

Antibiotic Sensitivity Profiles Determined with an *Escherichia coli* Gene Knockout Collection: Generating an Antibiotic Bar Code^{∇†}

Anne Liu, Lillian Tran, Elinne Becket, Kim Lee, Laney Chinn,
Eunice Park, Katherine Tran, and Jeffrey H. Miller*

Department of Microbiology, Immunology, and Molecular Genetics, and the Molecular Biology Institute,
University of California, and the David Geffen School of Medicine, Los Angeles, California 90095

Received 1 July 2009/Returned for modification 31 August 2009/Accepted 19 December 2009

We have defined a sensitivity profile for 22 antibiotics by extending previous work testing the entire KEIO collection of close to 4,000 single-gene knockouts in *Escherichia coli* for increased susceptibility to 1 of 14 different antibiotics (ciprofloxacin, rifampin [rifampicin], vancomycin, ampicillin, sulfamethoxazole, gentamicin, metronidazole, streptomycin, fusidic acid, tetracycline, chloramphenicol, nitrofurantoin, erythromycin, and triclosan). We screened one or more subinhibitory concentrations of each antibiotic, generating more than 80,000 data points and allowing a reduction of the entire collection to a set of 283 strains that display significantly increased sensitivity to at least one of the antibiotics. We used this reduced set of strains to determine a profile for eight additional antibiotics (spectinomycin, cephradine, aztreonam, colistin, neomycin, enoxacin, tobramycin, and cefoxitin). The profiles for the 22 antibiotics represent a growing catalog of sensitivity fingerprints that can be separated into two components, multidrug-resistant mutants and those mutants that confer relatively specific sensitivity to the antibiotic or type of antibiotic tested. The latter group can be represented by a set of 20 to 60 strains that can be used for the rapid typing of antibiotics by generating a virtual bar code readout of the specific sensitivities. Taken together, these data reveal the complexity of intrinsic resistance and provide additional targets for the design of codrugs (or combinations of drugs) that potentiate existing antibiotics.

The proliferation of antibiotic-resistant bacteria has reached the point where close to 100,000 infections occur in the United States per year that are caused by methicillin-resistant *Staphylococcus aureus* (MRSA), leading to 18,650 deaths in 2005 (44), a total that is greater than the 17,011 deaths the same year from HIV/AIDS according to the CDC (<http://www.cdc.gov/hiv/topics/surveillance/resources/reports/2005report/table7.htm>). The spread of resistance (2, 21, 51) has long stimulated efforts to find new antibiotics by a variety of methods, such as altering existing antibiotics, screening chemical (18) or peptide (32, 43) libraries for specific inhibitors (18), or targeting new proteins or processes (11, 32, 35, 47, 58). Recent efforts also have involved detecting new targets through genomics (66, 67), such as bioinformatics screening for novel producers of peptide antimicrobials (8) and finding new sources of antibiotics via metagenomics (68). Some authors have suggested that discovering new molecular scaffolds for antibiotics should be a priority (29). Another approach involves potentiating existing antibiotics by identifying targets for increasing susceptibility to specific antimicrobials (for a review, see reference 19), such as the AmgRS-mediated stress response to the action of aminoglycosides in *Pseudomonas aeruginosa* (49), or bacterial nitric oxide synthases (33). This has been achieved clinically in the case of inhibitors of β -lactamase with β -lactam antibiotics (see

the review by Buynak [15]). A related example is the imipenem-cilastatin combination (36, 38). Cilastatin is an inhibitor of DHP-1, a human renal dehydropeptidase-1 that hydrolyzes the β -lactam imipenem (36). In the laboratory, inhibitors of efflux pumps have been used together with tetracycline in *Escherichia coli* (63) and levofloxacin in *Pseudomonas aeruginosa* (56), but these have not been used successfully in a clinical setting. Tools now are available for more extensive systematic searches for targets for such potentiating codrugs. A transposon library has been used to detect genes responsible for increased sensitivity to one of a set of antibiotics in *Acinetobacter baylyi* (31), comprehensive transposon mutant libraries of *Pseudomonas aeruginosa* (41, 54) have been used to screen for sensitivity to tobramycin (49) and ciprofloxacin (13), and a somewhat less-defined transposon library has been used to detect sensitivity to one of a set of six antibiotics (28). A comprehensive transposon library also has been constructed for *Francisella novicida* (30). In yeast, a deletion library has been screened against a set of four DNA-damaging agents (74) and a set of more than 400 small molecules (37). In the work reported here, we have expanded our previous work (73) and used the high-throughput screening of an *Escherichia coli* knockout collection of close to 4,000 strains, each with a different gene inactivated (6), to look for mutants that are more susceptible to 1 of 14 different antibiotics (the antibiotics and their abbreviations are listed in Table 1). We then used a reduced set of 283 identified strains to examine an additional eight antibiotics with the intent of finding a core set of tester strains. Many mutants are more susceptible to a wide range of antibiotics and represent the basic intrinsic resistance framework, but others are more specific and allow us to define

* Corresponding author. Mailing address: Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA 90095. Phone: (310) 825-8460. Fax: (310) 206-3088. E-mail: jhmiller@microbio.ucla.edu.

† Supplemental material for this article may be found at <http://aac.asm.org/>.

[∇] Published ahead of print on 11 January 2010.

TABLE 1. List of antibiotics

Antibiotic	Category	Primary target	Process affected
Ciprofloxacin (CIP)	Fluoroquinolone	DNA gyrase	DNA replication
Enoxacin (ENX)			
Nitrofurantoin (NIT)	Nitrofurantoin	DNA	DNA
Metronidazole (MTR)	Nitroimidazole	DNA	DNA structure
Sulfamethoxazole (SFX)	Sulfonamide	Dihydropteroate synthetase	Folate synthesis
Rifampin (RIF)	Rifamycin	RNA polymerase	Transcription
Gentamicin (GEN)	Aminoglycoside	30S subunit	Protein synthesis
Tobramycin (TOB)			
Neomycin (NEO)			
Streptomycin (STR)			
Spectinomycin (SPT)	Aminocyclitol	30S subunit	Protein synthesis
Tetracycline (TET)	Polyketide	30S subunit	Protein synthesis
Vancomycin (VAN)	Glycopeptide	NAM/NAG peptides	Cell wall synthesis
Ampicillin (AMP)	Penicillin	Transpeptidase	Cell wall synthesis
Cephadrine (RAD)	Cephalosporin	Transpeptidase	Cell wall synthesis
Cefoxitin (FOX)			
Aztreonam (ATM)	Monobactam	Transpeptidase	Cell wall synthesis
Colistin (CST)	Polymyxin	Cytoplasmic membrane	Cell wall permeability
Chloramphenicol (CHL)	Phenicol	50S subunit	Protein synthesis
Erythromycin (ERY)	Macrolide	50S subunit	Protein synthesis
Fusidic acid (FUS)	Fusidic acid	Elongation factor G	Protein synthesis
Triclosan (TRI)	Chlorophenol	Enoyl-acyl carrier protein reductase	Fatty acid synthesis

so-called bar codes for the rapid typing of antibiotics with different target activities. These results also help to define new combinational drug targets, as most of the mutants do not result in growth inhibition in the absence of subinhibitory or sublethal concentrations of an antibiotic. Moreover, combining mutations with increased sensitivities would allow the design of more precise tests for the persistence of antibiotics in foods, milk, and various wastewaters.

MATERIALS AND METHODS

E. coli strains. The KEIO collection was described in Baba et al. (6) and made from the starting strain BW25113 (20). This strain (*lacI^q rrrB_{T14} ΔlacZ_{WJ16} hsdR514 ΔaraBAD_{AH33} ΔrhaBAD_{LD78}*) is the starting strain used in the experiments reported here unless otherwise stated.

E. coli genetic methods. Unless otherwise stated, all genetic methods are as described by Miller (62).

Use of the Deutz cryoreplicator. The Deutz cryoreplicator (23) contains 96 prongs on individual springs, allowing its frequent application to frozen glycerol cultures. The KEIO collection (4) is maintained on 45 96-well microtiter plates and stored at -80°C in glycerol. Material from frozen microtiter plates was transferred to microtiter wells with 0.5 ml of LB and were incubated overnight, and then the replicator was used to transfer a microdrop to microtiter wells with fresh LB medium containing 50 $\mu\text{g/ml}$ kanamycin to prevent the growth of contaminants. (All of the strains in the KEIO collection are Kan^r). After 3 to 4 h of growth, these plates were printed onto LB plates with different concentrations of different antibiotics. For the initial screening, kanamycin was present in the plates, but for retests and MIC determinations kanamycin was not present.

Determination of MICs. MICs (5) were determined by printing, with the Deutz replicator, approximately 2×10^5 to 4×10^5 CFU to an LB plate with the appropriate concentration of the desired antibiotic and then examining the spots after overnight incubation at 37°C . (This represents a minor deviation from the standard method in that LB medium was used and that somewhat more cells were applied to each spot.) All determinations were carried out on strain sets that were prepared from purified colonies. The tests were repeated up to six times in some cases and at various concentrations (see the supplemental material). The antibiotics used in the Tamae et al. study (73) were retested along with the new antibiotics, using additional concentrations and the somewhat different conditions, to standardize scoring. The concentrations reported for compounds such as the aminoglycosides might be different in the LB medium used here from those in other media, although we did not find reproducible differences when NaCl was left out of the LB medium in the two cases tested (tobramycin and gentamicin). Cross-resistance between some of the aminoglycosides, particularly

neomycin and the kanamycin resistance marker in the strains used, might alter the concentrations that are effective, but we found this to be the case only for neomycin. We found prominent patterns for neomycin at the concentrations reported.

Validation of the data set. A collection of 4,000 strains will contain some errors and some impure strains. The latter problem can be minimized by repurifying and retesting, as was done here. Also, in a number of cases, strains pick up suppressors that will attenuate the effects of the deletion knockouts. We have reisolated several strains to eliminate this effect. Thus, the copies of strains with *oxyR* and *recA* are somewhat different from those reported previously (73). Mori and coworkers (75) have subjected the KEIO collection to an intensive analysis aimed at uncovering errors in the collection that might arise from duplications of the target gene. They have generated a list of 14 mutants that are incorrect and another 9 that might be incorrect. We therefore have removed *coaE*, *glmM*, *parC*, *prfB*, *polA*, *rpoD*, and *rpsU* from our data sets. Ultimately, the most prudent use of such a large collection is to verify any mutants that are particularly important to the final results by PCR analysis and/or sequencing. We examined in detail some of the more important assignments. We took an expanded set of 12 of the mutants from the bar codes that are used to identify specific antibiotics (Table 2) (*recF*, *argO*, *degP*, *dacA*, *glpD*, *trxB*, *xseA*, *sapC*, *rplI*, *tufA*, *ycdZ*, and *dinB*), as well as *recA*, *oxyR*, *flgB*, *ppiD*, and *rpsF*. Using two primer pairs for each mutant, we verified in two PCR experiments for each that each carried a *kan* insert in place of the correct gene and that there was no presence of the original gene sequence elsewhere (via a duplication). In the case of *rpsF*, we carried out both PCR and sequencing. Moreover, an additional two from Table 2 can be identified by their

TABLE 2. Diagnostic gene knockout strains for specific antibiotics

Antibiotic	Strain
CIP-ENX.....	<i>xseA</i>
NIT.....	<i>recO</i>
MTR.....	<i>gshA</i> (<i>recO</i>)
SFX.....	<i>degP</i>
RIF.....	<i>trxB</i>
Aminoglycosides.....	<i>sapC ompF</i>
TET.....	<i>ycdZ</i>
VAN.....	<i>tufA</i>
β -lactams.....	<i>dacA</i>
CST.....	<i>vacJ</i>
CAM.....	<i>argO</i>
FUS.....	<i>rplI</i>
TRI.....	<i>glpD dnaJ</i>

phenotypes (*dam*, mutator; *lon*, mucoid formation). This constitutes a verification of 19 mutants, most of which are key indicators for specific antibiotics. We had reason to suspect the *recF* deletion mutant, and a similar analysis revealed that the *recF* deletion strain in the first copy of the KEIO strains is incorrect, although the *recF* strain in the second is correct, and this has been used in the work reported here. Regarding further validation, we have detected all four of the genes found through biochemical experiments that, when mutated, render the cell more sensitive to CPR (25) and all six of the genes for NIT (64).

Chemicals. Kanamycin, tetracycline, chloramphenicol, rifampin, vancomycin, sulfamethoxazole, gentamicin, ampicillin, spectinomycin, cephradine, colistin, streptomycin, aztreonam, erythromycin, fusidic acid, metronidazole, nitrofurantoin, neomycin, enoxacin, tobramycin, triclosan, and cefoxitin were purchased from Sigma (St. Louis, MO). Ciprofloxacin was purchased from ICN Biomedicals, Inc., Aurora, OH.

RESULTS AND DISCUSSION

Screening for antibiotic hypersensitivity. The entire KEIO collection of close to 4,000 strains (6) was screened for mutants that are more sensitive than the wild type to one or more of a set of 14 different antibiotics (Table 1). We first examined CIP, VAN, RIF, AMP, SFX, GEN, and MTR at several different subinhibitory concentrations (73), and we have expanded this to include an additional set of seven compounds (streptomycin, STR; fusidic acid, FUS; tetracycline, TET; chloramphenicol, CHL; nitrofurantoin, NIT; triclosan, TRI; and erythromycin, ERY; see Materials and Methods for details). This generated an initial set of close to 80,000 data points. Mutants showing increased susceptibility then were purified and retested, resulting in a set of 283 strains that displayed significantly increased susceptibility to at least 1 of the 14 antibiotics tested. We then used this set of 283 strains to test an additional eight antibiotics (spectinomycin, SPT; cephradine, RAD; aztreonam, ATM; colistin, CST; neomycin, NEO; enoxacin, ENX; tobramycin, TOB; and cefoxitin, FOX) (Table 1), as well as additional concentrations of the original 14 antibiotics, to show that a sensitivity profile can be determined from this smaller set of strains. Figures 1 and 2 give a broad overview of the data (for quantitative details, see the supplemental material) in which the strongest sensitivities are indicated in darker colors and sensitivities that are less strong are indicated in lighter colors, with different colors and numbers indicating different functions (Fig. 1). The functions were categorized and assigned numbers as follows: 1, DNA recombination, replication, and repair; 1A, DNA related; 2, permeation, membranes, and transport; 2A, chaperoning; 3, protein synthesis; 3A, RNA processing; 4, general metabolic reactions; 5, transcriptional control; 6, prophage encoded; and 7, unassigned functions. The intensity is shown in three levels. The darkest level for each group represents the strongest sensitivities. Many different types of functions are involved, including those concerned with DNA replication, recombination, and repair that are prevalent among the sensitivity profiles for agents that interact with DNA, as well as functions involved in the cell wall and cell membrane, chaperoning, protein synthesis, and general metabolism. Also, close to 30 genes encoding transcriptional regulators appear in Fig. 1. Figure 2 groups all of the detected susceptible mutants deleted for as-yet unassigned genes. In addition to providing a wealth of information on different functions involved in intrinsic resistance, this set of 283 strains can be used to generate a susceptibility fingerprint for antibiotics. For example, note how clearly the two fluoroquinolones CIP and ENX give very sim-

ilar profiles. However, as detailed below, one set of mutants in Fig. 1 and 2 is defective in functions involved in multidrug resistance, as the resulting phenotype includes increased sensitivity to a range of different antibiotics, while another set of hypersensitive mutants are partially or completely specific for each antibiotic or class of antibiotic. From the data shown in Fig. 1, it is possible to predict whether an antibiotic primarily affects DNA-related functions or not, but it is necessary to separate the different components of the strain set to enable the typing of antibiotics with a simpler set of mutants displaying more specific patterns.

Multidrug-resistant functions. Many of the 283 mutants shown in Fig. 1 and 2 display hypersensitivity to more than one antibiotic. We have selected those that show increased sensitivity to at least 8 of the 22 antibiotics tested (36%), as shown in Fig. 3: a total of 61 mutants, including genes involved in recombination and recombinational repair of double-strand breaks (*recABC*), the main efflux pump in *E. coli* (*acrA*, *acrB*, *tolC*), those involved in cell wall and cell membrane synthesis and integrity, and transporters and chaperones. Note that *yciM* mutants (Fig. 2) are more sensitive to 21 of the 22 antibiotics tested and *tolC* mutants to 19 (Fig. 1). Several genes encoding ribosomal proteins (*rplA*, *rpmE*, *rpmJ*) or their modification (*rimK*) confer intrinsic resistance to a varied set of antibiotics, as their deletion renders the cell more sensitive than the wild type. Figure 3 includes genes encoding partially (*ybgF*, *yfgC*) or completely (*yciM*, *ybeY*, *ycbW*, *yjjY*) uncharacterized functions. Interestingly, some genes encode proteins that are involved in the regulation of gene expression (*dksA*, *fur*, *hfq*, *hns*, *mfd*, *nusB*, *rseA*, *xapR*, *yciT*). In some (and perhaps all) cases, these regulators affect functions that already appear in Fig. 1, such as the DksA transcription factor that regulates genes involved in double-strand break repair (61), in addition to multiple target genes associate with many processes (65). The regulatory genes operate in different ways, such as Hfq, which binds many small noncoding RNAs and is involved in posttranscriptional gene regulation (50), or HNS, a DNA-binding protein involved in DNA topology and compaction, which plays a role in the regulation of many unrelated genes (70 and references therein), or RseA, an anti-sigma factor that inhibits the transcriptional activity of σ^E (1, 72). Additionally, *rfaH* mutants lacking an antitermination factor (7, 9) are more sensitive to six antibiotics (Fig. 1).

Specific sensitivities. While the data in Fig. 1 and 2 portray a sensitivity profile that is diagnostic for each antibiotic, the charts are complex. However, we can simplify this by selecting only those mutants that display more specific sensitivities to a particular antibiotic or class of antibiotic. Figure 4 shows the results from the latter set of mutants and displays a more useful set of mutants for typing antibiotics (also see the following sections). This selected set or subsets can be used to generate a bar code signature for each antibiotic; as more antimicrobial compounds are tested, they can be added. From Fig. 3 and 4, we can see both general and specific sensitivities within each functional category. For example, with regard to the sensitivities of knockouts of genes related to recombination and repair, the *recABC* genes appear general (Fig. 3), but many genes in the RecF pathway, as well as those involved in other DNA-related functions, are more specific. One should note that the RecBCD pathway carries out the repair of double-

Gene	CIP	ENX	NIT	MTR	SFX	RIF	GEN	TOB	NEO	STR	SPT	TET	VAN	AMP	RAD	FOX	ATM	CST	CHL	ERY	FUS	TRI	Categ	Gene Product Description	
<i>dam</i>																							1	DNA adenine methylase	
<i>dinB</i>																								1	DNA polymerase IV
<i>dinG</i>																								1	ATP-dependent helicase
<i>recA</i>																								1	DNA strand exchange and recombination protein
<i>recB</i>																								1	DNA helicase, dsDNA/ssDNA exonuclease
<i>recC</i>																								1	DNA helicase, dsDNA/ssDNA exonuclease
<i>recF</i>																								1	ssDNA and dsDNA binding, subunit of RecFOR complex
<i>recO</i>																								1	DNA repair protein; subunit of RecFOR complex
<i>recR</i>																								1	Recombination and repair, subunit of RecFOR complex
<i>recG</i>																								1	DNA helicase, resolution of Holliday junctions
<i>recJ</i>																								1	Single-stranded-DNA-specific exonuclease
<i>recN</i>																								1	Recombination and repair protein
<i>recQ</i>																								1	ATP-dependent DNA helicase
<i>rivA</i>																								1	Holliday junction DNA helicase
<i>rivC</i>																								1	Holliday junction nuclease
<i>uvrA</i>																								1	Excision nuclease subunit A
<i>uvrC</i>																								1	Excinuclease ABC, subunit C; UV damage repair
<i>uvrD</i>																								1	DNA-dependent ATPase I and helicase II
<i>xerC</i>																								1	Integrase/site-specific recombinase
<i>xseA</i>																								1	Exodeoxyribonuclease VII large subunit
<i>xseB</i>																								1	Exodeoxyribonuclease VII small subunit
<i>cmk</i>																								1A	Cytidylate kinase
<i>fts</i>																								1A	DNA-binding protein - chromosome compaction
<i>ftsP</i>																								1A	Cell division protein required during stress (suII)
<i>gor</i>																								1A	Glutathione reductase (NADPH)
<i>gshA</i>																								1A	Glutamate-cysteine ligase
<i>gshB</i>																								1A	Glutathione synthetase subunit
<i>hrpA</i>																								1A	ATP-dependent helicase
<i>tdK</i>																								1A	Thymidine kinase / deoxyuridine kinase
<i>yfgD</i>																								1A	Predicted oxidoreductase
<i>yglA</i>																								1A	Putative ligase
<i>yglS</i>																								1A	Predicted oxidoreductase, 4Fe-4S ferredoxin-type subunit
<i>minC</i>																								1A, 2	Septum site determining protein; cell division inhibitor
<i>acrA</i>																								2	AcrAB-TolC Multidrug Efflux transport, periplasmic lipoprotein subunit
<i>acrB</i>																								2	AcrAB-TolC Multidrug Efflux transport, inner-membrane pump
<i>tolC</i>																								2	AcrAB-TolC Multidrug Efflux transport, outer membrane subunit
<i>argO</i>																								2	Arginine outward transport
<i>bamB</i>																								2	Lipoprotein- outer membrane protein assembly (yfgL)
<i>cpxA</i>																								2	Sensor kinase-phosphotransferase
<i>csxG</i>																								2	Putative curli production protein
<i>cypA</i>																								2	Membrane protein required for colicin V production
<i>dacA</i>																								2	D-alanyl-D-alanine carboxypeptidase; penicillin-binding protein 5
<i>dacC</i>																								2	Dicarboxylate transporter
<i>mrcB</i>																								2	Penicillin-binding protein 1B
<i>dhlB</i>																								2	D-alanine-D-alanine ligase, B subunit
<i>dedD</i>																								2	Putative lipoprotein - inner membrane
<i>ecnB</i>																								2	Subunit of entericidin toxin-antitoxin complex
<i>emtA</i>																								2	Lytic murein transglycosylase E
<i>envC</i>																								2	Cytokinesis - murein hydrolase (yibP)
<i>envZ</i>																								2	Osmolarity sensor protein
<i>exbD</i>																								2	ExbD uptake of enterochelin
<i>fehB</i>																								2	subunit of ferric enterobactin ABC transporter
<i>fehC</i>																								2	Ferric enterobactin transport, ATP-binding subunit
<i>flgF</i>																								2	Flagellar basal-body rod protein FlgF
<i>fruB</i>																								2	Subunit of fructose PTS transporter
<i>glcA</i>																								2	GlcA glycolate transporter
<i>gltI</i>																								2	Subunit of GltJKL glutamate ABC transporter
<i>leuE</i>																								2	Leucine RhtB transporter
<i>lpxL</i>																								2	Lipid A biosynthesis lauroyl acyltransferase
<i>lpxM</i>																								2	Myristoyl-acyl carrier acyltransferase (msbB)
<i>mdjI</i>																								2	subunit of MdjI spermidine SMR transporter (ydgF)
<i>nlpC</i>																								2	NlpC-putative lipoprotein hydrolase
<i>ompF</i>																								2	Outer membrane protein F precursor; Porin
<i>pal</i>																								2	Peptidoglycan-associated lipoprotein precursor.
<i>phoU</i>																								2	PhoU phosphate transport system protein
<i>plsX</i>																								2	Fatty acid/phospholipid synthesis protein
<i>ppiB</i>																								2	Peptidyl-prolyl cis-trans isomerase B
<i>ppiD</i>																								2	Peptidyl-prolyl cis-trans isomerase D
<i>proV</i>																								2	Proline ABC transporter
<i>proW</i>																								2	Proline ABC transporter, inner membrane subunit
<i>pstA</i>																								2	Phosphate transport system permease, membrane subunit
<i>pstC</i>																								2	Subunit of phosphate ABC transporter
<i>pstS</i>																								2	Phosphate-binding periplasmic protein precursor
<i>qmcA</i>																								2	Predicted protease, membrane anchored (ybbK)
<i>rfaC</i>																								2	Lipopolysaccharide heptosyltransferase-1
<i>rfaD</i>																								2	ADP-L-glycero-D-manno-heptose-6-epimerase
<i>rfaE</i>																								2	Heptose 1-phosphate adenylyltransferase
<i>rfaG</i>																								2	Lipopolysaccharide glucosyltransferase I
<i>rfaP</i>																								2	Lipopolysaccharide core biosynthesis protein
<i>rfaQ</i>																								2	Lipopolysaccharide core biosynthesis protein; heptosyl transferase III
<i>rfaY</i>																								2	Lipopolysaccharide core biosynthesis protein
<i>rffA</i>																								2	dTDP-4-oxo-6-deoxy-D-glucose transaminase
<i>sapC</i>																								2	Peptide uptake ABC transporter
<i>secG</i>																								2	Protein-export membrane protein; Protein Secretion Complex
<i>smpA</i>																								2	Outer membrane lipoprotein/Protein Assembly Complex
<i>tatB</i>																								2	Sec-independent protein translocase

FIG. 1. Strains with sensitivity to one or more of the 22 antibiotics with three levels of intensities: stronger susceptibilities are in darker shades, medium susceptibilities are in lighter shades, and weak susceptibilities are in the lightest shade. The categories (categ.) are the following: 1, DNA replication, recombination, and repair; 1A, functions indirectly affecting category 1; 2, transport, efflux, cell wall, and cell membrane synthesis; 2A, chaperones and functions related to category 2; 3, protein synthesis; 3A, RNA processing; 4, central metabolic reactions; 5, regulation; and 6, prophage-carried genes and cell adhesion.

Gene	CIP	ENX	NIT	MTR	SFX	RIF	GEN	TOB	NEO	STR	SPT	TET	VAN	AMP	PRAD	FOX	ATM	CST	CHL	ERY	FUS	TRI	Categ	Gene Product Description	
<i>taoC</i>																							2	Sec-independent protein translocase	
<i>tolQ</i>																								2	Colicin transport; Tol-Pal Cell Envelope Complex
<i>tolR</i>																								2	Colicin transport; Tol-Pal system component
<i>tonB</i>																								2	Cytoplasmic membrane protein; energy transducer
<i>trxA</i>																								2	Thioredoxin I
<i>trxB</i>																								2	Thioredoxin reductase
<i>typA</i>																								2	LPS biosynthesis/GTP-binding protein
<i>vacJ</i>																								2	Predicted lipoprotein
<i>wbbL</i>																								2	Lipopolysaccharide biosynthesis protein, N-ter fragment
<i>ybfB</i>																								2	Predicted inner membrane protein
<i>ybgF</i>																								2	Subunit of The Tol-Pal Cell Envelope Complex
<i>ybjI</i>																								2	Predicted transporter
<i>ybjO</i>																								2	Predicted inner membrane protein
<i>ybjP</i>																								2	Predicted lipoprotein
<i>ycdZ</i>																								2	Predicted inner membrane protein
<i>yceK</i>																								2	Predicted lipoprotein
<i>yehM</i>																								2	Sulfate SulP transporter
<i>yeiS</i>																								2	Conserved inner membrane protein
<i>yefG</i>																								2	L-Ala-D/L-Glu epimerase
<i>ydcS</i>																								2	Putative ABC transporter periplasmic binding protein
<i>ydcX</i>																								2	Predicted inner membrane protein.
<i>yhdP</i>																								2	Transporter activity, membrane protein
<i>yheL</i>																								2	Subunit of sulfur transfer protein complex
<i>yiaA</i>																								2	Conserved inner membrane protein
<i>yiaD</i>																								2	Predicted outer membrane lipoprotein
<i>yidE</i>																								2	Predicted transporter
<i>ymfA</i>																								2	Predicted inner membrane protein
<i>yneE</i>																								2	Conserved inner membrane protein
<i>ynhG</i>																								2	L,D-transpeptidase YnhG
<i>degP</i>																								2A	Periplasmic serine protease and chaperone
<i>dnaJ</i>																								2A	Chaperone with DnaK; heat shock protein
<i>dnaK</i>																								2A	Chaperone protein Hsp70; DNA biosynthesis and heat shock
<i>hspB</i>																								2A	FKBP-typepeptidyl-prolyl cis-trans isomerase
<i>hspA</i>																								2A	Periplasmic chaperone (skp)
<i>hscA</i>																								2A	Chaperone, member of Hsp70 protein family
<i>hscB</i>																								2A	Hsc20 co-chaperone, Hsc66 IscU iron-sulfur cluster
<i>ihpB</i>																								2A	Small heat shock protein
<i>lon</i>																								2A	DNA-binding, ATP-dependent protease
<i>surA</i>																								2A	Peptidyl-prolyl cis-trans isomerase and chaperone
<i>ycbR</i>																								2A	Predicted periplasmic pilin chaperone
<i>ygeG</i>																								2A	Predicted chaperone
<i>yral</i>																								2A	Putative chaperone
<i>dusB</i>																								3	tRNA dihydrouridine synthase (yhdG)
<i>elaD</i>																								3	Deubiquitinating protease
<i>hflK</i>																								3	Regulator of FtsH protease
<i>mmmG</i>																								3	Protein involved in a rRNA modification pathway (gidA)
<i>pppB</i>																								3	Phosphatidylglycerophosphatase B.
<i>prfC</i>																								3	Peptide chain release factor 3
<i>rhlB</i>																								3	ATP-dependent RNA helicase of the RNA degradosome
<i>rhmK</i>																								3	Ribosomal protein S6 modification protein.
<i>rplA</i>																								3	50S ribosomal protein L1.
<i>rplI</i>																								3	50S ribosomal protein L9.
<i>rplR</i>																								3	Subunit of 50S ribosomal subunit and ribosome
<i>rpmE</i>																								3	50S ribosomal protein L31.
<i>rpmF</i>																								3	50S ribosomal protein L32.
<i>rpmG</i>																								3	Subunit of 50S ribosomal subunit
<i>rpmJ</i>																								3	50S ribosomal protein L36
<i>rpsF</i>																								3	30S ribosomal protein S6.
<i>rsgA</i>																								3	Ribosome small subunit-dependent GTPase A (yjeQ)
<i>rsmE</i>																								3	16S rRNA m3U1498 methyltransferase (yggJ)
<i>rsmF</i>																								3	16S rRNA m5C1407 methyltransferase (yebU)
<i>sirA</i>																								3	Sulfur transfer protein
<i>tufA</i>																								3	Elongation factor Tu
<i>tufB</i>																								3	Elongation factor Tu
<i>yheM</i>																								3	tRNA modification, sulfur transfer prot. Complex (tusC)
<i>yheN</i>																								3	tRNA modification, sulfur transfer prot. Complex (tusD)
<i>yigC</i>																								3	Predicted peptidase
<i>yidD</i>																								3	Predicted peptidase
<i>yihK</i>																								3	Subunit of predicted rRNA methylase
<i>pcnB</i>																								3A	poly(A) polymerase I
<i>ppp</i>																								3A	Subunit of polynucleotide phosphorylase and degradosome
<i>rhn</i>																								3A	Subunit of RNase BN
<i>rhmE</i>																								3A	23S rRNA m2U2552 methyltransferase
<i>rhmH</i>																								3A	23S rRNA m3Ψ1915 methyltransferase (ybeA)
<i>rppH</i>																								3A	RNA pyrophosphohydrolase (nudH)
<i>aceE</i>																								4	Subunit of pyruvate dehydrogenase
<i>aceF</i>																								4	Subunit of pyruvate dehydrogenase multienzyme complex
<i>ackA</i>																								4	Propionate kinase / acetate kinase
<i>acpT</i>																								4	Holo-[acyl carrier protein] synthase 2
<i>amiA</i>																								4	N-acetyl-muramoyl-L-alanine amidase 1
<i>atpG</i>																								4	ATP synthase, F1 complex, γ subunit
<i>cis</i>																								4	Cardiolipin synthetase
<i>cof</i>																								4	Pyridoxal phosphatase / HMP-PP hydrolase
<i>cvaA</i>																								4	Adenylate cyclase
<i>cvdB</i>																								4	Cytochrome bd-I terminal oxidase subunit II
<i>dapF</i>																								4	Diaminopimelate epimerase
<i>dcd</i>																								4	Deoxycytidine triphosphate deaminase

FIG. 1—Continued.

Gene	CIP	ENX	NIT	MTR	SFX	RIF	GEN	TOB	NEO	STR	SPT	TET	VAN	AMP	RAD	FOX	ATM	CST	CHL	ERY	FUS	TRI	Categ	Gene Product Description	
<i>eutM</i>																							4	Predicted carboxysome structural protein, ethanolamine utilization	
<i>fabF</i>																								4	β-ketoacyl-ACP synthase
<i>fdx</i>																								4	Ferredoxin
<i>fidB</i>																								4	Flavodoxin 2
<i>folB</i>																								4	Dihydroneopterin aldolase
<i>glnD</i>																								4	uridylyltransferase / uridylyl-removing enzyme
<i>glpD</i>																								4	Subunit of glycerol-3-phosphate dehydrogenase
<i>gmhB</i>																								4	D,D-heptose 1,7-bisphosphate phosphatase (yaeD)
<i>gntK</i>																								4	Superpathway of gluconate degradation
<i>gmY</i>																								4	Protein involved using DNA as carbon source
<i>gpmM</i>																								4	Phosphoglycerate mutase, cofactor independent (gpmI)
<i>hyfF</i>																								4	Hydrogenase-4 component F
<i>hyfJ</i>																								4	Hydrogenase 4 component J, putative protein processing element
<i>iscS</i>																								4	Cysteine desulfurase monomer
<i>iscU</i>																								4	Scaffold protein involved in iron-sulfur cluster assembly (<i>nifU</i>)
<i>lpdA</i>																								4	Lipoamide dehydrogenase, Glycine cleavage
<i>mraW</i>																								4	S-adenosyl-dependent methyltransferase
<i>nagA</i>																								4	N-acetylglucosamine-6-phosphate deacetylase
<i>ndbB</i>																								4	Dihydroneopterin triphosphate pyrophosphohydrolase
<i>mioM</i>																								4	Subunit of NADH:ubiquinone oxidoreductase I
<i>pgaC</i>																								4	UDP-N-acetyl-D-glucosamine β-1,6-N-acetyl-D-glucosaminyl transferase
<i>pta</i>																								4	Phosphate acetyltransferase
<i>rpe</i>																								4	Ribulose-phosphate 3-epimerase
<i>rpiA</i>																								4	Ribose-5-phosphate isomerase A
<i>rutA</i>																								4	Predicted coenzyme N5,10-methylenetetrahydromethanopterin reductase
<i>rutB</i>																								4	Predicted isochorismatase (ycdL)
<i>sucD</i>																								4	Subunit of succinyl-CoA synthetase
<i>tktA</i>																								4	Transketolase subunit, pentose phosphate pathway
<i>treA</i>																								4	Periplasmic trehalase
<i>ubiG</i>																								4	3-demethylubiquinone-8 3-methyltransferase
<i>ybgC</i>																								4	Esterase/thioesterase
<i>yegF</i>																								4	Predicted FAD-binding phosphodiesterase
<i>ycjU</i>																								4	β-phosphoglucosmutase
<i>yulB</i>																								4	Protein involved in detoxification of methylglyoxal
<i>ydhI</i>																								4	Predicted aldolase
<i>ycgO</i>																								4	Predicted 4Fe-4S cluster-containing protein
<i>ygfZ</i>																								4	Folate-binding protein
<i>yjgB</i>																								4	FMN phosphatase
<i>yjiW</i>																								4	Predicted pyruvate formate lyase activating enzyme
<i>yjC</i>																								4	Phosphoglycerate mutase 2 (gpmB)
<i>zwf</i>																								4	Glucose-6-phosphate 1-dehydrogenase.
<i>cedA</i>																								5	Cell division modulator
<i>espE</i>																								5	Transcription antiterminator and regulator of RNA stability
<i>cysB</i>																								5	Transcriptional dual regulator
<i>deaD</i>																								5	Cold-shock DEAD-box protein A (RNA helicase)
<i>dksA</i>																								5	RNA polymerase-binding transcription factor
<i>flhD</i>																								5	Subunit of FlhDC transcriptional dual regulator
<i>fur</i>																								5	Ferrie uptake regulation protein.
<i>gcvA</i>																								5	GcvA transcriptional dual regulator
<i>hdfR</i>																								5	HdfR transcriptional repressor
<i>hfg</i>																								5	RNA Chaperone involved in post-transcriptional regulation
<i>hipB</i>																								5	Subunit of HipB transcriptional repressor
<i>hms</i>																								5	H-NS transcriptional dual regulator
<i>ihfA</i>																								5	Integration host factor transcriptional dual regulator
<i>leuO</i>																								5	Transcriptional dual activator
<i>mfd</i>																								5	Transcription-repair coupling factor
<i>nusB</i>																								5	Transcription antitermination protein
<i>oxyR</i>																								5	Transcriptional dual regulator
<i>pdhR</i>																								5	PdhR transcriptional dual regulator
<i>phoP</i>																								5	Transcriptional regulatory protein
<i>rfaH</i>																								5	Transcriptional antiterminator
<i>rpoN</i>																								5	RNA polymerase sigma 54 factor.
<i>rseA</i>																								5	Negative regulator of sigma E
<i>seqA</i>																								5	Negative modulator of initiation of replication
<i>slyA</i>																								5	SlyA transcriptional activator
<i>xapR</i>																								5	Transcriptional activator
<i>ycaN</i>																								5	Putative transcriptional regulator LYSR-type
<i>yciT</i>																								5	DNA-binding transcriptional regulator (deoT)
<i>ydhH</i>																								5	Predicted DNA-binding transcriptional regulator
<i>ydhB</i>																								5	Protein with similarity to transcriptional regulators
<i>yidP</i>																								5	Predicted DNA-binding transcriptional regulator
<i>yihL</i>																								5	YihL putative transcriptional regulator
<i>zapB</i>																								5	Cell division factor (yiuU)
<i>ybcN</i>																								6	DLP12 prophage; DNA base-flipping protein
<i>ybcW</i>																								6	DLP12 prophage; predicted protein
<i>yfiR</i>																								6	CP4-57 prophage; predicted DNA-binding transcriptional regulator
<i>yicG</i>																								6	DLP12 prophage; predicted protein
<i>ymfT</i>																								6	e14 prophage; predicted DNA-binding transcriptional regulator

FIG. 1—Continued.

strand DNA breaks, while the RecF pathway is involved principally in the repair of single-strand breaks and gaps (4, 17, 54, 55, 71). The RecF pathway mutants *recF*, *recO*, *recR*, *recQ*, and *recJ*, along with *dinG* and *uvrC* mutants, are specifically sensitive to NIT and, usually to a lesser extent, MTR, the only two agents that are converted to forms that directly damage DNA.

Among these, *recQ* mutants are absolutely specific for NIT. Also, *xseA* gene knockouts, lacking an exonuclease, *exo VII* large subunit (16), and, to a somewhat lesser extent, *xseB* knockouts, are particularly sensitive to fluoroquinolones. Interestingly, mutants lacking the glutathione reductase system are fairly specific in their hypersensitivity to MTR. However,

Gene	CIP	ENX	NIT	MTR	SFX	RIF	GEN	TOB	NEO	STR	SPT	TET	VAN	AMP	PRAD	FOX	ATM	CST	CHL	ERY	FUS	TRI	Categ	Gene Product Description	
<i>rssA</i>																							7	Hypothetical	
<i>gfcC</i>																								7	Conserved protein (ymcB)
<i>JW2207</i>																								7	-
<i>JW5115</i>																								7	-
<i>JW5169</i>																								7	-
<i>JW5360</i>																								7	-
<i>JW5474</i>																								7	-
<i>yaiU</i>																								7	Predicted protein
<i>ybaB</i>																								7	Hypothetical
<i>ybdD</i>																								7	Conserved
<i>ybdN</i>																								7	Conserved
<i>ybeD</i>																								7	Hypothetical
<i>ybeY</i>																								7	Hypothetical
<i>ybgT</i>																								7	Conserved
<i>ybhT</i>																								7	Hypothetical
<i>ybjQ</i>																								7	Hypothetical
<i>yebK</i>																								7	Hypothetical
<i>yebW</i>																								7	Predicted protein
<i>yceD</i>																								7	Hypothetical
<i>yehJ</i>																								7	Hypothetical
<i>yeiM</i>																								7	Conserved
<i>yldK</i>																								7	Hypothetical
<i>yldD</i>																								7	Predicted peptidase
<i>ydhT</i>																								7	Hypothetical
<i>yebV</i>																								7	Predicted protein
<i>yfiH</i>																								7	Conserved protein
<i>yhhA</i>																								7	Conserved
<i>yidD</i>																								7	Predicted protein
<i>yihG</i>																								7	Predicted endonuclease
<i>yigA</i>																								7	Conserved
<i>yijY</i>																								7	Predicted protein
<i>ymbA</i>																								7	Conserved

FIG. 2. Hypothetical strains with sensitivity to one or more of the 22 antibiotics. All belong to category 7 for unassigned gene products.

mutants lacking the thioredoxin/thioredoxin reductase system (*trxA*, *trxB*) are sensitive specifically to RIF.

Antibiotic identification. We can designate a minimal set of 13 to 15 strains that allowed the identification of almost all of the antibiotics used here, as depicted in Table 2. In some cases, further testing with other mutants from Fig. 4 can distinguish between members of general groups (fluoroquinolones, aminoglycosides, and β -lactams). Of course, other combinations of strains are possible for a list of this type. The one exception is ERY, for which no single strain is specifically sensitive. However, one can use strains such as *pnp* or *rep*, which are hypersensitive to ERY, and then identify the other possibilities, such as RIF, with strains diagnostic for RIF.

Targets for codrugs or potentiators. Considering the studies reported here and elsewhere (13, 28, 31, 37, 73), can we use this type of information to design codrugs that potentiate existing antibiotics? The data in Fig. 1 to 4 offer an expanded set of targets for finding compounds that act by inactivating proteins that provide intrinsic resistance and, thus, could be used in combinational therapy with existing antibiotics. There is an emerging emphasis on considering combinational strategies (19), of which there are several types. One category includes combinations of antibiotics that are either in the same pathway (e.g., cotrimoxazole [14]) or in different pathways (e.g., isoniazid plus rifampin, pyrazinamide, and streptomycin for the treatment of tuberculosis; <http://www.cdc.gov>; also see reference 19) to yield a more effective treatment and often to overcome resistance to a single drug. Recently, Kishony and coworkers have described the functional classification of drugs according to their pairwise interactions (76). An additional class is represented by synergicid (quinupriostin-dalfopristin), a combination of two bacteriostatic drugs that together produce a bactericidal effect (3). A third category comprises drugs used with a codrug that inhibits a resistance pathway, as has been used clinically (15, 36, 38) and in the laboratory (56, 63).

Additional targets for codrugs (either small molecules or engineered bacteriophages [57]) involve different pathways of intrinsic resistance or persister cell formation or maintenance (52, 53). Of particular interest in the work reported here are targets for potentiators that involve specific responses to a particular antibiotic or class of antibiotic; focusing on these might yield a useful set of compounds to be used with specific antibiotics. These include the following.

(i) Rifampin. Finding inhibitors of the *trxA*- or *trxB*-encoded enzymes (thioredoxin 1 and thioredoxin reductase, respectively) (10, 22), the *rhlB*-encoded RNA helicase, or the *coaE*-encoded dephospho-coenzyme A kinase would sensitize cells to lower levels of RIF or increase the potency of RIF at higher levels.

(ii) Triclosan. TRI works by specifically inhibiting the *fabI* gene product that catalyzes a key step in fatty acid biosynthesis (60). Mutants with deletions of the *dam* gene lack adenine methylase and are almost uniquely sensitive to TRI. Mashhoon, Reich, and coworkers have identified a number of compounds that specifically inhibit bacterial methylases, including the Dam methylase (58, 59). Using these inhibitors in the presence of TRI on the wild-type strain would be equivalent to using a *dam* mutant strain with TRI and, thus, could be an effective drug combination. Because *glpD* mutants are specifically inhibited by TRI, the inhibitor of the GlpD enzyme, glycerol-3-phosphate dehydrogenase, also would be an effective codrug for TRI. Interestingly, Lewis has shown that GlpD is a key protein involved in the generation of persister cells in the population (52, 53).

(iii) Nitrofurantoin. An inhibitor of the RecQ helicase, a homolog of the human helicase lacking in Bloom's Syndrome patients (27), would be an effective and specific codrug for NIT. Yet another codrug candidate would be an inhibitor of the MdtJI spermidine SMR transporter.

Gene	CIP	ENX	NIT	MTR	SFX	RIF	GENT	TOB	NEO	STR	SPT	TET	VAN	AMP	RAD	FOX	ATM	CST	CHL	ERY	FUS	TRI	Categ	
<i>recA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	1
<i>recB</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	1
<i>recC</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	1
<i>acrA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>acrB</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>tolC</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>ddlB</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>envC</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>lpxL</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>lpxM</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>nlpC</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>proW</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>pstS</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>qmcA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>rfaC</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>rfaD</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>rfaE</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>rfaG</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>tatC</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>tolR</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>ybgF</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>ydcS</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2
<i>dnaK</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2A
<i>hlpA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2A
<i>hscA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2A
<i>surA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	2A
<i>rimK</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3
<i>rplA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3
<i>rpmE</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3
<i>rpmJ</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3
<i>rsmF</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3
<i>yheM</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3
<i>yheN</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3
<i>yfgC</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3
<i>rlmE</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	3A
<i>atpG</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>dapF</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>gmhB</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>gpmM</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>iscS</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>mraW</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>pgaC</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>rutA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>ycjU</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>ygeO</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	4
<i>dksA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>fur</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>hfq</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>hns</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>mfd</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>musB</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>rseA</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>xapR</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>yciT</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	5
<i>ylcG</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	6
<i>ybeD</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	7
<i>ybeY</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	7
<i>ybhT</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	7
<i>yebW</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	7
<i>yciM</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	7
<i>yjiY</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	7

FIG. 3. Sixty-one strains with sensitivity to eight or more of the 22 antibiotics.

(iv) **Aminoglycosides.** There are numerous functions that are specifically involved in protecting the cell from aminoglycosides (Fig. 4), and perhaps SapC and SecG are the best targets. (SecG has been suggested as a target for potentiators of aminoglycosides in experiments by others [46]).

(v) **β-Lactams.** Inhibitors of some of the penicillin binding proteins pinpointed in Fig. 4 (e.g., the *dacA*- and *mrcB*-encoded functions) would potentiate β-lactams.

Some of the functions identified in Fig. 1 and 2 represent pathways that are not directly connected to the presumed pri-

Gene	CIP	ENX	NIT	MTR	SFX	RIF	GEN	TOB	NEO	STR	SPT	TET	VAN	AMP	RAD	FOX	ATM	CST	CHL	ERY	FUS	TRI	Categ	Gene Product Description
DNA-related																								
<i>xseA</i>																							1	Exodeoxyribonuclease VII large subunit
<i>xseB</i>																							1	Exodeoxyribonuclease VII small subunit
<i>recN</i>																							1	Recombination and repair protein
<i>recG</i>																							1	DNA helicase, resolution of Holliday junctions
<i>fis</i>																							1A	DNA-binding protein - chromosome compaction
<i>uvrD</i>																							1	DNA-dependent ATPase I and helicase II
<i>ybjQ</i>																							7	Hypothetical protein
<i>recQ</i>																							1	ATP-dependent DNA helicase
<i>recJ</i>																							1	Single-stranded-DNA-specific exonuclease recJ
<i>dinG</i>																							1	ATP-dependent helicase
<i>recF</i>																							1	Subunit of RecFOR complex
<i>recO</i>																							1	DNA repair protein RecO
<i>recR</i>																							1	Recombination and repair , subunit of RecFOR complex
<i>uvrA</i>																							1	Excision nuclease subunit A
<i>uvrC</i>																							1	Excinuclease ABC, subunit C; UV damage repair
<i>dam</i>																							1	DNA adenine methylase
<i>gor</i>																							1A	Glutathione reductase (GRase).
<i>gshA</i>																							1A	Glutamate--cysteine ligase
<i>gshB</i>																							1A	Glutathione synthetase
<i>dinB</i>																							1	DNA polymerase IV
Aminoglycoside Sensitivity																								
<i>sapC</i>																							2	Peptide uptake ABC transporter
<i>secG</i>																							2	Protein-export membrane protein SecG
<i>hflK</i>																							3	Regulator of FtsH protease
<i>JW5360</i>																							7	
<i>ompF</i>																							2	Outer membrane protein F precursor; Porin ompF
<i>prfC</i>																							3	Peptide chain release factor 3 (RF-3).
<i>rffA</i>																							2	dTDP-4-oxo-6-deoxy-D-glucose transaminase
<i>yidD</i>																							7	Predicted protein YidD
<i>leuO</i>																							5	Transcriptional dual activator
<i>folB</i>																							4	Dihydroneopterin aldolase
<i>glnD</i>																							4	uridylyltransferase / uridylyl-removing enzyme
<i>nudB</i>																							4	Dihydroneopterin triphosphate pyrophosphohydrolase
<i>yidD</i>																							7	Predicted protein
<i>yidP</i>																							5	Predicted DNA-binding transcriptional regulator
B-lactam Sensitivity																								
<i>emtA</i>																							2	Lytic murein transglycosylase E
<i>dacA</i>																							2	Penicillin-binding protein 5 precursor
<i>rsgA</i>																							3	Ribosome small subunit-dependent GTPase A (yjeQ)
<i>mrcB</i>																							2	Penicillin-binding protein 1B
<i>yfiH</i>																							7	Conserved Protein
<i>ycbK</i>																							7	Conserved protein Ycbk
Other Specific Sensitivity																								
<i>lon</i>																							2A	DNA-binding, ATP-dependent protease
<i>gntY</i>																							4	Protein involved using DNA as carbon source
<i>deaD</i>																							5	Cold-shock DEAD-box protein A (RNA helicase)
<i>mdtJ</i>																							2	subunit of MdtJI spermidine SMR transporter (ydgF)
<i>degP</i>																							2A	Periplasmic serine protease and chaperone
<i>cedA</i>																							5	Cell division modulator CedA.
<i>trxA</i>																							2	Thioredoxin I
<i>trxB</i>																							2	Thioredoxin reductase
<i>rpmG</i>																							3	Subunit of 50S ribosomal subunit
<i>ydfH</i>																							5	Predicted DNA-binding transcriptional regulator
<i>rhlB</i>																							3	ATP-dependent RNA helicase of the RNA degradosome
<i>ybcN</i>																							6	DLP12 prophage; DNA base-flipping protein
<i>ycdZ</i>																							2	Predicted inner membrane protein
<i>ppiD</i>																							2	Peptidyl-prolyl cis-trans isomerase D
<i>tufA</i>																							3	Elongation factor Tu
<i>pgpB</i>																							3	Phosphatidylglycerophosphatase B.
<i>vacJ</i>																							2	Predicted lipoprotein
<i>flgF</i>																							2	Flagellar basal-body rod protein FlgF
<i>argO</i>																							2	Arginine outward transport
<i>rplI</i>																							3	50S ribosomal protein L9.
<i>dnaJ</i>																							2A	Chaperone with DnaK; heat shock protein
<i>glpD</i>																							4	Subunit of glycerol-3-phosphate dehydrogenase
<i>rsmE</i>																							3	16S rRNA m3U1498 methyltransferase (yggJ)

FIG. 4. Strains with sensitivities unique to a particular class of antibiotics and strains with specific sensitivities.

many target of some of the antibiotics and reflect the complexity of antibiotic action (34). Studying some of these pathways in depth should provide additional insights regarding antibiotic action. For instance, Collins and coworkers have argued that the generation of hydroxyl radicals leading to double-strand breaks is a major contributor to cell death for bactericidal antibiotics (26, 45, 46). From Fig. 1 it is evident that a number of strains lacking some aspect of the RecBCD system involved in the recombinational repair of double-strand breaks (*recA*, *recB*, or *recC*) are more sensitive to many antibiotics, and

mildly increased sensitivity to some of the four aminoglycosides tested is exhibited by mutants lacking the ability to respond to DNA damage or damaging agents (e.g., *dinG*, *xseB*, *gshB*). We can use the specific sensitivity profiles defined here to type new antibiotics with significantly less effort than that for microarray studies, particularly if we employ the reduced set of strains shown in Fig. 4 or in Table 2. In principle, these data complement the data from microarrays (12, 40, 42, 69), since each method has a different, although not totally independent, basis for scoring. Microarrays measure the change in gene

expression in response to a subinhibitory concentration of an antibiotic, whereas the profiles such as those in Fig. 1 to 4 measure specific phenotypes. Moreover, some of the strains shown in Fig. 4 can be used in the initial screening for specific antibiotics. Previous workers have used engineered strains to aid in the detection of antibiotics from soil isolates (24, 39).

As noted above, the data in this work further define the intrinsic resistome, as has work with other microorganisms (13, 28, 31, 37); as we dismantle the cell's intrinsic protection, the cell becomes more sensitive. We have shown that certain double mutants are even more sensitive than their parents alone (73). We can apply the knowledge of the property of double and triple mutants to construct improved strains that are useful for detecting the presence of antibiotics in the environment, such as in milk or hospital wastewater, as is done with the Delvotest SP-NT and the Copan milk test (48), and have already developed some preliminary tests.

ACKNOWLEDGMENTS

We thank Julian Davies and Lynn Silver for helpful discussions and comments on the manuscript.

E.B. was supported by the Whitcome Fellowship. This work was supported by a grant from the National Institutes of Health (ES0110875).

REFERENCES

- Alba, B. M., J. A. Leeds, C. Onufryk, C. Z. Lu, and C. A. Gross. 2002. DegS and YaeL participate sequentially in the cleavage of RseA to activate the σ^E -dependent extracytoplasmic stress response. *Genes Dev.* **16**:2156–2168.
- Alekshun, M. N., and S. B. Levy. 2007. Molecular mechanisms of antibacterial multidrug resistance. *Cell* **128**:1037–1050.
- Allington, D. R., and M. P. Rivey. 2001. Quinupristin/dalfopristin: a therapeutic review. *Clin. Ther.* **23**:24–44.
- Amundsen, S. K., and G. R. Smith. 2003. Interchangeable parts of the *Escherichia coli* recombinational machinery. *Cell* **112**:741–744.
- Andrews, J. M. 2001. Determination of minimum inhibitory concentrations. *Antimicrob. Agents Chemother.* **48**:5–16.
- Baba, T., T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K. A. Datsenko, M. Tomita, B. L. Wanner, and H. Mori. 2006. Construction of *Escherichia coli* K-12 in-frame single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2**:2006–2008.
- Bailey, M. J. A., C. Hughes, and V. Koronakis. 1997. RfaH and the *ops* element, components of a novel system controlling bacterial transcription elongation. *Mol. Microbiol.* **26**:845–851.
- Begley, M., P. D. Cotter, C. Hill, and R. P. Ross. 2009. Identification of a novel two-peptide lantibiotic, lichenicidin, following rational genome mining for LanM proteins. *Appl. Environ. Microbiol.* **75**:5451–5460.
- Beloin, C., K. Michaelis, K. Lindner, P. Landini, J. Hacker, J.-M. Ghigo, and U. Dobrindt. 2006. The transcriptional antiterminator RfaH represses biofilm formation in *Escherichia coli*. *J. Bacteriol.* **188**:1316–1331.
- Bjur, E., S. Eriksson-Ygberg, F. Aslund, and M. Rhen. 2006. Thioredoxin 1 promotes intracellular replication and virulence of *Salmonella enterica* serovar Typhimurium. *Infect. Immun.* **74**:5140–5151.
- Bowser, T. E., V. J. Bartlett, M. C. Grier, A. K. Verma, T. Warchol, S. B. Levy, and M. N. Alekshun. 2007. Novel anti-infection agents: small-molecule inhibitors of bacterial transcription factors. *Bioorg. Med. Chem. Lett.* **17**:5652–5655.
- Brazas, M. D., and R. E. W. Hancock. 2005. Using microarray gene signatures to elucidate mechanisms of antibiotic action and resistance. *Drug Discov. Today* **10**:1245–1252.
- Breidenstein, E. B., B. K. Khaira, I. Wiegand, J. Overhage, and R. E. Hancock. 2008. Complex ciprofloxacin resistome revealed by screening a *Pseudomonas aeruginosa* mutant library for altered susceptibility. *Antimicrob. Agents Chemother.* **52**:4486–4491.
- Brumfitt, W., and J. M. Hamilton-Miller. 1994. Limitations of and indications for the use of co-trimoxazole. *J. Chemother.* **6**:3–11.
- Buynak, J. D. 2006. Understanding the longevity of the β -lactam antibiotics and of antibiotic/ β -lactamase inhibitor combinations. *Biochem. Pharmacol.* **71**:930–940.
- Chase, J. W., B. A. Rabin, J. B. Murphy, K. L. Stone, and K. R. Williams. 1986. *Escherichia coli* exonuclease VII. Cloning and sequencing of the gene encoding the large subunit (*xseA*). *J. Biol. Chem.* **261**:14929–14935.
- Clark, A. J., and K. B. Low. 1988. Pathways and systems of homologous recombination in *Escherichia coli*, p. 155–215. In K. B. Low (ed.), *The recombination of genetic material*. Academic Press, San Diego, CA.
- Costi, M. P., A. Gelanin, D. Barlocco, S. Ghelli, F. Soragni, F. Reniero, T. Rossi, A. Ruberto, C. Guillou, A. Cavazzuti, C. Casolari, and S. Ferrari. 2006. Antibacterial agent discovery using thymidylate synthase biolibrary screening. *J. Med. Chem.* **49**:5958–5968.
- Cottarel, G., and J. Wierzbowski. 2007. Combination drugs, an emerging option for antibacterial therapy. *Trends Biotech.* **25**:547–555.
- Datsenko, K. A., and B. L. Wanner. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **97**:6640–6645.
- Davies, J. 2007. Microbes have the last word. *EMBO Rep.* **8**:616–621.
- Debarbieux, L., and J. Beckwith. 1998. The reductive enzyme thioredoxin 1 acts as an oxidant when it is exported to the *Escherichia coli* periplasm. *Proc. Natl. Acad. Sci. USA* **95**:10751–10756.
- Deutz, W. A., L. Rüedi, R. Hermann, K. O'Connor, J. Büchs, and B. Witholt. 2000. Methods for intense aeration, growth, storage, and replication of bacterial strains in microtiter plates. *Appl. Environ. Microbiol.* **66**:2641–2646.
- DeVito, J. A., J. A. Mills, V. G. Liu, A. Agarwal, C. F. Sizemore, Z. Yao, D. M. Stoughton, M. G. Cappiello, M. D. Barbosa, L. A. Foster, and D. L. Pompiliani. 2002. An array of target-specific screening strains for antibacterial discovery. *Nat. Biotechnol.* **20**:478–483.
- Drlica, K., and X. Zhao. 1997. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol. Mol. Biol. Rev.* **61**:377–391.
- Dwyer, D. J., M. A. Kohanski, and J. J. Collins. 2009. Role of reactive oxygen species in antibiotic action and resistance. *Curr. Opin. Microbiol.* **12**:482–489.
- Ellis, N. A., J. Groden, T. Z. Ye, J. Straughan, D. J. Lennon, S. Ciocci, M. Proytcheva, and J. German. 1995. The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* **83**:655–666.
- Fajardo, A., N. Martinez-Martin, M. Mercadillo, J. C. Galan, B. Ghysels, S. Matthijs, P. Cornelis, L. Wiehlmann, B. Tuemmler, F. Baquero, and J. L. Martinez. 2008. The neglected intrinsic resistome of bacterial pathogens. *PLoS One* **3**:e1619.
- Fischbach, M. A., and C. T. Walsh. 2009. Antibiotics for emerging pathogens. *Science* **325**:1089–1093.
- Gallagher, L. A., E. Ramage, M. A. Jacobs, R. Kaul, M. Brittnacher, and C. Manoil. 2007. A comprehensive transposon mutant library of *Francisella novicida*, a bioweapon surrogate. *Proc. Natl. Acad. Sci. USA* **104**:1009–1014.
- Gomez, M. J., and A. A. Neyfakh. 2006. Genes involved in intrinsic antibiotic resistance of *Acinetobacter baylyi*. *Antimicrob. Agents Chemother.* **50**:3562–3567.
- Gunderson, C. W., and A. M. Segall. 2006. DNA repair, a novel antibacterial target: Holliday junction-trapping peptides induce DNA damage and chromosome segregation defects. *Mol. Microbiol.* **59**:1129–1148.
- Gusarov, I., K. Shatalin, M. Starodubtseva, and E. Nudler. 2009. Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics. *Science* **325**:1380–1384.
- Hancock, R. E. W. 2007. The complexities of antibiotic action. *Mol. Syst. Biol.* **3**:142.
- Higgins, D. A., M. E. Pomianek, C. M. Cramel, R. K. Taylor, M. F. Semmelhack, and B. L. Bassler. 2007. The major *Vibrio cholerae* autoinducer and its role in virulence factor production. *Nature* **450**:883–886.
- Hikida, M., K. Kawashima, M. Yoshida, and S. Mitsuhashi. 1992. Inactivation of new carbapenem antibiotics by dehydropeptidase-1 from porcine and human renal cortex. *J. Antimicrob. Chemother.* **30**:129–134.
- Hillenmeyer, M. E., E. Fung, J. Wildenhain, S. E. Pierce, S. Hoon, W. Lee, M. Proctor, R. P. St. Onge, M. Tyers, D. Koller, R. B. Altman, R. W. Davis, C. Nislow, and G. Giaever. 2008. The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science* **320**:362–365.
- Hoffman, J., J. Trimble, and G. M. Brophy. 2009. Safety of imipenem/cilastatin in neurocritical care patients. *Neurocrit. Care* **10**:403–407.
- Hsieh, P., S. A. Siegel, B. Rogers, D. Davis, and K. Lewis. 1998. Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. *Proc. Natl. Acad. Sci. USA* **95**:6602–6606.
- Hutter, B., C. Schaab, S. Albrecht, M. Borgmann, N. A. Brunner, C. Freiberg, K. Ziegelbauer, C. O. Rock, I. Ivanov, and H. Loferer. 2004. Prediction of mechanisms of action of antibacterial compounds by gene expression profiling. *Antimicrob. Agents Chemother.* **48**:2838–2844.
- Jacobs, M. A., A. Alwood, I. Thaipisuttikul, D. Spencer, E. Haugen, S. Ernst, O. Will, R. Kaul, R. C. Levy, L. Chun-Rong, D. Guenther, D. Bovee, M. V. Olson, and C. Manoil. 2003. Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* **100**:14339–14344.
- Kaldalu, N., R. Mei, and K. Lewis. 2004. Killing by ampicillin and ofloxacin induces overlapping changes in *Escherichia coli* transcription profile. *Antimicrob. Agents Chemother.* **48**:890–896.
- Kepple, K. V., J. L. Boldt, and A. M. Segall. 2005. Holliday junction-binding peptides inhibit distinct junction-processing enzymes. *Proc. Natl. Acad. Sci. USA* **102**:6867–6872.
- Klevens, R. M., M. A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L. H. Harrison, R. Lynfield, G. Dumyati, J. M. Townes, A. S. Craig, E. R.

- Zell, G. E. Fosheim, L. K. McDougal, R. B. Carey, and S. K. Fridkin. 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* **298**:1763–1771.
45. Kohanski, M. A., D. J. Dwyer, B. Hayete, C. A. Lawrence, and J. J. Collins. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**:797–810.
 46. Kohanski, M. A., D. J. Dwyer, J. Wierzbowski, G. Cottarel, and J. J. Collins. 2008. Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. *Cell* **135**:679–690.
 47. Kuijl, C., N. D. L. Savage, M. Marsman, A. W. Tuin, L. Janssen, D. A. Egan, M. Ketema, R. van den Nieuwendijk, S. J. F. van den Eeden, A. Geluk, A. Poot, G. van der Marel, R. L. Beijersbergen, H. Overkleef, T. H. M. Ottenhoff, and J. Neeffjes. 2007. Intracellular bacterial growth is controlled by a kinase network around PKB/AKT1. *Nature* **450**:725–730.
 48. Le Breton, M.-H., M.-C. Savoy-Perroud, and J.-M. Diserens. 2007. Validation and comparison of the Copan milk test and Delvotest SP-NT for the detection of antimicrobials in milk. *Anal. Chim. Acta* **586**:280–283.
 49. Lee, S., A. Hinz, E. Bauerle, A. Angermeyer, K. Juhasova, Y. Kaneko, P. K. Singh, and C. Manoil. 2009. Targeting a bacterial stress response to enhance antibiotic action. *Proc. Natl. Acad. Sci. USA* **106**:14570–14575.
 50. Lee, T., and A. L. Feig. 2008. The RNA binding protein Hfq interacts specifically with tRNAs. *RNA* **14**:514–523.
 51. Levy, S. B., and B. Marshall. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* **10**:S122–S129.
 52. Lewis, K. 2007. Persister cells, dormancy, and infectious disease. *Nat. Rev. Microbiol.* **5**:48–56.
 53. Lewis, K. 2008. Multidrug tolerance of biofilms and persister cells. *Curr. Top. Microbiol. Immunol.* **322**:107–131.
 54. Liberati, N. T., J. M. Urbach, S. Miyata, D. G. Lee, E. Drenkard, G. Wu, J. Villanueva, T. Wei, and F. M. Ausubel. 2006. An ordered, nonredundant library of *Pseudomonas aeruginosa* strain PA14 transposon insertion mutants. *Proc. Natl. Acad. Sci. USA* **103**:2833–2838.
 55. Lloyd, R. G., and K. B. Low. 1996. Homologous recombination, p. 2236–2255. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli and Salmonella: cellular and molecular biology*, 2nd ed. ASM Press, Washington, DC.
 56. Lomovskaya, O., M. S. Warren, A. Lee, J. Galazzo, R. Fronko, M. Lee, J. Blais, D. Cho, S. Chamberland, T. Renau, H. Ishida, and V. J. Lee. 2001. Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. *Antimicrob. Agents Chemother.* **45**:105–116.
 57. Lu, T. K., and J. J. Collins. 2009. Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. *Proc. Natl. Acad. Sci. USA* **106**:4629–4634.
 58. Mashhoon, N., M. Carroll, C. Pruss, J. Eberhard, S. Ishikawa, R. A. Estabrook, and N. Reich. 2004. Functional characterization of *Escherichia coli* DNA adenine methyltransferase, a novel target for antibiotics. *J. Biol. Chem.* **279**:52075–52081.
 59. Mashhoon, N., C. Pruss, M. Carroll, P. H. Johnson, and N. O. Reich. 2006. Selective inhibitors of bacterial DNA adenine methyltransferase. *J. Biomol. Screen.* **11**:497–510.
 60. McMurry, L. M., M. Oethinger, and S. B. Levy. 1998. Triclosan targets lipid synthesis. *Nature* **394**:531–532.
 61. Meddows, T. R., A. P. Savory, J. I. Grove, T. Moore, and R. G. Lloyd. 2005. RecN protein and transcription factor DksA combine to promote faithful recombinational repair of DNA double-strand breaks. *Mol. Microbiol.* **57**:97–110.
 62. Miller, J. H. 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria, p. 194–195. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
 63. Nelson, M. L., and S. B. Levy. 1999. Reversal of tetracycline resistance by different bacterial tetracycline resistance determinants by an inhibitor of the Tet(B) antiport protein. *Antimicrob. Agents Chemother.* **43**:1719–1724.
 64. Ona, K. R., C. T. Courcelle, and J. Courcelle. 2009. Nucleotide excision repair is a predominant mechanism for processing nitrofurazone-induced DNA damage in *Escherichia coli*. *J. Bacteriol.* **191**:4959–4965.
 65. Paul, B. J., M. B. Berkmen, and R. L. Gourse. 2005. DksA potentiates direct activation of amino acid promoters by ppGpp. *J. Bacteriol.* **102**:7823–7828.
 66. Payne, D. J., M. N. Gwynn, D. J. Holmes, and M. Rosenberg. 2004. Genomic approaches to antibacterial discovery. *Methods Mol. Biol.* **266**:231–259.
 67. Pucci, M. J. 2007. Novel genetic techniques and approaches in the microbial genomics era: identification and/or validation of targets for the discovery of new antibacterial agents. *Drugs R D* **8**:2010–2212.
 68. Schloess, P. D., and J. Handelsman. 2003. Biotechnological prospects from metagenomics. *Curr. Opin. Biotechnol.* **14**:303–310.
 69. Shaw, K. J., N. Miller, X. Liu, D. Lerner, J. Wan, A. Bittner, and B. J. Morrow. 2003. Comparison of the changes in global gene expression of *Escherichia coli* induced by four bactericidal agents. *J. Mol. Microbiol. Biotechnol.* **5**:105–122.
 70. Skoko, D., D. Yoo, H. Bai, B. Schnurr, J. Yan, S. M. McLeod, J. F. Marko, and R. C. Johnson. 2006. Mechanism of chromosome compaction and looping by the *Escherichia coli* nucleoid protein Fis. *J. Mol. Biol.* **364**:777–798.
 71. Spies, M., and S. C. Kowalczykowski. 2005. Homologous recombination by RecBCD and RecF pathways, p. 389–403. *In* N. P. Higgins (ed.), *The bacterial chromosome*. ACM Press, Washington, DC.
 72. Tam, C., B. Collinet, G. Lau, S. Raina, and D. Missiakia. 2002. Interaction of the conserved region 4.2 of σ^E with the RseA anti-sigma factor. *J. Biol. Chem.* **277**:27282–27287.
 73. Tamae, C., A. Liu, K. Kim, D. Sitz, J. Hong, E. Becket, A. Bui, P. Solaimani, K. P. Tran, H. Yang, and J. H. Miller. 2008. Determination of antibiotic hypersensitivity among 4,000 single gene knockout mutants of *Escherichia coli*. *J. Bacteriol.* **190**:5981–5988.
 74. Workman, C. T., H. C. Mak, S. McCuine, J. Tagne, M. Agarwal, O. Ozier, T. J. Begley, L. D. Samson, and T. Ideker. 2006. A systems approach to mapping DNA damage response pathways. *Science* **312**:1054–1059.
 75. Yamamoto, N., K. Nakahigashi, T. Nakamichi, M. Yoshino, Y. Takai, Y. Touda, A. Furubayashi, S. Kinjo, H. Dose, M. Hasegawa, K. A. Datsenko, T. Nakayashiki, M. Tomita, B. L. Wanner, and H. Mori. 2009. Update on the Keio collection of *Escherichia coli* single-gene deletion mutants. *Mol. Syst. Biol.* **5**:335–338.
 76. Yeh, P., A. I. Tschumi, and R. Kishony. 2006. Functional classification of drugs by properties of their pairwise interactions. *Nat. Genet.* **28**:489–494.