RESEARCH REPORT
PORK SAFETY

TITLE: FURTHER CHARACTERIZATION OF SALMONELLA AND CAMPYLOBACTER ISOLATES FROM NARMS STUDY**: NPB#13-258**

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INDUSTRY SUMMARY:

The primary objective of this research was to examine the potential impact of normal lairage practices on Salmonella, Campylobacter and E. coli antimicrobial resistance patterns in swine following evisceration in an abattoir environment. Fecal samples were collected upon arrival at the abattoir and later from the cecum within cohort groups. Also non-cohort pig and environmental samples were collected from the abattoir for comparison purposes. Isolates of Salmonella, Campylobacter, and E. coli tested for resistance to a panel of antimicrobials as well as characterized by serotype, phenotype and genetic patterns as appropriate. The results indicate a wide variety of antimicrobial resistance and other patterns that were not consistent between arrival and post slaughter samples. The conclusion is that normal lairage practices do impact bacterial populations in the gut in a manner which alters their antimicrobial resistance profiles. Thus resulting antimicrobial resistance patterns from samples collected post slaughter are not useful measures for monitoring on-farm resistance since they are even farther removed from the source then arrival samples. These results will aid the swine industry and inform research and regulatory communities as they seek to develop and enhance surveillance systems which reflect the impact of on-farm antimicrobial use.

KEYWORDS: SALMONELLA, CAMPYLOBACTER, E. COLI, ANTIMICROBIAL RESISTANCE, ABATTOIR SAMPLES

SCIENTIFIC ABSTRACT

There is a public health concern related to the use of antimicrobials in agriculture and in response the FDA has taken certain actions including the voluntary phase out of the use of antimicrobials for production purposes. Surveillance systems such as National Antimicrobial Resistance Monitoring System (NARMS) have been in place for many years. While NARMS does monitors trends in antimicrobial resistance among foodborne bacteria from humans, retail meats, and animals, it was not designed to correlate on-farm antimicrobial use practices with observed antimicrobial resistance trends. Therefore, a surveillance system which can correlate antimicrobial resistance patterns to antimicrobial use would be very helpful in assessing the impact of FDA judicious antimicrobial use policies. Since USDA's Food Safety and Inspection Service does collect samples at abattoirs for microbiological and antimicrobial susceptibility testing, it seems logical to explore the possibility of using these results as a means to evaluate antimicrobial resistance patterns from known sources with antimicrobial use practices. However, studies have shown changes in Salmonella population patterns during transport to and lairage within abattoirs which would affect the relationship between antimicrobial resistance patterns and antimicrobial use practices seen from samples collected following slaughter. Therefore, an in-depth comparison of

antimicrobial resistance patterns of Salmonella, Campylobacter and E. coli isolates collected from pigs at different points of slaughter would be useful to determine if abattoir samples are appropriate for monitoring the impact of changes in on-farm antimicrobial use practices. In this study fecal samples were collected upon arrival at the abattoir and later from the cecum following evisceration within cohort groups. Also non-cohort pig and environmental samples were collected from the abattoir for comparison purposes. Isolates of Salmonella, Campylobacter, and E. coli tested for resistance to a panel of antimicrobials as well as characterized by serotype, phenotype and genetic patterns as appropriate. A total of 358 pigs were sampled (arrival, n=139; post-slaughter, n=140, lairage environment, n=79). For Salmonella, 1163 isolates were collected, serotyped, and tested for antimicrobial susceptibility to a panel of drugs. Similarly 303 Campylobacter and 703 E. coli isolates were collected and tested. The results suggest that samples collected post-slaughter are not reflective of antimicrobial resistance patterns observed at arrival for any of these organisms. Therefore, if patterns at arrival are not reflected by post-slaughter partners, this suggests sample collected post-slaughter are not useful as measures for monitoring on-farm resistance, as on-farm resistance is even more distal geographically and temporally then arrival samples. These results are important to the swine industry as well as researchers and regulators and they explore options and make decisions regarding implementation of appropriate antimicrobial resistance surveillance systems to meet the needs of changing antimicrobial use practices.

INTRODUCTION

The National Antimicrobial Resistance Monitoring System (NARMS) has been in place for nearly 20 years which monitors the trends in antimicrobial resistance (AR) among foodborne bacteria from humans, retail meats, and animals. A primary function of NARMS is to serve the Food and Drug Administration (FDA) as a post-approval safety monitoring system for food animal antibiotics. Animal isolates available for testing in the NARMS have come several sources but principally from animal samples collected at slaughter through the United Stated Department of Agriculture's Food Safety and Inspection Service (USDA FSIS). Additionally the animal bacteria targeted by NARMS include foodborne pathogens such as Salmonella, Campylobacter, and Escherichia coli. There have been growing public health concerns related to AR in foodborne pathogens and the contribution of on-farm antimicrobial use in the AR for these organisms. Part of the FDA strategy to combat AR has been to promote judicious use of medically important antimicrobial drugs in food animals by working with industry to phase out the use of these drugs for production purposes (Guidance for Industry #209: The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals, 2012). The need to assess the effect of FDA efforts with this strategy has been recognized and the question of whether samples tested by NARMS would provide insight on the effect of phasing out drugs for production purposes on AR has been asked. Previous studies have shown changes in Salmonella prevalence and serotype patterns during transport to and lairage within abattoirs (Hurd *et al.*, 2001; Larsen *et al.*, 2004; Rostagno *et al.*, 2005; Wang *et al.*, 2010) which suggest that the stress of transport and lairage and potential exposure to other Salmonella prior to slaughter alters Salmonella colonization patterns. One would expect that this alteration of would also effect the AR patterns found in samples collected at the farm versus at some point in the slaughterhouse. Indeed, some longitudinal studies indicate that the slaughterhouse environment has considerable effect on carcass contamination with Salmonella (Kich *et al.*, 2011; Keelara *et al.*, 2013). Yet these studies present evidence that some Salmonella AR patterns in carcass samples reflect persistence from the farm. Less has been done to determine if possible farm-to-slaughter relationships with Campylobacter and E. coli AR patterns exist. Therefore, an in-depth comparison of AR patterns of Salmonella, Campylobacter and E. coli isolates collected from pigs at different points of slaughter (i.e. initial arrival and post evisceration) with the same cohort of pigs is needed to address the question of whether current monitoring systems could be used to assess on-farm antimicrobial use patterns.

OBJECTIVES

The specific objectives of this study using cecal populations as the determinate measure were: (1) to examine the potential impact of normal lairage practices on Salmonella, Campylobacter, and E. coli antimicrobial resistance patterns post evisceration; (2) to determine differences in Salmonella, Campylobacter and E. coli populations based on prevalence, serotype, molecular, or phenotypic pattern changes post evisceration.

METHODS AND MATERIALS

Study design

The study design is an observational quasi-experiment. Pigs were sampled upon arrival at the abattoir and then pigs from the same group of animals were sampled again at slaughter. The term quasi experiment is poorly defined but in human health the term has been used for studies designed to assess the impact of laws that apply to an entire group, such a motorbike helmet or seatbelt laws. Such studies have no parallel control group but compare the prevalence of an outcome at two points. Here we are comparing characteristics (serotype prevalence, antimicrobial resistance patterns, PFGE patterns, E. coli phenotypic patterns) at two time points with the intervention between the time points being exposure to the abattoir environment.

Variables

The exposure of interest was the abattoir environment, as the hypothesis was that the abattoir environment and all that it entails include lairage, cross contamination and the slaughter process would result in changes in the outcomes of interest. The primary outcomes of interest were Salmonella prevalence, serotype prevalence, and antimicrobial resistance patterns overall, by serotype and by sample source. In addition, the prevalence by sample source, antimicrobial resistance pattern by sample source, and PFGE pattern by sample source of Campylobacter and phenotype and antimicrobial resistance patterns overall and by sample source of E. coli were secondary outcomes of interest.

Setting and Participants

The samples were collected from a large, Midwestern US abattoir, which processes approximately 18,000 finishing pigs daily. On each collection day truckload lots (150+ hogs) arriving at the abattoir were selected by convenience from two farms. Eligible truckloads were direct from the farm and of US origin non-export, conventional hogs. After identifying the pen locations where pigs would be placed for lairage rest, the sampler(s) collected two fecal samples per pen (for a total of 4 samples out of 10) prior to pen loading [if possible]. Then the sampler(s) took 6 additional samples from other pens or holding areas to get a distributed sampling pattern. The sampler(s) attempted to follow each sample period with the same processes. These samples are referred to as lairage pig samples and represent the Salmonella diversity in the lairage.

Once pens were loaded, the sampler(s) collected 10 fecal swabs from recently voided manure pats as quickly as possible. Random selection approach was employed. All fecal samples were collected from the center of the pat to avoid contamination or drying. The samples were stored in ice packed coolers while waiting for the cecal collection to be completed. These samples are referred to as arrival samples and may represent the Salmonella from the farm as well as what could have been obtained from a contaminated truck.

When the lairage rest was completed (minimum of 2 hours) each lot of animals was processed by the plant as a single group (normally groups at the participating processor are mixed together) with a gap in between test lots to facilitate lot differentiation. The sampler(s) watched the carcasses at an access point to spot the correct lots. After a time period indicated by the processor to equal the time required for animals to travel from the access point to the sample collection point, the sampler(s) removed 10 ceca from the line. These ceca were immediately placed in plastic bags and in carts to minimize contamination. Cecal samples were collected by clipping the cecal tip and expressing contents into a 50 ml capped tube. New scissors were used for each collection and the sampler changed gloves each time. The sampler(s) then replaced the viscera back onto the line for further processing. When sample collection was completed, the cecal samples were added to the ice pack cooler and the samples were transported directly back to Ames and submitted to ISU VDL.

Data sources/ measurement

Sample handling in the laboratory

For Salmonella and Campylobacter culture, samples were set up in the appropriate media upon receipt at the laboratory.

For E. coli, samples received on 2013-09-03 were set up upon receipt at the ISU VDL. Subsequent samples were sent frozen or fresh on ice packs to USDA ARS BEAR Research Unit, Athens, GA.

Sample identification

Each individual sample was assigned a sample ID number and a descriptor ID number (lot) for each group. The sample ID number is 6 digits. The 1st two digits indicate the pig group and are sequential i.e. 1st group is 10xxxx, 2nd is 11xxxx, 3rd is 12xxxx etc. The second two digits indicate sample type xx01xx is always initial fecals, xx02xx is always cecals and xx03xx is always environmental fecals. The last two digits indicate collection order xxxx01-n (max 99). The lot ID is 4 digits and was assigned by the plant when the samples were pig groups and created by the collector when the samples were lairage pig samples. This number is connected to collection characteristics such as group ID and sampling location. The sample ID number was maintained throughout testing and a relational database was built to combine the information.

Salmonella culture, isolation and identification

Salmonella culture and isolation was performed as previously described (Standardization, 2007) with some exceptions. Briefly, approximately 10 gram samples were pre-enriched in buffered peptone water (BPW) at 1/10 dilution and incubated at 35 °C for 24h. Subsequently a 0.1 ml aliquot BPW culture was transferred to a tube containing 10 ml of Rappaport Vassiliadis (RV) broth and 1ml of the BPW culture to Tetrathionate-Hajna (TET) broth. The inoculated RV and TET broth were incubated at 41.5 °C for 24 h. After incubation aliquots of RV and TET culture were inoculated onto selective media (XLT4 agar and Brilliant Green with Novobiocin (BGN)) agar and were incubated at 35 °C for 24 h. For confirmation, one colony considered being typical or suspect for Salmonella was selected from each enrichment/selective agar combination: RV XLT4, RV BG, HJ XLT4, HJ BG. If growth was not visible on all plates, multiple colonies could be selected from same plate to achieve goal of at least 4 colonies per sample. The selected colonies were streaked onto the surface of Hekton-Enteric (HE) agar plates in a manner, which allowed well-isolated colonies to develop. These plates were incubated at 37 °C for 24 h. This process was repeated to ensure pure cultures for identification and subsequent testing.

Campylobacter culture, isolation and identification

Samples were swabbed directly onto Campylobacter Mueller-Hinton (CMH) media and incubated at 42 °C for 48 h in a microaerophilic environment. After incubation the plates were examined for the presence of typical Campylobacter colonies. For confirmation, 1-4 colonies considered to be typical or suspect were selected. The selected colonies were streaked onto CMH and incubated 42 °C for 48 h. This process was repeated to ensure pure cultures for identification and subsequent testing.

Escherichia coli culture, isolation and identification

Iowa State University Veterinary Diagnostic Laboratory, Ames IA

Samples were swabbed directly onto blood agar and Tergitol-7 media and incubated at 35 °C for 24h. After incubation the plates were examined for the presence of typical E. coli colonies. For confirmation, 1-3 colonies considered to be representative colonies for various phenotypes were selected, if available. Therefore the might have been 12 E coli isolates per sample, but general there were fewer ~ 6-7 isolates per sample. The E.coli phenotypes are characterized as rough, smooth, mucoid, or hemolytic. Rough forms are rather granular in appearance with uneven margins (fried egg appearance). Colonies of smooth strains are convex and glistening with even margins. Mucoid colonies are large, moist, and often coalesce. Hemolytic colonies produce a clear zone on blood agar and on Tergitol-7 hemolytic E. coli produce a small red or burgundy colony (tetrazolium reducers). Selected colonies were streaked onto blood agar or Tergitol-7 media and incubated at 35 °C for 24h. This process repeated to ensure pure cultures for identification and subsequent testing.

USDA ARS BEAR Research Unit, Athens, GA

Upon receipt all fresh and frozen samples were kept frozen at -80 °C until initial culture. Each sample was then allowed to thaw overnight at 4 °C. Subsequently each bag was diluted with EE Broth, Mossel (Acumedia, Lansing, MI) broth 9:1 and incubated overnight in at 42°C. From each bag a swab of the broth was isolated onto Chromagar™ ECC (DRG International, Paris, France) and incubated overnight at 36°C. From each Chromagar™ ECC, plate, 2 to 3 representative E. coli colonies were isolated and streaked onto second Chromagar™ ECC plate. These plates were incubated overnight at 36°C.

From the second Chromagar™ ECC plate, each colony was inoculated into 5 mls of Tryptone water, vortexed, and incubated overnight at 36°C. From the same plate a TSA slant was inoculated with a colony of the sample and incubated at 36°C overnight. Each Tryptone water sample was inoculated with 2 to 3 drops of Kovacs Indole Reagent to confirm E.coli identification. A positive sample presented a cherry red coloration at the top of the liquid. No color presented if sample was negative. For all negative samples, the TSA slant was discarded. All positive samples were then shipped on TSA slants to Iowa State VDL for phenotypic identification and frozen at BEAR Research Unit with 30% glycerin in LB broth and stored at -80 °C prior to further characterization.

Bacterial identification
Confirmation of all selected colonies was conducted using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) (Bruker Daltonics, Billerica, MA) prior to antimicrobial susceptibility testing. All recovered isolates were frozen with 50% glycerin in BHI broth and stored at -80 °C at the ISU VDL.

Antimicrobial susceptibility testing for Salmonella, E. coli and Campylobacter
Antimicrobial susceptibility testing for all recovered isolates in the study was performed at ISU VDL from December 2013 through August 2014. Antimicrobial susceptibility testing was accomplished by broth microdilution using minimum inhibitory concentrations (MIC) as described in CLSI M31-A3 (Clsi., 2008). NARMS breakpoints are used for interpretive criteria to determine susceptible, intermediate or resistant status(Cdc., 2014) . The TREK Sensititre system and NARMS (CMV2AGNF, CMV3AGNF) panels for Salmonella and E. coli or CAMPY plates for Campylobacter were used in this study. On the CMV2AGNF plates, Salmonella and E. coli isolates were tested for susceptibility to amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Salmonella and E. coli isolates tested using CMV3AGNF plates included the same antimicrobials with the exception of kanamycin. Campylobacter isolates were tested for susceptibility to azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin, and tetracycline.

Serotype determination for Salmonella isolates
All Salmonella isolates were serotyped at the Bear Research Unit, Athens, GA as described (Leader *et al.*, 2009) with the following modification. Whole cell template for each isolate was prepared for PCR by streaking isolates from the transport agar slant to Brilliant Green Sulfa agar plate and incubating the plate at 37C 18-20 hours. One well isolated colony was selected then struck to a Trypticase Soy Agar plate with 5% sheep blood plate and incubating at 37C for 18-20 hours. One well isolated colony was placed in a sterile 0.5ml microcentrifuge tube containing 100l sterile distilled de-ionized water, vortexed thoroughly and stored at +4C prior to use. Samples were stored at -20C if they were not tested immediately.

Campylobacter pulse-field gel electrophoresis (PFGE)
All isolates were prepared and restricted using the PulseNet protocol (http://www.cdc.gov/pulsenet/ PDF/campylobacter-pfge-protocol-508c.pdf) with minor modifications as necessary (Ribot *et al.*, 2001) Isolates were restricted with 40U of SmaI per sample as per the protocol with incubation at 25C for 2 h. Restricted samples were prepared and run on a PFGE gel for 18 h on a Chef Mapper (BioRad) with initial switch times of 6.76s and a initial switch time of 35.8s. Following the PFGE run, gels were stained using EtBr and images captured as TIFF _les. All images were imported into BioNumerics software (Ver 6.6) and banding patterns identified. Band matching and cluster analysis was performed using an unweighted pair group method with arithmetic averages (UPGMA) and the Dice coefficient with 0.5% optimization and 1% tolerance levels for the fingerprint patterns with the antimicrobial resistance patterns included, and PFGE fingerprints alone.

Data management
Sample ID numbers and results were entered and maintained in the ISU VDL Laboratory Information System (LIMS). Results were reviewed by the principal investigator. Data was extracted from LIMS and combined with additional Salmonella serotype information from Bear Research Unit or Campylobacter PFGE information from Dr. Logue's Research Laboratory as appropriate. Data was kept in excel spreadsheets that were laid out/designed for relational database use to be imported into an access database for verification, manipulation, and basic analysis. In the Access database all record types were checked for duplicates, missing records, and other errors using SQL functions primarily through access queries. These combined data were reviewed by principal investigator and analysis group.

Bias
The major source of bias is the absence of a control group, therefore we cannot make interference that the changes in patterns observed are due to abattoir. For such inference it would be necessary to have a parallel control group that did not undergo the abattoir experience to make such inference. This is issue is discussed further in the discussion. To reduce potential for selection bias (that the differences observed are due to the approach to selecting pigs in both groups), it would have been preferably to have identified the same pigs for sample collection throughout the process but this was not possible. Instead, we matched on group, making the assumption that patterns from the 10 haphazardly collected pigs

would be reflective of the group. Similarly it would have been desirably to randomly select pigs from the group, however this is not feasible in a working abattoir as no sampling frame is available. Several steps were taken to reduce misclassification of the Salmonella, E Coli and Campylobacter status of the pigs and these quality controls are included in the description of the methods of culture.

Study size

The study size was determine by the availability of funds, costs of travelling and organizing visits to abattoirs and a pragmatic balance between obtaining a reasonable number of pigs per group and a reasonable number of groups. The sample size involved two decisions the number of groups and the number of pigs within n group to be sample. The number of groups was based on a pragmatic decision that staff could process based on weekly visits to the abattoir in 3 months and while at the plant resources were available to select 10 pigs per group.

Quantitative variables

For the MIC obtained for all isolates, the MIC were converted to interpretive criteria (resistant, intermediate and susceptible) using breakpoints from 2012 National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Final Report (Cdc., 2014)

Statistical Methods

Exploratory data analysis

For the remainder of the report the following terminology is used. A sample refers to a fecal/cecal/environmental sample, and there is one sample per pig at each sampling location (arrival, post-lairage, post-slaughter). The term isolate is used to refer to a sub-sample that was tested for serotype and antibiotic resistance phenotype. Samples may have more than one isolate and at most 4 Salmonella and Campylobacter isolates and at most 12 E. coli isolates.

The exploratory data analysis consisted of cross tabulation of outcomes. For the analysis we classified isolates as "resistant" if they were resistant to at least one antibiotic. The descriptive analysis aimed to describe the prevalence of Salmonella, Campylobacter and E coli isolates at the three sampling points, and over time. The analysis was conducted at the sample and isolate level based on relevance. For E coli, we also presented resistance data for mucoid, smooth, rough and hemolytic phenotypes. As numerous patterns were present, we presented only the more common patterns. The definition of common varied by bacteria and was arbitrary based on presentation considerations.

Hypothesis testing data analysis

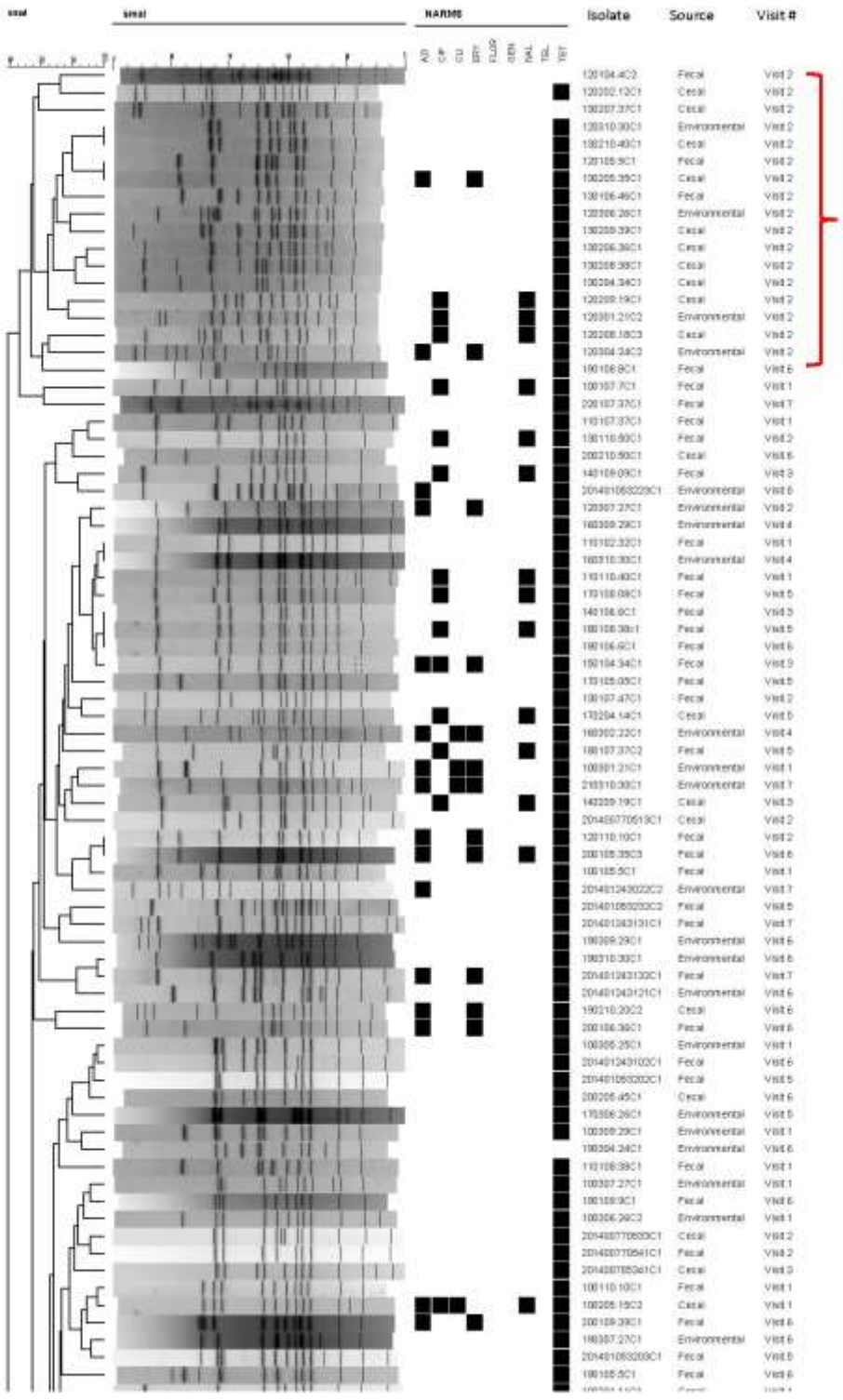
Although these data are from a quasi-experiment, we did conduct a hypothesis testing analysis. We tested if the prevalence of isolates resistant to at least one antimicrobial was different between post-slaughter and arrival samples. The rationale for this approach was that if we could reject the hypothesis that the prevalence of resistant patterns was the same, this would suggest that post-slaughter samples would not adequately reflect the arrival samples. We used Chi-squares test for proportions to compare prevalence of isolates resistant to at least one antimicrobial was different between post-slaughter and arrival samples. When the outcome was rare, we used Fisher's exact test. For one organism, too few pan-susceptible isolates where available for a sensible test, so we compared the prevalence of the most common resistant pattern at post-slaughter and arrival. We did not conduct any hypothesis tests comparing lairage samples to post-slaughter as these were not part of the a priori hypothesis.

RESULTS

Salmonella

Distribution of samples by Salmonella positive samples by location and date

Of the 358 samples included in the analysis, Salmonella was not detected in 51 samples (negative) and was detected in 307 samples (positive). The frequency of isolation of Salmonella from each sample based on location and date is provided


HARMS
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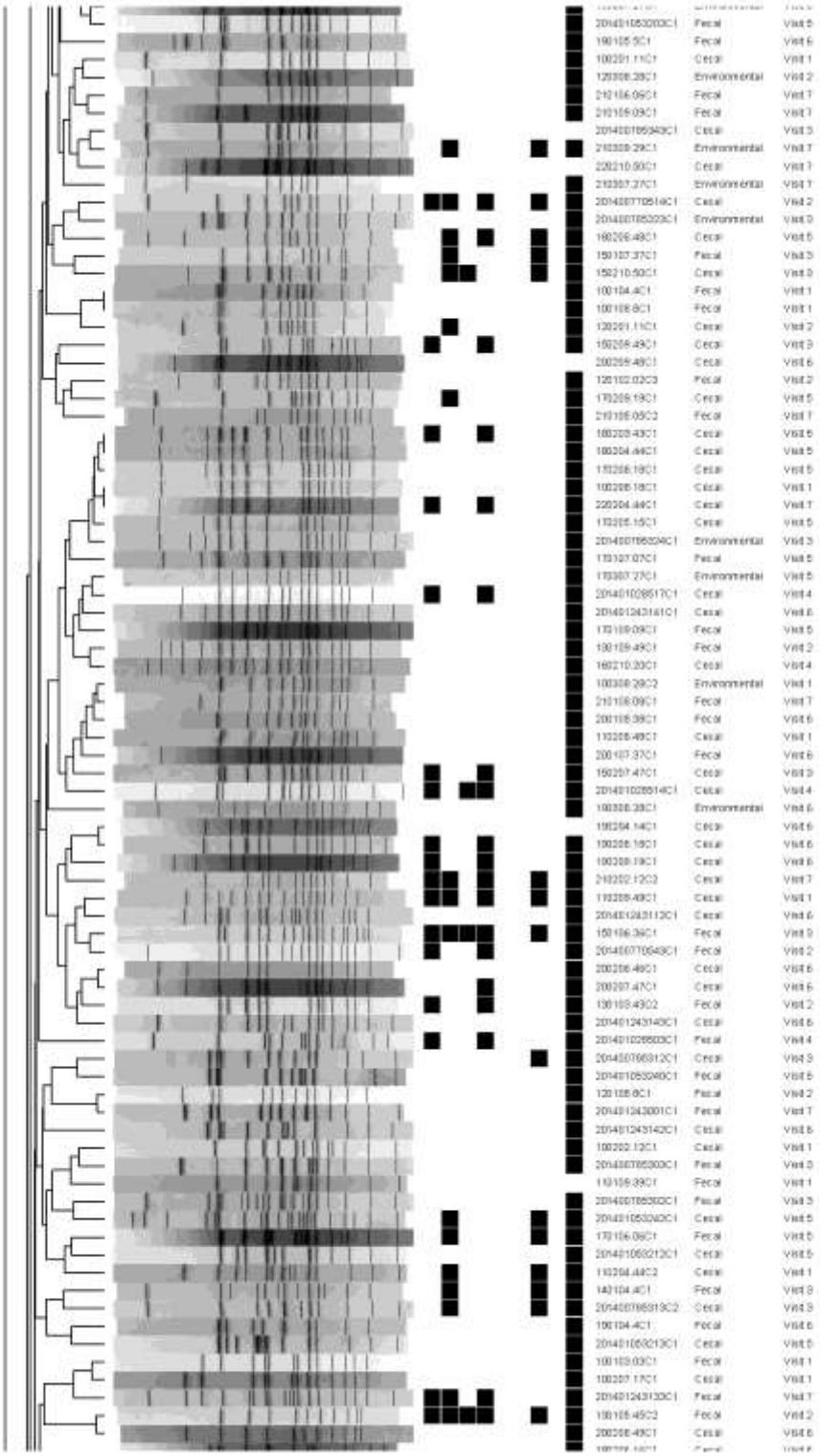
20140105300C1 Fecal Visit 5
190105.5C1 Fecal Visit 6
100201.11C1 Cecal Visit 1
120308.28C1 Environmental Visit 2
210106.09C1 Fecal Visit 7
210105.03C1 Fecal Visit 1
20140078540C1 Cecal Visit 3
210309.29C1 Environmental Visit 7
220210.50C1 Cecal Visit 7
210307.27C1 Environmental Visit 7
20140077051 4C1 Cecal Visit 2
20140078520C1 Environmental Visit 0
160206.49C1 Cecal Visit 5
150107.37C1 Fecal Visit 5
150210.50C1 Cecal Visit 0
100104.4C1 Fecal Visit 1
100108.8C1 Fecal Visit 1
120201.11C1 Cecal Visit 2
160206.49C1 Cecal Visit 3
200209.49C1 Cecal Visit 6
120102.02C3 Fecal Visit 2
170209.19C1 Cecal Visit 5
210105.09C3 Fecal Visit 7
160209.43C1 Cecal Visit 5
160304.44C1 Cecal Visit 5
150208.18C1 Cecal Visit 5
100208.18C1 Cecal Visit 1
220304.44C1 Cecal Visit 7
110205.15C1 Cecal Visit 5
20140078520 4C1 Environmental Visit 3
110107.07C1 Fecal Visit 5
110307.27C1 Environmental Visit 5
20140102851 7C1 Cecal Visit 4
20140124316 1C1 Cecal Visit 6
170109.09C1 Fecal Visit 5
190109.49C1 Fecal Visit 2
160210.20C1 Cecal Visit 4
100309.29C2 Environmental Visit 1
210108.09C1 Fecal Visit 7
200108.38C1 Fecal Visit 6
110208.49C1 Cecal Visit 1
200107.37C1 Fecal Visit 6
150207.47C1 Cecal Visit 3
20140102851 4C1 Cecal Visit 4
100308.28C1 Environmental Visit 6
190204.14C1 Cecal Visit 6
190208.19C1 Cecal Visit 6
190209.19C1 Cecal Visit 6
210202.12C2 Cecal Visit 7
110209.49C1 Cecal Visit 1
20140124311 2C1 Cecal Visit 6
150106.36C1 Fecal Visit 3
20140077064 9C1 Fecal Visit 2
200206.49C1 Cecal Visit 6
200207.47C1 Cecal Visit 6
130103.43C2 Fecal Visit 2
20140124314 0C1 Cecal Visit 6
20140102860 3C1 Fecal Visit 4
20140078631 2C1 Cecal Visit 3
20140105304 0C1 Fecal Visit 5
120108.6C1 Fecal Visit 2
20140124300 1C1 Fecal Visit 7
20140124314 2C1 Cecal Visit 6
100202.12C1 Cecal Visit 1
20140078520 3C1 Fecal Visit 3
110109.39C1 Fecal Visit 1
20140078636 0C1 Fecal Visit 3
20140105302 0C1 Cecal Visit 5
170106.09C1 Fecal Visit 5
20140105321 2C1 Cecal Visit 5
110204.44C2 Cecal Visit 1
140104.4C1 Fecal Visit 0
20140078691 9C2 Cecal Visit 3
190104.4C1 Fecal Visit 6
20140105321 3C1 Cecal Visit 0
100103.03C1 Fecal Visit 1
100207.17C1 Cecal Visit 1
20140124313 0C1 Fecal Visit 7
190105.45C2 Fecal Visit 2
200208.49C1 Cecal Visit 6

Table 1 and Table 2 respectively. Salmonella was isolated from samples each day of sampling (Table 2). 1163 isolates were obtained from the 307 Salmonella positive samples. The percentage of times that 1, 2, 3 or 4 isolates were obtained from a sample is shown in Table 3. It was most common to isolate 4 Salmonella from a sample i.e., four isolates were obtained from 76% (233 of 307) of the positive samples. Table 4 shows the distribution of the number of Salmonella positive isolates at each point and the serotypes identified. 524 of the Salmonella positive isolates were identified from the arrival samples, 367 at post-slaughter samples and 272 from the lairage environment samples.

Distribution of isolates by Salmonella serotype at sampling points

The most common serotype overall and at each sampling point was Derby, followed by I 4,[5],12:i:-. The third most common isolate varied based on the sampling point, as illustrated by the data in Table 4. For example, *Salmonella* Typhimurium was the third most common serotype isolated at arrival, however post –slaughter the third most common serotype was *Salmonella* Agona. Figure 1 provides an illustration of the distribution of the serotype patterns over time. It can be seen that some serotypes were only identified on a single day e.g. Adelaide, Johannesburg, Litchfield, Liverpool, London, Mbandaka, Muenster, Uganda. Riseen was only detected on two days. Other serotypes were isolated on multiple sampling days, Agona, Derby, Heidelberg, I 4,[5],12:i:-, Infantis, and Typhimurium.

Figure 2 provides a more detailed summary of similar data for the serotype *Salmonella* Derby only. *Salmonella* Derby was consistently detected on all days at arrival, lairage environment, and post-slaughter.

It was also of interest to know if the same isolate was consistently detected within each sample. Table 4 describes the distribution of Salmonella serotype for all positive samples. Table 5 provides similar information however this only relates unique serotypes within a sample. The total observations in Table 5 is 424. If we considered that each time the same serotype was obtained from the same sample that it was clonal, these data could be interpreted as the number of unique isolates within the 1163 isolates. For example, on 32 occasions the serotype Derby was isolated once from the sample, and on 85 occasions all 4 isolates from the sample were serotype Derby. For example, if a sample had 4 Derby isolates, this would represent 4 data points in Table 4 but only 1 data point in Table 5**Error! Reference source not found.** Similarly, if a sample had 2 *Salmonella* Derby isolates and 2 *Salmonella* Infantis, this would represent 4 data points in Table 4 but only 2 data point in Table 5, a *Salmonella* Derby and *Salmonella* Infantis positive sample. Table 5 provides the frequency and percentages of unique serotype by sampling location. The 6 most common isolates (Agona, Derby, Heidelberg, I 4,[5],12:i:-, Infantis, and Typhimurium).

Distribution of isolates by antibiotic resistance patterns

The distribution of antibiotic resistance patterns combining data from all isolates that were resistant to at least one antimicrobial tested are provided in Table 6. 898 of the 1163 (77%) isolates were resistant to at least one antimicrobial tested. The Chi-square test of the null hypothesis that the proportion of isolates resistant to at least one antimicrobial tested was the same at arrival and post-slaughter was rejected (χ^2 squared = 4.3485, df = 1, p-value = 0.03704). Subsequent tables provide information about the distribution of commonly observed resistance patterns across the following serotypes: *Salmonella* DerbyTable 7, *Salmonella* I 4,[5],12:i:- Table 8. *Salmonella* Infantis Table 9, *Salmonella* Typhimurium Table 10 and *Salmonella* Agona Table 11. The eight most common patterns are reported and other patterns are combined into other. For all of these serotypes, the null hypothesis that the proportion of isolates resistant to at least one antimicrobial tested was the same at arrival and post-slaughter was rejected *Salmonella* Derby (χ^2 -squared = 24.7593, df = 1, p-value = 6.495e-07) *Salmonella* I 4,[5],12:i:-((χ^2-squared = 10.9913, df = 1, p-value = 0.0009154, *Salmonella* Infantis (fishers exact test -p-value = 0.5916, *Salmonella* Typhimurium (χ^2-squared = 9.45, df = 1, p-value = 0.002111) and *Salmonella* Agona (χ^2-squared = 16.5632, df = 1, p-value = 4.706e-05). Note that for *Salmonella* Infantis, the power of the test to detect differences may have been limited as pan-susceptible isolates were so common.

Campylobacter

Campylobacter isolation

Of the 359 samples collected, Campylobacter was not detected in 17 samples (negative) and was detected in 342 samples (positive). All isolates were identified as Campylobacter coli. For Campylobacter, the detection of organisms in samples based on location is provided in Table 12

Campylobacter pulse-field gel electrophoresis and antimicrobial resistance patterns

Two hundred ninety (290) isolates were recovered for PFGE analysis and 288 isolates were available for antimicrobial susceptibility testing. Combined PFGE results and antimicrobial resistance patterns are shown in Figure 4. Shared patterns were observed over the multiple visits of the study, these patterns were shared for cecal, fecal and environmental isolates. The most homogeneous pattern sharing was observed for visit 2 (red bracket), where the same pattern was found for fecal, cecal and environmental isolates examined, suggesting this was probably a dominant pattern or clone that existed at the farm and in the pigs. However the drug resistance patterns of these isolates were not all identical suggesting that genotype and phenotype did not correlate. No other clusters in the dendrogram were as distinct as this first cluster suggesting that there are many different types and clones of Campylobacter circulating in pig production and clones may not be exclusive to one site/farm. None of isolates examined from any of the other visits cluster exclusively suggesting that the patterns observed were independent of the visit date. Analysis of the antimicrobial resistance patterns observed found that 17 different resistance patterns were observed for 288 *Campylobacter* isolates included in the analysis (Table 13).Similar antimicrobial resistance patterns were found on different visits. Three (3)% of isolates were pan susceptible while 51% were resistant to one drug (TET); about 27% were resistant to 3 drugs with the patterns AZI, ER, TET or CIP, NAL TET being dominant; 2% were resistant to 6 drugs (AZI, CIP, CLIN, ERY, NAL and TET). No resistance was observed for gentamicin, telithromycin or florfenicol. Quinolone, Fluoroquinolone, Macrolide and Tetracycline resistance were observed at relatively low levels but some strains were resistant to all classes simultaneously suggesting these strain types may be emerging. Further follow up to determine the genetic nature of the resistance is warranted as these resistance traits are usually associated with plasmids (*tetO*) and mutations of the 23S rDNA (macrolide) and *gyrA* (quinolone). Table 14 shows the levels of multidrug resistance observed among isolates examined (n=288). Only 3% of isolates were pan-susceptible. The majority of *Campylobacter* isolates were resistant to 1 drug (TET), and 27% resistant to 3 drugs; about 2% were resistant to 6 drugs.

Table 15 shows the prevalence of resistance according to drug classes examined. Classes are defined as a group of similar drugs; for the purposes of this analysis, classes of drugs were defined by CLSI as Aminoglycosides – Gentamicin (GEN); Lincosamides – Clindamycin (CLIN); Macrolides – Azithromycin (AZI) and Erythromycin (ERY); Ketolides – Telithromycin (TEL); Phenicols – Florfenicol (FFN) and Chloramphenicol (CHL); Fluoroquinolones – Ciprofloxacin (CIP); Quinolones – Nalidixic acid (NAL); and Tetracyclines – Tetracycline (TET). The majority (51.7%) of isolates examined were resistant to one class of drugs, which in this study were the tetracyclines. 18 and 19% of the *Campylobacter* isolates examined were resistant to two and three classes of drugs respectively which included groups such as the quinolones, fluoroquinolones, tetracyclines, and macrolides.

Escherichia coli

Escherichia coli isolation

Of the 359 samples collected, E. coli was not detected in 14 samples (negative) and was detected in 345 samples (positive). By location, E. coli was isolated from 138 arrival samples, 131 post-slaughter samples and 76 lairage samples. Nine hundred and fifty three (953) isolates were recovered from positive samples of which 813 isolates could be classified by one of the 4 phenotypes. The number of E. coli isolates by phenotype included: 531 rough, 197 smooth, 62 mucoid, and 23 hemolytic.

Escherichia coli antibiotic resistance patterns

Although 953 isolates were identified, only seven hundred and thirty six (736) E. coli isolates were recovered for antimicrobial susceptibility testing. The common E coli patterns by sample location (arrival and post-slaughter) are presented in Table 6. The most common E. coli patterns by phenotype and sample location are presented in Table 7. Most isolates were resistant to at least one antimicrobial hypothesis test on these data. However, it is likely worth noting that several patterns were identified post-slaughter that were not identified pre-slaughter, and visa versa. This would suggest that the overall patterns of antimicrobial resistance observed post-slaughter do not reflect the patterns at arrival.

IX. Discussion and Conclusion

Overall the aim of this study was to determine if the patterns of antimicrobial resistance observed post-slaughter from swine cecal contents were reflective of patterns observed in the same cohort of pigs at arrival. The rationale for the study was that if post-slaughter samples were reflective of arrival samples, then post-slaughter samples might be suitable as proxy measures of on-farm anti-microbial resistance patterns if it could subsequently be documented that resistance patterns at arrival reflect samples collected at the farm. Of course, this is a two-step hypothesis, however by testing the 1st concept, it may or may not be necessary to validate the 2nd concept. Such an approach to the question is an efficient and pragmatic approach to testing the idea of post-slaughter samples as proxies for on-farm samples.

The results suggest that samples collected post-slaughter are not reflective of antimicrobial resistance patterns observed at arrival, for the common Salmonella serotypes and E coli. For Campylobacter the patterns of resistance observed have little relevance since there was no clear pattern of clonal retention from arrival to post-slaughter based on the PFGE analysis. In the analysis where antimicrobial resistance data is included the antimicrobial resistance patterns are shared between visits and sources suggesting that antimicrobial resistance patterns could not be used alone as an indicator of source or status. If patterns at arrival are not reflected by post-slaughter partners, this suggest sample collected post-slaughter are not useful as measures for monitoring on-farm resistance, as on-farm resistance is even more distal geographically and temporally then arrival samples and therefore we would naturally expect even larger differences between post-slaughter and true on farm prevalence than post-slaughter and arrival samples.

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Figure 1: The frequency of collection of Salmonella serotypes for collection day serotype. Samples were collected on 8 days (x axis).

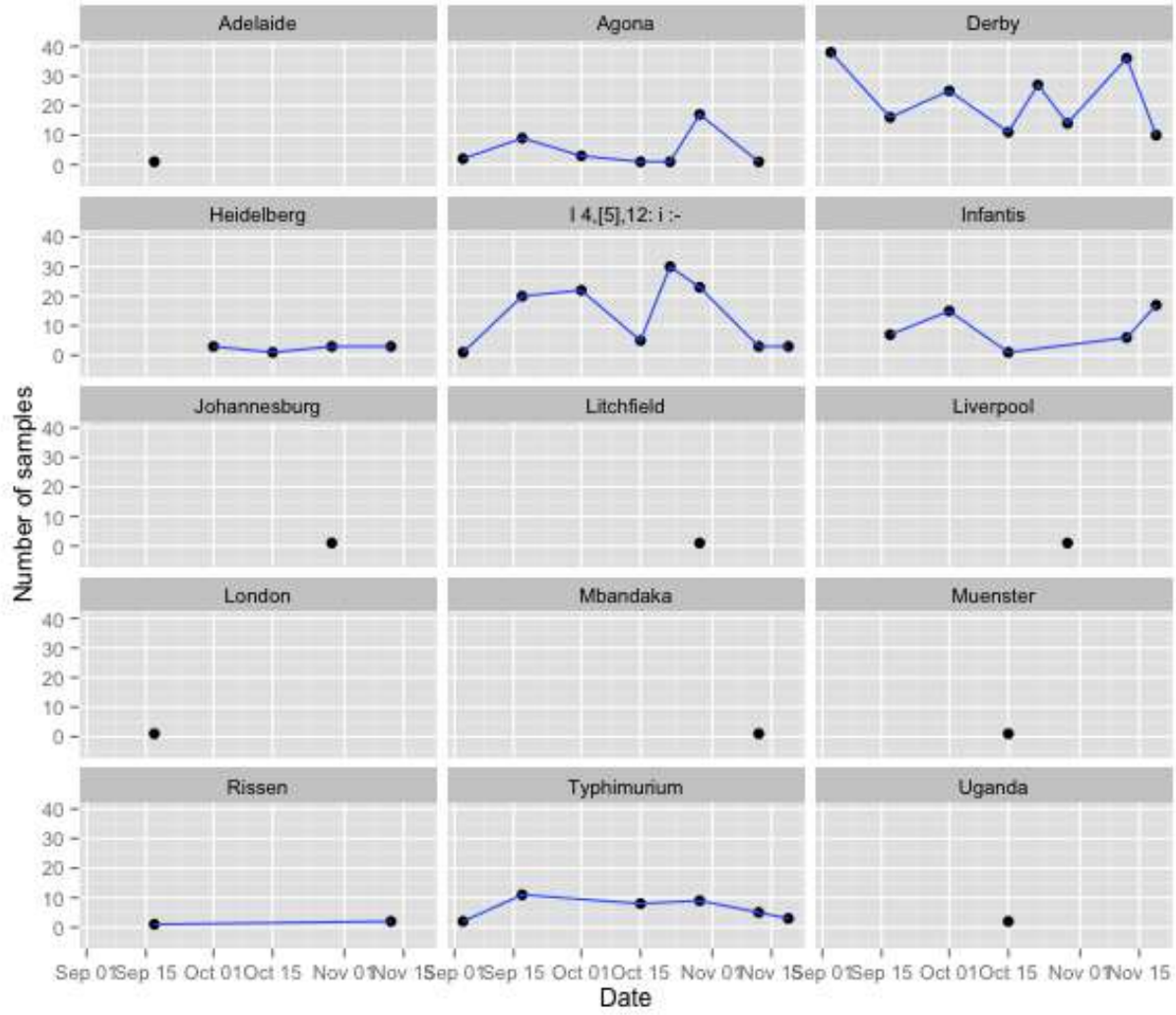


Figure 2: Total number of samples by day for Derby

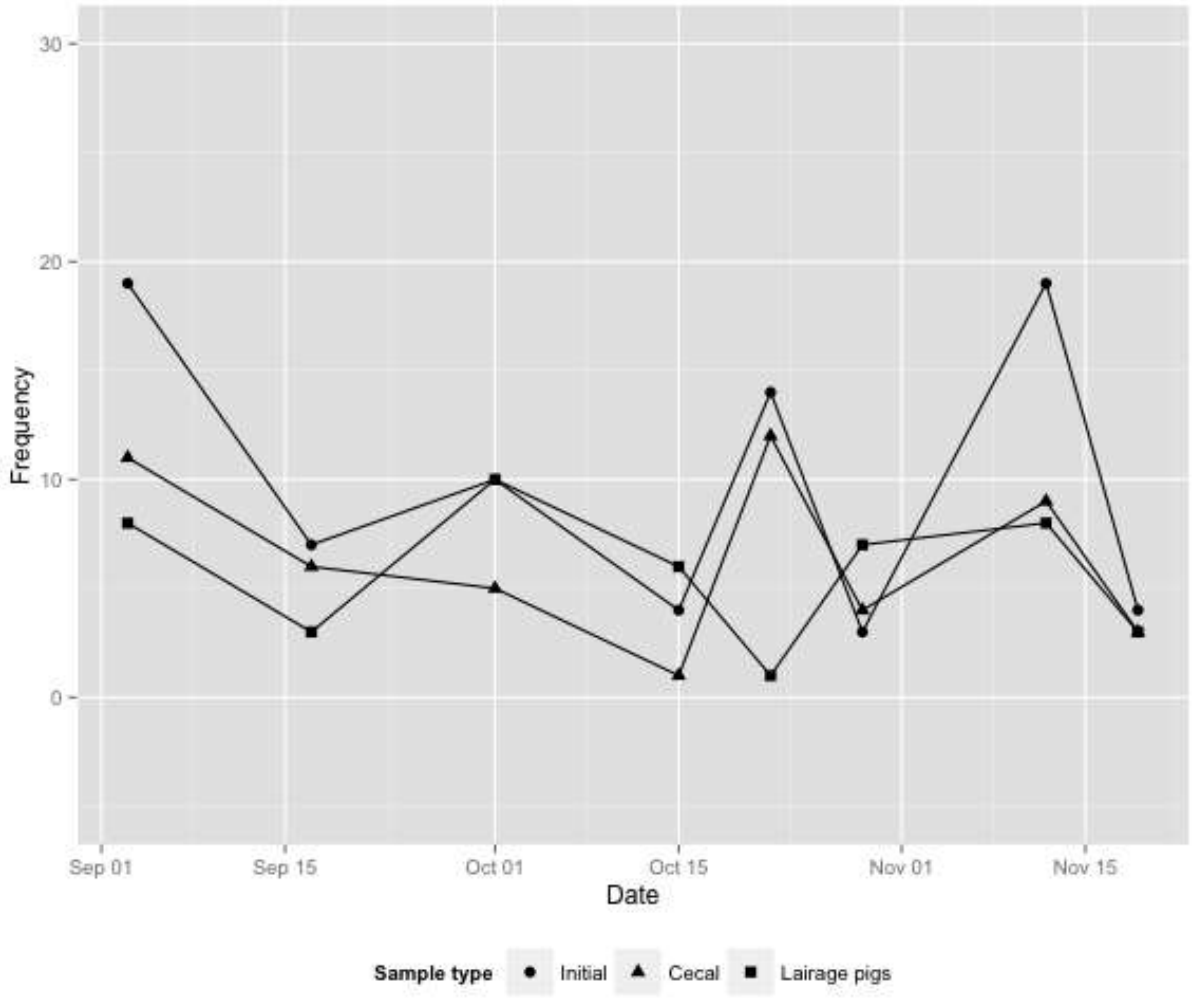


Figure 3: Subsample distribution for each sample by serotype. If a sample had the same serotype for all 4 samples, then the color is consistent across the row. When the color changes this indicates that the isolates had different serotypes.

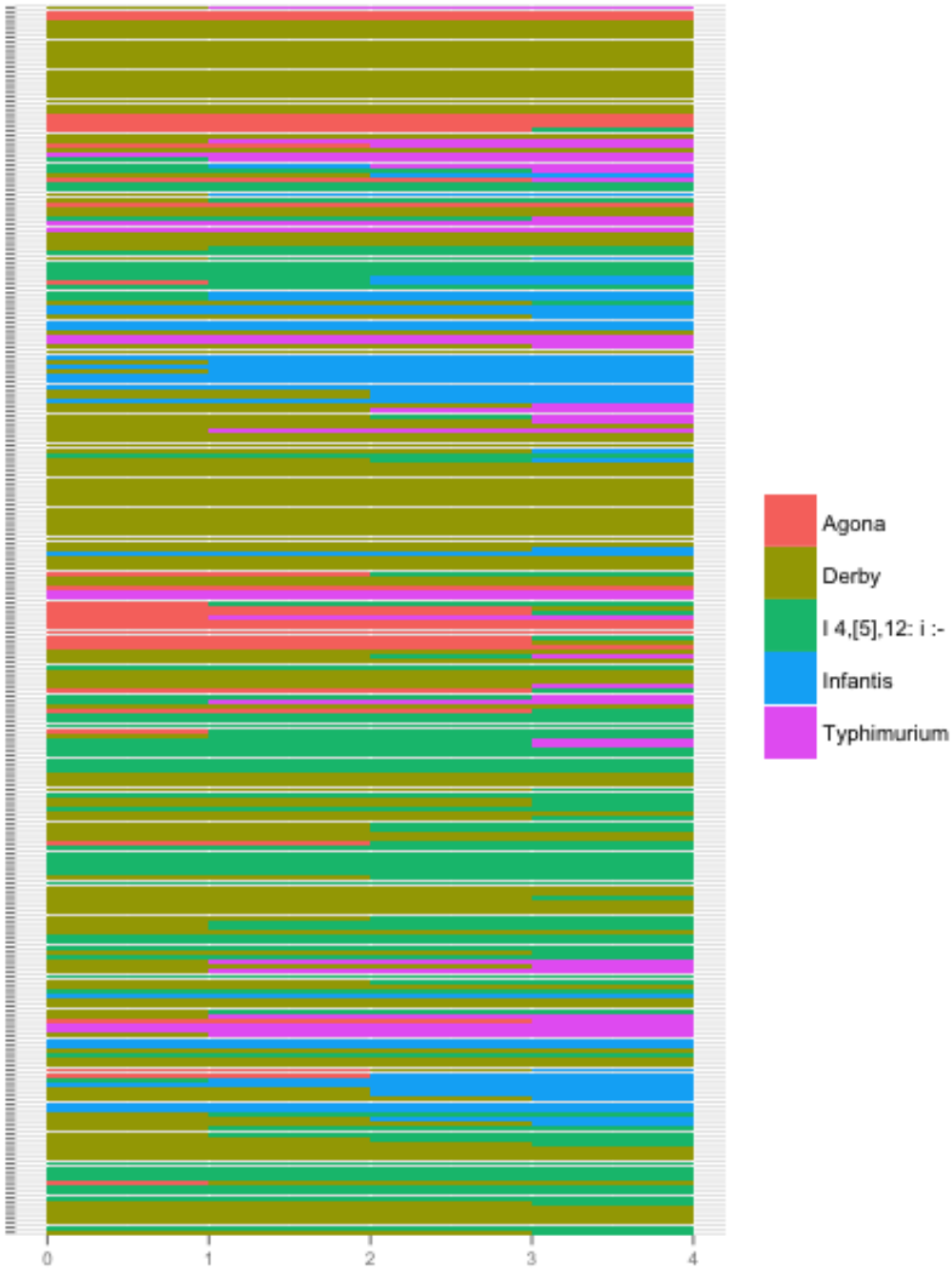
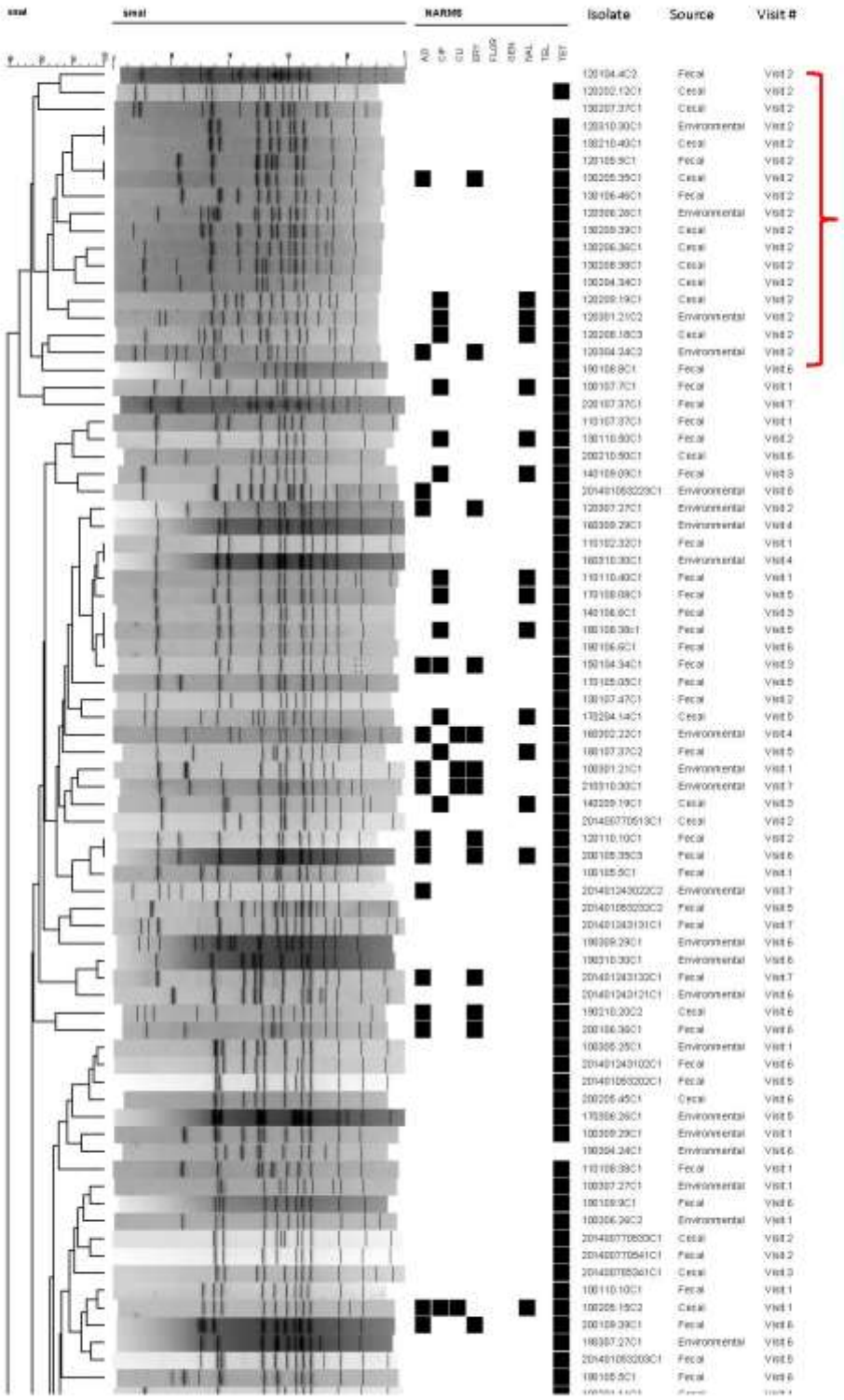
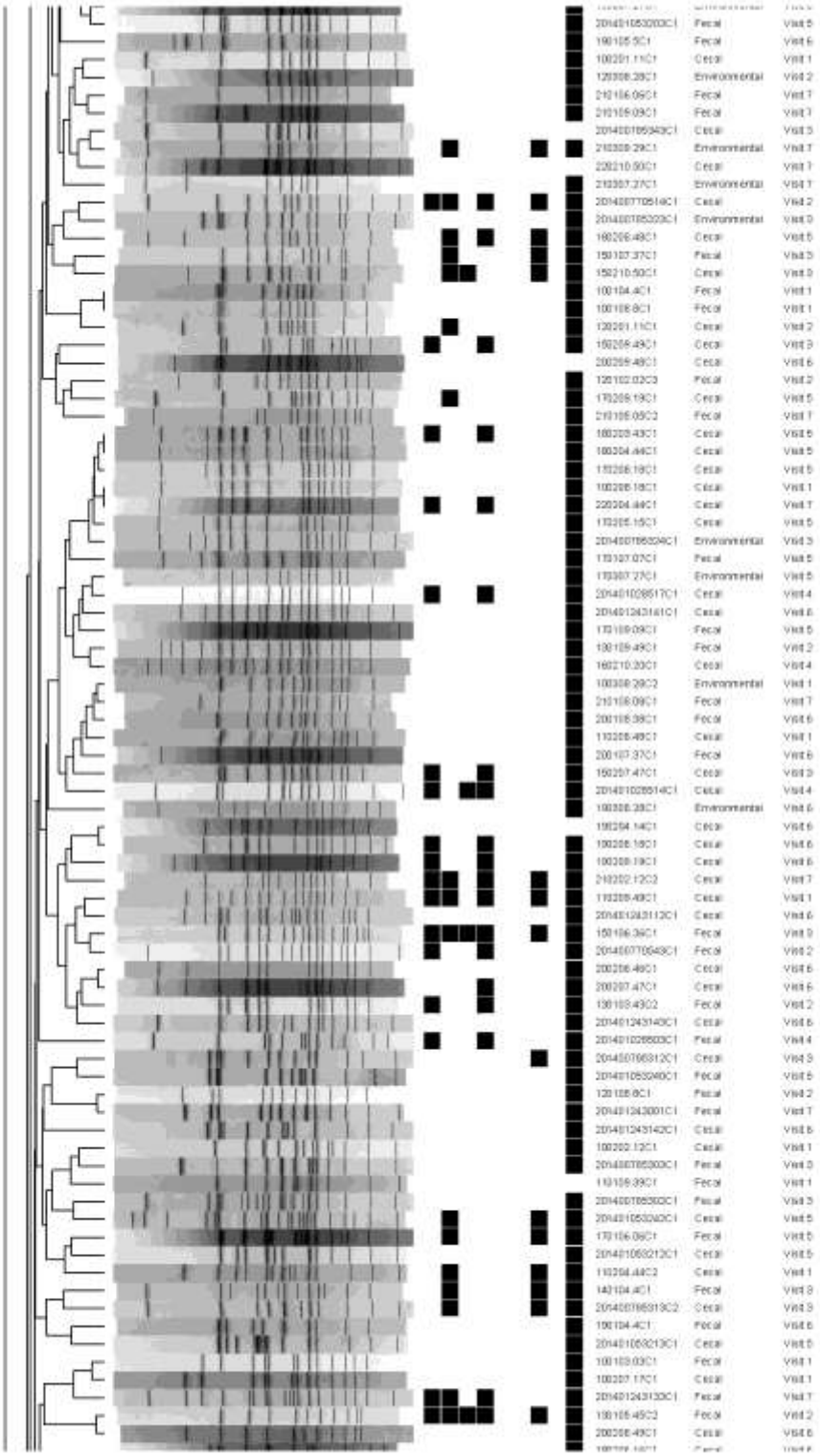


Figure 4: PFGE fingerprints and antimicrobial resistance pattern of Campylobacter coli isolates.




20140105300C1 Fecal Visit 5
190105.5C1 Fecal Visit 6
100201.11C1 Cecal Visit 1
120308.28C1 Environmental Visit 2
210106.09C1 Fecal Visit 7
210105.03C1 Fecal Visit 1
20140078540C1 Cecal Visit 3
210309.29C1 Environmental Visit 7
220210.50C1 Cecal Visit 7
210307.27C1 Environmental Visit 7
20140077051 4C1 Cecal Visit 2
20140078520C1 Environmental Visit 0
160206.49C1 Cecal Visit 5
150107.37C1 Fecal Visit 5
150210.50C1 Cecal Visit 0
100104.4C1 Fecal Visit 1
100108.8C1 Fecal Visit 1
120201.11C1 Cecal Visit 2
160206.49C1 Cecal Visit 3
200209.49C1 Cecal Visit 6
120102.02C3 Fecal Visit 2
170209.19C1 Cecal Visit 5
210105.09C3 Fecal Visit 7
160209.43C1 Cecal Visit 5
160304.44C1 Cecal Visit 5
150208.18C1 Cecal Visit 5
100208.18C1 Cecal Visit 1
220304.44C1 Cecal Visit 7
110205.15C1 Cecal Visit 5
20140078520 4C1 Environmental Visit 3
110107.07C1 Fecal Visit 5
110307.27C1 Environmental Visit 5
20140102851 7C1 Cecal Visit 4
20140124316 1C1 Cecal Visit 6
170109.09C1 Fecal Visit 5
190109.49C1 Fecal Visit 2
160210.20C1 Cecal Visit 4
100309.29C2 Environmental Visit 1
210108.09C1 Fecal Visit 7
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110208.49C1 Cecal Visit 1
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20140102851 4C1 Cecal Visit 4
100308.28C1 Environmental Visit 6
190204.14C1 Cecal Visit 6
190208.19C1 Cecal Visit 6
190209.19C1 Cecal Visit 6
210202.12C2 Cecal Visit 7
110209.49C1 Cecal Visit 1
20140124311 2C1 Cecal Visit 6
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200207.47C1 Cecal Visit 6
130103.43C2 Fecal Visit 2
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20140102860 3C1 Fecal Visit 4
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20140105302 0C1 Cecal Visit 5
170106.09C1 Fecal Visit 5
20140105321 2C1 Cecal Visit 5
110204.44C2 Cecal Visit 1
140104.4C1 Fecal Visit 0
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20140105321 3C1 Cecal Visit 0
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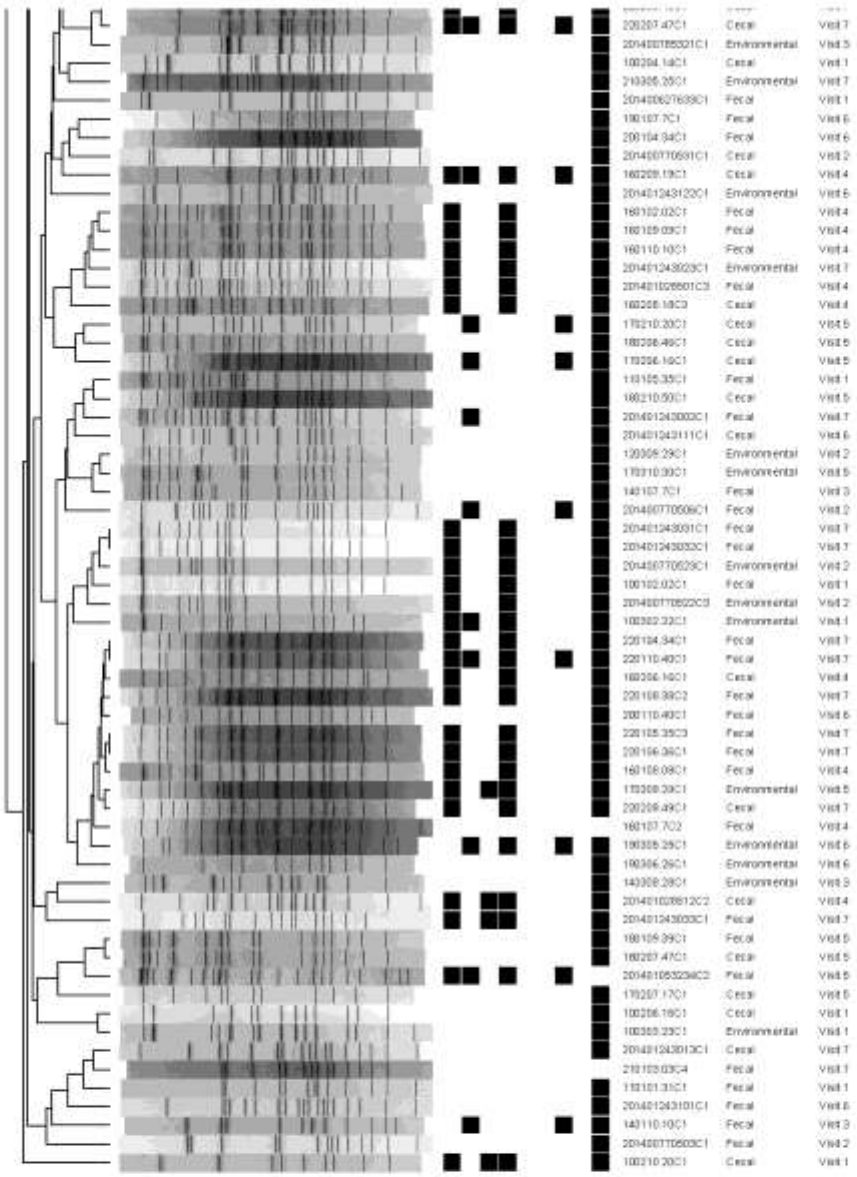

Table 1: Salmonella status of samples by sampling location

Sample status	Arrival	Post-Slaughter	Lairage Environment	Total
Negative	4	38	9	51

Positive	135	102	70	307
Total	139	140	79	358

Table 2: Salmonella status of samples by day

Sample collection date	Negative	Positive	Total
2013-09-03	8	41	49
2013-09-17	9	41	50
2013-10-01	2	48	50
2013-10-15	10	20	30
2013-10-22	5	44	49
2013-10-29	2	48	50
2013-11-12	9	41	50
2013-11-19	6	24	30
Total	51	307	358

Table 3: Percentage of samples with 1, 2, 3 and 4 isolates.

Isolates	**Percentage**
1	17%
2	3%
3	4%
4	76%

Table 4: Distribution of serotypes of all Salmonella positive isolates by sampling location and serotype (n= 1163)

Serotype	Arrival	Post-Slaughter	Lairage Environment	Total
Adelaide	1	0	0	1
Agona	43	38	11	92
Derby	220	165	141	526
Heidelberg	2	28	0	30
I 4,[5],12: i :-	140	72	69	281
Infantis	76	18	28	122
Johannesburg	0	4	0	4
Litchfield	0	0	1	1
Liverpool	0	3	0	3
London	1	0	0	1
Mbandaka	1	0	0	1
Muenster	1	0	0	1
Rissen	3	4	0	7
Typhimurium	35	35	18	88
Uganda	1	0	4	5
Total	524	367	272	1163

Table 5: Frequency and percentages of unique isolates serotype and sampling location, (percentages refer to row totals) (n= 424)

Salmonella Serotype	Arrival	Post-Slaughter	Lairage Environment	Total
Adelaide	1(100%)	0(0%)	0(0%)	1
Agona	17(50%)	13(38%)	4(12%)	34
Derby	80(45%)	51(29%)	46(26%)	177
Heidelberg	2(20%)	8(80%)	0(0%)	10
I 4,[5],12: i :-	53(50%)	27(25%)	27(25%)	107
Infantis	30(65%)	5(11%)	11(24%)	46
Johannesburg	0(0%)	1(100%)	0(0%)	1
Litchfield	0(0%)	0(0%)	1(100%)	1
Liverpool	0(0%)	1(100%)	0(0%)	1
London	1(100%)	0(0%)	0(0%)	1
Mbandaka	1(100%)	0(0%)	0(0%)	1
Muenster	1(100%)	0(0%)	0(0%)	1
Rissen	2(67%)	1(33%)	0(0%)	3
Typhimurium	17(45%)	13(34%)	8(21%)	38
Uganda	1(50%)	0(0%)	1(50%)	2

Table 6: Frequency and percentage of resistance pattern for all Salmonella that were resistant to at least one antimicrobial (% are for the row)

Resistance Patterns [1]	**Arrival (n=524)**	**Post-Slaughter (n=367)**	**Lairage Environment (n=272)**
Amox.Ampi.Cefo.Cefti.Ceftr.Chlor.Stre.Sulf.Tetr	7(16%)	31(69%)	7(16%)
Amox.Ampi.Cefo.Cefti.Ceftr.Stre.Sulf.Tetr	34(51%)	11(16%)	22(33%)
Ampi.Chlor.Stre.Sulf.Tetr	11(25%)	20(45%)	13(30%)
Ampi.Stre.Sulf.Tetr	109(50%)	56(26%)	54(25%)
Stre.Sulf.Tetr	47(35%)	50(37%)	39(29%)
Stre.Tetr	6(35%)	5(29%)	6(35%)
Tetr	93(37%)	100(40%)	58(23%)
Other [2]	43(36%)	59(50%)	17(14%)
Total resistant to at least one antimicrobial	350	332	216
Pan-susceptible	174	35	56

[1]: Isolates with a resistant pattern to at least one of the antimicrobials included in the panel

[2]: Patterns with fewer than 6 observations

Table 7: Frequency and percentage of resistance pattern by sample location for *Salmonella* Derby serotype (n= 448) (% represent rows)

Resistance Patterns [1]	**Arrival (n=220)**	**Post-Slaughter (n=165)**	**Lairage Environment (n=141)**
Amox.Ampi.Cefo.Cefti.Ceftr.Chlor.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Amox.Ampi.Cefo.Cefti.Ceftr.Stre.Sulf.Tetr	34(51%)	10(15%)	22(34%)
Ampi.Chlor.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Ampi.Stre.Sulf.Tetr	0(0%)	0(0%)	1(100%)
Stre.Sulf.Tetr	45(34%)	50(37%)	39(29%)
Stre.Tetr	0(0%)	0(0%)	4(100%)
Tetr	78(35%)	91(41%)	54(24%)
Other [2]	10(50%)	6(30%)	4(20%)
Total resistant to at least one antimicrobial	167	157	124
Pan-susceptible	53	8	17

[1]: Isolates with a resistant pattern to at least one of the antimicrobials included in the panel

[2]: Patterns with fewer than 6 observations

Table 8: Frequency and percentage of resistance pattern row totals by sample location for I4 serotype

Resistance Patterns [1]	**Arrival (n=140)**	**Post-Slaughter (n=72)**	**Lairage Environment (n=69)**
Amox.Ampi.Cefo.Cefti.Ceftr.Chlor.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Amox.Ampi.Cefo.Cefti.Ceftr.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Ampi.Chlor.Stre.Sulf.Tetr	0(0%)	1(25%)	3(75%)
Ampi.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Sulf.Tetr	1(100%)	0(0%)	0(0%)
Tetr	12(48%)	9(36%)	4(16%)
Other [2]	105(47%)	62(27%)	59(26%)
Total resistant to at least one antimicrobial	118	72	66
Pan-susceptible	22	0	3

[1]: Isolates with a resistant pattern to at least one of the antimicrobials included in the panel

[2]: Patterns with fewer than 6 observations

Table 9: Frequency and percentage of resistance pattern row totals by sample location for *Salmonella infantis* serotype

Resistance Patterns [1]	**Arrival (n=76)**	**Post-Slaughter (n=18)**	**Lairage Environment (n=28)**
Amox.Ampi.Cefo.Cefti.Ceftr.Chlor.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Amox.Ampi.Cefo.Cefti.Ceftr.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Ampi.Chlor.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Ampi.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)
Sulf.Tetr	6(75%)	0(0%)	2(25%)
Tetr	0(0%)	0(0%)	0(0%)
Other	0(0%)	0(0%)	0(0%)
Total resistant to at least one antimicrobial	6	0	2
Pan-susceptible	70	18	26

[1]: Isolates with a resistant pattern to at least one of the antimicrobials included in the panel

Table 10: Frequency and percentage of resistance pattern row totals by sample location for *Salmonella* Typhimurium serotype (n= 78)

Resistance Patterns [1]	**Arrival**	**Post-Slaughter**	**Lairage Environment**
Amox.Ampi.Cefo.Cefti.Ceftr.Chlor.Stre.Sulf.Tetr	0(0%)	0(0%)	3(100%)
Amox.Ampi.Cefo.Cefti.Ceftr.Stre.Sulf.Tetr	0(0%)	1(100%)	0(0%)
Ampi.Chlor.Stre.Sulf.Tetr	9(24%)	19(50%)	10(26%)
Ampi.Stre.Sulf.Tetr	6(100%)	0(0%)	0(0%)
Stre.Sulf.Tetr	1(100%)	0(0%)	0(0%)
Stre.Tetr	0(0%)	0(0%)	0(0%)
Tetr	0(0%)	0(0%)	0(0%)
Other	9(31%)	15(52%)	5(17%)
Total resistant to at least one antimicrobial	25	35	18
Pan-susceptible	10	0	0

[1]: Isolates with a resistant pattern to at least one of the antimicrobials included in the panel

Table 11: Frequency and percentage of resistance pattern row totals by sample location for *Salmonella* Agona serotype (n= 54)

Resistance Patterns [1]	Arrival	Post-Slaughter	Lairage Environment	Total
Amox.Ampi.Cefo.Cefti.Ceftr.Chlor.Stre.Sulf.Tetr	7(17%)	31(74%)	4(9%)	0
Amox.Ampi.Cefo.Cefti.Ceftr.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)	0
Ampi.Chlor.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)	0
Ampi.Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)	0
Stre.Sulf.Tetr	0(0%)	0(0%)	0(0%)	0
Stre.Tetr	0(0%)	0(0%)	0(0%)	0
Tetr	3(100%)	0(0%)	0(0%)	0
Other [2]	6(67%)	1(11%)	2(22%)	0
Total resistant to at least one antimicrobial	16	32	6	
Pan-susceptible	27	6	5	

[1]: Isolates with a resistant pattern to at least one of the antimicrobials included in the panel

[2]: Patterns with fewer than 2 isolates

Table 12: Total number of samples by sample location and sample result for Campylobacter

Sample location	Negative	Positive	Positive -No isolate to test for AMR	Total
Arrival	7	122	10	139
Post-Slaughter	3	115	22	140
Lairage environment	7	66	7	80
Total	17	303	39	359

Table 13: Antimicrobial Resistance Patterns observed among Campylobacter isolates examined[1].

Resistance pattern	Number of isolates displaying pattern	Prevalence %
Susceptible	9	3.12%
Tet	149	51.73%
Cip, Tet	3	1.04%
Nal, Tet	1	0.34%
Azi, Tet	3	1.04%
Ery, Tet	2	0.69%
Azi, Ery, Tet	45	15.63%
Cip, Nal, Tet	35	12.15%
Cip, Ery, Nal, Tet	2	0.69%
Cip, Clin, Nal, Tet	1	0.34%
Azi, Cip, Ery, Nal	1	0.34%
Azi, Cip, Ery, Tet	1	0.34%
Azi, Ery, Nal, Tet	1	0.34%
Azi, Clin, Ery, Tet	15	5.21%
Azi, Cip, Clin, Nal, Tet	1	0.34%
Azi, Cip, Ery, Nal, Tet	10	3.47%
Azi, Cip, Clin, Ery, Nal, Tet	6	2.08%

[1]Total tested = 288

Table 14: Levels of multi drug resistance observed among Campylobacter isolates examined[1].

Number of Drugs	Number of Strains	Prevalence %
0	9	3.12%
1	149	51.73%
2	9	3.13%
3	80	27.77%
4	24	8.33%
5	11	3.82%
6	6	2.08%

[1]Total tested = 288

Table 15: Number of isolates resistant to classes of drugs among Campylobacter isolates examined[1].

Number of Classes of Drugs	Number of Isolates in Class	Prevalence %
1	149	51.73%
2	54	18.75%
3	56	19.44%
4	13	4.52%
5	7	2.43%
Susceptible	9	3.13%

[1]Total tested = 288

Table 16: Total number of subsamples by resistant pattern and sample location (E. coli, n=703)

Resistance patterns [1]	Arrival	Post-Slaughter	Lairage Environment	Total
Tetr	103	111	55	269
Stre.Tetr	39	52	31	122
Ampi.Stre.Tetr	57	31	14	102
Ampi.Tetr	23	28	7	58
Stre.Sulf.Tetr	11	9	8	28
Chlor.Stre.Sulf.Tetr	6	4	8	18
Ampi.Sulf.Tetr	2	6	4	12
Ampi.Stre.Sulf.Tetr	6	3	2	11
Ampi.Cefo.Cefti.Tetr	3	5	2	10
Stre.Sulf.Tetr.Trim	3	1	4	8
Other[2]	23	31	11	65
Total	276	281	146	703
Pan-susceptible	21	8	4	33

[1]: Isolates with a resistant pattern to at least one of the antimicrobials included in the panel

[2]: Patterns with fewer than 8 isolates

Table 17: Total number resistant pattern and sample location for common phenotypes for E. coli, (n=663 isolates with either hemolytic, rough, mucoid, or smooth phenotypes)

Phenotype and resistant pattern [1]	Arrival	Post-Slaughter	Lairage Environment	Total
Hemolytic				
Tetr	3	3	3	9
Stre.Tetr	1	1	0	2
Ampi.Sulf.Tetr	0	1	0	1
Ampi.Tetr	0	0	1	1
Stre.Sulf.Tetr	2	0	0	2
Total	6	5	4	15
Mucoid				
Tetr	2	11	3	16
Ampi.Tetr	0	6	0	6
Ampi.Stre.Tetr	7	2	0	9
Stre.Sulf.Tetr	2	2	0	4
Ampi.Cipr.Nali.Stre.Sulf.Tetr.Trim	2	2	0	4
Ampi.Chlor.Cipr.Gent.Nali.Stre.Sulf.Tetr.Trim	0	2	0	2
Chlor.Stre.Tetr	0	1	1	2
Chlor.Stre.Sulf.Tetr	0	1	0	1
Ampi.Cefo.Cefti	0	1	0	1
Ampi.Stre.Sulf.Tetr	1	0	0	1
Ampi.Gent.Stre.Sulf.Tetr	1	0	0	1
Total	15	28	4	47
Rough				
Tetr	73	67	36	176
Stre.Tetr	31	32	25	88
Ampi.Stre.Tetr	42	21	9	72
Ampi.Tetr	13	13	5	31
Stre.Sulf.Tetr	6	3	3	12
Ampi.Stre.Sulf.Tetr	5	3	2	10
Ampi.Stre.Sulf.Tetr.Trim	0	3	2	5
Ampi.Cefo.Chlor.Stre.Sulf.Tetr.Trim	0	2	0	2
Chlor.Stre.Sulf.Tetr	6	1	4	11
Stre.Sulf.Tetr.Trim	2	1	4	7
Ampi.Sulf.Tetr	0	1	3	4
Sulf.Tetr.Trim	2	1	1	4
Ampi.Cefo.Cefti.Tetr	0	1	1	2
Sulf.Tetr	2	1	0	3
Ampi.Cefo.Cefti.Stre.Sulf.Tetr	0	1	0	1
Ampi.Cefo.Cefti.Stre.Sulf.Tetr.Trim	0	1	0	1
Ampi.Chlor.Stre.Tetr	0	1	0	1
Chlor.Stre.Sulf.Tetr.Trim	0	1	0	1

Chlor.Stre.Tetr	0	1	0	1
Stre	2	0	1	3
Ampi.Cefo.Cefti.Chlor.Stre.Sulf.Tetr.Trim	0	0	1	1
Ampi.Chlor.Gent.Stre.Sulf.Tetr	0	0	1	1
Cefo.Tetr	0	0	1	1
Chlor.Stre.Sulf	0	0	1	1
Ampi.Cefo.Cefti.Stre.Tetr	2	0	0	2
Ampi.Cefo.Cefti.Chlor.Stre.Sulf.Tetr	1	0	0	1
Ampi.Cipr.Gent.Nali.Stre	1	0	0	1
Total	188	155	100	443
Smooth				
Tetr	21	22	10	52
Stre.Tetr	6	13	6	25
Ampi.Tetr	8	8	1	17
Ampi.Stre.Tetr	7	6	3	16
Stre.Sulf.Tetr	1	4	4	9
Ampi.Sulf.Tetr	2	4	1	7
Ampi.Cefo.Cefti.Tetr	3	2	1	6
Ampi.Chlor.Cipr.Gent.Nali.Stre.Sulf.Tetr.Trim	0	2	0	2
Chlor.Stre.Sulf.Tetr	0	1	3	4
Chlor.Stre.Sulf.Tetr.Trim	1	1	1	3
Ampi.Stre.Sulf.Tetr.Trim	1	1	0	2
Ampi	0	1	0	1
Ampi.Cefo.Cefti.Stre.Tetr	0	1	0	1
Ampi.Gent.Stre.Sulf.Tetr	0	1	0	1
Chlor.Nali.Sulf.Tetr	0	1	0	1
Stre.Sulf	0	1	0	1
Sulf.Tetr	0	1	0	1
Ampi.Chlor.Stre.Sulf.Tetr.Trim	1	0	1	2
Ampi.Cefo.Stre.Sulf.Tetr	1	0	0	1
Ampi.Chlor.Nali.Stre.Sulf.Tetr.Trim	1	0	0	1
Ampi.Chlor.Stre.Tetr	1	0	0	1
Ampi.Nali.Tetr	1	0	0	1
Chlor.Stre.Tetr	1	0	0	1
Stre.Sulf.Tetr.Trim	1	0	0	1
Total	57	70	31	158

[1]: Isolates with a resistant pattern to at least one of the antimicrobials included in the panel