Published in final edited form as:

Mol Cell Probes. 2010 December ; 24(6): 325–345. doi:10.1016/j.mcp.2010.07.004.

Comparison of phenotypic and genotypic antimicrobial profiles in *Escherichia coli* and *Salmonella enterica* from the same dairy cattle farms

Joy Scaria¹, Lorin D Warnick¹, John B. Kaneene², Katherine May², Ching-Hao Teng³, and Yung-Fu Chang¹

¹Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

²Center for Comparative Epidemiology, Michigan State University, East Lansing, Michigan

³Institute of Molecular Medicine, National Cheng Kung University Medical College, Tainan, Taiwan

Abstract

Transmission of antimicrobial drug resistance from resistant bacteria to non-resistant strains is an important public health issue. In this study, we have examined the possibility of multiple resistance gene transfer between *Escherichia coli* and *Salmonella* in the natural setting. Bacteria isolated from calves concurrently shedding *E. coli* and *Salmonella* showed similar antimicrobial drug resistance patterns as measured by a broth dilution method. However, microarray analysis of the antibiotic resistance at the gene level revealed several differences in resistance gene profile. Resistance profiles of *E. coli* isolated from different farms were closer than the profile of *E. coli* and *Salmonella* isolated from the same farm. This shows that the chance of multiple resistance gene transfers between these species is unlikely.

Keywords

Salmonella; *Escherichia coli*; Microarray; antibiotic resistance

Introduction

Increased prevalence of antibiotic resistant bacteria is a cause of concern for human and animal health. It is widely accepted that the use of antibiotics either for medical or agricultural purposes selects for resistant bacteria[1,2]. Many drug resistance genes reside on self-transmissible genetic elements like conjugative plasmids and transposons, which enable the transmission of resistance to other species[3,4]. The presence of antibiotics, even in subinhibitory concentrations, may select for resistant bacteria harboring such elements, which then have the potential to disseminate resistance to susceptible strains. Once acquired, these antibiotic resistance genes are slow to be lost even in the absence of selecting antibiotic[5,6]. This along

© 2010 Elsevier Ltd. All rights reserved.

*Corresponding author. Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, 14853. Phone: (607) 253-3675. Fax: (607) 253-3943, yc42@cornell.edu (YFC).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

with transfer of resistance determinants to unrelated species, makes it a serious threat to both human and animal health. For example, evidence of extensive interspecies transfer of integron mediated antimicrobial resistance genes among multidrug-resistant enterobacteriaceae has been reported [7]. Similarly, transfer of beta-lactam resistance between *Klebsiella pneumoniae* and *E. coli* in clinical settings is also known [8]. These examples of resistance transfer between relatively distantly related species raise the possibility of much more frequent exchange of resistance determinants between closely related bacterial species. For example, *E. coli* and *Salmonella* are closely related members of enterobacteriaceae sharing a common ancestor that diverged roughly 150 million years ago [9]. *In vitro* experiments have demonstrated that ampicillin resistance could be transferred from *Salmonella enterica* serovar *Typhimurium* DT104 to *E. coli* K12 [10]. However such studies do not accommodate the complexity of microbial systems in nature under which such transfers take place. High-throughput technologies like microarrays are being increasingly used for the detection and typing of their virulence factors and antibiotic resistance profile [11–13]. In this study, to investigate the possibility of multiple antibiotic resistance gene transfer between *E. coli* and *Salmonella* we have used microarrays to analyze the antimicrobial resistance and virulence gene profiles in *E. coli* and *Salmonella* isolated from bovine fecal samples from dairy farms that had concurrent shedding of both bacterial species.

Materials and Methods

Sample collection, isolation and identification of *E. coli* and *Salmonella*

To test the possibility that there could be a large scale transfer of antibiotic resistance genes between closely related species, we analyzed the antibiotic and virulence gene profile of *E. coli* and *Salmonella* isolated from bovine fecal samples with concurrent shedding of these two species. The samples (total 97 isolates) analyzed in this study were selected from a total number of 2088. Starting from September 2003, samples were collected over a period of 15 months as part of a milk replacer study from non-organic dairy farms from Michigan [14]. The antibiotic profile of isolates on the phenotypic level was analyzed using MIC testing and on the genotypic level using microarrays.

Farm selection for collection of fecal samples, selection of animals for testing, sample collection and laboratory methods for *E. coli* and *Salmonella* isolation and identification were described by Kaneene et al [14]. The details of the strains used in this study are given in Appendix 1.

Antimicrobial susceptibility testing

Commercially prepared microbroth dilution antimicrobial panels, with a prepared range of antibiotics were used for antimicrobial susceptibility testing for *E. coli* and *Salmonella* (Trek Diagnostics, CMV7CNCD)^h. For all plates, *E. coli* ATCC 25922 was used as quality control (QC). QC results were reviewed with each batch of tests run and if these were not within acceptable limits, all tests were re-run. All QC results were in expected range for results reported here. Except for ceftiofur and streptomycin, isolates were classified as resistant based on Clinical Laboratory Standards Institute (CLSI) break points.

Microarray development, hybridization and data analysis

Previously we have reported development of microarray for molecular typing of *Salmonella* serovars [13] and for differentiation of *E. coli* pathotypes [12]. These two arrays contained probes (70mers) designed using genus specific, species specific and virulence genes of *E. coli* pathotypes and *Salmonella* serovars. In this study we pooled all the probes from these two arrays and added another 114 probes designed on different classes of antimicrobial resistance genes. This new array contains a total of 891 probes and in addition to detecting the virulence

genes, could also detect the antimicrobial resistance profile of the strains. The complete details of the probes and genes represented in the array is given in Appendix 2. DNA isolation for microarray, microarray printing, slide processing, hybridization, scanning and data analysis was carried out as per protocols described by Scaria et al [13] and each experiment was carried out in duplicate.

Results

Antimicrobial resistance profiles from MIC data

A total of 61 *E. coli* and 36 *Salmonella* isolates were subcultured to pure culture from samples collected on three farms. For both the species, resistance to multiple antibiotics was observed (Table 1). All the *E. coli* strains were resistant to streptomycin. 95% of the strains were resistant to tetracycline. This was followed by 91% and 47% resistance to kanamycin and ampicillin respectively. All the strains were susceptible to amikacin, ciprofloxacin and nalidixic acid. Except for four isolates all other isolates were susceptible to all other antibiotics tested (Fig. 1).

While *E. coli* showed a diverse resistance pattern, *Salmonella* isolates showed a more skewed resistance pattern. All *Salmonella* isolates were susceptible to amikacin, ciprofloxacin and nalidixic acid. While 95% of *E. coli* isolates showed to tetracycline, streptomycin and kanamycin, only 80% of *Salmonella* isolates exhibited resistance to these antibiotics. The remaining 6 were susceptible to all antibiotics tested. In contrast to the 47% resistance shown in *E. coli* for ampicillin, all *Salmonella* except for the six universally susceptible isolates were resistant. Multidrug resistance in *Salmonella* was much more common than *E. coli* isolates. 33% of *Salmonella* isolates were resistant to ten out of sixteen antibiotics tested. The heat map representation of MIC data for *E. coli* and *Salmonella* is given in Fig.1.

Antimicrobial resistance profile of the isolates in the gene level

The array used in this work contained probes targeting most prevalent antibiotic resistance genes belonging to β -lactams, tetracyclines, aminoglycosides, macrolides and trimethoprim allowing higher resolution of resistance gene detection than MIC testing. In the macrolide class of resistance genes, *ermD* was the most prevalent gene present in *E. coli* (49%) and *Salmonella* (58%). Other genes in this class that were present in more than one *E. coli* isolate were *oleB* and *mphC* (<3%). One *Salmonella* isolate (#6170) was positive for most of the genes in all classes of antibiotics. If this isolate is excluded, all other *Salmonella* isolates were negative for all other macrolide genes. In the β -lactam category, *BIL*(55%), *IRT*(38%), *TEM1*(35%) and *carA* (30%) were most prevalent genes in *E. coli* isolates. *Salmonella* isolates also showed similar trend for these genes; *BIL*(47%), *IRT*(50%) and *TEM1*(36%). However, the striking difference was that all *Salmonella* isolates except for the highly resistant strain was negative for *carA* gene. Among tetracyclines, both species although showed similar trend in MIC level, the gene level pattern was strikingly different. Prevalent genes in *E. coli* for tetracycline's were *tetA*(38%) and *tetB*(64%). *tetA*(80%) was prevalent in *Salmonella* with *tetB* being a rare instance; *tetM* was frequent (30%) in *Salmonella* isolates, but absent in *E. coli*. Among aminoglycoside resistance genes *aph(6)-Id* (78%), *aph(3')-Ic* (63%) and *ant(3'')-Ia''* (8%) was most common in *Salmonella*. Pattern for aminoglycoside's in *E. coli* was similar but more diverse; *aph(6)-Id*(60%), *aph(3')-Ic*(35%), *ant(3'')-Ia''*(30%), *aph(3')-Ib*(17%), *aac(6')-IIa* (7%), *aac(3)-IIIa*(10%) and *aac(3)-Ia*(10%). Most common trimethoprim resistance gene was *hdfXII* in both *Salmonella* (33%) and *E. coli* (5%). However, *E. coli* isolates contained another additional trimethoprim gene *hdfXV* in equal frequency to that of *hdfXII* which was absent in *Salmonella* isolates. A heat map representation of full antibiotic resistance gene profile of all *E. coli* and *Salmonella* isolates is given in Fig. 2 and Fig. 3, respectively. This profile, although very similar to that of MIC data shows striking differences. Most prominent genes

that were present in *E. coli* but absent in *Salmonella* isolates were *carA* (β -lactam resistance, 30%), *tetB* (tetracycline resistance, 64%) and *aac(3)-IIIa* (10%). *mefE* gene conferring resistance to macrolides was very common in *Salmonella* (72%) but was absent in all *E. coli* isolates. Another difference between MIC data and microarray profile was the detection of resistance genes in the strains where MIC data showed susceptibility to all antibiotics. Microarray results were positive for several genes for the following strains which showed no resistance at MIC level; 6133 (*ermD*, *tetA*, *BIL*), 6134 (*mefE*, *tetA*, *TEM*, *aph(6)-ID*, *aph(3)-Ic*), 6136 (*ermD*, *mefE*, *tetA*, *IRT*, *TEM1*, *aph(6)-Id*, *aph(3)-Ic*), 6141 (*ermD*), *mefE*, *tetA*) and 6278(*mefE*, *tetA*, *IRT*, *aph(6)-ID*, *aph(3)-Ic*). However the strain 6161 was negative for all genes in array and was susceptible to all antibiotics at MIC level.

Hierarchical clustering (HCL) of strains was performed with Manhattan median method as the distance matrix (Fig.4). The clustering pattern obtained did not show any correlation between location of farm and genetic resistance profile. Rather all *E. coli* isolates tended to cluster together while *Salmonella* isolates formed separate clusters (Fisher exact test p-value < 0.0001). When considering *E. coli* and *Salmonella* together, there was an association between farm and cluster (p-value =0.002). However, for *E. coli* alone, there was no significant association (p-value = 0.2). For *Salmonella* alone there was a significant association (p-value < 0.0001). This is consistent with *E. coli* cluster types being more evenly distributed among the farms while some *Salmonella* cluster types tended to be more farm specific. This shows that the resistance profiles of all *E. coli* isolates from different farms are closer than that of the profile of an *E. coli* and *Salmonella* isolated from the same farm. Different clustering methods did not alter the cluster configuration significantly.

Analysis of virulence genes

Analyzing the genetic relatedness of the isolates would be a better indicator of the strain dispersion and this could reveal clonality. When samples were hierarchically clustered based on array results from virulence genes, all the clusters formed contained isolates from all farms, indicating that these are not clonal and there is widespread strain dispersion (Fig.5). The only instance of samples from one farm forming a sub-cluster within a main cluster was observed with the group of samples 6318, 6291, 6166, 6582, 6162. These samples had also formed one tight cluster in HCL tree of antibiotic resistance genes. However, for all these samples gene variability was detected for *bcf* fimbrial genes, *sth* fimbrial genes, *fels* elements, phage-5 genes and among genes in SPI-3 region. This variability in multiple loci is a clear evidence for the fact that none of these isolates were clonal.

The probes for *E. coli* genes also included common genes and virulence genes from different pathotypes. When HCL tree based on these was built for the *E. coli* isolates (Fig. 6), the trend was similar to that of the *Salmonella*. All the clusters formed were containing isolates from all the farms, again indicating that there is a large-scale dispersion of these strains. Also, there was a large number of variability with respect to the virulence gene content.

Discussion

The global rise in antimicrobial resistance is not new, but the number of resistant species, the geographical locations affected and the increase in multidrug resistant bacteria is cause for concern [5]. One of the major reasons for the large scale dissemination of resistance is the horizontal transfer of resistance genes between closely related and even to unrelated bacterial species. The continuous presence of antibiotic in an ecological niche promotes the selection of resistant forms and then the possibility of resistant genes being transferred from the resistant bacteria to susceptible bacteria is very high. The large-scale agricultural use of antibiotics is such an example[15]. Reviews have suggested that of the 22.7 million Kg of antibiotics produced in United States, approximately 17.8% were used in animal production[16]. This

point to the possibility of selection and dissemination of resistance genes between closely related species like *Salmonella* and *E. coli* in agricultural settings. To test this possibility, we analyzed the antimicrobial resistance profiles of *E. coli* and *Salmonella* isolates from dairy farms in Michigan. Farms were initially selected based on using oxytetracycline and neomycin in milk replacer fed to calves and the presence of tetracycline-resistant enteric bacteria. Use of these antibiotics in calf feed may select for the resistant forms of bacteria. Farm selection for collection of fecal samples was previously described [14]. All the isolates of both species were susceptible to amikacin, ciprofloxacin and nalidixic acid. Almost absolute resistance was shown for tetracycline, kanamycin and streptomycin. There were several multidrug resistant strains. Previous surveys of antimicrobial resistance in *E. coli* and *Salmonella* from dairy farms in USA reported mixed levels of resistance [17–20]. However, the most prevalent resistance was seen for tetracycline, streptomycin and ampicillin [19,20] which is in consensus with our results. Analysis of MIC data showed that the overall resistance patterns for the two species were similar. A similar conclusion was drawn after a study of *E. coli* and *Salmonella* isolates from 21 states in the US [19]. Just the phenotypic level of analysis of resistance alone would then support the theory of large-scale resistance gene transfer between *E. coli* and *Salmonella*. Although MIC testing shows the phenotypic and expressed levels of resistance, it does not provide any information about the underlying genes responsible for the resistance. Microarrays provide an excellent means of detecting this in a high throughput manner. The occurrence profile of *ermD* (macrolide resistance), *BIL*, *IRT*, *TEM1*, *carA* (all conferring β -lactam resistance), *tetA* (tetracycline resistance), *aph(6)-Id*, *aph(3')-Ic* and *ant(3'')-Ia* (aminoglycoside resistance) genes were similar in *E. coli* and *Salmonella*. Based on such similarities, other studies have indicated the possibility of gene transfer between *E. coli* and *Salmonella* [21]. Although the resemblance in the gene profiles supported the possibility of gene exchange, the possibility of large scale transfers appeared less likely due to the differences antibiotic gene profiles detected. Most striking differences were the presence of *carA*, *tetB* and *aac(3)-IIIa* genes in *E. coli* but absent in *Salmonella*. *mefE* (macrolide resistance) which was present in 72% of *Salmonella* was absent in all *E. coli* isolates. If there was large-scale gene transfer between these two species, then it would be expected that at least one of these high prevalence genes in one species would have been transferred to the other. The three major mechanisms by which bacteria acquire genes are conjugation, transformation and transduction. However, several barriers of gene transfer even between closely related bacteria like *E. coli* and *Salmonella* is shown to be operational [22]. These barriers include surface exclusion, restriction-modification system and mismatch-repair [23]. Surface exclusion is a barrier to conjugation and is the major mechanism that prevents conjugation between unrelated species. It has been shown that mismatch-repair is major barrier to recombination between *E. coli* and *Salmonella* [22]. The success of antibiotic resistance gene transfer between these species then would depend on mechanism of exchange. For example, conjugative transfer of donor plasmid from *Salmonella enterica* serovar Typhimurium DT104 strain to a recipient *Escherichia coli* K12 strain has demonstrated [10]. Such plasmid mediated mechanisms could account for the similarities in resistance gene profile found in our study. Since we used total DNA from isolates for microarray, the difference between a plasmid and genomic DNA mediated mechanism could not be delineated. Another common route of multiple antibiotic resistance gene transfer are integrons [24]. Integrons provide bacteria with a gene-capture system perfectly adapted for the challenges of multiple-antibiotic treatment regimes [25]. However, since array used in this study did not contain probes for integrons or integron associated genes, we could not ascertain the possibility of involvement of integron mediated resistance gene transfer.

Evidence against large scale gene transfer also come from the HCL tree constructed based on the antibiotic gene profile of *Salmonella* and *E. coli*. In all the clusters formed, all *E. coli* grouped together while other clusters were composed of *Salmonella* only. Had there been large-scale gene transfers between them, *E. coli* and *Salmonella* from one farm would have had similar profiles and would have formed clusters based on origin of isolation. The HCL tree

constructed based on common and virulence genes shows that there is widespread dissemination of strains across the farms and the strains are not clonal.

When compared to MIC data, use of microarray yielded higher resolution of resistance gene detection. For instance, the samples 6133, 6134, 6136, 6141 and 6278 showed no resistance at MIC level, but showed the presence of resistance genes in microarray analysis, indicating the possibility of strains harboring these genes but not expressing them. Thus this study shows that microarray could be used as a complimentary tool MIC testing to extract additional information like presence of silent resistance genes and the genetic relatedness of stains. This study also suggests that the rate of dissemination of resistant *E. coli* or *Salmonella* to different geographical locations is greater than the rate of resistance gene exchange between them.

Acknowledgments

This project was supported with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institute of Health, Department of Health and Human Services under contract, N01-AI-30054, Project No. ZC002-03, the Federal Formula Fund from the Cornell University Agricultural Experiment Station,

References

- Walton J. In vivo transfer of infectious drug resistance. *Nature* 1966;211:312–313. [PubMed: 5337905]
- Roberts MC. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiol Rev* 1996;19:1–24. [PubMed: 8916553]
- Oyarzabal OA, Rad R, Backert S. Conjugative transfer of chromosomally encoded antibiotic resistance from *Helicobacter pylori* to *Campylobacter jejuni*. *J Clin Microbiol* 2007;45:402–408. [PubMed: 17135441]
- Sedgley CM, Lee EH, Martin MJ, Flannagan SE. Antibiotic resistance gene transfer between *Streptococcus gordonii* and *Enterococcus faecalis* in root canals of teeth ex vivo. *J Endod* 2008;34:570–574. [PubMed: 18436036]
- Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med* 2004;10:S122–S129. [PubMed: 15577930]
- Pallecchi L, Bartoloni A, Paradisi F, Rossolini GM. Antibiotic resistance in the absence of antimicrobial use: mechanisms and implications. *Expert Rev Anti Infect Ther* 2008;6:725–732. [PubMed: 18847408]
- Leverstein-van Hall MA, Box AT, Blok HE, Paauw A, Fluit AC, Verhoef J. Evidence of extensive interspecies transfer of integron-mediated antimicrobial resistance genes among multidrug-resistant *Enterobacteriaceae* in a clinical setting. *J Infect Dis* 2002;186:49–56. [PubMed: 12089661]
- Bidet P, Burghoffer B, Gautier V, et al. In vivo transfer of plasmid-encoded ACC-1 AmpC from *Klebsiella pneumoniae* to *Escherichia coli* in an infant and selection of impermeability to imipenem in *K. pneumoniae*. *Antimicrob Agents Chemother* 2005;49:3562–3565. [PubMed: 16048985]
- Euzeby JP. Revised Salmonella nomenclature: designation of Salmonella enterica (ex Kauffmann and Edwards 1952) Le Minor and Popoff 1987 sp. nov., nom. rev. as the neotype species of the genus *Salmonella* *Lignieres* 1900 (approved lists 1980), rejection of the name *Salmonella choleraesuis* (Smith 1894) Weldin 1927 (approved lists 1980), and conservation of the name *Salmonella typhi* (Schroeter 1886) Warren and Scott 1930 (approved lists 1980). Request for an opinion. *Int J Syst Bacteriol* 1999;49(Pt 2):927–930. [PubMed: 10319519]
- Walsh C, Duffy G, Nally P, O'Mahony R, McDowell DA, Fanning S. Transfer of ampicillin resistance from *Salmonella Typhimurium* DT104 to *Escherichia coli* K12 in food. *Lett Appl Microbiol* 2008;46:210–215. [PubMed: 18028324]
- Frye JG, Jesse T, Long F, et al. DNA microarray detection of antimicrobial resistance genes in diverse bacteria. *Int J Antimicrob Agents* 2006;27:138–151. [PubMed: 16427254]
- Palaniappan RU, Zhang Y, Chiu D, et al. Differentiation of *Escherichia coli* pathotypes by oligonucleotide spotted array. *J Clin Microbiol* 2006;44:1495–1501. [PubMed: 16597882]

13. Scaria J, Palaniappan R, Chiu D, et al. Microarray for molecular typing of *Salmonella enterica* serovars. *Molecular and Cellular Probes*. 2008
14. Kaneene JB, Warnick LD, Bolin CA, Erskine RJ, May K, Miller R. Changes in Tetracycline Susceptibility of Enteric Bacteria Following Switching to Non-Medicated Milk Replacer in Dairy Calves. *J Clin Microbiol*. 2008
15. Silbergeld EK, Graham J, Price LB. Industrial food animal production, antimicrobial resistance, and human health. *Annu Rev Public Health* 2008;29:151–169. [PubMed: 18348709]
16. Mathew AG, Cissell R, Liamthong S. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathog Dis* 2007;4:115–133. [PubMed: 17600481]
17. Blau DM, McCluskey BJ, Ladely SR, et al. *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005;68:696–702. [PubMed: 15830658]
18. Fluckey WM, Loneragan WG, Warner R, Brashears MM. Antimicrobial drug resistance of *Salmonella* and *Escherichia coli* isolates from cattle feces, hides, and carcasses. *J Food Prot* 2007;70:551–556. [PubMed: 17388041]
19. Lundin JI, Dargatz DA, Wagner BA, et al. Antimicrobial drug resistance of fecal *Escherichia coli* and *Salmonella* spp. isolates from United States dairy cows. *Foodborne Pathog Dis* 2008;5:7–19. [PubMed: 18260811]
20. Ray KA, Warnick LD, Mitchell RM, et al. Prevalence of antimicrobial resistance among *Salmonella* on midwest and northeast USA dairy farms. *Prev Vet Med* 2007;79:204–223. [PubMed: 17215055]
21. van Essen-Zandbergen A, Smith H, Veldman K, Mevius D. Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter* spp. in the Netherlands. *J Antimicrob Chemother* 2007;59:746–750. [PubMed: 17307772]
22. Rayssiguier C, Thaler DS, Radman M. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* 1989;342:396–401. [PubMed: 2555716]
23. Thomas CM, Nielsen KM. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nat Rev Microbiol* 2005;3:711–721. [PubMed: 16138099]
24. Stokes HW, Hall RM. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. *Mol Microbiol* 1989;3:1669–1683. [PubMed: 2560119]
25. Mazel D. Integrons: agents of bacterial evolution. *Nat Rev Microbiol* 2006;4:608–620. [PubMed: 16845431]

Fig. 1. Heat map representation of MIC data for all *E. coli* (panel A) and *Salmonella* (panel B) strains tested. Column labels represent the antibiotic and row label represent each strain the color scheme is as follows: red – susceptible, green – resistant, black – Not interpreted.

Fig. 2.

Heat map representation of microarray data for antibiotic resistance genes of all *E. coli* strains. Column labels represent the strains and row label represent genes. The color scheme is as follows: red – gene absent, green – gene present, black – uncertain.

Fig. 4. Hierarchical Clustering of *Salmonella* and *E. coli* isolates based on antibiotic resistance gene profile. Genes were clustered using Manhattan median method as the distance matrix with average linkage rule. Red areas = gene absence, grey areas = uncertain, green areas = gene presence. F1E = Farm1 *E. coli*, F2E = Farm2 *E. coli*, F3E = Farm 3 *E. coli*, F1S = Farm 1 *Salmonella*, F2S = Farm 2 *Salmonella*, F3S = Farm 3 *Salmonella*.

Fig. 5. Hierarchical Clustering of *E. coli* isolates based on Virulence gene profile. Genes were clustered using Manhattan median method as the distance matrix with average linkage rule. Genes were clustered using Pearson absolute method as the distance matrix with average linkage rule. Red areas = gene absence, grey areas = uncertain, green areas = gene presence. F1 = Farm 1, F2 = Farm 2 and F3 = Farm 3.

Fig. 6.

Hierarchical Clustering of *Salmonella* isolates based on virulence gene profile. Genes were clustered using Manhattan median method as the distance matrix with average linkage rule. Genes were clustered using Pearson absolute method as the distance matrix with average linkage rule. Red areas = gene absence, grey areas = uncertain, green areas = gene presence. F1 = Farm 1, F2 = Farm 2 and F3 = Farm 3.

Table 1

Common antimicrobial resistance patterns of *Salmonella* and *E. coli* isolated from calves shedding these bacteria concurrently. Antibiotic names are abbreviated as follows; Amp- Ampicillin, Amox- AmoxClav, Cef – Ceftriaxone, Cefo – Cefoxitin, Ceft- Ceftiofur, Cep – Cephalothin, Chl – Chlamphenicol, Gen- Gentamycin, Kan – Kanamycin, Strep – Streptomycin, Sulf- Sulfamethoxazole, Tet – Tetracycline, Tri- Trimethoprim

Resistance pattern	Number of Isolates	Organism
Amp, Kan, Strep, Sulf, Tet	17	<i>E. coli</i>
Gen, Kan, Strep, Tet	2	<i>E. coli</i>
Amox, Amp, Ceft, Cep, Cef, Cefo, Gen, Kan, Strep	3	<i>E. coli</i>
Kan, Strep, Sulf	13	<i>E. coli</i>
Kan, Strep, Sulf, Tet	7	<i>E. coli</i>
Kan, Strep, Sulf, Tet, Tri	2	<i>E. coli</i>
Amp, Amox, Ceph, Chl, Gen, Kan, Strep, Sulf, Tet, Ceft	16	<i>Salmonella</i>
Amp, Kan, Strep, Tet	10	<i>Salmonella</i>
Amp, Amox, Kan, Strep, Tet	1	<i>Salmonella</i>
Non Resistant	7	<i>Salmonella</i>
Ungrouped	19	<i>E.coli</i> , <i>Salmonella</i>

Strain ID	Organism	Strain	Serotype
6129	<i>Escherichia coli</i>	E.coli 80-53	NA
6130	<i>Escherichia coli</i>	E.coli 104-69	NA
6131	<i>Escherichia coli</i>	E.coli 77-67	NA
6132	<i>Salmonella</i>	Sal 2-3	reading
6133	<i>Salmonella</i>	Sal 3-21	bovis-morbificans
6134	<i>Salmonella</i>	Sal 2-19	bovis-morbificans
6135	<i>Escherichia coli</i>	E.coli 33-57	NA
6136	<i>Salmonella</i>	Sal 3-77	bovis-morbificans
6137	<i>Escherichia coli</i>	E.coli 26-9	NA
6138	<i>Salmonella</i>	Sal 8-5	oranienburg
6139	<i>Escherichia coli</i>	E.coli 101-21	NA
6140	<i>Escherichia coli</i>	E.coli 28-25	NA
6141	<i>Salmonella</i>	Sal 2-49	bovis-morbificans
6143	<i>Escherichia coli</i>	E.coli 105-29	NA
6146	<i>Escherichia coli</i>	E.coli 85-41	NA
6147	<i>Escherichia coli</i>	E.coli 38-11	NA
6148	<i>Escherichia coli</i>	E.coli 35-47	NA
6149	<i>Escherichia coli</i>	E.coli 87-75	NA
6150	<i>Escherichia coli</i>	E.coli 87-37	NA
6151	<i>Escherichia coli</i>	E.coli 110-59	NA
6152	<i>Escherichia coli</i>	E.coli 11-13	NA
6153	<i>Escherichia coli</i>	E.coli 112-71	NA
6154	<i>Escherichia coli</i>	E.coli 86-33	NA
6158	<i>Escherichia coli</i>	E.coli 32-63	NA
6159	<i>Escherichia coli</i>	E.coli 101-15	NA
6160	<i>Salmonella</i>	Sal 9-53	oranienburg
6161	<i>Salmonella</i>	Sal 3-63	bovis-morbificans
6162	<i>Salmonella</i>	Sal 2-67	oranienburg
6163	<i>Escherichia coli</i>	E.coli 81-9	NA
6164	<i>Salmonella</i>	Sal 2-67	bovis-morbificans
6165	<i>Escherichia coli</i>	E.coli 109-65	NA
6166	<i>Salmonella</i>	Sal 4-59	oranienburg
6168	<i>Salmonella</i>	Sal 3-41	enteritidis
6169	<i>Salmonella</i>	Sal 9-53	oranienburg
6170	<i>Salmonella</i>	Sal 4-79	oranienburg
6174	<i>Escherichia coli</i>	E.coli 38-59	NA
6175	<i>Escherichia coli</i>	E.coli 95-31	NA
6176	<i>Escherichia coli</i>	E.coli 93-27	NA
6177	<i>Escherichia coli</i>	E.coli 99-75	NA
6178	<i>Escherichia coli</i>	E.coli 92-47	NA
6179	<i>Escherichia coli</i>	E.coli 38-29	NA

Strain ID	Organism	Strain	Serotype
6180	<i>Escherichia coli</i>	E.coli 9-15	NA
6181	<i>Escherichia coli</i>	E.coli 39-67	NA
6182	<i>Escherichia coli</i>	E.coli 39-73	NA
6183	<i>Escherichia coli</i>	E.coli 115-9	NA
6184	<i>Escherichia coli</i>	E.coli 116-75	NA
6185	<i>Escherichia coli</i>	E.coli 4-11	NA
6186	<i>Escherichia coli</i>	E.coli 96-15	NA
6187	<i>Escherichia coli</i>	E.coli 115-27	NA
6188	<i>Escherichia coli</i>	E.coli 4-13	NA
6264	<i>Escherichia coli</i>	E.coli 121-51	NA

Probe name	Gene name
eco1	F165F
eco3	focF
eco4	focG
eco5	focH
eco6	afaD1
eco7	focC
eco8	focD
eco9	fsoE
eco10	fimB
eco12	rfc
eco13	afaD
eco14	iucD=AerA
eco15	hlyA
eco16	hlyC
eco17	papG1
eco18	papGII
eco19	traT
eco20	drbE122
eco21	cdtC
eco22	afaE -5
eco23	kpsM
eco24	nfaE
eco25	cnF1
eco26	cdtA
eco27	papC
eco28	papE
eco29	papG3
eco30	cdtB
eco31	cvi
eco32	cnf2
eco33	kfiB
eco34	afaC
eco35	afaB nfaE (S61968)
eco36	usp
eco37	F165Afimbriae
eco37	focG
eco38	F165G
eco39	F165F
eco40	hlyB

Probe name	Gene name
eco41	hlyD
eco42	papX
eco43	papF
eco44	papK
eco45	papJ
eco46	papH
eco47	papA
eco48	papI
eco49	sat
eco54	papH_2
eco56	senB
eco57	senA