Comparison of Molecular Subtyping and Antimicrobial Resistance Detection Methods Used in a Large Multi-State Outbreak of Extensively Drug-Resistant Campylobacter jejuni Infections Linked to Pet Store Puppies


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Running title: Molecular epidemiology of Campylobacter jejuni

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ABSTRACT

Campylobacter jejuni is a leading cause of enteric bacterial illness in the United States. Traditional molecular subtyping methods, such as pulsed-field gel electrophoresis (PFGE) and 7-gene multilocus sequencing typing (MLST), provided limited resolution to adequately identify C. jejuni outbreaks and separate out sporadic isolates during outbreak investigations. Whole genome sequencing (WGS) has emerged as a powerful tool for C. jejuni outbreak detection. In this investigation, 45 human and 11 puppy isolates obtained during a 2016-2018 outbreak linked to pet store puppies were sequenced. Core genome multilocus sequence typing (cgMLST) and high-quality single nucleotide polymorphism (hqSNP) analysis of the sequence data separated the isolates into the same two clades containing minor within clade differences; however, cgMLST analysis does not require selection of an appropriate reference genome making this method preferable to hqSNP analysis for Campylobacter surveillance and cluster detection. The isolates were classified as ST2109—a rarely seen MLST sequence type. PFGE was performed on 38 human and 10 puppy isolates; PFGE patterns did not reliably predict clustering by cgMLST analysis. Genetic detection of antimicrobial resistance determinants predicted that all outbreak-associated isolates would be resistant to six drug classes. Traditional antimicrobial susceptibility testing (AST) confirmed a high correlation between genotypic and phenotypic antimicrobial resistance determinations. WGS analysis linked C. jejuni isolates in humans and pet store puppies even when canine exposure information was unknown, aiding the epidemiological investigation during this outbreak. WGS data were also used to quickly identify the highly drug-resistant profile of these outbreak-associated C. jejuni isolates.
INTRODUCTION


C. jejuni is the most common Campylobacter species associated with human illness (1-2).

Though Campylobacter cases are usually sporadic, previous Campylobacter outbreaks have been linked to consumption of raw milk, contaminated water, chicken meat, and raw peas (3-7). Exposure to pets such as dogs also carries a risk of Campylobacter infection (1-3, 8-9). During 2012 to 2017, 13 canine-associated Campylobacter outbreaks were reported through the National Outbreak Reporting System (NORS, https://wwwn.cdc.gov/norsdashboard/) to the Animal Contact Outbreak Surveillance System. Studies have shown C. jejuni may cause diarrhea in dogs, especially puppies, or that dogs can carry C. jejuni asymptomatically (9).

Pulsed-field gel electrophoresis (PFGE) and 7-gene multilocus sequence typing (MLST) have been used historically to differentiate sporadic from outbreak C. jejuni isolates (4-5, 10-11). However, whole genome sequencing (WGS) has been shown to provide superior resolution and concordance with epidemiologic data when compared with PFGE and MLST during these outbreak investigations (12-16). WGS provides information across a broader range of the genome, and is therefore more informative than methods such as PFGE and MLST that provide information from limited parts of the genome (17-18). WGS analysis methods used in Campylobacter outbreak investigations can include: i. high quality single nucleotide...
polymorphism (hqSNP) analysis, which compares isolate genomes to a closely related reference to derive SNP differences; ii. core genome (cg)MLST analysis, which examines allele differences at core genome loci of the isolates (those loci are found in 95% of the reference organism strains); and iii. whole genome (wg)MLST analysis, which examines allele differences between core and accessory genome loci of the isolates (13, 19-21). Since cgMLST examines allele differences in genes that are common to all isolates being compared, it should be well-suited for surveillance. If further resolution between potential outbreak isolates is needed, then hqSNP or wgMLST can be used (22-23).

Most patients recover from Campylobacter infections without antibiotic treatment; however, antibiotic treatment is recommended in patients with severe illness or risk factors for severe disease such as age >65 years, pregnancy or immune suppression (24). Broth microdilution is the preferred method used for antimicrobial susceptibility testing (AST) of Campylobacter isolates (25). This method is not performed by many laboratories and is time consuming for Campylobacter isolates, which can take 48 hours to grow and requires a microaerophilic environment (26). WGS analysis is a timely, cost-effective method to identify resistance determinants and accurately predict the corresponding phenotypes (23, 27-29). Recent work with Campylobacter has demonstrated a strong correlation between resistance as determined by AST and resistance as predicted through the identification of known genetic resistance determinants from WGS data (25).

In this study, we examined sequences from 56 outbreak-associated C. jejuni isolates obtained from 45 human and 11 puppy fecal specimens during a multistate outbreak linked to pet store puppies (30). We compared the ability of PFGE, 7-gene MLST, cgMLST, and hqSNP analyses to
identify outbreak isolates after conclusion of the investigation. We identified resistance determinants using WGS data and inferred a predicted resistance pattern, which was compared with phenotypic AST results for a subset of isolates.

MATERIALS AND METHODS

Isolates. From January 2016 through February 2018, C. jejuni isolates from 51 stool specimens and 23 puppy fecal specimens were obtained; however, only 45 human and 11 puppy isolates were consistent with the case definition described by Montgomery et al (30). Human stool specimens or isolates were submitted to the public health laboratories (PHLs) from physician offices or clinical laboratories per state-specific requirements. In addition, puppy feces specimens were collected by PHL and agriculture officials from 29 pet stores in six states.

Isolate information is listed in Supplemental Table 1.

Pulsed-field gel electrophoresis. PFGE was performed by PHLs or at CDC for a subset of isolates obtained from human and puppy feces specimens using the Standard Operating Procedure for PulseNet PFGE of Campylobacter jejuni (17). The DNA was digested with restriction enzyme SmaI (48 isolates) and in some cases DNA was also digested with restriction enzyme KpnI (35 isolates). PFGE patterns were analyzed in BioNumerics v6.6.10 (Applied Maths, Sint-Martens-Latem, Belgium) and uploaded to the PulseNet Campylobacter National Database.

Whole genome sequencing and analysis. Genomic DNA was extracted from overnight cultures of the isolates listed in Supplemental Table 1 and the DNA was sequenced by PHLs or CDC according to the Standard Operating Procedure for PulseNet Nextera XT Library Prep and Run Setup for the Illumina Miseq (https://www.cdc.gov/pulsenet/pdf/pnl32-miseq-nextera-xt.pdf).

The sequences were analyzed in BioNumerics v7.6.3 (Applied Maths, Sint-Martens-Latem,
Belgium) and cgMLST was performed on assemblies that were 1.6-1.8 Mbp in length, had ≥ 49X basepair coverage, and had 88-98% of core genome (1168—1316) loci identified which are within PulseNet quality control thresholds (1.4-2.2 Mbp length, ≥ 20X basepair coverage, ≥ 85% of core genome loci identified). The cgMLST scheme, available at [http://pubmlst.org/campylobacter](http://pubmlst.org/campylobacter) in the BioNumerics database contains 1343 C. jejuni/C. coli loci (20). Cluster analysis using cgMLST allele calls from loci identified in all 56 sequences (categorical values) was used to generate an unweighted pair group method with arithmetic mean (UPGMA) dendrogram. Loci without allele calls were ignored in pairwise sequence comparisons used to generate the tree.

To generate a closed reference sequence, genomic DNA from a representative isolate included in the largest cgMLST clade (2017D-0132) was also extracted from an overnight culture using the Promega Wizard Genomic DNA Purification Kit (Madison, WI). Library size selection was completed using Bluepippin (Sage Science, Beverly, MA). The library was sequenced on the Pacific Biosciences (PacBio, Menlo Park, CA) RSII instrument using one SMRT cell and P6-C4 chemistry for 360 minutes. The sequence was initially assembled using the PacBio Hierarchical Genome Assembly Process version 3 (31). The mean coverage of the PacBio sequence was 488.3X and the chromosomal contig (1.67 Mbp sequence length, 30.6 %GC) and two plasmid contigs (contig 1 = 55.9 Kbp sequence length and 28.4 %GC, contig 2 = 31.9 Kbp sequence length and 28.3 %GC) were circular and complete. Unicycler-polish v0.4.4 with Pilon v1.22 ([https://github.com/rrwick/Unicycler](https://github.com/rrwick/Unicycler)) was used to polish this assembly using the Illumina sequence reads for this isolate (32-33).
HqSNP analysis was performed by trimming the Illumina sequence reads using fastx_trimmer three bases from the 5’ ends and mapping the sequences to the assembled chromosomal PacBio sequence using Lyve-SET version 1.1.4f with SMALT (34-35). SNPs were called using VarScan (36) at >20X coverage, >95% read support, and allowed flanking set to 100 bp (21). To provide a side-by-side visual comparison of the cgMLST and hqSNP sequence analysis, a tanglegram was generated in R v3.5.1 (https://github.com/rstudio/rstudio) using the dendextend package (37). Several layout optimization methods were evaluated (DendSer, labels, ladderize, random, step1side, and step2side) and among these the step2side method produced the best result and was used for optimization (https://github.com/jchen232/CampyPuppyTangle).

In silico 7-gene MLST analysis of sequences was performed using a feature in BioNumerics v7.6 which provides the MLST sequence types (STs) from the Campylobacter MLST database (http://pubmlst.org/campylobacter).

Acquired and mutational antimicrobial resistance determinants in these isolates were detected by analyzing the assembled sequences and through read-based approaches as previously described (38). Briefly, resistance genes were detected using Megablast in ncbi-blast+ v. 2.3.0 and the ResFinder database (updated on 03-02-2018) using cutoffs of 90% identity and 50% coverage (39). Read mapping to 23S rRNA and gyrA reference sequences (NCBI accession numbers Z29326.1, and NC_002163.1 respectively) in CLC Genomics Workbench v11.0 (Qiagen, Redwood City, CA) was used to detect mutations in these genes. Predicted phenotypes were assigned based on the presence/absence of resistance determinants. Lastly, predicted resistance heatmaps based on the cgMLST UPGMA tree constructed in BioNumerics v7.6 were generated in R v3.5.1 using the gheatmap function in the ggtree package (40).
Antimicrobial Susceptibility Testing. The minimum inhibitory concentrations (MICs) of nine antimicrobials (azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, nalidixic acid, telithromycin, tetracycline) belonging to 7 antimicrobial classes on the National Antimicrobial Resistance Monitoring System (NARMS) Campylobacter Panel were determined for 19 human and nine puppy isolates (denoted on Figure 2) using a standard broth microdilution assay (Sensititre; Thermo Fisher Scientific, Waltham, MA) and the results were interpreted using clinical breakpoints or epidemiologic cutoff values from The European Committee on Antimicrobial Susceptibility Testing (EUCAST, http://www.eucast.org/clinical_breakpoints).

Resistance Classification. Isolates are reported as “susceptible” and “resistant” throughout this manuscript according to the EUCAST criteria (http://www.eucast.org/, accessed 02/15/2020). Briefly, isolates were categorized as “susceptible” if they had either an MIC in the susceptible range according to clinical breakpoints or a wild-type MIC according to epidemiological cutoff value (ECOFF). Isolates were categorized as “resistant” if they had either a resistant result according to clinical breakpoints or had an MIC above the ECOFF (not wild-type). Although there is not currently an accepted definition for what constitutes extensive drug resistance in C. jejuni, there is growing awareness of the need to distinguish strains with resistance to multiple antimicrobials (increasingly common in the United States) from those for which treatment options are limited to broad-spectrum antibiotics rarely required for the management of Campylobacter infections (41). Here, we use the term extensively drug-resistant (XDR) to refer to strains that are resistant to macrolides and fluoroquinolones (the antimicrobial classes...
recommended for treatment of *Campylobacter* and three or more additional antimicrobial classes (42).

**Accession numbers.** Illumina sequence reads were deposited into the Sequence Read Archive at NCBI with the accession numbers shown in Supplemental Table 1. The closed reference genome of *C. jejuni* isolate 2017D-0132 (Biosample SAMN07615386) was deposited into GenBank at NCBI with the accession numbers shown in Supplemental Table 1. The closed reference genome of *C. jejuni* isolate 2017D-0132 (Biosample SAMN07615386) was deposited into GenBank at NCBI with the accession numbers shown in Supplemental Table 1. The closed reference genome of *C. jejuni* isolate 2017D-0132 (Biosample SAMN07615386) was deposited into GenBank at NCBI with the accession numbers shown in Supplemental Table 1.

**RESULTS**

**PFGE Analysis.** *SmaI* PFGE performed on a subset of 38 human and 10 puppy isolates resulted in seven different patterns designated A—G (Figures 1 and 2). *KpnI* PFGE performed on 25 human and 10 puppy isolates resulted in three different patterns designated 1—3 and 10 different *SmaI/KpnI* pattern combinations (Figures 1 and 2). Isolates not analyzed by *KpnI* were provided a *KpnI* pattern designation 0 and isolates not analyzed by PFGE were designated NP for PFGE 'Not Performed' (Figure 2).

**MLST, cgMLST, hqSNP Analysis.** Using *In silico* MLST, we determined that all of the sequenced isolates (45 human and 11 puppy isolates) were ST2109. These isolates formed two distinct clades (Clades 1 and 2) by cgMLST analysis; each clade contained isolates from both cases with and without known exposures to pet stores and puppies with clade allelic differences ranging from 0 to 40 (Figure 2). Clades 1 and 2 were differentiated from each other by 201-241 alleles. Several isolates that were separated by two-enzyme PFGE clustered together by cgMLST analysis. Isolates with pattern combinations A2 and B1 fell into cgMLST Clade 1 only, isolates producing pattern combinations C3 and D3 fell into cgMLST Clade 2 only, and only one isolate...
each produced pattern combinations B3, E3, F3, and G3 (located in Clade 2 only). In addition, isolates in cgMLST Clade 1 had four different PFGE pattern combinations (A1, A2, A3, and B1) and isolates in cgMLST Clade 2 had seven different PFGE pattern combinations (A3, B3, C3, D3, E3, F3, and G3). Conversely, several isolates that were indistinguishable by Smal/KpnI PFGE pattern combinations were dispersed into different clades by cgMLST. Isolates with pattern combination A3 were found in Clades 1 and 2. The sequences were also examined using hqSNP analysis. A tanglegram demonstrated that the isolates formed the same two clades as observed by cgMLST analysis (0—152 and 0—41 SNPs) and hqSNP analysis differentiated clades 1 and 2 by 716—1048 SNPs; however, minor differences in within clade placement of isolates were observed (Figure 3).

**Antimicrobial resistance.** All 56 outbreak isolates contained genes or mutations encoding resistance to kanamycin/amikacin, streptomycin, tetracycline, ciprofloxacin, nalidixic acid, erythromycin, azithromycin, telithromycin, and clindamycin. Furthermore, 49 isolates contained a gene encoding resistance to gentamicin (Table 1). The 28 isolates that were further characterized by AST using the NARMS Campylobacter panel showed resistance to tetracycline, ciprofloxacin, nalidixic acid, erythromycin, azithromycin, telithromycin, and clindamycin and 26/28 isolates had resistance to gentamicin (Table 1). The correlations between the resistance phenotypes and genotypes for the tested drugs were 100% except for florfenicol (Table 1). Seven of 28 isolates were phenotypically resistant to florfenicol; however, no genetic determinants associated with florfenicol resistance were found in these isolates (Table 1, Figure 2). Kanamycin, amikacin, and streptomycin are not on the NARMS Campylobacter Panel so the
correlation between resistances phenotypes and genotypes for these drugs could not be determined (Table 1).
DISCUSSION

WGS data analysis methods have previously been shown to provide greater concordance with epidemiologic data during outbreak investigations, and demonstrate higher throughput, reproducibility, and sensitivity when compared to results generated by 7-gene MLST and PFGE (12-16, 43-44). In this investigation, we showed that cgMLST analysis of WGS data was highly concordant with epidemiologic information linking cases to a *Campylobacter* outbreak associated with puppies. Additionally, cgMLST identified some cases genetically related to the outbreak strain where epidemiologic linkage could not be determined or cases were lost to follow up. Two main clades of isolates were identified using cgMLST analysis; whereas, 7-gene MLST grouped all isolates into the same ST. Specifically, all isolates in this outbreak investigation were ST2109, which is a rare sequence type in the ST45 clonal complex (45-48). However, eight additional ST2109 isolates (from three chicken samples and five clinical specimens) were not genetically related to the isolates in cgMLST Clades 1 and 2 (> 50 alleles different), had unknown epidemiologic linkage to the outbreak isolates, and did not produce the XDR resistance patterns necessary for outbreak inclusion (data not shown) suggesting that 7-gene MLST alone is not discriminatory enough to be used during *Campylobacter* outbreak investigations. PFGE analysis, another traditional subtyping technique, resulted in an array of diverse patterns among the epidemiologically linked cases, and thus could not provide the strong concordance that was provided by WGS for confirmed cases in this puppy-linked *Campylobacter* outbreak. The diverse PFGE patterns may due to most isolates exhibiting partial digestion using Smal and no digestion using KpnI suggesting the presence of methylases which affects the interpretation of the PFGE data. In addition, point mutations, recombinations,
insertions, and deletions can lead to the loss or gain of restriction sites causing related isolates to have distinct PFGE banding patterns (49). Therefore, it is not surprising that sequences generated from isolates with diverse PFGE patterns clustered together by cgMLST analysis. The C. jejuni isolates identified over a two-year period fell into two distinct cgMLST clades with high taxon diversity within and between clades. Animal contact Campylobacter outbreaks have been shown to contain isolates with a large amount of genetic diversity suggesting that host-adapted evolution of strains may occur or that multiple strains of the same pathogen may infect a host animal (4-5, 11). Conversely, Campylobacter outbreaks associated with raw milk have been shown to contain clinical isolates with limited genetic diversity (6-7, 16, 50). As sequence-based analysis methods such as cgMLST become more widely used to investigate Campylobacter outbreaks, a better understanding of the potential genetic diversity of isolates will develop.

There were some differences in the ordering of the isolates within the individual clades in the cgMLST compared with the hqSNP trees, which is expected as cgMLST and hqSNP methods measure relatedness of isolates differently (i.e. alleles vs. SNPs); however, the isolates grouped the same within each clade. The range of SNP differences among isolates within each clade and between clades were greater than range of allele differences identified using cgMLST. This may be due to several reasons including SNPs in the intergenic region, multiple SNPs within a single gene, and SNPs detected in genomic regions outside the cgMLST scheme. HqSNP analysis is dependent on a priori knowledge of isolates to select an appropriate reference genome; whereas, only the genus and species of the isolates is needed before they can be analyzed by
cgMLST against an organism-specific allele database making cgMLST preferable for surveillance and cluster detection of *Campylobacter*.

The *C. jejuni* isolates included in this outbreak investigation were predicted to be resistant to 10-11 drugs across six drug classes including azithromycin and ciprofloxacin, the antimicrobials recommended by the Infectious Diseases Society of America for the treatment of *Campylobacter*-infected patients (42). These resistance patterns had been rarely seen among *C. jejuni* isolates previously; of more than 12,000 *C. jejuni* surveillance isolates submitted to NARMS during 2004—2015, only 1% had similar resistance patterns as those observed among isolates in this outbreak ([https://wwwn.cdc.gov/narmsnow/](https://wwwn.cdc.gov/narmsnow/)). Interestingly, many puppies associated with this outbreak were administered prophylactic antibiotics to which the strains were resistant including macrolides, tetracyclines, quinolones, and aminoglycosides (30); this practice may have provided a selective advantage for the XDR *C. jejuni* strains.

As mentioned previously, all of the *C. jejuni* outbreak isolates were ST2109 and had similar XDR patterns implying that the XDR profile is a distinguishing feature of this ST. However, a recent study found that a ST2109 isolate from a dog was resistant to azithromycin, clindamycin, erythromycin, gentamicin, telithromycin, and tetracycline, but susceptible to quinolones and florfenicol (45). In addition the eight ST2109 isolates unrelated to this outbreak were not XDR (data not shown) suggesting that identification of ST2109 alone would not be sufficient to predict the XDR resistance pattern of *Campylobacter* isolates. Selective pressure from antibiotic use in puppies where ST2109 is found should be explored as a possible explanation for emergence of XDR ST2109.
Predicted resistance using WGS and antimicrobial susceptibility testing were concordant for all drugs except florfenicol for which both WGS and MIC data were available. None of the 283 isolates examined contained a known florfenicol resistance determinant, but seven isolates were phenotypically florfenicol resistant, suggesting a possible unknown resistance mechanism which is being further investigated. The correlation between predicted resistance and AST results in this study is similar to results from a 2016 study where correlation between WGS-derived antimicrobial genotypes and corresponding AST phenotypes were examined among 114 Campylobacter isolates (25). Specifically, there was 100% agreement for tetracycline, ciprofloxacin, nalidixic acid, erythromycin, and 95-99% agreement for gentamicin, azithromycin, clindamycin, and telithromycin resistance. However, all isolates in that study were also florfenicol susceptible by AST and no genes for florfenicol resistance were found (25).

In conclusion, we demonstrated that we were better able to link C. jejuni isolates from humans and pet store puppies using WGS-based analysis methods compared to traditional subtyping methods. WGS data were used to quickly identify XDR profiles and resistance determinants in a broader set of isolates than those that would be tested phenotypically. These results demonstrate the power of WGS to aid in C. jejuni outbreak investigations and predict the antimicrobial resistance phenotype of the isolates.

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**FIGURE LEGENDS**

**Figure 1.** Representative *Sma*I (A) and *Kpn*I (B) PFGE patterns of the *Campylobacter jejuni* isolates associated with this outbreak. Different PFGE patterns are designated A-G (*Sma*I) or 1-3 (*Kpn*I). Molecular weights (Kb) are displayed at the top of the images.

**Figure 2.** CgMLST and antimicrobial resistance analysis of *Campylobacter jejuni* isolates.

UPGMA dendrogram containing sequences from 45 human and 11 puppy isolates constructed from cgMLST loci (1316 loci) in BioNumerics v7.6.3. *Sma*I and/or *Kpn*I pattern designations of isolates as provided from Figure 1 are shown in the PFGE column of the figure and the PFGE pattern designation key is to the left of the figure. Median [minimum-maximum] allele differences among sequences within clades 1-2 and between clades are shown on the figure.

Percent similarities of allele calls from the sequences is denoted by the scale at the top of the figure. Isolates from human cases with epidemiologic link to puppies from pet stores ( ), human cases without any epidemiologic information available ( ) and puppy feces ( ) are indicated on the figure.

Predicted antimicrobial resistance derived from *in silico* resistance determinant detection are indicated to the right of the figure: azithromycin (AZM), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), florfenicol (FLN), gentamicin (GEN), nalidixic acid (NAL), telithromycin (TEL), tetracycline (TET). ■ indicates that the isolate was predicted resistant to the antimicrobial drug and □ indicates that the isolate was predicted susceptible to the antimicrobial drug.

Traditional antimicrobial susceptibility testing was also performed on isolates indicated by . ■ indicates that the isolate was predicted susceptible to the antimicrobial drug; however, the isolate was determined to be resistant to the antimicrobial drug by traditional AST.
Figure 3. Side by side comparison of cgMLST and hqSNP dendrograms generated from the sequences of the *Campylobacter jejuni* outbreak isolates. The tanglegram was created with R v3.5.1 using the dendextend package and layout was optimized using the step2side method. The cgMLST dendrogram is displayed on the left and the hqSNP dendrogram is displayed on the right. Identical strains are linked between trees using straight lines that are colored according to the cgMLST clade: clade 1, clade 2. Minimum-maximum allele or SNP differences among sequences within clades 1-2 and between clades are shown on the figure.
Figure 2

PFGE Pattern Designation Key
NP: PFGE Not Performed
A0: SmaI Pattern A, KpnI Not Performed
A1: SmaI Pattern A, KpnI Pattern 1
A2: SmaI Pattern A, KpnI Pattern 2
A3: SmaI Pattern A, KpnI Pattern 3
B0: SmaI Pattern B, KpnI Not Performed
B1: SmaI Pattern B, KpnI Pattern 1
B2: SmaI Pattern B, KpnI Pattern 2
B3: SmaI Pattern B, KpnI Pattern 3
C0: SmaI Pattern C, KpnI Not Performed
C1: SmaI Pattern C, KpnI Pattern 1
C2: SmaI Pattern C, KpnI Pattern 2
C3: SmaI Pattern C, KpnI Pattern 3
D0: SmaI Pattern D, KpnI Pattern 3
E0: SmaI Pattern E, KpnI Pattern 3
F0: SmaI Pattern F, KpnI Pattern 3
G0: SmaI Pattern G, KpnI Pattern 3

231 [201-241]

Clade 1
7 [0-40]

Clade 2
4 [0-28]
<table>
<thead>
<tr>
<th>Drug class</th>
<th>Drug</th>
<th>Presence of gene(s) or mutation(s) conferring resistance (n=56)</th>
<th>No. isolates phenotypic resistant (n = 28)</th>
<th>Correlation between genotype and phenotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracyclines</td>
<td>Tetracycline</td>
<td>tet (O) (n = 56)</td>
<td>28</td>
<td>28/28 (100%)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Kanamycin/Amikacin</td>
<td>aph (3')-III (n = 56)</td>
<td>NT *</td>
<td>NT *</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Streptomycin</td>
<td>aad E (n = 56)</td>
<td>NT *</td>
<td>NT *</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Gentamicin</td>
<td>aph (2'')-Ih (n = 49)</td>
<td>26</td>
<td>28/28 (100%)</td>
</tr>
<tr>
<td>Phenicol</td>
<td>Florfenicol</td>
<td>None</td>
<td>7</td>
<td>21/28 (75%)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Ciprofloxacin</td>
<td>gyr A T86I (n = 56)</td>
<td>28</td>
<td>28/28 (100%)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Nalidixic Acid</td>
<td>gyr A T86I (n = 56)</td>
<td>28</td>
<td>28/28 (100%)</td>
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<tr>
<td>Macrolides</td>
<td>Azithromycin</td>
<td>23S rRNA A2075G (n = 56)</td>
<td>28</td>
<td>28/28 (100%)</td>
</tr>
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<td>28/28 (100%)</td>
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<tr>
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<td>28/28 (100%)</td>
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<tr>
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<td>Clindamycin</td>
<td>23S rRNA A2075G (n = 56)</td>
<td>28</td>
<td>28/28 (100%)</td>
</tr>
</tbody>
</table>

* NT = Not tested; kanamycin/amikacin and streptomycin are not tested on the NARMS AST Panel.