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WGS accurately predicts antimicrobial resistance in *Escherichia coli*

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Abstract

Objectives: The objective of this study was to determine the effectiveness of WGS in identifying resistance genotypes of MDR *Escherichia coli* and whether these correlate with observed phenotypes.

Methods: Seventy-six *E. coli* strains were isolated from farm cattle and measured for phenotypic resistance to 15 antimicrobials with the Sensititre® system. Isolates with resistance to at least four antimicrobials in three classes were selected for WGS using an Illumina MiSeq. Genotypic analysis was conducted with in-house Perl scripts using BLAST analysis to identify known genes and mutations associated with clinical resistance.

Results: Over 30 resistance genes and a number of resistance mutations were identified among the *E. coli* isolates. Resistance genotypes correlated with 97.8% specificity and 99.6%

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Transparency declarations

G. H. L. has provided scientific advice to various pharmaceutical companies that market antimicrobial drugs for administration to animals. He has on occasion billed for his service. G. H. L. has also received honoraria for service on advisory boards and presentations from pharmaceutical companies. All other authors: none to declare.

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Supplementary data

Tables S1 to S4 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

sensitivity to the identified phenotypes. The majority of discordant results were attributable to the aminoglycoside streptomycin, whereas there was a perfect genotype–phenotype correlation for most antibiotic classes such as tetracyclines, quinolones and phenicols. WGS also revealed information about rare resistance mechanisms, such as structural mutations in chromosomal copies of *ampC* conferring third-generation cephalosporin resistance.

Conclusions: WGS can provide comprehensive resistance genotypes and is capable of accurately predicting resistance phenotypes, making it a valuable tool for surveillance. Moreover, the data presented here showing the ability to accurately predict resistance suggest that WGS may be used as a screening tool in selecting anti-infective therapy, especially as costs drop and methods improve.

Introduction

Technological advancements and decreased sequencing costs may soon allow WGS to replace a number of traditional microbiology laboratory methods.[1,2] While the operational processes for WGS are relatively undemanding, the management and analysis of these large datasets require specialized expertise and software tools. As a result, most clinical and diagnostic laboratories still rely on phenotypic measures to identify bacterial antibiotic resistance.[3] Although in general phenotypic testing is reproducible, interlaboratory variability can be problematic.[4,5] Thus genotypic methods, which rely on the identification of specific genes and mutations, may result in more clear-cut and consistent measures of resistance and thus play a useful role in resistance surveillance practices.

Although *Escherichia coli* is part of normal intestinal flora, several strains can cause a variety of enteric and extraintestinal infections, some of which can be life-threatening and require antimicrobial therapy. In addition, the rising antibiotic resistance of *E. coli* and other enteric pathogens is a critical public health issue worldwide.[6] In *E. coli*, this problem is largely mediated by the acquisition of exogenous genes through transmissible plasmids, integrons and transposons.[7,8] To better understand the origins, sources and spread of antimicrobial-resistant *E. coli*, it is important to catalogue and compare resistance genes in isolates from different sources. Further, correlating genotype and phenotype is a necessary aspect of uncovering novel resistance mechanisms and understanding the relative contribution of known resistance genes and their allelic variants. Therefore, we sought to correlate the phenotypic resistance patterns of *E. coli* with the genetic determinants that contribute to resistance.

Previous genotypic studies have used focused approaches with PCR tests or microarrays for detection of specific genes.[9,10] These techniques can be useful to discover a number of features of isolates, including resistance and virulence determinants.[11] Nevertheless, these methods only detect particular genes and are unable to uncover new or rare resistance mechanisms. Just as importantly, testing for specific genes for large numbers of isolates is more costly and laborious, as well as less informative, than what can be gleaned from WGS. Recently, several groups have used WGS to correlate resistance genotypes with phenotypes in various bacteria,[12] including *E. coli*.[4,13] These studies demonstrated the high sensitivity and specificity of the approach, but included large numbers of low-resistance isolates. To

better evaluate the correlation of genotype and phenotype, we focused on MDR isolates and examined for the presence of hundreds of resistance genes and resistance-associated mutations.

Materials and methods

Bacterial strains, culture and MIC testing

MDR *E. coli* strains (n = 76) were selected from 2668 *E. coli* isolated from farm cattle in 2011. Samples were isolated as part of a pilot farm project in which caecal sample *E. coli* isolates were obtained from cattle sent for slaughter at facilities throughout the USA inspected by the US Department of Agriculture. Susceptibility testing was performed by broth microdilution using a Sensititre system (Trek Diagnostic Systems, Cleveland, OH, USA) according to standardized protocols, in which bacteria were incubated for 18 h at 35°C in antibiotic-containing plates (CMV2AGNF).[14] CLSI interpretive criteria[15] were used and the resistance breakpoints for each antibacterial agent were as follows: gentamicin, ≥16 mg/L; kanamycin, ≥64 mg/L; streptomycin, ≥64 mg/L; amoxicillin/clavulanic acid, ≥32/16 mg/L; ceftriaxone, ≥4 mg/L; cefoxitin, ≥32 mg/L; ceftiofur, ≥8 mg/L; trimethoprim/sulfamethoxazole, ≥4/76 mg/L; sulfisoxazole, ≥512 mg/L; azithromycin, ≥32 mg/L; ampicillin, ≥32 mg/L; chloramphenicol, ≥32 mg/L; ciprofloxacin, ≥4 mg/L; nalidixic acid, ≥32 mg/L; and tetracycline, ≥16 mg/L. Note that there are no CLSI interpretive criteria for streptomycin, azithromycin or the veterinary drug ceftiofur for *E. coli*, so interpretive criteria defined by the National Antimicrobial Resistance Monitoring System were used.[14] Isolate-level susceptibility data are shown in Table S1 (available as Supplementary data at *JAC* Online).

Genome sequencing and analysis

Genomic DNA was extracted with a DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA) per the manufacturer's instructions. DNA concentrations were measured using a Qubit fluorometer (Life Technologies, MD, USA) to determine DNA input from each isolate. WGS was performed using the MiSeq platform using v3 reagent kits with paired-end 2×300 bp reads (Illumina, San Diego, CA, USA). Libraries were prepared by following the Illumina Nextera XT sample preparation guide. Sequences for individual strains were demultiplexed by MiSeq Reporter version 2.5.1. Reads were trimmed by removing ambiguous nucleotides and those with Phred scores of <20. Assembly without scaffolding was performed *de novo* for each isolate with CLC Genomics Workbench version 7.5 (CLC bio, Aarhus, Denmark) using de Bruijn-based assembly with automatic word-size determination, discarding contigs of <200 bp. Contigs with low coverage (<10% of the average genome coverage) were also removed from final genome sequences. Genomes were annotated using the National Center for Biotechnology Information's Prokaryotic Genome Automated Pipeline version 2.9.[16] Among the 76 samples, there was a median of 154 contigs (range: 79–394) and 62-fold coverage (range: 34–114) per genome. Isolate-level sequencing data results are listed in Table S2. Whole-genome sequences and antibiogram data of the 76 *E. coli* isolates were deposited into GenBank under BioProject accession number PRJNA266657. Accession numbers for individual isolates are listed in Table S2.

Resistance genotype identification

Resistance genes were identified using Perl scripts to perform local BlastX with an in-house resistance gene database containing 2546 resistance genes and gene variants across all major antibiotic classes (Table S3; database version 1 October 2014). Hits were identified by having ≥85% amino acid identity and ≥50% sequence length to known resistance proteins. Hits of <100% identity and/or sequence length were analysed by additional manual BLAST analysis to identify the appropriate resistance genes.

For analysis of chromosomal structural gene mutations, Perl scripts were used to extract *gyrA*, *gyrB*, *parC*, *parE* and *ampC* genes, which were analysed for quinolone resistance-determining regions (QRDRs) or promoter mutations, as appropriate, with alignment by ClustalW in Mega version 6.06.[17]

Genotype was determined to match phenotype when a strain had phenotypic resistance in addition to known resistance genes or mutations or had phenotypic susceptibility in the absence of resistance genes or mutations. Intermediate phenotypes were counted as susceptible in this analysis. When mismatches occurred, antimicrobial susceptibility testing was repeated. If discrepancies still remained, then sequencing was reperformed using the same plate used for the repeated susceptibility testing. Any remaining discrepancies are shown at the isolate level in Table S4. CIs for genotype–phenotype correlations were calculated using OpenEpi version 3.03 with Fleiss quadratic continuity correction.[18]

Results

Antimicrobial susceptibility profiles

From cattle faecal samples, 76 *E. coli* isolates were selected that were resistant to at least four antimicrobials and three classes of antibiotics, determined by testing isolates for susceptibility to 15 antimicrobial compounds in nine classes (Figure 1). The most common resistances among these isolates were to sulfisoxazole (89.5%), tetracycline (88.2%), ampicillin (72.4%), chloramphenicol (72.4%) and streptomycin (68.4%). In contrast, there was less resistance to azithromycin (1.3%), gentamicin (3.9%) and ciprofloxacin (7.9%). Isolate-level resistance prevalence was in between these levels for the remaining seven antimicrobials tested.

Among these MDR isolates, 55.3% had resistance to at least five classes of antibiotics and 17.1% had resistance to at least seven of the nine classes tested. WGS-based genotypic analysis was performed to correlate resistance genotypes and phenotypes. For this analysis, Perl scripts were used to perform local BlastX to query genome sequences for genes similar to those in our in-house resistance gene database. A complete list of each strain and its resistance phenotype and genotype is shown in Table S4, with MIC values from susceptibility testing in Table S1.

Aminoglycosides

Streptomycin resistance genes were present in 84.2% of isolates. The most frequently identified resistance genes were *strA* [*aph(3″)-Ib*] and *strB* [*aph(6″)-Id*], which were always

present together in the same strains (75% of all strains). Also common were *aadA* genes, present in 21.1% of all strains (Table 1). Although most strains had *strA*/*strB* and/or *aadA* genes, only 81.3% containing at least one resistance gene had phenotypic resistance to streptomycin. Resistance was more common among strains with *strA*/*strB* genes as 51/57 (89.5%) were resistant. In contrast, only 10/16 (62.5%) strains with at least one *aadA* gene were resistant, including just 1/7 (14.3%) that did not have *strA*/*strB* genes as well. All of the *strA*/*strB* and *aadA* genes that did not confer phenotypic resistance had intact coding regions and promoters, including some with 100% identity to genes present in isolates with observed resistance.

E. coli isolates were also tested for susceptibility to other aminoglycosides, gentamicin and kanamycin. Resistance to each was uncommon, with just 13.2% resistant to kanamycin and 3.9% resistant to gentamicin. Each isolate with kanamycin resistance possessed an *aph(3′)-Ia* gene, as did one isolate without phenotypic resistance that possessed a truncated gene. All gentamicin-resistant isolates had *aac* genes, which were not encoded by any isolates lacking resistance.

β-Lactams

We detected the presence of three main β-lactamases that contributed to resistance phenotypes in our isolates: $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$ and $bla_{\text{CMY-2}}$. TEM-1 confers ampicillin resistance and did so for each strain that had the gene (43.4%; Table 1). This was also the case for the one isolate that expressed OXA-1. In contrast, CMY-2, expressed by 30.3% of strains, confers expanded resistance to potentiated β-lactams as well as third-generation cephalosporins such as ceftiofur and ceftriaxone. Each isolate except one with this gene demonstrated resistance to ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur and ceftriaxone. The one isolate that differed was not resistant to the veterinary cephalosporin ceftiofur, instead having an intermediate phenotype (MIC 4 mg/L).

In addition to these well-characterized resistance genes, overexpression of chromosomal *ampC* β-lactamase also confers clinical resistance.[19] We identified four isolates with −42 thymine-to-cytosine transitions in their promoters, which is known to cause increased *ampC* expression (Table 1).[19] Three of the isolates had β-lactam resistance in the absence of other resistance genes (N35912PS, N36834PS and N33633PS), while the other isolate (N33552PS) carried $bla_{\text{CMY-2}}$, which masked any potential resistance conferred by the *ampC* mutation. Overproduction of AmpC is predicted to confer resistance to ampicillin, amoxicillin/clavulanic acid and cefoxitin.[20] However, two of the three isolates had only intermediate susceptibility to cefoxitin (MIC 16 mg/L). One of these (N36834PS) had additional unanticipated resistance to ceftiofur and ceftriaxone (Table S4). This is likely due to an S287R amino acid substitution, as other substitutions of this residue have been shown to confer expanded β-lactam resistance.[21]

Folate synthesis inhibitors

Resistance to sulfisoxazole was common (89.5%), with each resistant isolate encoding dihydropteroate synthase *sul* genes.[22] Each *sul* gene conferred the appropriate resistance and there was no resistance shown by any isolates lacking these genes. In addition, trimethoprim/

sulfamethoxazole resistance was found in 17.1% of strains, with dihydrofolate reductase (*dfrA*) genes being responsible in each instance. There was no unexpected resistance, but one isolate had a truncated *dfrA1* gene that resulted in its antibiotic susceptibility.

Macrolides

In our panel of MDR *E. coli*, there was only one isolate with resistance to the macrolide azithromycin, mediated by an *mphA* gene, which encodes a macrolide phosphotransferase.[23] No additional isolates had any macrolide resistance genes.

Phenicols

Chloramphenicol resistance was found in 72.4% of the MDR *E. coli*. Resistance was predominantly mediated by the *floR* gene, present in 94.5% of resistant isolates. However, a minority of strains were also found to have other resistance genes, including *cmlA*, *catA1* and *catB3*. Each isolate with at least one resistance gene demonstrated chloramphenicol resistance, while all isolates lacking resistance genes were susceptible to chloramphenicol.

Quinolones

We found that 27.6% of isolates were resistant to nalidixic acid, with 7.9% being resistant to ciprofloxacin. Isolates with a single mutation in the QRDR of *gyrA* typically have nalidixic acid resistance, with two mutations being required for ciprofloxacin resistance.[24] These genotypes correlated well in our study, as there were 21 isolates with *gyrA* mutations (S83L, S83F, D87G and D87N amino acid changes), all of which were resistant to nalidixic acid (Table S4). Six isolates had multiple *gyrA* mutations, with each isolate being resistant to both nalidixic acid and ciprofloxacin. Only strains with multiple mutations had ciprofloxacin resistance, consistent with our genotypic prediction. Eight of the isolates with *gyrA* mutations also had mutations in *parC*, while three had mutations in *parE* (Table S4). Mutations of each have also been associated with increased quinolone resistance, but no isolates had *parC* or *parE* mutations in the absence of *gyrA* mutations.[25] None of the *E. coli* was found to contain relevant *gyrB* mutations, which can also alter susceptibility to quinolones.[26]

In addition to mutation of gyrase and topoisomerase genes, three isolates also contained *qnr* genes that can confer quinolone resistance.[27] Individual isolates had *qnrB2*, *qnrB6* or *qnrS2* genes, yet each was still clinically susceptible to both nalidixic acid and ciprofloxacin. This is consistent with previous reports that these *qnr* genes do not confer actual resistance, but instead elevated MIC levels of both antibiotics.[28–30] All three isolates encoding *qnr* genes had elevated ciprofloxacin and nalidixic acid MICs of ≥0.25 and 8 mg/L, respectively (Table S1). Although this still resulted in these isolates being classified as susceptible to each drug, the only other isolates with MICs at least that high had at least one *gyrA* QRDR mutation. This confirms the capability of these genes to confer decreased quinolone susceptibility.

Tetracyclines

Tetracycline resistance was widespread among our isolates, with 88.2% of isolates demonstrating resistance. The most prevalent resistance gene was *tet*(A) (in 80.6% of tetracycline-resistant strains), although *tet*(B)-, *tet*(C)- and *tet*(D)-mediated resistance was

also common (Table 1). The *tet*(M) gene was found in several isolates, each of which also had *tet*(A). Genotype was an excellent predictor of phenotype, as each isolate that had at least one resistance gene was resistant to tetracycline. Furthermore, there were no isolates lacking *tet* genes that had resistance.

Resistance elements

In addition to the identification of resistance determinants, WGS can also provide information about elements that contain multiple resistance genes, such as integrons or resistance islands. For example, we found that 35 isolates (46.1%) had the resistance genes *tet*(A), *strA*/*strB* and *sul2* together on the same contig. Of these 35, 11 also had a *floR* gene present, 8 had a $bla_{\text{TEM-1}}$ gene and 8 had both (Figure 2). These numbers are likely underestimates, since only isolates with these genes on individual contigs were included and many additional isolates had these genes on multiple contigs.

Discussion

Here, we report on the utility of WGS for accurately predicting antibiotic resistance. Overall, >30 resistance genes were identified from 76 MDR *E. coli* isolates, along with a number of resistance-associated mutations (Table 1 and Table S4). In addition, the resultant resistance genotypes correlated with 99.6% sensitivity (95% CI 98%–100%) and 97.8% specificity (95% CI 96%–99%) to resistance phenotypes (Table 2). Although our analysis only included MDR isolates, this demonstrates the robustness of WGS in predicting resistance phenotypes. Overall, among 1140 phenotypic resistance tests, there were only 17 discrepancies between genotype and phenotype, with each remaining after phenotypes were retested (Table 3).

Among the discordant results, 12 (70.6%) were from isolates with streptomycin resistance genes that lacked phenotypic resistance. There is no CLSI-defined streptomycin breakpoint for *E. coli*, although resistance is often demarcated by an MIC of ≥64 mg/L.[31] In our dataset, a vast majority of *strA*/*strB*-encoding isolates did have streptomycin MICs ≥64 mg/L, although this was not true of strains with *aadA* genes. This confirms the previous results of Sunde and Norstrom,[32] who demonstrated that *strA*/*strB* genes confer higher resistance than *aadA* genes. Although a reduction in the MIC cut-off may result in better genotype–phenotype correlation, even optimized streptomycin breakpoints can result in substantial discrepancies with genotypic data.[33] Thus, the presence of these genes could instead be used as an indicator of resistance potential and may present a reasonable alternative to conventional phenotypic testing.

Besides streptomycin, some minor discrepancies were observed for β-lactam antibiotics (Table 3). One strain carried $bla_{\text{CMY-2}}$ and exhibited an intermediate phenotype for ceftiofur (MIC 4 mg/L). Similarly, two strains with *ampC* promoter mutations displayed intermediate phenotypes to cefoxitin (MIC 16 mg/L). The only two resistance phenotypes not predicted by genotype were from strain N36834PS, which had unexpected *ampC*-mediated resistance to ceftiofur and ceftriaxone. Although not previously described, the S287R substitution of N36834PS AmpC likely resulted in its resistance to the third-generation cephalosporins. This was confirmed by the fact that the S287R amino acid change is the only difference between it and AmpC from N33633PS, which does not have resistance to these cephems.

Although our results show a high degree of correlation between resistance genotypes and phenotypes for most antibiotic classes, this study does have some drawbacks. For example, the use of whole-genome shotgun sequencing results in fragmentary genomes with sequences assembled into contigs. Although this did not appear to hinder our analysis, it complicates the identification of resistance plasmids and cassettes. In addition, strains with multiple resistance genes for a single antimicrobial may demonstrate phenotypic resistance, but it is unclear which gene(s) confer this resistance. Furthermore, genotypic prediction of resistance relies on curated databases of known resistance determinants, so currently unknown resistance mechanisms would not be identified by this approach. Since any resistance gene database is necessarily incomplete, this means that errors cannot always be avoided.

In general, WGS accurately predicted the vast majority of resistance phenotypes from MDR *E. coli* strains. Additional studies based on this work may focus on utilizing genotypic methods to predict resistance profiles in the absence of phenotypic information. Subsequent phenotypic susceptibility testing can then be used to validate the approach.

Although phenotypic testing is the current norm for determining antibiotic resistance, WGS can provide additional useful information. This is because WGS detected resistance mechanisms such as *qnr* genes that result in elevated MICs without meeting clinical resistance thresholds, providing information that may be of use in patient treatment. Since resistance genes are either present or absent and do not have breakpoints, this makes WGS a more unbiased and consistent method for determining at least the genotypic potential for antibiotic resistance. In many cases, phenotypic testing is still faster and cheaper than WGS-based genotypic analysis. However, this is changing and once WGS data are obtained, hundreds or thousands of resistance genes can be simultaneously identified in an automated process. This simplifies the detection of rare resistance genes for phenotypes that may not be tested in a cost-effective manner and supports the use of WGS-based genotypic analysis in resistance surveillance programmes. WGS can also be used to identify the relatedness of bacterial isolates and therefore support the investigation of bacterial source attribution. Thus, WGS could supplement existing scientific and clinical techniques while gaining new insight into resistance patterns, virulence genes and emerging outbreaks.

Overall, we used WGS to successfully identify the resistance genotypes for 76 MDR *E. coli* strains. These genotypes correlated well with resistance phenotypes, demonstrating the potential of WGS-based techniques to replace phenotypic indicators of resistance. The vast amount of data gained by WGS also potentiates its use for a number of additional research and clinical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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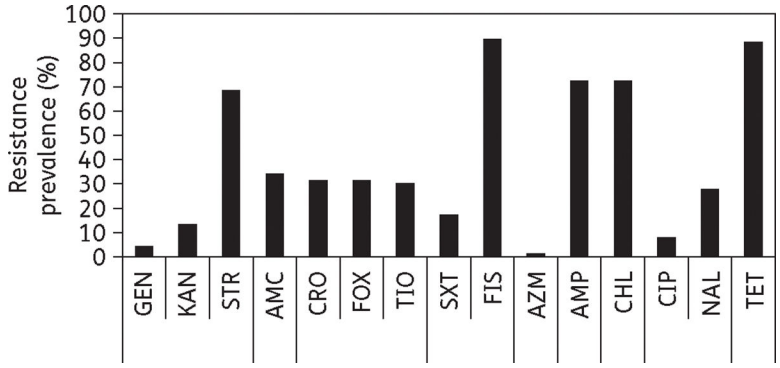

Figure 1.

Isolate-level prevalence of resistance among *E. coli* isolates. The percentage of resistant isolates is depicted for each antibiotic tested. Resistance is grouped by classes, separated by lines, in the order of: aminoglycosides, β-lactam/β-lactam inhibitors, cephems, folate synthesis inhibitors, macrolides, penicillins, phenicols, quinolones and tetracyclines. GEN, gentamicin; KAN, kanamycin; STR, streptomycin; AMC, amoxicillin/clavulanic acid; CRO, ceftriaxone; FOX, cefoxitin; TIO, ceftiofur; SXT, trimethoprim/sulfamethoxazole; FIS, sulfisoxazole; AZM, azithromycin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline.

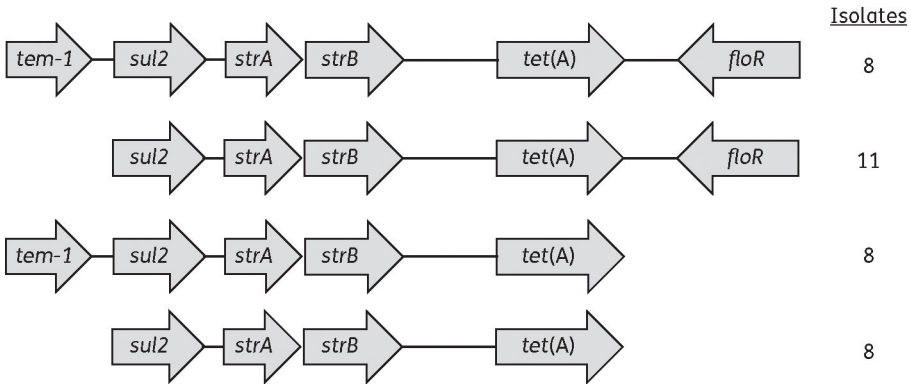

Figure 2.

Depiction of resistance elements containing contiguous antibiotic resistance genes. The values indicate the number of isolates with each resistance element.

Table 1.

List of resistance genes and their prevalence

Resistance gene	Antibiotic class	Resistance phenotype	Prevalence (%)
strA [*aph(3')-Ib*]	aminoglycosides	STR	75.0
strB [*aph(6')-Id*]	aminoglycosides	STR	75.0
aadA1	aminoglycosides	STR	9.2
aadA2	aminoglycosides	STR	9.2
aadA5	aminoglycosides	STR	2.6
aadA7	aminoglycosides	STR	1.3
aadA24	aminoglycosides	STR	1.3
aph(3')-Ia	aminoglycosides	KAN	14.5
aac(3')-VI	aminoglycosides	GEN	2.6
aac(3')-IId	aminoglycosides	GEN	1.3
$bla_{\text{TEM-1}}$	β-lactams	AMP	43.4
$bla_{\text{OXA-1}}$	β-lactams	AMP	1.3
$bla_{\text{CMY-2}}$	β-lactams	AMC, AMP, CRO, FOX, TIO	30.3
ampC (−42 T→C)	β-lactams	AMC, AMP, FOX	5.3
sul1	folate synthesis inhibitors	FIS	17.1
sul2	folate synthesis inhibitors	FIS	78.9
sul3	folate synthesis inhibitors	FIS	2.6
dfrA1	folate synthesis inhibitors	SXT	5.3
dfrA5	folate synthesis inhibitors	SXT	1.3
dfrA12	folate synthesis inhibitors	SXT	9.2
dfrA17	folate synthesis inhibitors	SXT	2.6
mphA	macrolides	AZM	1.3
floR	phenicols	CHL	68.4
cmlA	phenicols	CHL	2.6
catA1	phenicols	CHL	3.9
catB3	phenicols	CHL	1.3
qnrB2 [a]	quinolones	NAL, CIP	1.3

Resistance gene	Antibiotic class	Resistance phenotype	Prevalence (%)
qnrB6 [a]	quinolones	NAL, CIP	1.3
qnrS2 [a]	quinolones	NAL, CIP	1.3
gyrA mutations	quinolones	NAL, CIP	27.6
parC mutations	quinolones	NAL, CIP	10.5
parE mutations	quinolones	NAL, CIP	3.9
tet(A)	tetracyclines	TET	71.1
tet(B)	tetracyclines	TET	22.4
tet(C)	tetracyclines	TET	21.1
tet(D)	tetracyclines	TET	19.7
tet(M)	tetracyclines	TET	9.2

STR, streptomycin; KAN, kanamycin; GEN, gentamicin; AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CRO, ceftriaxone; FOX, cefoxitin; TIO, ceftiofur; FIS, sulfisoxazole; SXT, trimethoprim/sulfamethoxazole; AZM, azithromycin; CHL, chloramphenicol; NAL, nalidixic acid; CIP, ciprofloxacin; TET, tetracycline.

[a]Only confers reduced susceptibility.

Table 2.

Evaluation of genotypic analysis for the prediction of resistance phenotypes

	Phenotype: susceptible		Phenotype: resistant			
Antibiotic	**genotype: resistant**	**genotype: susceptible**	**genotype: resistant**	**genotype: susceptible**	**Sensitivity (%)**	**Specificity (%)**
GEN	0	73	3	0	100	100
KAN	0	66	10	0	100	100
STR	12	12	52	0	100	50
AMC	0	50	26	0	100	100
CRO	0	52	23	1	95.8	100
FOX	2	50	24	0	100	96.1
TIO	1	52	22	1	95.6	98.1
SXT	0	63	13	0	100	100
FIS	0	8	68	0	100	100
AZM	0	75	1	0	100	100
AMP	0	21	55	0	100	100
CHL	0	21	55	0	100	100
CIP	0	70	6	0	100	100
NAL	0	55	21	0	100	100
TET	0	9	67	0	100	100
Overall	15	677	446	2	99.6	97.8

GEN, gentamicin; KAN, kanamycin; STR, streptomycin; AMC, amoxicillin/clavulanic acid; CRO, ceftriaxone; FOX, cefoxitin; TIO, ceftiofur; SXT, trimethoprim/ sulfamethoxazole; FIS, sulfisoxazole; AZM, azithromycin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline.

Table 3.

List of resistance genotype-phenotype discrepancies

Strain(s)	Drug(s)	Gene(s)	Predicted genotype	Phenotype	Explanation
N33707PS	TIO	*bla*$_{CMY-2}$	R	I	unknown; intermediate susceptibility
N35912PS, N36834PS	FOX	*ampC*	R	I	unknown; intermediate susceptibility
N36834PS	CRO, TIO	*ampC*	S	R	*ampC* coding mutations
Six strains	STR	*aadA* genes	R	S	unknown; high resistance threshold
Six strains	STR	*strA*+*strB*	R	S	unknown; high resistance threshold

TIO, ceftiofur; FOX, cefoxitin; CRO, ceftriaxone; STR, streptomycin; S, susceptible; I, intermediate; R, resistant.