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Supplementary Methods

Derivation of resistant laboratory bacterial strains: *E. coli* laboratory strain J53 with defined fluoroquinolone-resistant chromosomal mutations in gyrA (*gyrA1* - G81D; *gyrA2* - S83L) were obtained from the Hooper lab, Massachusetts General Hospital, Boston, MA. Plasma-mediated quinolone resistance determinants (*oqxAB*, *qnrB*, *aac6-Ib*) were purified from clinical isolates previously determined to contain these plasmids. Electrocompetent cells of *E. coli* parent strain J53 was transformed with these plasmids, and their presence was confirmed with PCR.

Sample processing: For Gram negative isolates in liquid culture, 5 – 20 µl of culture was added directly to 100 µl RLT buffer and vortexed. For Gram-positive isolates, the samples were additionally mechanically disrupted with bead beating after the addition of RLT. For spiked blood samples, $1x10^7$ bacteria were added per ml of blood. Samples were then added to PAXgene blood RNA tubes (PreAnalytiX), and processed according to the manufacturer's protocol through the first centrifugation. Supernatant was then aspirated, pellets were resuspended in 350 µl RLT buffer, and 4 µl were used directly in hybridizations. For spiked urines and clinical specimens, 5-20 µl of urine was added directly to 5 volumes of RLT buffer. For mycobacteria, 1.5 ml of culture was centrifuged, then resuspended in Trizol (Gibco) with or without mechanical disruption by bead beating, and the initial aqueous phase was collected for analysis. Viral and parasite RNA were similarly prepared using Trizol and chloroform. For all lysates, 3-5 µl were used directly in hybridizations according to standard nCounter protocols. For organism identification, raw scores were normalized to internal hybridization controls. For antibiotic susceptibility determination, raw counts were normalized to the mean of the middle 50% of all probes for a sample, and fold induction for each gene was determined by comparing antibiotic-treated to untreated samples.

Selection of organism identification probes: To select nCounter probes for differential detection of organisms, we compared all publically available sequenced genomes for relevant organisms. We identified genes conserved within each species by selecting coding sequences (CDS) having at least 50% identity over at least 70% of the CDS length for all sequenced genomes for pathogenic strains of that species available at the time that the probe sets were generated. For a complete list of included genomes for each organism see Supplementary Table S9. We broke the CDS into overlapping 50-mers and retained only those 50-mers with 100% identity within a species and having no greater than 50% identity to a CDS in any other species in the study. Available published expression data in Gene Expression Omnibus was reviewed, and genes with good expression under most conditions were selected. To identify unique *M. tuberculosis* probes, published microarray data was used to identify highly expressed genes falling into one of two classes: those unique to the *M. tuberculosis* complex (>70% identity to any other gene in the non-redundant database using BLASTN and conserved across all available *M. tuberculosis* and *M. bovis* genomes), as well as those with >85% identity across a set of clinically relevant mycobacteria including *M. tuberculosis, M. avium,* and *M. paratuberculosis. C. albicans* probes were designed against 50-mer segments of *C. albicans* genome unique in comparison with the complete genomes of ten additional pathogenic organisms that were included in its probe set. Viral probes were designed against highly conserved genes within a virus (i.e. all HSV-2 or HIV-1 isolates) that were less conserved among viruses within the same family, (i.e. between HSV-1 and HSV-2). *Plasmodium falciparum probes* were designed against genes expressed abundantly in each of the blood stages of the parasite life cycle. All probes were screened to avoid cross hybridization with human RNA.

Description of Probe Sets: For Gram-negative organism identification, a pooled probe-set containing probes for *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were used. For mycobacterial organism identification, species-specific probes for *M. tuberculosis* and broader mycobacterial genus probes were among a larger set of probes against microbial pathogens. For expanded bacterial and fungal organism identification, probe sets included probes to *S. pyogenes*, *S. aureus*, *S. pneumoniae*, *S. mitis*, *S. agalactiae*, *H. influenzae, S. proteamaculans*, *A. baumanii*, S. *maltophilia*, *C. albicans*, and *P. mirabilis*. For viral and parasitic organism identification, probe sets included probes to *HSV-1*, *HSV-2*, *influenza A*, *HIV-1*, *P. falciparum*, and *M. tuberculosis*. Unless otherwise indicated, non-organism probes consist of all other organism identification probes in the probe set.

nCounter data analysis and calculation of distance metric mean squared distance for drug-sensitivity: To transform qualitative expression signatures into a binary outcome of sensitive or resistant we developed an algorithm that collapses the response signature of each sample into a single value. This metric, which we term the Squared Projected Distance, or SPD, is calculated as follows:

1. First, variation in sample amount is corrected for by normalizing raw nCounter values to the geometric mean of the middle fifty percent of all probes in a sample.
2. The expression levels for each strain before or after drug treatment were denoted C_{i,P_j}^{before} or C_{i,P_j}^{after}, where i indicates the sample index and P_j denotes the jth probe measured.
3. Next the "log induction ratio", denoted *LIR*, is computed for each probe:

$$LIR_{P_j}^{i} \equiv \ln\left[C_{i,P_j}^{before} / C_{i,P_j}^{after} \right]$$

   Probes that do not change expression levels upon drug treatment will possess an *LIR* near zero, whereas induced or repressed genes with possess *LIR* scores greater or less than zero, respectively.

4. We group all drug sensitive strains, which number N^S, and compute the average *LIR* for each probe P_j:

$$\overline{LIR_{P_j}^{S}} \equiv \frac{\sum_{i=1}^{N^S} LIR_{P_j}^{i}}{N^S}$$

   This represents a one-dimensional vector with a number of elements equal to the number of probes.

5. The same process is repeated to find the center of the N^R drug resistant samples:

$$\overline{LIR_{P_j}^{R}} \equiv \frac{\sum_{i=1}^{N^R} LIR_{P_j}^{i}}{N^R}$$

6. A vector $\vec{A}$ that points from $\overline{LIR_{P_j}^{S}}$ to $\overline{LIR_{P_j}^{R}}$ is defined as:

$$\vec{A} \equiv \overline{LIR_{P_j}^{R}} - \overline{LIR_{P_j}^{S}}$$

Similarly a vector $\overrightarrow{B^i}$ is calculated that points from $\overline{LIR^S_{P_j}}$ to each sample $LIR^i_{P_j}$:

$$\overrightarrow{B^i} \equiv LIR^i_{P_j} - \overline{LIR^S_{P_j}}$$

These vectors are schematically represented in SI appendix Fig S1.

7. The projection of $\overrightarrow{B^i}$ in the direction of $\vec{A}$ is next calculated for every sample:

$$P^i = \vec{A} \bullet \overrightarrow{B^i} \,/\, \vec{A} \bullet \vec{A}$$

where here dots signify vector dot products.

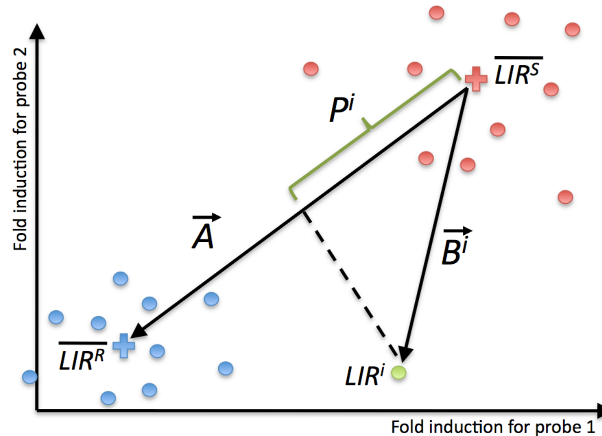
8. Multiplying P^i by its absolute value to preserve sign information results in the Squared Projected Distance, our chosen metric:

$$SPD^i = P^i \left| P^i \right|$$

Here vertical bars represent the absolute value. All strains are transformed in this manner.

9. Finally, to assess the significance of the observed scores, all SPD are converted to z-scores, defined to be the number of standard deviations a sample lies relative to all the drug-sensitive strains in our data set.

A graphical representation of this methodology is shown below:



Schematic in two dimensions of methodology for collapsing signatures into a single value, the squared projected distance (SPD). Red circles represent the log induction ratio of sensitive samples, blue circles represent the log induction ratio for resistant samples. The red and blue plus signs represent the centers of these two populations and define $\overline{LIR^S}$, $\overline{LIR^R}$, and the vector A between them. The green circle represents an unknown sample of index i to be interrogated and defines the vector B^i. P^i is the projection of B^i in the direction of A.

The acquisition of clinical materials enabled us to experimentally evalute the predictive capabilities of this assay. To do this, we treated the data obtained from known isolates that had been spiked into urine (shown in Fig. 5E) as a training data set, and the clinical samples as true unknowns. The training data (spiked urines) were used to define the sensitive and resistant centroids. Hence, in the analysis of the clinical specimens, the vector A is identical to that used in

the computation of the SPD scores in Fig. 5E. The SPD scores of the clinical samples reflect their projected distance from the centroid of the sensitive spiked urines along this vector.

Microbiological evaluation of clinical urine specimens: For independent microbial identification and determination of antibiotic susceptibilities, 5 µl of a 1:1000 dilution of the urine specimen was spotted onto HardyCHROM™ Urine Biplates, Red (R) Gram negative colonies are confirmatory of *E. coli*, thus no further testing of these samples was done. Blue (B) or white (W) Gram negative colonies required additional testing, so samples were streaked for individual colonies and tested using API 20 E system (BioMérieux). Blue (B) Gram positive colonies observe on HUrBi plates indicated the co-presence of *Enterococcus faecalis*, and white (W) Gram-positive colonies were identified as *Staphylococcus aureus* by the production of red pigment on HardyCHROM (Hardy Diagnostics) *Staphylococcus aureus* plates. MICs were determined by microtiter broth dilution.

Organism identification sensitivity determination: *Klebsiella* or *Pseudomonas* cultures were inoculated in LB media from single colonies. Cultures were grown to an OD600 of approximately 1, and serially diluted 10-fold in LB media. 10µl of each dilution were then lysed in 40µl of RLT buffer; 4µl of lysate was used directly in the nCounter assay according to the manufacturer's protocol.

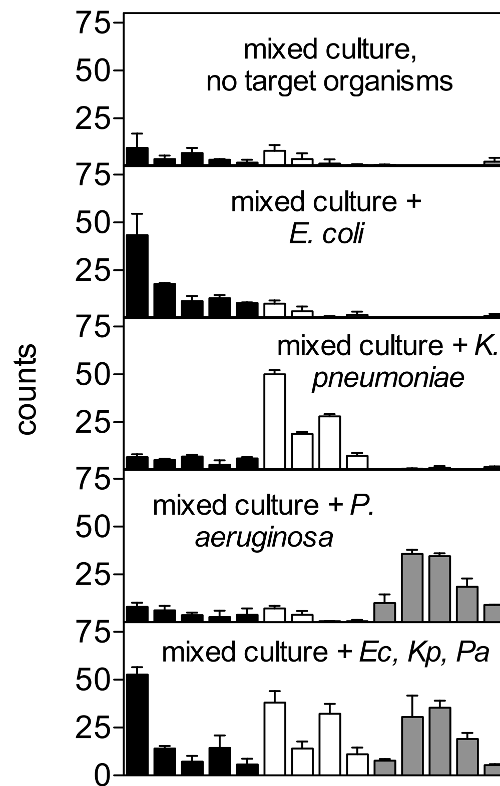


Figure S1. Organism identification in mixed culture. Bacterial cultures were lysed and analyzed with nCounter probes for species-specific transcripts. Y-axis: transcript raw counts; X-axis: gene. Probes for *E. coli* (black), *K. pneumoniae* (white), *P. aeruginosa* (grey). *E. coli*, *K. pneumoniae*, or *P. aeruginosa* were grown to log phase, then mixed in equal amounts with 8 additional bacterial species, i.e. 1 part *E. coli* to 8 parts mixed culture (Mixed culture = equal numbers of *Providencia stuartii*, *Proteus mirabilis*, *Serratia marcescens*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Morganella morganii*, *Klebsiella oxytoca*, *Citrobacter freundii)*

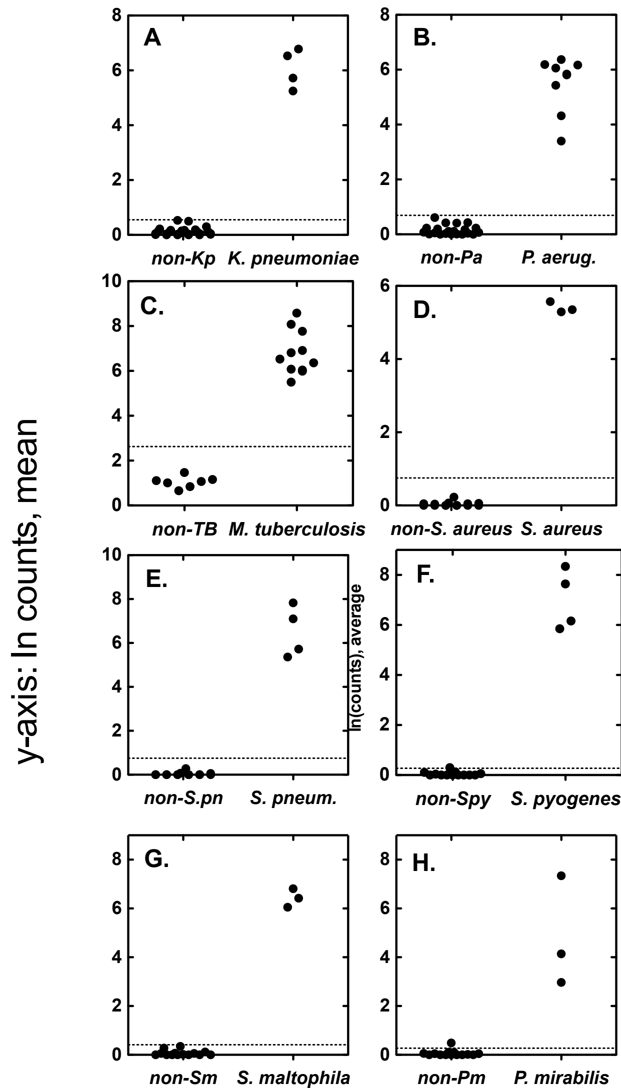


Figure S2. Organism identification: clinical isolates. Bacterial cultures were lysed and probes designed to detect species-specific transcripts were added, hybridized, and detected by standard nCounter protocol. A pooled probe-set containing probes for *E. coli*, *K. pneumoniae*, or *P. aeruginosa* was used in A and B. In C, species-specific probes for *M. tuberculosis* were among a larger set of probes against microbial pathogens. For (D-H), a pooled probe-set containing probes for the five organisms shown (*Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Stenotrophomonas maltophilia,* and *Proteus mirabilis*) along with six other organism (not tested) was used. Y-axis: sum of the log-transformed, normalized counts from 1-5 independent transcripts for each organism; X-axis: species tested. To account for any variability in assay efficiency, prior to log transformation, endogenous assay control probe counts were used to normalize the raw counts for each sample; based the observed counts for the highest of 5 endogenous control probes were normalized to an expected count value of 10,000. The dashed line indicates 3 standard deviations from the mean of the control ("non-organism") samples. "Non-organism" samples indicate samples tested that contained other bacterial organisms but where the defined organism was absent. For (A) and (B), non-*E. coli* isolates are *K. pneumoniae* and *P. aeruginosa*, non-*K. pneumoniae* strains are *E. coli* and *P. aeruginosa*, and non-*P. aeruginosa* strains are *E. coli* and *K. pneumoniae.* For (C), non-TB samples were non-tuberculous mycobacteria including *M. intracellulare, M. paratuberculosis, M. abscessus, M. marinum, M. gordonae, and M. fortuitum.* Each point represents a single clinical isolate tested once. Numbers of strains and clinical isolates tested are shown in Table S1 and genes used for pathogen identification are listed in Table S2.

Table S1. Genes used for bacterial organism identification.

Organism	Gene	Annotated function
E. coli	*murC*	Peptidoglycan synthesis
	putP	Sodium solute symporter
	uup	Subunit of ABC transporter
	opgG	Glucan biosynthesis
K. pneumoniae	*mraW*	S-adenosyl-methyltransferase
	ihfB	DNA-binding protein
	clpS	Protease adaptor protein
	lrp	Transcriptional regulator
P. aeruginosa	*mpl*	Ligase, cell wall synthesis
	proA	Gamma-glutamyl phosphate reductase
	dacC	Carboxypeptidase, cell wall synthesis
	lipB	Lipoate protein ligase
	sltB1	Transglycosylase
Conserved *Mycobacterium*	*carD*	Transcription factor
	infC	Translation initiation factor
M. tuberculosis	*Rv1398c*	Hypothetical protein
	mptA	Immunogenic protein 64
	hspX	Heat shock protein
P. mirabilis	*ackA*	Acetate metabolism
	ftnA	Iron storage
	pta	Acetate metabolism
	secD	Protein secretion
S. aureus	*ileS*	tRNA synthetase
	ppnK	NAD kinase
	pyrB	Aspartate carbamoyltransferase
	rocD	Ornithine aminotransferase
S. pneumoniae	*arcB*	Arginine deiminase
	murZ	Peptidoglycan synthesis
	phoP	Two-component regulator
	prsA	Foldase
S. pyogenes	*birA*	Biotin-protein ligase
	cysM	Cysteine synthase
	hpt	Hypoxanthine-guanine phosphoribosyltransferase
	scrR	Transcriptional repressor
S. maltophilia	*clpP*	Protease
	dnaK	Chaperone
	purC	Phosphoribosylaminoimidazolesuccinocarboxamide synthase
	sdhA	Succinate dehydrogenase

Table S2. Numbers of laboratory and clinical bacterial isolates tested with organism identification probes.

Organism	Laboratory strains tested	Clinical isolates tested
E. coli	2	17
K. pneumoniae	0	4
P. aeruginosa	1	9
M. tuberculosis	1	10
P. mirabilis	0	3
S. aureus	0	3
S. pneumoniae	0	4
S. pyogenes	0	4
S. maltophilia	0	3

Table S3. Laboratory and clinical isolates tested for susceptibility profiling. Clinical isolates are designated CI.

Organism	Antibiotic	Sensitive (S) or Resistant (R)	Strain	MIC*	SPD culture	SPD blood	SPD urine
E. coli	Ciprofloxacin	S	K12	30 ng/ml	-0.029		0.071
		S	J53	30 ng/ml	0.001		
		S	CIEC9955	< 0.1 mg/ml	0.032	0.049	0.002
		S	CICr07	<0.1mg/ml		-0.003	-0.026
		S	CICr08	<0.1mg/ml	0.030		
		S	CICr10	<0.1mg/ml		-0.098	-0.071
		S	CICr11	<0.1mg/ml		-0.060	0.007
		R	CIEC1686	50 mg/ml	1.018	0.973	1.020
		R	CIEC9779	50 mg/ml	0.877	1.147	1.125
		R	CIEC0838	50 mg/ml	1.113	0.825	0.829
		R	CIEC1801	>100 mg/ml			0.940
		R	CIEC2219	>100 mg/ml			1.002
		R	CIEC4940	25 mg/ml			1.076
		R	CIEC8040	100 mg/ml			1.049
E. coli	Gentamicin	S	K12	8 mg/ml	-0.055		
		S	CIEC1676	8 mg/ml	0.054		
		S	CIEC9955	16 mg/ml	0.018		
		S	CIEC1801	8 mg/ml	-0.010		
		R	CIEC4940	>250 mg/ml	0.982		
		R	CIEC9181	>250 mg/ml	0.926		
		R	CIEC2219	125 mg/ml	1.083		
		R	CIEC0838	>250 mg/ml	1.026		
E. coli	Ampicillin	S	K12	4 mg/ml	-0.015		
		S	J53	4 mg/ml	-0.025		
		S	DH5a	8 mg/ml	0.095		
		R	CIEC9955	>250 mg/ml	2.414		
		R	CIEC2219	>250 mg/ml	0.669		
		R	CIEC0838	>250 mg/ml	0.818		
		R	CIEC9181	>250 mg/ml	0.594		
P. aeruginosa	Ciprofloxacin	S	PAO-1	1 mg/ml	0.009		
		S	CIPA2085	0.4 mg/ml	0.007		
		S	CIPA1189	0.4 mg/ml	0.001		
		S	CIPA9879	0.4 mg/ml	-0.023		
		R	CIPA2233	50 mg/ml	0.958		
		R	CIPA1839	25 mg/ml	1.452		
		R	CIPA1489	25 mg/ml	0.692		

Table S3 (cont.)

Organism	Antibiotic	Sensitive (S) or Resistant (R)	Strain	MIC*	SPD culture	SPD blood	SPD urine
M. tuberculosis	Isoniazid	S	H37Rv	0.05 mg/ml	-0.013, 0.000 (1, 0.2 μg/ml)		
		S	CIAS1	<0.2 mg/ml	-0.009		
		S	CIAS2	<0.2 mg/ml	-0.058		
		S	CIAS3	<0.2 mg/ml	0.075		
		S	CIAS4	<0.2 mg/ml	-0.007		
		S	CIAS5	<0.2 mg/ml	-0.129		
		S	CIAS10	<0.2 mg/ml	0.293		
		S	CIJ2	<0.2 mg/ml	0.059		
		S	CIJ3	<0.2 mg/ml	0.058		
		S	CIJ4	<0.2 mg/ml	0.170		
		R	CIJ5	>5 mg/ml	0.962		
		R	CIJ7	>5 mg/ml	1.077		
		R	CIJ12	>5 mg/ml	1.037		
		R	CIJ13	>5 mg/ml	0.896		
		R	C4A50	>6.25 mg/ml	1.141, 0.917 (1, 0.2 μg/ml)		
		R	C1A10	>6.25 mg/ml	0..820		
		R	C2A10	>6.25 mg/ml	1.005		
		R	BAA-812	0.4 mg/ml	0.041, 1.036 (1, 0.2 μg/ml)		
M. tuberculosis	Ciprofloxacin	S	$mc^2$6020	0.5 mg/ml	-0.060		
		S	CIAS1	<1 mg/ml	0.000		
		S	CIAS2	<1 mg/ml	-0.018		
		S	CIAS3	<1 mg/ml	0.200		
		S	CIAS4	<1 mg/ml	0.038		
		S	CIAS5	<1 mg/ml	0.032		
		S	CIAS10	<1 mg/ml	-0.082		
		R	C5A15	16 mg/ml	1.079		
		R	0.5D5	16 mg/ml	0.864		
M. tuberculosis	Streptomycin	S	H37Rv	1 mg/ml	0.059		
		S	CIAS1	<2 mg/ml	-0.017		
		S	CIAS2	<2 mg/ml	-0.005		
		S	CIAS3	<2 mg/ml	0.002		
		S	CIAS4	<2 mg/ml	-0.015		
		S	CIAS5	<2 mg/ml	0.001		
		R	C2A15	>32 mg/ml	1.137		
		R	C3L3-50	>32 ug/ml	0.759		

Table S4. Genes used for viral organism identification.

Organism	Gene	Annotated function
Herpes simplex virus-2	US4	Envelope glycoprotein G, unknown function
Influenza A	M1/M2	Matrix, Ion channel in viral envelope
HIV-1	Gag	Core structural proteins of virus
	Rev	Exports viral mRNA from nucleus

Table S5. Genes used for *Plasmodium falciparum* organism identification.

Probe name/ stage	Gene	Annotated function
Ring/troph 1	PFI1020c	Inosine-5'-monophosphate dehydrogenase
Ring/troph 2	PFA_0660w	Protein with DNAJ domain
Troph/early schizont 1	PFC0800w	Band 7 related protein
Troph/early schizont 2	PFE0660c	Putative purine nucleotide phosphorylase
Schizont 1	PF07_0128	Erythrocyte binding antigen 175
Schizont 2	PF13_0233	Myosin A
Early ring 1	PFD1170c	Plasmodium exported protein, unknown function
Early ring 2	PFA0110w	Ring-infected erythrocyte surface antigen precursor

Table S6. Genes used for *Candida albicans* organism identification.

Gene	Annotated function
CaJ7_0103	Hypothetical protein
CaJ7_0146	Hypothetical protein
CaJ7_0197	Hypothetical protein
CaJ7_0339	Hypothetical protein

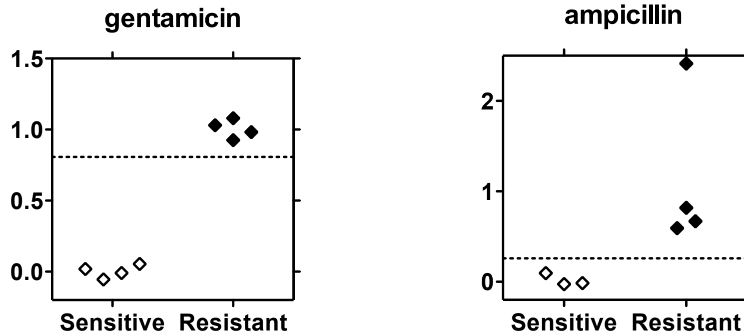


Figure S3. Squared projected distance (SPD) comparison of gentamicin or ampicillin-sensitive and -resistant *E. coli* strains. Y-axis: SPD of each sample relative to the centroid of the response of known sensitive strains. For gentamicin, the dashed line indicates three standard deviations from the mean of the resistant samples. For ampicillin, the dashed line indicates one standard deviation from the mean of the resistant samples. Strains tested are listed in Table 1, and the genes that define the sensitive signature are listed in Table S3.

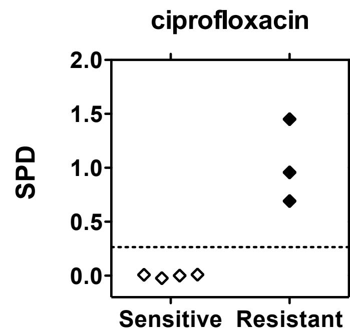


Figure S4. Squared projected distance (SPD) comparison of ciprofloxacin-sensitive and -resistant *P. aeruginosa* strains. Y-axis: SPD of each sample relative to the centroid of the response of known sensitive strains. The dashed line indicates two standard deviations from the mean of the resistant samples. Strains tested are listed in Table 1, and the genes that define the sensitive signature are listed in Supplementary Table 3.

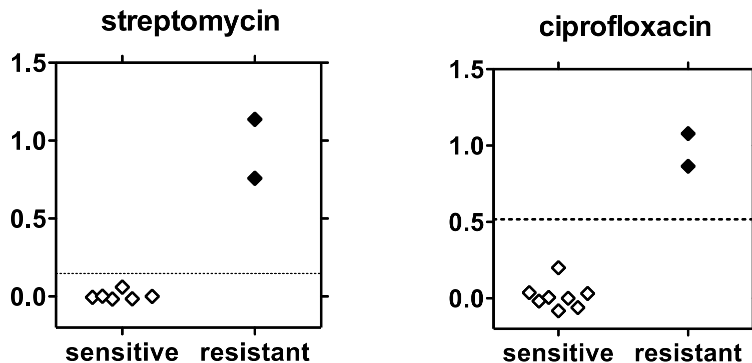


Figure S5. Squared projected distance comparison of streptomycin or ciprofloxacin-sensitive and -resistant *M. tuberculosis* strains. Y-axis: SPD of each sample relative to the centroid of the response of known sensitive strains. The dashed line indicates three standard deviations from the mean of the resistant samples. Strains tested are listed in Table 1, and the genes that define the sensitive signature are listed in Table S3.

Table S7. Genes associated with bacterial antibiotic sensitivity signatures

Organism	Antibiotic	Gene	Annotated function
E. coli	Ciprofloxacin (broth or blood)	*dinD*	DNA-damage-inducible protein D
		recA	DNA repair, SOS response
		uvrA	ATPase and DNA damage recognition protein
		uup	predicted subunit of ABC transporter
		argB	acetylglutamate kinase
	Ciprofloxacin (urine)	*dinD*	DNA-damage-inducible protein D
		recA	DNA repair, SOS response
		ftsQ	Cell division
		murC	UDP-N-acetylmuramyl-l-alanine ligase
	Gentamicin	*dinD*	DNA-damage-inducible protein D
		recA	DNA repair, SOS response
		b1649	lipopolysaccharide biosynthesis
		ftsQ	cell division
	Ampicillin	*uup*	ATP-binding protein
		proC	pyrroline reductase
		opgG	glucan biosynthesis
		secA	preprotein translocase subunit
P. aeruginosa	Ciprofloxacin	*PA_4175*	probable endoprotease
		mpl	peptidoglycan biosynthesis
		proA	glutamate-semialdehyde dehydrogenase
		lexA	Regulator of SOS response
M. tuberculosis	Ciprofloxacin	*lhr*	helicase
		rpsR	ribosomal protein S18-1
		ltp1	lipid transfer
		alkA	base excision repair
		efpA	efflux pump
		accD6	mycolic acid synthesis
		fadD32	mycolic acid synthesis
		moaB2	Molybdopterin biosynthesis
		bcpB	bacterioferritin comigratory protein
	Isoniazid	*efpA*	efflux pump
		kasA	mycolic acid synthesis
		accD6	mycolic acid synthesis
		fadD32	mycolic acid synthesis
		Rv3675	Possible membrane protein
	Streptomycin	*Rv0813*	conserved hypothetical protein
		groEL	heat shock protein
		lhr	helicase
		ltp1	lipid transfer
		efpA	efflux pump

Table S8. Microbiological evaluation of clinical urine specimens assayed for organism identification.

Specimen	Contents	HUrBI Gram-	HUrBI Gram+	API 20E profile	API 20E profile 2
110415-04	*Citrobacter koseri*	B	-	3340513	3344513
110415-05	*Enterobacter aerogenes*	B	-	5305773	
110415-06	*Klebsiella pneumoniae* ssp. *pneumoniae*	B	-	1215773	
110415-07	*Escherichia coli*	R	-	ND	
110415-08	*K. oxytoca, C. freundii*	B	-	5245773	1204572
110415-10	*E. cloaceae. Enterococcus faecalis*	B	B	3305573	
110415-11	*Proteus vulgaris, E. faecalis*	B	B	2774521	6774311
110415-13	*E. coli*	R	-	ND	
110415-16	*E. coli, E. faecalis*	W	B	4144532	
110415-17	*E. coli, K. pneumo., E. faecalis*	B	B	5044572	7315773
110415-19	*K. pneumoniae* ssp. *pneumoniae*	B	-	1215773	
110415-20	*E. coli*	R	-	ND	
110415-21	*E. coli*	R	-	ND	
110415-22	*C. freundii, E. coli*	B	-	5144512	3604773
110415-23	*P. aeruginosa*	W	-	2202000	
110415-31	*E. coli*	R	-	ND	
110415-32	*E. coli*	R	-	ND	
110518-49	*E. coli*	R	-	ND	
110518-51	*E. coli*	R	-	ND	
110518-56	*K. pneumoniae* ssp. *pneumoniae*	B	-	5215773	
110518-59	*E. coli*	R	-	ND	
110518-60	*E. coli*	R	-	ND	
110518-61	*E. coli*	R	-	ND	
110518-64	*E. coli*	R	-	ND	
110519-49	*E. cloacae/agglomerans*	B	-	3205573	
110519-54	*E. coli*	R	-	ND	
110519-55	*Staphylococcus aureus*	-	W	ND	
110519-56	*S. aureus*	-	W	ND	
110519-58	*K. pneumoniae ssp. pneumoniae*	B		5215773	
110519-60	uninfected	-	-	ND	
110519-65	*E. coli, E. faecalis*	R	B	ND	
110519-67	uninfected	-	-	ND	
110519-72	*K. pneumoniae ssp. pneumoniae*	B		5215773	7215773
110519-74	*E. coli*	R	-	ND	

Table S9. *E. coli* isolates from clinical urine specimens assayed for ciprofloxacin sensitivity

isolate	Sensitive (S) or Resistant (R)	CIP MIC	SPD, CIP
110415-20	R	64 µg/ml	0.755
110415-21	S	<125 ng/ml	0.036
110415-31	R	>64 µg/ml	0.841
110415-32	R	>64 µg/ml	0.917
110518-49	S	<125 ng/ml	0.464
110518-51	R	>32 µg/ml	1.31
110518-59	S	<125 ng/ml	0.122
110518-60	S	<125 ng/ml	-0.031
110518-61	S	<125 ng/ml	0.265
110518-64	R	>32 µg/ml	1.07
110519-54	S	<125 ng/ml	0.340
110519-65	R	>32 µg/ml	0.869
110519-74	S	<125 ng/ml	0.626

Table S10. Genomes included in algorithm for organism identification probe selection.

Organism	Genome name
Escherichia coli	*Escherichia_coli*_0127_H6_E2348_69
	*Escherichia_coli*_536
	Escherichia_coli _55989
	Escherichia_coli _BW2952
	Escherichia_coli _C_ATCC_8739
	Escherichia_coli _CFT073
	Escherichia_coli _E24377A
	Escherichia_coli _IAI39
	Escherichia_coli _K_12_substr__DH10B
	Escherichia_coli _K_12_substr__MG1655
	Escherichia_coli _K_12_substr__W3110
	Escherichia_coli _O157H7
	Escherichia_coli _O157_H7_EC4115
	Escherichia_coli _O157H7_EDL933
	Escherichia_coli _S88
Klebsiella pneumoniae	*Klebsiella pneumoniae* 342
	Klebsiella pneumoniae NTUH-K2044
	Klebsiella pneumoniae subsp. *pneumoniae* MGH 78578

Table S10 (cont.)	
Organism	**Genome name**
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* LESB58
	Pseudomonas aeruginosa PA7
	Pseudomonas aeruginosa PAO1
	Pseudomonas aeruginosa UCBPP-PA14
Staphylococcus aureus	*Staphylococcus aureus* 04-02981
	Staphylococcus aureus ED98
	Staphylococcus aureus RF122
	Staphylococcus aureus subsp. *aureus* COL
	Staphylococcus aureus subsp. *aureus* JH1
	Staphylococcus aureus subsp. *aureus* JH9
	Staphylococcus aureus subsp. *aureus* MRSA252
	Staphylococcus aureus subsp. *aureus* MSSA476
	Staphylococcus aureus subsp. *aureus* MW2
	Staphylococcus aureus subsp. *aureus* Mu3
	Staphylococcus aureus subsp. *aureus* Mu50
	Staphylococcus aureus subsp. *aureus* N315
	Staphylococcus aureus subsp. *aureus* NCTC 8325
	Staphylococcus aureus subsp. *aureus* USA300_FPR3757 FPR3757
	Staphylococcus aureus subsp. *aureus* USA300_TCH1516
	Staphylococcus aureus subsp. *aureus* str. Newman
Stenotrophomonas maltophilia	*Stenotrophomonas maltophilia* K279a
	Stenotrophomonas maltophilia R551-3
Streptococcus pneumoniae	*Streptococcus pneumoniae* 70585
	Streptococcus pneumoniae ATCC 700669
	Streptococcus pneumoniae CGSP14
	Streptococcus pneumoniae D39
	Streptococcus pneumoniae G54
	Streptococcus pneumoniae Hungary19A-6
	Streptococcus pneumoniae JJA
	Streptococcus pneumoniae P1031
	Streptococcus pneumoniae R6
	Streptococcus pneumoniae TIGR4
	Streptococcus pneumoniae Taiwan19F-14
Streptococcus pyogenes	*Streptococcus pyogenes* MGAS10270
	Streptococcus pyogenes MGAS10394
	Streptococcus pyogenes MGAS10750
	Streptococcus pyogenes MGAS2096
	Streptococcus pyogenes MGAS315
	Streptococcus pyogenes MGAS5005
	Streptococcus pyogenes MGAS6180

Table S10 (cont.)	
Organism	**Genome name**
Streptococcus pyogenes (cont.)	*Streptococcus pyogenes* MGAS8232
	Streptococcus pyogenes MGAS9429
	Streptococcus pyogenes NZ131
	Streptococcus pyogenes SSI-1
	Streptococcus pyogenes str. Manfredo
Proteus mirabilis	*Proteus mirabilis* HI4320
Candida albicans	*Candida albicans* WO-1
	Candida albicans SC5314

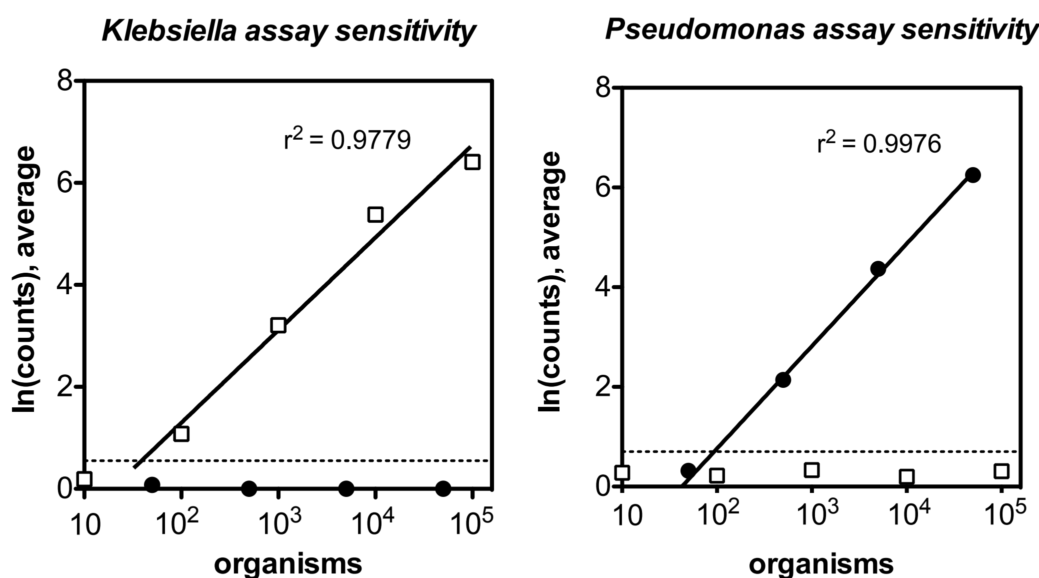


Figure S6. Limits of organism identification assay sensitivity. Klebsiella: open squares, Pseudomonas: solid circles. *Klebsiella pneumoniae* or *Pseudomonas aeruginosa* was grown in LB media to an OD600 of approximately 1. Culture was then serially diluted in LB media, lysed in RLT buffer, and lysates were used in the nCounter assay. Dashed line indicates 3 standard deviations from the mean score of "non-organism" samples (same as Figure S1). Solid line indicates linear regression of log-transformed data. Limit of sensitivity is defined as the X-value where the fitted line crosses the dashed line. For Klebsiella, this equates to 39 organisms; for Pseudomonas, this equates to 93 organisms.