effect. As β-lactamase production by both Gram-positive and Gram negative pathogens become a clinically relevant issue, efforts were made to develop an orally bioavailable broad spectrum penicillin that was also effective against these strains, resulting in the combination of amoxicillin and clavulanic acid, the combination with clavulanic acid increases the effect of amoxicillin and inhibits the development of resistance in β-lactamases (Kaur *et al*., 2011). This is seen in the farms, except for with Farm 2, the only farm that uses Amoxicillin on its own as well as with Clavulanic acid.

4.8 MIC study

The need for clinical breakpoints for veterinary pathogens is needed (Supre *et al.,* 2014). The need for accurate clinical breakpoints for veterinary pathogens is high (Supre *et al*., 2014). Oxytetracycline, Marbofloxacin and Cefquinome are common antimicrobials in veterinary healthcare, their clinical breakpoints are yet to be determined. A breakpoint setting requires integrated knowledge of Wild Type (WT) distribution of MICs and an assessment of pharmacodynamics and pharmacokinetics (Turnbridge *et al.,* 2011). This can be achieved by performing a population study focussing on the distribution of inhibition zones diameters on the condition that a large number of strains are tested (Supre *et al*., 2014). This study begins to examine this by comparing the ZD with the MICs and looking at WT distribution with the focus driven towards determining ECOFFs. ECOFF values help determine which strains are part of the WT population and which are not. This information alongside breakpoints and MICs can collectively start to create an analysis are to what resistance can be seen. ECOFFs are often unavailable for studying veterinary pathogens by disc diffusion or other methods, and in addition, ECOFFs and clinical breakpoints and not necessarily linked (Schwarz, 2010). It is important to note that this information alone, and the data collected and shown in secntion 3.4, cannot conclusively determine resistance profiles but it is the first steps in determining breakpoints are providing food distribution analysis of the population.

MIC variation between the four antibiotics shows strain variance to susceptibility of antibiotics. The Bacteria showed most susceptibility to Marbofloxacin with the lowest MIC values, Oxytetracycline saw much higher MICs –of greater than 32mg/l. For Cefquinome and Ampicillin there was more variation with MICs falling towards lower and high concentrations. Where this potentially begins to show differences in the antibiotics, MIC distribution is not enough to determine susceptibility or resistance of organisms. This is why using ZD values adjoined to MIC for comparison is important to show potential clinical

success or failure. Clinical testing enables analysis of the relationship between MIC values of the infectious organisms and the clinical and microbiological results of treatment (Pai *et al*., 2007).

MIC values are not comparable against other MIC values; the concentration only represents the MIC for that particular patient, antibiotic and origin (patient or animal). However, when using the ZD and MIC together it is able to compare the data. MIC values represent inhibition of growth of a strain, similar to a ZD, which represents the area of media where bacteria are unable to grow due to the presence of antibiotic. In other words, the drug presence is potentially impeding the growth of the organism. Therefore, low MIC values indicate that less drug is required for inhibiting the growth of the organism. This means those *E. coli* strains are potentially more susceptible to the antibiotic. A clear example of this is that data seen with Marbofloxacin. The MIC did not exceed 1mg/l (Figure 5). Likewise, high ZD values show that more organisms perish when exposed to this concentration. A high ZD value and low MIC value are associated with each other.

Marbofloxacin is a third generation cephalosporin, seen as one of the critically important antimicrobials not to be used to stop resistance. The lack of use of the antimicrobials is echoed in the results for the MIC and ZD. The ECOFF value for Marbofloxacin is low at 0.5mg/l and the ZD values were generally quite high. These results show a population that is highly susceptible to the antimicrobial with little variation in the species population. This supports the notion that lack of exposure to an antimicrobial will mean the organism may have either lost the resistance genes required to survive in the current environmental setting or not have developed the resistance initially. Epidemiological studies show a direct

correlation between overuse of antimicrobials and emergence of resistance (Ventola, 2015). Therefore, the opposite should also be true, and this evidence supports this.

Likewise, high MIC concentrations an low ZD values will be associated with one another, but this time would show potentially resistant strains. This is seen with Oxytetracycline (Figure 6) , where multiple strains of *E. coli* had an MIC concentration >32mg/l and a ZD that was less than 9mm. Further tests are needed at higher concentrations to determine the exact MIC value. This is similar to the some of the results for Ampicillin and Cefquinome yet the same cannot be said for Marbofloxacin.

Oxytetracycline and Ampicillin showed the same ECOFF at 16mg/l (figure 6 and 8 respectively). This was higher for Ampicillin that suggested by EUCAST (2016) at 8mg/l (figure 8) However, it is important to consider that this is for strains taken from humans and not from cattle. Whilst these two antibiotics have the same values, many more strains fell above this value and so would be considered part of the WT population and instead show resistance. This is also supported by the high number of strains having a ZD value lower than 9mm and many strains forming a cluster at the bottom right of the graph; having both high MIC values and low ZD.

Some strains showed high ZD values and also high MICs, also some strains showed low values for both. To have these set of values contradict what the values represent. If a sample is to have a low MIC values and so show high susceptibility, it is not then expected to have a low ZD value representative of a resistant strain. While there are strains that show this relationship, they still belong amongst the WT population, as shown by the ECOFF value. This is mostly seen with Cefquinome with data that have low MICs and low ZD values. This

suggests that the strains are showing variance in the sensitivity towards the antibiotic, yet no resistance can be detected with these values for the strain. Strains that have a higher MIC than the ECOFF value have a low ZD and high MIC and can suggest potential resistance. These types of results help distinguish WT from non-WT and thereby recognise potential resistance in a clinical practice (Bruin *et al.,* 2013). Therefore, although the breakpoints need to determine strains that are resistant are not available, most potentially seen with Oxytetracycline, these results can determine a potential resistance profile in a clinical practice. Another explanation for strains with these values is the presence of small colony variants. Small colony variants are a slow growing subpopulation of bacteria with distinctive phenotypic and pathogenic traits. Clinically, small colony variants are better able to persist within the mammalian cell population and less susceptible to antibiotics than their wild type counterparts, and can cause latent or recurrent infections on emergence from protective environments of the host cell (Proctor *et al*., 2006). This can explain why bacteria are showing counteractive values, they have the ability to hold high MIC values but when tested ZD; they have large diameters showing large susceptibility. The bacteria show different phenotypic traits within the WT population.

No differences were seen between the samples from the BG and ENV. This suggests that if further surveillance was to be conducted, through choosing an environmental location that represented the microbial activity of the whole farm. Surveillance is the main method for understanding the spread of resistance and monitoring how resistance in pathogens rises and falls. By showing that samples for data such as the results shown here can be obtained by using environmental samples will allow for a simpler method that can be as effective. Further research is needed to depict which environmental location can represent the herd and farm profile.

4.9 Statistical Analysis

The results of the statistical analysis did not show enough statistical signifagance which meant only assumptions could be made for the data. Statistical signifigance of the data could be achieved by increasing the population size of the data. This would be done through conducting more surveillance studies over longer periods of time.

5.0 Conclusion

Surveillance of resistance and antibiotic use is one of the main methods that can be used to help tackle the issue of antibiotic resistance. Information obtained from antimicrobial surveillance is important for establishing trends in pathogen antimicrobial resistance and for identifying emerging pathogens across all environments (Masterton, 2008).

The resistance profiles have shown what levels of resistance can be found at one point during the year for the herd and surrounding environment. There were clear differences between the samples taken from the BG and from the ENV within one farm and then again between all three farms enrolled. Differences within the targeted species were also seen and species on one farm would not have the same profile as found on the other two. What was evident within the results, however, were that high levels of resistance are present both on farm and in the herd themselves. The MIC results started to show a pattern between the MIC and ZD and that it is possible to determine a resistant trend without the use of set clinical breakpoints. For example, there was a high level of resistance towards Oxytetracycline, with very small ZD values and high MIC concentration which when correlated together, highlighted the difference between mutant and WT strains and the prevalence of resistance against that organism. Meanwhile, the exact opposite was able to be determined with Marbofloxacin.

The use of prescription data, shown in 3.1, gave a small insight as to how prescription patterns can be linked to trends into resistance but more research is needed to determine how antibiotics react to one another as well as cause resistance as it becomes evident that herds in the cattle industry can be regularly exposed to different antibiotics at differing doses. This exposure pattern differs greatly to that seen in human healthcare. Human healthcare allows precise recording of antibiotic use and this is not seen in animal healthcare. This should be introduced to allow better understanding of the spread of resistance organisms. The inability to identify trends in exposure to antibiotics and resistance stops the opportunity for a better understanding as to how antibiotic use affects resistance in food –producing animals, and so stops relating the research to its effect on human healthcare. This study has shown the important and relevance of One Health. The One Health initiative aims to equalise the work and research effort to combine and understand relevant issues between specialists. The antimicrobial resistance that is seen in animal and human healthcare support the movement of organisms from on to the other and also impact the environment. Combining the efforts and research can improve surveillance of the resistant organisms: understanding their origin and evolution and so finding how to stop their spread of infection.

5.1 Future work

This study can only be considered as a snapshot of the profiles of resistance that can be found on farm. It is clear that routine monitoring of antibiotic resistance levels on farm is necessary. Relating these findings to antibiotic use on farm can help monitor antibiotic resistance profile in the healthy herd and so be of us in limiting the rise of resistant bacterial strains on farm locations and in individual animals. The overall aim with regard to

antimicrobial resistance is to determine effective use of antibiotics, to ensure to animal welfare is still kept to a high level and antibiotics are used prudently.

Longitudinal studies will also help research to see how these resistance profiles change over time, and whether the movement of resistant organisms can be monitored and possibly predicted in the future. By understanding the movement of resistance on farm, a better understanding as to how resistance moves from animal to human could also be achieved. This means that the risks seen in human healthcare will also potentially become less. At the moment the rise of resistance and uncertainty of resistance origin has dictated how antibiotics are used. This leaves multiple classes of antibiotics to no longer be available of use for animal healthcare due to the limited treatment options in human healthcare. If these studies can show how resistance evolves over time it will allow better understanding of the effects of antibiotic use in a lot more detail.

This study could have been further improved by als enrolling beef farms into the study. This would allow a comparison between the different types of farming among the cattle industry. Enrolling a beef farm will also allow species variation, as different species of cattle are used between the beef and dairy industry, and a potential increase in the number of organisms isolated and identified. The MIC study could further improve by looking at the MICs for all species across the three farms. Looking at the MIC variation between the different species and farm will also add to determining clinical breakpoints for those set antibiotics. MICs are not a set value and can change over a few months, tracking the changes will help to understand how susceptibility to the antibiotic changes. Further work with the environmental samples can determine how weather affects the resistance found in the environment.

Many countries have seen a pressure to decrease the number of antibiotics prescribed. The movement from DCT to SDCT is one example of this. However, antibiotic treatment is still effective; it is only through misuse that it becomes ineffective. Antibiotic treatment should still be seen as a form of therapy and prudent use of antimicrobials should be welcomed. This can be achieved by recognising those that follow guidelines for correct antibiotic use. A proposed charter would allow recognition for correct antimicrobial use while increasing public awareness of the issue regarding antimicrobial resistance. Those who qualify to be awarded the charter that shows responsible antibiotic use will be recognised as a business that used the antibiotics correctly and so are helping to improve the issue of resistance. The Responsible Antibiotic Use Charter (RUAC) can be awarded after a series of steps are achieved, for example a workshop teaching farmers about what the different antibiotics are and importance of correct dosing and treatment. Farms involved in the RUAC can be part of the longitudinal study the looks at resistance over time across different farm locations in different geographical areas. Conducting this study on a much larger scale will improve the efficacy of the study and allow a better understanding of on farm resistance.

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