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Whole-Genome Sequencing for Detecting Antimicrobial Resistance in Nontyphoidal *Salmonella*

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Laboratory-based *in vitro* antimicrobial susceptibility testing is the foundation for guiding anti-infective therapy and monitoring antimicrobial resistance trends. We used whole-genome sequencing (WGS) technology to identify known antimicrobial resistance determinants among strains of nontyphoidal *Salmonella* and correlated these with susceptibility phenotypes to evaluate the utility of WGS for antimicrobial resistance surveillance. Six hundred forty *Salmonella* of 43 different serotypes were selected from among retail meat and human clinical isolates that were tested for susceptibility to 14 antimicrobials using broth microdilution. The MIC for each drug was used to categorize isolates as susceptible or resistant based on Clinical and Laboratory Standards Institute clinical breakpoints or National Antimicrobial Resistance Monitoring System (NARMS) consensus interpretive criteria. Each isolate was subjected to whole-genome shotgun sequencing, and resistance genes were identified from assembled sequences. A total of 65 unique resistance genes, plus mutations in two structural resistance loci, were identified. There were more unique resistance genes ($n = 59$) in the 104 human isolates than in the 536 retail meat isolates ($n = 36$). Overall, resistance genotypes and phenotypes correlated in 99.0% of cases. Correlations approached 100% for most classes of antibiotics but were lower for aminoglycosides and beta-lactams. We report the first finding of extended-spectrum β-lactamases (ESBLs) ($bla_{\text{CTX-M1}}$ and bla_{SHV2a}) in retail meat isolates of *Salmonella* in the United States. Whole-genome sequencing is an effective tool for predicting antibiotic resistance in nontyphoidal *Salmonella*, although the use of more appropriate surveillance breakpoints and increased knowledge of new resistance alleles will further improve correlations.

In the United States, the National Antimicrobial Resistance Monitoring System (NARMS) was established to track resistance in *Salmonella*, *Campylobacter*, and other foodborne bacteria by comparing strains from food-producing animals, raw retail meats, and human infections. This "One Health" approach to integrated surveillance provides information to assess the nature and magnitude of resistance in bacteria moving through the food supply and causing illnesses in humans. This information is needed to make science-based decisions to preserve antibiotic effectiveness for animals and humans.

Antibiotic susceptibility tests generate data using routine *in vitro* measurements that have changed little over the past 90 years (1). They consist mainly of measuring MICs of antibiotics arrayed in 2-fold serial dilutions or the diameter of inhibition zones around disks containing standard amounts of antibiotic (2). These techniques are a familiar and proven way to help select appropriate anti-infective therapy. Despite the familiarity of these methods, they have several well-known disadvantages, such as a lack of harmonization that impedes interlaboratory comparison of data, the absence of valid methods for many organisms, practical limitations on the number of agents that can be tested, and shifting interpretive standards (3). For public health surveillance systems, such as the NARMS, that are used to evaluate resistant foodborne hazards and to assess risks associated with agricultural antibiotic use (4), susceptibility data alone may be insufficient in some circumstances. For risk analysis, it is often necessary to perform additional genetic testing to compare alleles and strain types among isolates from different environments (5).

With the advent of affordable whole-genome sequencing (WGS) technology, it is now possible to determine and evaluate the entire DNA sequence of a bacterium at low costs in just a few days, making it an ideal tool for surveillance (6). By providing definitive genotype information, WGS offers the highest practical resolution for characterizing an individual microbe. This includes the full complement of resistance determinants (7, 8), including resistance to compounds not routinely tested phenotypically. Bacteria that have identical resistance patterns caused by different mechanisms can also be differentiated by WGS.

To evaluate the ability of WGS to predict antimicrobial resistance in *Salmonella*, we evaluated human and food isolates with various resistance patterns. Our data show that acquired resistance is very highly correlated with the presence of known resistance determinants. This work helps lay the foundation for further trials to evaluate how WGS data can be used to characterize foodborne microbial hazards and to assess risks related to drug use in food animal production and whether WGS data can be used to inform clinical decision-making regarding patients with salmonellosis.

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MATERIALS AND METHODS

Bacterial isolates. A total of 640 *Salmonella* isolates encompassing 43 serotypes from human clinical cases ($n = 104$) and retail meats ($n = 536$) collected by the NARMS program were selected for WGS analysis. The retail meat strains of *Salmonella* used in this study were selected to capture the range of resistance patterns in the NARMS culture collection and to encompass diverse serotypes, pulsed-field gel electrophoresis (PFGE) profiles, food sources, and geographic origins among isolates collected in 2011-2012. In addition, we included all of the NARMS retail meat isolates from sample year 2013. Among food isolates, 172 were pansusceptible isolates. The human *Salmonella* strains were from 2011-2012 and were selected to contain all multidrug resistance (MDR) patterns observed during this period, along with 24 pansusceptible isolates for comparison.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed for 14 antimicrobials, including gentamicin, streptomycin, ampicillin, amoxicillin-clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, azithromycin, chloramphenicol, nalidixic acid, ciprofloxacin, sulfisoxazole, trimethoprim-sulfamethoxazole, and tetracycline. MICs were determined by broth microdilution using dehydrated panels CMV2AGNF and CMV3AGNF (Thermo Fisher Scientific, Waltham, MA) according to standard protocols (9). Resistance was defined using CLSI criteria, except for streptomycin (≥64 mg/liter) and azithromycin (≥32 mg/liter), for which there are no clinical breakpoints. Susceptibility data for each isolate are shown in Tables S1 and S2 in the supplemental material for retail meat and human isolates, respectively.

WGS and assembly. Sequencing libraries were prepared according to the Illumina Nextera XT sample preparation guide. Genomic DNA was purified using the Qiagen DNeasy kit (Qiagen, Valencia, CA), and DNA concentrations were measured using a Qubit fluorometer (Life Technologies, MD). Whole-genome sequencing (WGS) was performed using v2 or v3 chemistry with paired-end 2- by 25- or 2- by 300-bp reads on the MiSeq platform (Illumina, San Diego, CA). Read trimming and assembly were performed as previously described (10), with *de novo* assembly using automated parameters in CLC Genomics Workbench version 7.5. Genomes were annotated using the National Center for Biotechnology Information's Prokaryotic Genome Automated Pipeline version 2.9 (11). Among the 640 samples, there was a median of 83 contigs (range: 33 to 715) and 63-fold coverage (range: 21 to 156) per genome.

Identification of resistance genes. To ensure a comprehensive analysis using all known resistance determinants, we combined publicly available resistance gene databases (ResFinder [Center for Genomic Epidemiology, DTU], ARG-ANNOT [IHU Méditerranée Infection], and CARD [McMaster University]) with additional unique genes present in GenBank, resulting in a reference data set containing 2,546 resistance genes (10). Resistance genes were identified using Perl scripts to perform local BLASTX with the in-house resistance gene database. Resistance determinants were identified if they fit the criteria of ≥85% amino acid identity and ≥50% sequence length identity to known resistance proteins. Sequences showing <100% identity and/or sequence length were analyzed by additional BLAST analysis to identify the appropriate resistance genes.

For analysis of chromosomal structural gene mutations, Perl scripts were used to extract *gyrA*, *gyrB*, *parC*, and *parE* genes, which were analyzed for quinolone resistance-determining region (QRDR) mutations, with alignment by ClustalW in Mega version 6.06 (12).

Correlation of susceptibility phenotypes and genotypes. A total of 8,960 phenotypic data points were generated from the 640 isolates by *in vitro* antimicrobial susceptibility testing. Each interpretation of resistant or susceptible to a given antimicrobial agent was compared with the presence or absence of a known corresponding resistance gene(s) and/or structural gene mutations. Intermediate phenotypes were counted as susceptible in this analysis. In initial testing, approximately 10% of isolates had instances where one or more susceptibility phenotype did not correlate with genotype. In all cases of discordance, both WGS and *in vitro* susceptibility testing were repeated. The remaining 88 discrepancies are shown at the isolate level in Tables S1 and S2 in the supplemental material. Using the phenotypic results as the reference outcome, sensitivity was calculated by dividing the number of isolates that were genotypically resistant by the total number of isolates exhibiting clinical resistance phenotypes. Specificity was calculated by dividing the number of isolates that were genotypically susceptible by the total number of isolates with susceptible phenotypes.

Accession number(s). WGS data of all 640 *Salmonella* isolates were submitted to the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA242614. Accession numbers for individual isolates are listed in Tables S1 and S2 in the supplemental material.

RESULTS

Among the 640 total strains we characterized, the most common resistances were to tetracycline (55%; $n = 349$), streptomycin (41%; $n = 260$), sulfisoxazole (38%; $n = 245$), and ampicillin (38%; $n = 242$), followed by ceftriaxone (18%; $n = 116$), amoxicillin-clavulanate (18%; $n = 116$), ceftiofur (18%, $n = 113$), gentamicin (16%; $n = 105$), and cefoxitin (15%; $n = 95$). We detected low levels of resistance to chloramphenicol (7%; $n = 44$), trimethoprim-sulfamethoxazole (3%; $n = 22$), nalidixic acid (2%; $n = 15$), ciprofloxacin (0.6%; $n = 4$), and azithromycin (0.2%; $n = 1$). (To compare resistances in *Salmonella* isolates by source over time, please see the NARMS annual reports at http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm.)

Among the strains selected, 31% ($n = 196$) had no resistance to any of the tested antimicrobial compounds. Eighteen percent ($n = 116$) displayed resistance to at least five classes of antibiotics, and 3% ($n = 20$) had resistance to at least seven of the nine classes tested. Isolate-level data for the retail meat and human isolates used in this study are displayed in Tables S1 and S2 in the supplemental material, respectively.

Correlation of phenotypic resistance with known resistance genes. Overall, phenotypic resistance correlated highly with the presence of known resistance determinants, with genotype agreeing with phenotype for 8,872 of 8,960 phenotypic tests. This resulted in an overall concordance between the methods of 99.0%. In total, 1,727 of the 8,960 broth dilution tests indicated resistance, and associated genes or mutations were predicted to cause resistance in all but 20 instances, 9 of which were related to aminoglycosides, 5 to beta-lactams, 4 to sulfisoxazole or trimethoprim-sulfamethoxazole, and 2 to quinolones. This resulted in an overall sensitivity of 98.8% (1,707/1,727) (Table 1). For retail meats, sensitivity was 99.2% and specificity 99.3%, whereas for humans, sensitivity was 97.6% and specificity 98.0%. This information is in Tables S3 and S4 in the supplemental material.

Among the 7,232 phenotypically susceptible test results, there were 68 occasions where resistance genes were detected by WGS. Among these resistance genes, 40 encoded aminoglycoside resistance, 27 beta-lactam resistance, and 1 chloramphenicol resistance (Table 1). This resulted in an overall specificity of 99.1% (7,164/7,232) (Table 1). This information for retail meat and human isolates is depicted separately in Tables S3 and S4, respectively, in the supplemental material.

Resistance genes. A total of 65 unique resistance genes were identified, most of which are associated with clinical resistance or decreased susceptibility to one of the 14 tested drugs (see Table S1 in the supplemental material). Genes were also identified that are

TABLE 1 Genotype and phenotype comparison of *Salmonella* isolates from humans and retail meat, 2011 to 2013[a]

Antibiotic	No. of test results				Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Phenotype: resistant		Phenotype: susceptible					
	Genotype: resistant	Genotype: susceptible	Genotype: resistant	Genotype: susceptible				
Aminoglycosides								
GEN	99	6	5	530	94.3	99.1	95.2	98.9
STR	257	3	35	345	98.8	90.8	88.0	99.1
Beta-lactam/beta-lactam inhibitor								
AMC	114	2	0	524	98.3	100.0	100	99.6
Cephems								
FOX	93	2	21	524	97.9	96.1	81.6	99.6
TIO	113	0	4	523	100.0	99.2	96.6	100
CRO	116	0	1	523	100.0	99.8	99.1	100
Penicillin								
AMP	241	1	1	397	99.6	99.7	99.6	99.7
Folate pathway inhibitors								
FIS	244	1	0	395	99.6	100.0	100	99.7
SXT	19	3	0	618	86.4	100.0	100	99.5
Macrolide								
AZM	1	0	0	639	100.0	100.0	100	100
Phenicol								
CHL	44	0	1	595	100.0	99.8	97.8	100
Quinolones								
CIP	4	0	0	636	100.0	100.0	100	100
NAL	13	2	0	625	86.7	100.0	100	99.7
Tetracycline								
TET	349	0	0	291	100.0	100.0	100	100
Total	1,707	20	68	7,164	98.8	99.1	96.2	99.7

[a] Abbreviations: GEN, gentamicin; STR, streptomycin; AMC, amoxicillin-clavulanic acid; FOX, cefoxitin; TIO, ceftiofur; CRO, ceftriaxone; AMP, ampicillin; FIS, sulfisoxazole; SXT, trimethoprim-sulfamethoxazole; AZM, azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline; PPV, positive predictive value; NPV, negative predictive value.

predicted to confer resistance to additional compounds (kanamycin, bleomycin, hygromycin B, lincomycin, and disinfectants). The diversity of resistance gene alleles was higher among the 104 human isolates ($n = 59$ alleles) than among the 536 retail meat strains ($n = 36$ alleles) (see Table S5 in the supplemental material). A list of the resistance genes and their frequencies among isolates are shown in Table S6. A synopsis is presented below.

Tetracycline resistance genes. Of the 46 distinct *tet* alleles described to date (13), eight were identified in our strain set, with the highest prevalence being efflux pumps encoded by *tetA* (35%), *tetC* (20%), *tetB* (19%), and *tetD* (16%). Retail meat strains carried only *tetA* through *tetD*, with the exception of five strains of *Salmonella enterica* serotype Typhimurium, which carried *tetG*. The *tetG* gene was detected in 12 isolates in total (7 human and 5 retail meat, with 11 of serotype Typhimurium and 1 of serotype Braenderup), with each of the *S.* Typhimurium isolates producing the well-known ACSSuT penta-resistance pattern of serotype Typhimurium DT104 (ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline) (14). Each of the 11 *tetG*-containing *S.* Typhimurium isolates also carried *sul1*, bla_{carB-2}, *aadA2*, and *floR*, with some human isolates containing additional aminoglycoside resistance genes [*aadA1*, *aac(3)-IV*, *aac(3)-VI*, *aph(3′)-Ia*, *aph(4)-Ia*, *strA*, and *strB*].

Relatively rare were ribosomal protection mechanisms conferred by *tetM* ($n = 2$) and *tetO* ($n = 1$), as well as a single instance of *tetX*, which encodes a tetracycline-degrading enzyme. These three genes were found only among human isolates. In all but one case, these genes were present in isolates with additional tetracycline resistance genes, so their contribution to the Tet^r phenotype in *Salmonella* was not certain. One isolate with *tetM* had no other identified tetracycline resistance genes and was tetracycline resistant, suggesting that *tetM* may be a functional resistance determinant in *Salmonella*. Among all antibiotic resistance phenotypes, tetracycline resistance was most common, and there were no cases in which phenotype and genotype did not agree (100% sensitivity and specificity) (Table 1).

Aminoglycoside resistance. Twenty distinct aminoglycoside resistance alleles were detected, with 14 different genes among food isolates and 18 in human isolates. Genes unique to human isolates included *aadA5*, *aadA7*, *aadA12*, *aadA24*, and *aac(3)-Id*, whereas those unique to food isolates included *aac(6′)-Ib* and *aadA13*. Overall, the most common aminoglycoside resistance genes were *strA* and *strB*, both of which were present in 30% of resistant strains, followed by *aadA1* (15%) and *aph(3′)-Ia* (9%). Sensitivity and specificity for genotypic-phenotypic correlations were 97.5% and 95.6%, respectively.

Sulfonamide and trimethoprim resistance. Sulfisoxazole resistance in both retail meat and human strains was predominantly encoded by *sul1* or *sul2* (present in 39% and 66% of resistant isolates, respectively), with 2% of isolates containing *sul3*. Among the detected dihydrofolate reductase resistance alleles, *dfrA15* was unique to food isolates, while 7 alleles (*dfrA1*, *dfr5*, *dfr7*, *dfrA8*,

dfrA12, *dfrA14*, and *dfrA17*) were present only in human isolates. Genotypic prediction for resistance to the folate synthesis inhibitors resulted in a sensitivity of 98.5%, with specificity being 100%.

Beta-lactam resistance. Among beta-lactam-resistant strains, a total of 10 genes encoding beta-lactamases were identified, with the most common being bla_{TEM-1} (19.7%) and bla_{CMY-2} (17.7%), followed by a diversity of minor genes, each present in less than 2% of isolates. Genes present only in human isolates included $bla_{CTX-M-14b}$, bla_{FOX-6}, bla_{LAP-1}, and bla_{OXA-2}, with only retail meat isolates expressing bla_{SHV-2a} and $bla_{CTX-M-1}$. For beta-lactams, genotypes predicted phenotypes with 99.3% sensitivity and 98.9% specificity. Results by drug are depicted in Table 1.

Quinolone resistance. Quinolone resistance is typically mediated by mutation of the quinolone resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, *parC*, and *parE* (15–17) and/or the acquisition of plasmid-mediated quinolone resistance (PMQR) genes (18). QRDR mutations usually confer nalidixic acid resistance, with multiple mutations being required for ciprofloxacin resistance. Strains containing PMQR genes do not typically exhibit clinical resistance to ciprofloxacin or nalidixic acid, unless QRDR mutations or additional PMQR genes are present. In this study, 21 isolates had either QRDR mutations or PMQR genes, all of which were from human clinical cases. Each isolate with a *gyrA* mutation(s) was resistant to nalidixic acid, with three isolates with *gyrA* mutations also possessing *parC* mutations that contributed to ciprofloxacin resistance (19). A total of nine isolates carried various plasmid-mediated *qnr* genes, which function by protecting DNA gyrase from quinolone binding (18). Most isolates with these *qnr* genes did not have nalidixic acid or ciprofloxacin MICs that reached resistance breakpoints, although isolates with *qnrB19* genes tended to have higher MICs (0.5 to 1 mg/liter) than those with other *qnr* genes, such as *qnrS* and *qnrA* (MIC, 0.12 to 0.25 mg/liter). One isolate was resistant to both nalidixic acid and ciprofloxacin, and it was found to carry both a *qnr* gene and the PMQR genes *oqxA* and *oqxB* (20). Genotypic sensitivity for quinolone antibiotics was 89.5%, with specificity being 100%; results for individual drugs are shown in Table 1.

Macrolide resistance. For macrolide analysis, we tested only azithromycin, and only a single isolate displayed non-wild-type susceptibility. This isolate contained the *mphA* gene, which encodes a macrolide phosphotransferase shown to confer azithromycin resistance in *Escherichia coli* (21). Additional macrolide resistance genes were identified (*mphB*, *mphE*, and *mel*), but these are only known to confer resistance to erythromycin.

DISCUSSION

Affordable WGS technologies are quickly becoming a routine part of laboratory medicine, promising a single workflow to supplant several traditional procedures that require specialized training and reagents (22). Diagnostic tests using DNA sequences and other culture-independent methods accurately identify bacterial species and are becoming more common (23). Open-access tools for *Salmonella* serotyping based on WGS obviate traditional methods and the expense of maintaining quality typing antisera (24). WGS has proven very effective in identifying the source of outbreaks (25–27), has greatly improved trace-back studies (28), and has been used in regulatory action to recall contaminated food products (29). Theoretically, any phenotypic feature of an organism can be derived from the genome sequence. To date, few studies have conducted large-scale sequencing projects to explore the utility of WGS to augment or replace *in vitro* antimicrobial susceptibility as part of a routine laboratory workflow.

To assess the power of WGS to identify antimicrobial resistance in salmonellae, we compared the associations between clinical resistance and the presence of known resistance determinants. A total of 640 strains of *Salmonella* representing 43 serotypes recovered from human and food sources in the NARMS program were analyzed. We show very high concurrence between MICs at or above clinical breakpoints and the presence of known resistance genes, with the two techniques agreeing in 99% of cases. Additional genes associated with resistance to compounds not tested phenotypically were also identified (see Table S6 in the supplemental material), demonstrating another benefit to the approach.

Despite the high level of concordance between genotypic and phenotypic methods, there were some instances of disagreement. Most notably, there were 35 isolates that carried streptomycin resistance genes but were phenotypically susceptible. Streptomycin is included in the NARMS surveillance design because this class of drugs has been commonly used historically in food-producing animals and therefor can serve as a marker for resistant strains moving through the food supply. Because streptomycin is not used to treat enteric infections, there is no CLSI-defined clinical breakpoint for streptomycin. The NARMS program has long used ≥64 mg/liter to define resistance and detected it with a narrow dilution range (32 mg/liter to 64 mg/liter) around the breakpoint. Further testing using a broader dilution range (2 mg/liter to 64 mg/liter) revealed that ≥32 mg/liter better reflects the presence of resistance genes in *Salmonella* and *E. coli* (30), as has been found by others (31). Based on these data, the NARMS will change interpretive criteria for streptomycin resistance in *Salmonella* in future reports.

After streptomycin, the highest number of incongruities was seen with cefoxitin. Cefoxitin resistance is tracked to indicate certain types of beta-lactamases in *Salmonella* and *E. coli*. First- and second-generation cephalosporin susceptibility results are not reported in clinical medicine for *Salmonella* because the drugs may appear active *in vitro* but are not therapeutically effective (2). In our analysis, 21/545 susceptible strains carried resistance genes for cefoxitin whose presence was not revealed phenotypically (Table 1). In 20 of the 21 instances, MICs were a single dilution below the clinical breakpoint for cefoxitin resistance, resulting in intermediate phenotypes. Using the intermediate MIC cutoff (16 mg/liter) to identify resistance would have considerably improved genotype-phenotype correlations for cefoxitin. Similarly, a small number of discrepancies were observed for ceftiofur and ceftriaxone, for which isolates containing known resistance genes had MICs that did not reach the breakpoints.

Among beta-lactam-resistant strains, $bla_{CTX-M-14b}$, bla_{FOX-6}, bla_{LAP-1}, and bla_{OXA-2} genes were present only in human isolates and bla_{SHV-2a} and $bla_{CTX-M-1}$ were found only in retail meat isolates. The latter are interesting findings, as ESBL genes are relatively rare among *Salmonella* isolates causing human infections in the United States (32), with only one ESBL present among the human isolates in this study. To our knowledge, this is the first report of ESBL genes identified from *Salmonella* isolated from retail meats in the United States.

All salmonellae with quinolone resistance mechanisms in this study were isolated from humans. While there were relatively few isolates with phenotypic resistance to nalidixic acid, 2/15 test re-

sults did not correlate. The resistance breakpoint for ciprofloxacin is ≥1 μg/ml. It was noticed that the five isolates that carried the *qnrB19* gene had higher MICs of ciprofloxacin (0.5 to 1 mg/liter) than isolates carrying other *qnr* genes, such as *qnrS* and *qnrA* (MIC, 0.12 to 0.25 mg/liter). Although relatively few isolates with these mechanisms were present, this preliminary analysis suggests that some *qnr* genes may be more effective than others at decreasing fluoroquinolone susceptibility. Interestingly, all isolates except one (17/18) with an intermediate MIC (0.12 to 0.5) for ciprofloxacin either had a single QRDR mutation or carried a *qnr* gene, illustrating that WGS can be used to detect decreased susceptibility to some antibiotics.

Our overall finding that resistance phenotype and genotype correlate highly for *Salmonella* is consistent with the work of Zankari et al., who examined 50 strains of *Salmonella* from swine and found complete agreement between the results of phenotypic profiles and that were predicted from the resistome (33). Tyson et al. observed similar high correlations between phenotypic and genotypic resistance in a study of 76 *E. coli* strains from cattle, where resistance genotypes correlated with 97.8% specificity and 99.6% sensitivity to the identified phenotypes (10). As with the results presented here for *Salmonella*, most of the discrepant *E. coli* results related to streptomycin testing. For *Campylobacter*, Zhao et al. examined the susceptibility profiles of 114 strains from human, retail meat, and animal sources against a panel of nine antimicrobials and found an overall correlation of 99.2% (34). These data all show that a WGS-based analytical workflow can include antimicrobial resistance gene surveillance reporting for the major target bacteria in the NARMS and similar integrated resistance monitoring programs.

Among the few other large-scale studies investigating genetic susceptibility testing, Gordon et al. examined 501 *Staphylococcus aureus* isolates and found that the overall sensitivity and specificity of WGS were 97% compared to standard methods (7). In a study of 74 *E. coli* and 69 *K. pneumoniae* bacteremia isolates tested against seven antimicrobial agents, Stoesser et al. showed sensitivity and specificity above 96% (8). As more genera are examined, and the contribution of specific genes to MICs is resolved, it appears likely that the genomic approach will prove a powerful alternative to traditional methods of tracking antimicrobial-resistant bacteria in surveillance programs (6).

We found that WGS analysis of *Salmonella* is as reliable at identifying clinically resistant strains for most antibiotics as is the measurement of MICs using standardized broth microdilution methods. Discrepancies were highest for aminoglycosides (gentamicin and streptomycin) and cefoxitin. Despite the many benefits of WGS technologies, there are several important issues to address and limitations to consider. For example, consensus on the minimum sequence data quality standards is needed to govern the reporting of WGS data. For evaluating resistance from a risk-ranking perspective, the presence of silent genes, multicopy genes, and their genetic context needs to be better understood. Because of the design of the NARMS susceptibility testing panel, we could not evaluate the concordance between genotype and MIC (a high proportion of isolates were at the extremes of the dilution range). This is a necessary step to understand the limits of resistome typing as a guide to therapy. Shifting of phenotypic breakpoint thresholds from CLSI clinical values to epidemiologically based cutoff values can also drastically alter the sensitivity and specificity of the approach. Furthermore, some of the discrepancies observed may have been due to unidentified resistance mechanisms. This points to an additional limitation of the method, in that it only detects previously identified mechanisms. As new resistance mechanisms are discovered and added to the database, this should reduce the number of isolates expressing phenotypic resistance but for which no gene is identified, thereby improving sensitivity of the test. This requires that some form of phenotypic antimicrobial susceptibility testing continue for surveillance and clinical testing.

The antibiotic resistance database used in this study, similar to other available resistance databases (Resfinder, ARG-ANNOT, and CARD), contains the total complement of known antibacterial resistance genes, not just those in *Salmonella*. This database was used in previously published studies showing a high correlation between phenotypic resistance and the presence of resistance genes in *Campylobacter* spp. (34) and *Escherichia coli*. Not all the determinants in the database have been demonstrated to affect susceptibility in *Salmonella*. At the same time, this approach permits the identification of new alleles that have crossed species and ecological barriers, such as those moving from Gram-positive bacteria to *Salmonella* or those previously found only in certain environments or geographic regions. As WGS data accumulate along with accompanying phenotypic susceptibility data, we will attain a better understanding of which resistance determinants are functional in different bacteria, their contribution to MICs in strains with various acquired resistances, and their distribution in different environments.

Despite some remaining limitations, the comprehensive information provided by WGS will greatly enhance the monitoring of antimicrobial-resistant strain types and genes circulating in humans, foods, animals, and environments. In addition, genomic data will bolster efforts to understand sources of infection, identify and characterize outbreaks, and better understand the consequences of antibiotic use. The improved ability to monitor the resistome in different ecosystems can be used to identify emerging resistance hazards more quickly and help to implement timely control strategies designed to mitigate risks to public health.

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