Abstract

The rise in carbapenem-resistant Enterobacteriaceae (CRE) infections has created a global health emergency, underlining the critical need to develop faster diagnostics to treat swiftly and correctly. Although rapid pathogen-identification (ID) tests are being developed, gold-standard antibiotic susceptibility testing (AST) remains unacceptably slow (1–2 d), and innovative approaches for rapid phenotypic ASTs for CREs are urgently needed. Motivated by this need, in this manuscript we tested the hypothesis that upon treatment with \(\beta\)-lactam antibiotics, susceptible Enterobacteriaceae isolates would become sufficiently permeabilized, making some of their DNA accessible to added polymerase and primers. Further, we hypothesized that this accessible DNA would be detectable directly by isothermal amplification methods that do not fully lyse bacterial cells. We build on these results to develop the polymerase-accessibility AST (pol-aAST), a new phenotypic approach for \(\beta\)-lactams, the major antibiotic class for gram-negative infections. We test isolates of the 3 causative pathogens of CRE infections using ceftriaxone (CRO), ertapenem (ETP), and meropenem (MEM) and demonstrate agreement with gold-standard AST. Importantly, pol-aAST correctly categorized resistant isolates that are undetectable by current genotypic methods (negative for \(\beta\)-lactamase genes or lacking predictive genotypes). We also test contrived and clinical urine samples. We show that the pol-aAST can be performed in 30 min sample-to-answer using contrived urine samples and has the potential to be performed directly on clinical urine specimens.

Introduction

The evolution and global spread of carbapenem-resistant Enterobacteriaceae (CRE) threatens to disrupt modern healthcare systems, which rely heavily on \(\beta\)-lactams (especially...
reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the U.S. Government. This work was also supported by a Burroughs Wellcome Fund Innovation in Regulatory Science Award, an NIH National Research Service Award (NRSA) [5T32GM07616NSF] to N.G.S., NIH NIGMS Predoctoral Training Grants [GM008042] to A.W. and E.J.L., a grant from the Joseph J. Jacobs Institute for Molecular Engineering for Medicine, and a fellowship (to E.S.S.) from Joan and Jerry Doren. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: The technology described in this publication is the subject of a patent application filed by Caltech. R.F.I. has a financial interest in Talis Biomedical Corp.

Abbreviations: AMP, ampicillin; AST, antibiotic susceptibility test/testing; AUC, area under the curve; BHI, Brain Heart Infusion Broth; CDC, Centers for Disease Control and Prevention; CML, Clinical Microbiology Laboratory; Cq, quantitation cycle; CRE, carbapenem-resistant Enterobacteriaceae; CRO, ceftriaxone; CT, time-to-contrast; CT, time-to-contrast; ETP, ertapenem; FDA, Food and Drug Administration; ID, identification; LAMP, loop-mediated isothermal amplification; MEM, mephenoxam; MHB, Mueller Hinton II Broth; MIC, minimum inhibitory concentration; NA, nucleic acid; NEB, New England Biolabs; NF-H2O, nuclease-free H2O; nuc-aAST, nuclease-accessibility AST; PCR, polymerase chain reaction; POC, point of care; pol-aAST, polymerase-accessibility AST; qPCR, quantitative PCR; RFU, relative fluorescent unit; ROC, receiver operating characteristic; RPA, recombinase polymerase amplification; TTP, time-to-positive; TTPD, time-to-positive difference; TTPD_{CT}, TTPD, control to treated; TTPD_{Υ}, TTPD, lysed-control to treated; UTI, urinary tract infection; WHO, World Health Organization.

carbapenems, the last-resort treatments) to control bacterial infections [1–3]. Mortality rates for CRE infections are as high as 30%–49% [4–6], and thus the global emergence and spread of CRE infections represents a public health emergency [7–9]. The Centers for Disease Control and Prevention (CDC) places CRE in its highest (“urgent”) category of antimicrobial-resistant pathogen threats [8,10], and the World Health Organization (WHO) labels CRE as a critical-priority pathogen [7]. Escherichia coli, Klebsiella pneumoniae, and Enterobacter spp. compose the majority of CRE infections and are the most commonly monitored Enterobacteriaceae [8,11–13].

To halt the further spread of CRE, patients need to be treated swiftly and correctly at the point of care (POC); however, there is no fast and general method for determining antibiotic susceptibility [14–16]. The current clinical workflow for treatment of bacterial infections consists of an identification (ID) step followed by an antibiotic susceptibility test (AST). Although progress is being made to develop faster ID tests [17–19] and a rapid 20-min ID test is on the horizon [20–22], the gold-standard for AST remains a culture-based workflow using broth or agar dilution that requires 1 to 2 d and is thus far too slow [23,24]. Because AST results are so delayed, healthcare providers usually treat empirically, leading to inappropriate prescriptions and even life-threatening outcomes [25], as well as the further spread of resistance. To improve treatment and promote antibiotic stewardship, healthcare providers need a rapid phenotypic AST [26–29].

ASTs are either genotypic or phenotypic. Genotypic tests predict resistance by measuring the presence of genes known to be involved in resistance. Genotypic tests can be fast [30] but often have limited clinical utility because they target defined mechanisms of resistance. For example, rapid genotypic methods to detect gram-negative β-lactamase genes have been developed [31–34], but these tests only detect one of the many known β-lactamase classes and still require 30 to 40 min (estimated from described methods). Similarly, the commercial Cepheid Xpert Carba-R assay (Cepheid, Sunnyvale, CA), which detects 5 β-lactamase gene families, was shown to detect 50% of resistant isolates and took 88 min [35]. Moreover, although Carba-R is Food and Drug Administration (FDA) approved, its utility in treatment scenarios is limited (i.e., negative results are not actionable), so when prescribing antibiotics, it must be used in conjunction with a phenotypic AST [36,37]. Rapid methods for measuring the activity of specific β-lactamases also exist [38–42]. However, these tests only detect one mechanism of resistance, and sample-to-answer times have not been reported.

Phenotypic ASTs are ideal because they determine susceptibility directly by exposing the sample to antibiotics and measuring the target organism’s response. The gold-standard AST (broth microdilution [23,24]) is a phenotypic test. Most phenotypic tests require the growth of viable organisms isolated from patient samples, a process that requires days and is thus too slow for the POC. Innovative, faster phenotypic tests for β-lactams were developed based on in situ nucleic-acid staining or fluorescence measurements [43–45], flow cytometry [46], microscopy [47–49], optical density [50,51], and mass spectrometry [52]. However, the majority of the currently proposed methods still require 60- to 180-min antibiotic-exposure steps in addition to the time needed to perform the assay, and no method has emerged that achieves short (approximately 15 min) antibiotic exposure and short (approximately 15 min) assay time but does not require excessively complex or delicate instrumentation so the method can be deployed at the POC.

Rapid phenotypic methods based on quantification of nucleic acids (NAs) have shown great promise for a rapid POC AST due to the speed, specificity, and robustness of NA detection [53–58]. There is an additional advantage to using NA quantification as a readout of the bacterial response to antibiotic: because rapid pathogen ID from clinical samples is commonly performed via NA analysis, it would likely be easier to integrate an NA-based phenotypic AST
into a combined ID/AST workflow performed from the same clinical sample. Additionally, the use of NA-based methods provides molecular specificity towards the target pathogen, which is important in clinical samples that can contain multiple organisms. For antibiotics that directly or indirectly impact NA replication on short timescales, we have demonstrated that the quantification of DNA [59,60] or RNA [61] can be used to rapidly (30 min) and reliably determine susceptibility to nitrofurantoin and ciprofloxacin. Subsequent efforts have targeted the β-lactam class (the most widely prescribed class of antibiotic [1,2]) using these methods [62]. However, because β-lactams do not directly impact NA replication on short timescales, this direct translation of the existing NA-based technique required a 2-h antibiotic exposure, which is not sufficiently rapid for the POC. For a POC AST to impact management of CRE infections, it must (i) determine susceptibility to β-lactams, including carbapenems; (ii) be rapid (<30-min sample-to-answer) [63,64]; and (iii) be phenotypic [26,27]. As discussed subsequently, rapid pathogen ID technologies are becoming available, and therefore pathogen ID is not the focus of this work.

Here, we hypothesized that a new NA-based approach could be used to develop a rapid phenotypic AST for multiple β-lactams. We hypothesized that upon treatment with β-lactam antibiotics, susceptible Enterobacteriaceae isolates would become sufficiently permeabilized so some of their DNA would become accessible to added polymerase and primers. Further, we hypothesized that this accessible DNA would be detectable directly by isothermal amplification methods that do not fully lyse bacterial cells. To differentiate between resistant and susceptible organisms, rather than measuring how total NA concentration is impacted by antibiotic exposure (as in previous NA-based ASTs), we hypothesized that we could measure the accessibility of NAs to polymerase following a short antibiotic exposure. Here, we test these hypotheses and use them to design a new AST method, termed polymerase-accessibility AST (pol-aAST). To validate the method, we performed 82 ASTs using clinical isolates of 3 major CRE pathogens exposed to each of 3 commonly prescribed β-lactams for gram-negative infections: ceftriaxone (CRO), ertapenem (ETP), and meropenem (MEM). To further demonstrate that this method has potential to be used clinically in POC-relevant timescales, we (i) performed timed sample-to-answer experiments using contrived urine samples to ensure that the whole assay can be performed in <30 min, and (ii) we performed a pilot study on clinical urine samples from patients with urinary tract infections (UTIs).

Results
The pol-aAST relies on differential accessibility of NAs to polymerases as a result of antibiotic exposure. In this manuscript, we define differential accessibility to polymerase as a difference in the measured rate of amplification between control and antibiotic-treated samples. In the first step of pol-aAST, a single sample is split into control and treated aliquots of equal volume, and the treated aliquot is exposed to a β-lactam. Antibiotic exposure is a critical step in any phenotypic AST because phenotypic tests measure the response of bacteria to antibiotics. If the bacteria in the sample are resistant, we hypothesized that no differences in NA amplification would be observed between control and treated aliquots. If the bacteria are susceptible, we hypothesized that antibiotic treatment would lead to a compromised peptidoglycan cell wall (Fig 1A) and partial release of NAs (Fig 1B). We hypothesized that both the compromised cell wall and partial release of NAs would increase the accessibility of NAs to polymerase in a treated antibiotic-susceptible aliquot. In the second step of pol-aAST, control and treated aliquots are exposed to polymerase in amplification conditions (Fig 1C), and the rate of amplification is measured.
To successfully differentiate susceptible and resistant samples, ideal amplification conditions must (i) not fully lyse cells, (ii) enhance alterations (damage) to the cell wall caused by exposure to β-lactams, and (iii) increase NA release only from antibiotic-damaged cells. The rate of amplification is dependent on the concentration of polymerase-accessible NA. In susceptible samples, more NAs are released in the treated aliquot, leading to faster amplification in susceptible treated aliquots (Fig 1D) relative to the controls. Resistant samples are not affected by the antibiotic, so control and treated aliquots have similar NA release and time-to-positive (TTP). In these samples, the low concentration of naturally occurring extracellular DNA is ultimately amplified, but at a slower rate. Amplification rate in an isothermal amplification reaction is quantified by measuring the TTP, the time it takes the reaction fluorescence to reach a predetermined threshold. We found that using pol-aAST, isolates susceptible to the β-lactam being tested show increased accessibility of NAs to polymerase, manifesting in an earlier TTP relative to the control. The TTPs of any two samples, such as the control and treated aliquots, can be compared to generate a TTP difference (TTPD) value, which can then be used to determine susceptibility by comparing to a susceptibility threshold. Here, we used the DNA polymerase *Bst* 3.0 (New England Biolabs [NEB], Ipswitch, MA) under loop-mediated isothermal amplification (LAMP) conditions.

We hypothesized that the chemical environment in which amplification occurs would significantly impact the result of pol-aAST and that—for pol-aAST to differentiate susceptible and resistant samples—amplification conditions should not be fully lysing. To test this, we performed pol-aAST using LAMP, as well as quantitative PCR (qPCR) (Fig 2). LAMP is performed at a single temperature (70˚C), which we hypothesized would not be fully lysing, whereas qPCR is a thermocycled amplification technique reaching a maximum temperature of

| antibiotic | bacterial gDNA | amplicon | outer membrane | peptidoglycan | polymerase |
|------------|----------------|----------|----------------|---------------|------------|

**Fig 1. Overview of the pol-aAST shown for susceptible and resistant samples exposed to β-lactams.** (a) Treated aliquots are exposed to a β-lactam. In susceptible samples, β-lactams compromise cell wall integrity. (b) NAs are released from compromised cells, increasing NA accessibility to polymerase. (c) Released NAs in the susceptible treated aliquot amplify faster than NAs from intact cells in the control aliquot, resulting in a difference in TTP. No difference in amplification between control and treated aliquots is observed in resistant samples. (d) TTPD between control and treated aliquots is used to assess susceptibility. ABX, antibiotic; AST, antibiotic susceptibility testing; gDNA, genomic DNA; NA, nucleic acid; pol-aAST, polymerase-accessibility AST; R, resistant; RFU, relative fluorescent units; S, susceptible; TTP, time-to-positive; TTPD, time-to-positive difference.

https://doi.org/10.1371/journal.pbio.3000652.g001
95˚C, which we hypothesized would be fully lysing. Indeed, we observed that pol-aAST was successful in differentiating susceptible and resistant isolates when performed using LAMP, but not when performed using qPCR (Fig 2). We tested qPCR with a total of 2 susceptible and 2 resistant isolates, none of which showed a statistically significant difference in quantitation cycle (Cq) between control and treated samples. When using LAMP, detectable differences were observed between control and treated aliquots when using isolates susceptible to the target β-lactam (TTPD = 1.02 min). Additionally, the presence of cells not lysed during LAMP is evidenced by the shorter TTPs seen when an aliquot of the same sample is lysed using an extraction buffer prior to performing LAMP (explained in more detail subsequently). These differences confirm that choice of amplification chemistry is critical to the success of pol-aAST and are consistent with previous work evaluating thermal lysis [65].

To investigate the mechanism of pol-aAST, we performed experiments to separate free NAs from NAs contained within structurally intact cells or associated with cell debris. Susceptible and resistant clinical isolates were exposed to one or more β-lactams in parallel for 15 min, then filtered through 0.2 μM filters to remove cells from free NAs. NAs in the sample and eluate were then quantified using droplet digital PCR (ddPCR). We observed that following exposure to β-lactams, susceptible isolates treated with β-lactams released a significantly larger

---

**Fig 2.** The pol-aAST requires non-lytic amplification conditions. (a–b) Thermal profiles of LAMP and PCR. (c–d) LAMP and PCR amplification curves for a susceptible *E. coli* isolate exposed to ETP for 15 min. Blue and black lines are the average of triplicate samples. Grey lines represent standard deviation of triplicates. A difference in TTP for control and treated aliquots is observed for susceptible isolates when quantifying NAs using LAMP, but not PCR. Raw data are provided in **S5 Table.** AST, antibiotic susceptibility test/testing; Cq, quantitation cycle; ET, ertapenem; LAMP, loop-mediated isothermal amplification; NA, nucleic acid; PCR, polymerase chain reaction; pol-aAST, polymerase-accessibility AST; RFU, relative fluorescent units; TTP, time-to-positive.

https://doi.org/10.1371/journal.pbio.3000652.g002
percentage of DNA than resistant samples (Fig 3). The amount of DNA released depended on the antibiotic being tested. Exposure to MEM resulted in an average of 21% of DNA being released from susceptible isolates, with a slightly smaller average percent (15%) released as a result of exposure to ETP. Interestingly, susceptible samples only released an average of 6% of DNA when exposed to CRO, demonstrating that NA release is dependent on choice of antibiotic and not, e.g., a universal stress response. These results also demonstrate that the magnitude of the effect of a β-lactam on cell wall integrity can be measured and is different depending on the antibiotic used, even on short exposure timescales.

To validate the pol-aAST method, we first performed 82 ASTs using 12 clinical isolates of *E. coli*, 8 clinical isolates of *K. pneumoniae*, 9 clinical isolates of 2 species of Enterobacter (*E. aerogenes* and *E. cloacae*, collectively “Ebs”), and the β-lactams CRO, ETP, and MEM. The set included isolates from each genus that were susceptible and isolates that were resistant to each of the 3 antibiotics. In addition to isolates obtained from the UCLA Clinical Microbiology Laboratory (CML; see Methods), those tested included *E. coli* and *K. pneumoniae* isolates from the CDC Enterobacteriaceae Carbapenem Breakpoint panel [66], as well as all available Enterobacter spp. isolates from the same panel. All samples were amplified using quantitative LAMP, and categorical agreement was compared to gold-standard broth microdilution AST. Two approaches for determining susceptibility were investigated in all pol-aASTs performed.
The first approach we investigated was to compare the difference in TTP values of the control and treated aliquots in each pol-aAST. The TTP (in minutes) of the control and treated aliquots are used to calculate TTPD_{CT}. The pol-aAST results using *E. coli* (b), *K. pneumoniae* (c), and *Enterobacter* spp. (d) isolates exposed to CRO, ETP, and MEM for 15 min. Red points represent isolates with either no detectable carbapenemase genes (*Ec* and *Kp* isolates) according to a published genotypic assay [67] and commercial assay [68] or no predictive genotype (*Ebs* isolates) according to the whole genome sequencing by the CDC [66]. S/R thresholds (dashed lines) were set halfway between the lowest susceptible and the highest resistant TTPD_{CT} values. Raw data are provided in S3 Table. +ABX, antibiotic-treated; AST, antibiotic susceptibility testing; CDC, Centers for Disease Control and Prevention; CRO, ceftiraxone; CT, control to treated; ctrl, control; *Ebs*, *E. aerogenes* and *E. cloacae* collectively; *Ec*, *E. coli*; ETP, ertapenem; *Kp*, *K. pneumoniae*; MEM, meropenem; pol-aAST, polymerase-accessibility AST; R, resistant; RFU, relative fluorescent units; S, susceptible; TTP, time-to-positive; TTPD, time-to-positive difference; TTPD_{CT}, TTPD control to treated.

https://doi.org/10.1371/journal.pbio.3000652.g004

The first approach we investigated was to compare the difference in TTP values of the control and treated aliquots in each pol-aAST. This difference was defined as TTPD control to treated (TTPD_{CT} (Fig 4A)). Using the TTPD_{CT} method, we obtained 100% categorical agreement with gold-standard AST for all antibiotics tested with *E. coli* (Fig 4B), *K. pneumoniae* (Fig 4C), and *Enterobacter* spp. (Fig 4D) isolates. The values of TTPD_{CT} were well-separated between susceptible and resistant isolates in all CRE-antibiotic combinations. Note that the threshold values separating TTPD_{CT} of susceptible and resistant isolates depend on the antibiotic used (e.g., CRO gives a smaller response and therefore requires a lower threshold), as well as the pathogen tested (e.g., *K. pneumoniae* gives stronger response and requires a higher threshold). The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was 1.00 for all isolates and antibiotics tested. There were no errors relative to gold-standard AST when determining susceptibility by TTPD_{CT}.

The second approach we investigated was to compare the difference in TTP values of a fully lysed aliquot and the antibiotic-treated aliquot in each pol-aAST. The fully lysed aliquot was created by extracting NA from the antibiotic-treated sample using a single-step, LAMP-compatible extraction buffer. This difference was defined as TTPD lysed-control to treated (TTPD_{LT}) (Fig 5A). It is important to note that TTPD_{LT} only requires an antibiotic-treated sample during the exposure step (the method does not require the use of a no-antibiotic control during exposure), meaning that the original sample does not have to be split prior to exposure. Again, the thresholds were defined individually for each antibiotic and pathogen. Using the TTPD_{LT} method, we obtained 100% categorical agreement with gold-standard AST for all antibiotics tested only with *E. coli* (Fig 5B) and *K. pneumoniae* (Fig 5C) isolates, and with resistant isolates for which the genotypic tests fail to correctly predict the resistance phenotype (red points in Fig 5). When testing *Ebs* (Fig 5D) isolates, we observed 2 errors in which an isolate classified as CRO resistant was called susceptible, resulting in an overall categorical agreement of 88%. Because of these errors, the AUC for *Ebs* isolates tested with CRO was 0.94. Aside from these errors, susceptible and resistant isolates were well separated in all cases, with AUC = 1.00 for all antibiotics tested with *E. coli* and *K. pneumoniae*. Although we observed 2
errors, using the TTPD
LT
metric still gave excellent agreement with gold-standard AST and
required no splitting of the sample prior to exposure.

To demonstrate one of the major differences between pol-aAST, a phenotypic method, and
existing genotypic methods, we challenged the assay with 5 previously characterized isolates
that had either (i) no detectable \( \beta \)-lactamase genes or (ii) lacked any genotypic signature pre-
dictive of \( \beta \)-lactam resistance. We tested 2 \textit{E. coli} and 2 \textit{K. pneumoniae} isolates with no detect-
able \( \beta \)-lactamase genes as measured by both a published genotypic assay designed to screen for

Fig 5. Validation of the pol-aAST method using lysed control and antibiotic-treated aliquots. (a) Example calculation of TTPD between the lysed control and antibiotic-treated aliquots (TTPDLT). The TTP (in minutes) in the lysed control and antibiotic-treated aliquots are used to calculate TTPDLT. (b–d) The pol-aAST results using \textit{E. coli} (b), \textit{K. pneumoniae} (c), and \textit{Enterobacter} spp. (d) isolates exposed to CRO, ETP, and MEM for 15 min. Red points represent isolates with either no detectable carbapenemase genes (\textit{Ec} and \textit{Kp} isolates) according to a published genotypic assay \cite{67} and commercial assay \cite{68}, or no predictive genotype (\textit{Ebs} isolates) according to the CDC \cite{66}. S/R thresholds (dashed lines) were set halfway between the lowest susceptible and the highest resistant TTPDLT values except in the case of \textit{Enterobacter} spp. treated with CRO (see text). Raw data are provided in S3 Table. +ABX, antibiotic-treated; AST, antibiotic susceptibility testing; CDC, Centers for Disease Control and Prevention; ctrl, control; CRO, ceftriaxone; \textit{Ebs}, \textit{E. aerogenes} and \textit{E. cloacae} collectively; \textit{Ec}, \textit{E. coli}; ETP, ertapenem; \textit{Kp}, \textit{K. pneumoniae}; lc, lysed control; MEM, meropenem; pol-aAST, polymerase-accessibility AST; R, resistant; RFU, relative fluorescent units; S, susceptible; TTP, time-to-positive; TTPD, time-to-positive difference; TTPDLT, TTPD lysed-control to treated.

Fig 6. Timed sample-to-answer pol-aAST using contrived urine samples spiked with either \textit{Ec} or \textit{Kp}. (a) Because minimal sample handling is required for pol-aAST, all 4 contrived urine samples were run in parallel. (b) Urine samples were split into control and antibiotic-treated aliquots and incubated at 37°C for 13 min. A timer was started immediately after sample splitting. (c) All samples were added to pre-made LAMP mix and run in technical triplicate. (d) Samples were amplified using LAMP, and the fluorescence of reactions was monitored in real time. Once total fluorescence passed a predetermined threshold (indicating successful amplification), reactions were stopped and TTP values ported into an automated data-analysis spreadsheet. The timer was stopped as soon as the spreadsheet gave susceptibility calls. (e) Comparison of susceptibility calls with gold-standard AST categorization. Total assay time was 29.5 min. Raw data are provided in S3 Table. ABX, antibiotic; AST, antibiotic susceptibility test/testing; ctrl, control; Ec, \textit{E. coli}; ETP, ertapenem; \textit{Kp}, \textit{K. pneumoniae}; LAMP, loop-mediated isothermal amplification; pol-aAST, polymerase-accessibility AST; R, resistant; RFU, relative fluorescent units; rt, real-time; S, susceptible; TTP, time-to-positive; TTPD, time-to-positive difference.

https://doi.org/10.1371/journal.pbio.3000652.g005

https://doi.org/10.1371/journal.pbio.3000652.g006
6 β-lactamase gene families [67], as well as the Cepheid Xpert Carba-R test (a commercial, FDA-approved genotypic assay designed to screen for 5 β-lactamase gene families) [68]. These 4 isolates did not test positive in either assay because they lack the genes these assays screen for, despite being resistant (as determined by gold-standard broth microdilution). These 4 tested isolates were resistant to CRO and ETP, and one isolate from each genus was also resistant to MEM. Additionally, we tested a single resistant Ebs isolate from the CDC Enterobacteriaceae Carbapenem Breakpoint Panel (AR-Bank #0007). Whole genome sequencing of this isolate (performed by the CDC) revealed no known resistance markers [66], meaning that the mechanism of resistance was uncharacterized. The pol-aAST performed excellently in all cases, and all 5 isolates were correctly categorized as resistant (Figs 4 and 5, red points).

To investigate the sample-to-answer time of the pol-aAST, we performed timed experiments using contrived urine samples (Fig 6). Sample-to-answer time is a critical metric for any assay designed to be used at the POC but is often not reported at all, even for methods claiming to be rapid. In timed experiments, we (i) reduced the exposure time from 15 to 13 min to ensure that all handling could be performed during the 15 min allocated for exposure and (ii) used an automated data-analysis spreadsheet to provide a susceptibility call as soon as the LAMP reactions reached a predetermined threshold (indicating successful amplification). At the initiation of pol-aAST, a timer was started that ran for the duration of the experiment and was stopped once a susceptibility call had been made. The susceptibility of 4 isolates to ETP was tested simultaneously (Fig 6A). The pol-aAST consists of only 3 simple handling steps (Fig 6B–6D), which allowed us to perform pol-aAST in a total time of just 29.5 min, with results in agreement with gold-standard AST (Fig 6E).

We next ran the pol-aAST on clinical urine samples from patients diagnosed with UTI. These samples were confirmed to be Enterobacteriaceae-positive UTIs by the UCLA CML, and the pol-aASTs were run 3 to 5 d after collection. Initial experiments running the pol-aAST directly on clinical urine samples revealed an insufficient response to antibiotics in some samples. Because we analyzed urine samples that had been stored in a chemical preservative (see Methods) for 3 to 5 d after collection, some variation in the response to antibiotics was expected. However, we wished to test whether the delays in the response were indeed due to the phenotypic state of bacteria in these archived samples, and not due to the intrinsic biology of the bacterial strains in these samples. To test, we obtained 25 clinical urine specimens that exhibited an expected heterogeneity, as indicated by the wide range of urinalysis findings (see S2 Table): pH ranged from <5 to 8, specific gravities ranged from <1.005 to >1.060 (above and below the ranges detected in standard urinalysis), and protein, ketone, and bilirubin contents ranged from absent to the maximum measurable by urinalysis. Some samples contained red blood cells, leukocytes, and squamous epithelial cells. Two of the samples were polymicrobial. To ensure a response from bacteria in these specimens, we added a 30-min pre-incubation step of urine with media and increased the duration of antibiotic exposure to 45 min (see Methods). We did not optimize these conditions and did not attempt to identify the shortest possible incubation or exposure time. Eight samples were tested for ampicillin (AMP) susceptibility, and 17 samples were tested for ETP susceptibility. Prior to testing clinical samples using AMP, we tested 5 E. coli isolates using AMP (S1 Fig). Despite the heterogeneity in the urine matrix and the likely nutrient-deprived condition of the bacteria in the urine samples, pol-aAST experiments yielded clean separation between AMP-sensitive and -resistant E. coli. Additionally, we were able observe a response to ETP in 14 of 17 ETP-sensitive urine samples tested. Overall, we obtained 100% categorical agreement for determination of AMP susceptibility (4/4 susceptible and 4/4 resistant; Fig 7) and observed a response indicating susceptibility to ETP in 14 of 17 (82.4%) confirmed-susceptible samples (Fig 7), including the 2
polymicrobial samples. None of the samples received for testing by the pol-aAST method were ETP-resistant.

Discussion

The pol-aAST method enables rapid, organism-specific measurement of susceptibility to β-lactams—the most important class of antibiotic for gram-negative infections—thus providing the critically missing piece needed to develop a POC AST for this global health threat. The genera of isolates and the β-lactams used in this proof-of-concept study were intentionally chosen—E. coli, K. pneumoniae, and Ebs—and are responsible for the majority of CRE infections globally [8,11–13] (in some areas of the US, K. pneumoniae is responsible for up to 90% of CRE infections [5]). It is for this reason that E. coli, K. pneumoniae, and Ebs together make up the majority of isolates in the CDC’s Enterobacteriaceae Carbapenem Breakpoint panel, a collection of isolates designed specifically to challenge carbapenem-susceptibility tests in Enterobacteriaceae [66]. CRO, used broadly for a variety of infections because of its broad coverage and tolerability, was chosen as a representative third-generation cephalosporin. Similarly, ETP and MEM were chosen as clinically representative carbapenems [69]. When testing clinical samples, AMP was chosen because of its high resistance prevalence and thus availability of resistant samples (55.8% of clinical urine samples received by the UCLA CML are AMP resistant [70]). We chose ETP as a representative carbapenem.
The pol-aAST has 2 important requirements: (i) amplification conditions that are not fully lytic and (ii) release of NAs only from cells that are susceptible to the β-lactam to which they are exposed. If cells fully lyse, as they do in PCR, there is no difference in amplification between control and treated aliquots in susceptible isolates (Fig 2). It is only under partial-lysis conditions, as in LAMP, that cell integrity is preserved long enough to yield a substantial TTPD. Cell integrity, and rate and degree of lysis, will also depend on the identity of the organism, as well as its growth rate. In partial-lysis conditions, most NAs are still protected inside cells in the control aliquot, whereas a significant portion of NAs are released and start amplifying immediately in the treated aliquot. We know from previous work [60] that the speed of an optimized bulk LAMP reaction makes it difficult to linearly correlate TTP and NA concentration, unless very sensitive real-time measurements are made. Based on the magnitude of the differences in TTP observed here and the results measuring NA release (Fig 3), we suspect that both the state of NAs (inside intact cells versus inside or outside damaged cells) and the differences in concentration of free NAs contribute to the TTPDs observed. Cell-wall defects and damage are also likely to increase the penetration of amplification reagents into DNA trapped inside the remains of susceptible treated cells especially under the elevated temperature of the amplification reaction. We chose LAMP because we have shown previously that it is a rapid and specific isothermal amplification chemistry [60]. However, other non-lytic isothermal amplification chemistries could also be investigated. Additionally, DNA release (Fig 3) could be measured to determine susceptibility using PCR if combined with a filtration step; we have not evaluated the pros and cons of this approach in this paper. Lastly, alternative or modified accessibility-based AST approaches will likely need to be developed for different organisms, as we have done for Neisseria gonorrhoeae [71].

To demonstrate the flexibility of the pol-aAST method and the simplicity of the workflow, we investigated 2 approaches for determining susceptibility. The first, measuring TTPD_{CT}, gave 100% categorical agreement and uses a standard antibiotic-exposure step wherein one aliquot serves as the control and the other aliquot is exposed to an antibiotic. The second, measuring TTPD_{LT}, differs in that only a single aliquot of the original sample is used during the antibiotic-exposure step. After exposure, this aliquot is compared with a fully lysed control aliquot, which could be extracted at any point during the assay. Using only a single aliquot of the original sample during exposure reduces the challenges of fluid handling and metering, which will be valuable when developing fully integrated devices. When using a control and treated aliquot, both aliquots must have precisely metered volumes, and the heating required during exposure must be performed on both aliquots. Both methods showed excellent categorical agreement with gold-standard broth microdilution, and the choice of approach will be dictated by future device architecture.

To illustrate the value of phenotypic approaches, we evaluated pol-aAST using isolates that tested negative for β-lactamase genes and isolates that lack a predictive genotype (e.g., no β-lactamase production, no modified porins, no modified penicillin-binding proteins), based on published and commercial genotypic assays [67], and CDC classification based on the ResFinder database [72], respectively. The antibiotic susceptibility of isolates lacking β-lactamas cannot be detected by current, FDA-approved genotypic methods, yet bacteria that do not produce β-lactamas can constitute 11% to 71% of CRE infections [4,73,74]. Using pol-aAST, all 5 of these isolates were correctly categorized as resistant.

Sample-to-answer time directly reflects the speed of diagnostics in practice and is a major factor in how likely a diagnostic is to be adopted. In general, the shorter the sample-to-answer time, the more valuable the test is and the more feasible for use at the POC. With urine as the contrived sample matrix, pol-aAST was able to be completed in <30 min. This timescale is on par both with suggested time-frames for rapid POC diagnostics [63,64] and measured times of
patient visits [75]. Additionally, because urine involves relatively simple sample-handling steps, we were able to perform 4 ASTs in parallel when testing contrived samples. The ability to run several samples in parallel demonstrates the potential to multiplex multiple antibiotics, which will be important for the next steps, including the design of integrated devices.

We have demonstrated direct testing of 25 clinical UTI samples using the pol-aAST with changes to the workflow (see Methods). However, even with the heterogeneity of clinical urine specimens (see urinalysis in S2 Table), including 2 polymicrobial samples that were correctly classified as ETP-S, the pol-aAST demonstrated good agreement with gold-standard broth dilution. The ability to handle polymicrobial samples was predictable based on the molecular specificity of NA-based methods. We expect this work to set the foundation for future improvements when using clinical samples.

The pol-aAST method demonstrates a rapid NA-based phenotypic AST for β-lactams and CREs. As with any academic report of an innovative diagnostic technology development, this work has limitations in the breadth of its scope and level of technological maturity. The following work would further extend the clinical applicability of this study and will be necessary for translation into a system suitable for regulatory approval and clinical use. First, the pol-aAST needs to be further developed and evaluated with fresh clinical urine samples from patients; here, we have used chemically preserved samples that were 3 to 5 d old, which likely decreased the response time of bacteria to antibiotics. We expect fresh clinical samples to show more rapid and consistent responses; this hypothesis remains to be tested. We note that many state-of-the-art phenotypic AST methods are initially published without validation of performance directly on clinical samples, e.g. a recent breakthrough demonstrating phenotypic AST on isolates and on blood cultures [58]. Urine is a relevant matrix for a CRE diagnostic because UTIs are the most common source of CRE isolates [76], and because of the large number of hospital-acquired infections that involve catheters or other long-term indwelling medical devices [11], where CRE infections cause major problems. Second, to expand the scope of this approach, other sample types such as blood and blood cultures should be tested (in combination with appropriate pathogen-isolation and pathogen-enrichment technologies). Third, only categorical (S/R) agreement with the gold-standard method was tested here. While in the majority of cases a rapid categorical AST is clinically actionable, testing samples with a range of minimum inhibitory concentrations (MICs), including those with intermediate resistance, would further broaden the scope of the method. Fourth, we have not tested pol-aAST against heteroresistant samples. However, these are more common in gram-positive organisms [77] and are not common in gram-negative organisms. Fifth, the pol-aAST chemistry should be integrated with microfluidic devices so the AST can be performed directly on clinical samples with minimal user intervention. Sixth, the performance of these integrated devices will need to be evaluated in preclinical and clinical studies.

We emphasize that the specific pol-aAST described in this paper, just like other innovative rapid ASTs [60,78–81], is not intended to be the sole test to guide treatment. Even though pol-aAST is based on detection of pathogen-specific NAs and can therefore provide pathogen ID, we anticipate that in a clinical workflow pol-aAST would be performed after a separate rapid pathogen ID step [17,18,20]. This ID step would then allow an unambiguous choice of the appropriate rapid AST. Furthermore, pol-aAST would likely be combined with rapid AST for other antibiotics, such as fluoroquinolones that can be used to treat CRE infections. AST methods that rely on similar underlying chemistries are more likely to be successfully integrated together. Isothermal amplification of pathogen-specific NAs appears to be a promising approach for AST, and we have already shown how a rapid fluoroquinolone AST can be performed in 30 min using digital LAMP [60]. Integration of pol-aAST with these complementary methods and translation to a distributable diagnostic will enable (i) improved antibiotic
stewardship by reducing empiric use of carbapenems for Enterobacteriaceae, (ii) improved patient outcomes by detecting CRE infections for which carbapenems would be ineffective, and (iii) more cost-effective surveillance of CRE outbreaks.

We envision that exploratory and mechanistic research inspired by pol-aAST will lead to a new generation of AST diagnostics. Additional mechanistic studies, such as those involving visualizing bacterial response to antibiotics [82,83], would clarify the effects of different antibiotics on the responses measured in pol-aAST for different pathogens. To evaluate whether pol-aAST can be broadened beyond CREs and β-lactams, these studies would include organisms with cell envelopes that differ from Enterobacteriaceae (e.g., gram-positives) and other antimicrobials that affect the cell envelope, such as antimicrobial peptides [84] or vancomycin. It would also be desirable to evaluate pol-aAST with more amplification chemistries, including modified LAMP assays [85,86] and other isothermal chemistries [87–89], such as recombinase polymerase amplification (RPA), that are actively being developed and can be performed at lower temperatures. Ultimately, this new generation of AST diagnostics will be integrated with the rapid ID methods being developed [17,18,20] and with future rapid NA-based AST methods for additional antibiotics and pathogens. For example, we have developed the nuclease-accessibility AST (nuc-aAST) [71], which measures accessibility of DNA to nucleases and was used to perform a rapid test of antibiotic susceptibility on the fastidious organism *N. gonorrhoeae*. In contrast to the pol-aAST, the nuc-aAST enhances antibiotic-induced damage using surfactants after the antibiotic-exposure step and performs full cell lysis. Ultimately, to address the broad diversity of antibiotic-resistant pathogens, it is clear that integrated, multiplexed POC devices that incorporate multiple rapid phenotypic AST methods are needed. Innovative methods based on antibiotic-induced accessibility of NAs to enzymes are promising for generating such ASTs for multiple antibiotics and pathogens in an approach that is intrinsically compatible with other rapid AST methods [60] and with rapid pathogen ID [17,18,21,22].

**Methods**

**Ethics statement**

Remnant urine samples from patients with confirmed UTI were received by UCLA CML and released to the Caltech researchers under UCLA IRB #19–001098. The UCLA IRB waived the requirement for informed consent and/or assent and/or parent permission under 45 CFR 46.116 (d) for the entire study. No identifying information was obtained by the Caltech team, and the research was determined to be exempt by Caltech IRB (applications #18–0858 and #19–0909).

**Study design**

The objective of this study was to develop a rapid phenotypic AST for β-lactams based on DNA accessibility to polymerase for use with Enterobacteriaceae. To calculate the sample size necessary to validate the method (Figs 4 and 5), the Methods and Equation 5 from Banoo and colleagues [90] were used as described previously [60], namely, we suspected that the specificity and sensitivity of the nuc-aAST method would be 95% with a desired margin of error of ±10%. Under these conditions, 18.2 (or 19) samples must be tested with the nuc-aAST method and compared to the gold standard. We performed 36 ASTs with isolates susceptible to the antibiotic being tested and 46 ASTs with isolates resistant to the antibiotic being tested.

**Isolates, growth conditions, and antibiotic exposure conditions**

We obtained 25 de-identified clinical isolates from the UCLA CML and the CDC’s Enterobacteriaceae Carbapenem Breakpoint panel [66]. In the case of isolates obtained from the UCLA
CML, MICs were determined as described previously [59]. Genotypic testing of the 2 E. coli and 2 K. pneumoniae isolates selected for their lack of known β-lactamase genes was performed by UCLA CML using a previously published assay [67] and separately at the Keck School of Medicine of USC using the FDA-approved Cepheid Xpert Carba-R test. Whole genome sequencing of the single Ebs isolate selected for its lack of known resistance genes was performed by the CDC [66]. All isolates were stored as glycerol stocks at −80°C. Glycerol stocks were streaked onto Trypticase Soy Agar with 5% sheep’s blood (Becton Dickinson, Franklin Lakes, NJ) and grown overnight at 37°C or resuspended directly in liquid media. Prior to experiments, a small clump of cells was resuspended from plates or glycerol stocks in 2 mL Brain Heart Infusion Broth (BHI; Becton Dickinson) at 37°C + 5% CO₂ with 500 rpm shaking for 2 to 4 h until visibly turbid. OD₆₀₀ of the cultures was then measured, and working cultures were prepared at an OD₆₀₀ of 0.01–0.07 and grown for 50–145 min at 37°C + 5% CO₂ with 500 rpm. Working cultures were then diluted 10X into control and treated aliquots for antibiotic exposure. For validation experiments, antibiotic exposure was performed in 100 μL volumes consisting of 80 μL Mueller Hinton II Broth (MHB; Becton Dickinson), 5 μL nuclease-free H₂O (NF-H₂O), 5 μL 20X antibiotic stock solution, and 10 μL of working culture. In control aliquots, antibiotic stock solution was replaced with NF-H₂O. For filtration experiments, antibiotic exposure was performed in 100 μL volumes consisting of 65 μL MHB (Becton Dickinson), 21 μL NF-H₂O, 4 μL 25X antibiotic stock solution, and 10 μL of working culture. In control aliquots, antibiotic stock solution was replaced with NF-H₂O.

**Antibiotic stocks**

CRO disodium salt hemi(heptahydrate) (Sigma, St. Louis, MO), ETP sodium salt (Research Products International, Prospect, IL), and MEM trihydrate (TCI, Portland, OR) were used to create 1.0 mg/mL antibiotic stock solutions in NF-H₂O based on manufacturer-reported purity, aliquoted, and stored at −80°C. AMP sodium salt (Sigma, St. Louis, MO) was used to create 10.0 mg/mL antibiotic stock solutions in NF-H₂O based on manufacturer-reported purity, aliquoted, and stored at −80°C. Aliquots were only thawed and used once on the days of experiments.

**Comparison of amplification methods**

In order to compare amplification using LAMP and PCR, E. coli isolates were exposed to 0.5 μg/mL ETP for 15 min. Samples were then transferred directly into either PCR or LAMP mix on ice. Amplification was started immediately. qPCR was performed on a Roche LightCycler 96 using SsoFast EvaGreen Supermix (BioRad, Hercules, CA); 10 μL reactions were used. 10% of the final reaction volume was template. Published primers targeting the 23S rRNA genes of Enterobacteriaceae were used [91] at a final concentration of 500 nM. Cycling conditions consisted of 3.0 min at 95°C, followed by 35 cycles of 95°C for 10 s, 60°C for 10 s, and 72°C for 15 s. Fluorescence was measured using the SYBR Green channel after each 72°C extension step. LAMP was performed on a BioRad CFX96 using the following conditions: 10 μL reaction volume containing 1X Isothermal Reaction Buffer II (NEB), 5 mM MgSO₄ (NEB), 1.4 mM dNTPs (NEB), 320 U/mL Bst 3.0 (NEB), and 2 μM Syto-9 (Thermo Fisher); 10% of the reaction volume was template. Primer sequences (designed to target the 23S rRNA genes of Enterobacteriaceae) and concentrations have been described previously [60]. Cycling conditions consisted of 2.0 min at 12°C (while lid was heating), followed by 120 cycles of 70°C for 10 s. Fluorescence was measured using the SYBR Green channel every 10 s (after each cycle). We also ran an analogous LAMP reaction in the absence of Tween-20 (which is normally present in Isothermal Reaction Buffer II; NEB), to test for a potential difference in lysis.
efficiency; however, the resulting reaction rates were substantially lower than when Tween-20 was included.

**Filtration experiments**

Filtration experiments were performed using *E. coli* isolates exposed to 0.5 μg/mL ETP for 15 min. Immediately following exposure, cultures were passed through 0.22 μm, 1.5 mL cellulose acetate centrifuge tube filters (Corning Costar Spin-X, Corning, NY). DNA retention by the filters was <7% when measured by quantifying purified Lambda phage DNA (NEB) before and after filtration. Quantification was performed using ddPCR (QX200, BioRad). In filtration experiments, 50 μL of sample was added to the filter and centrifuged for 4 min at 1,000 rcf. DNA was extracted from both the feed and filtrate using QuickExtract DNA Extraction Solution (Lucigen, Middleton, WI). Samples were diluted 10X into extraction buffer and extracted according to manufacturer instructions. The concentration of the single copy *E. coli* *uidA* gene was then quantified in the feed and filtrate extractions. The percentage of *E. coli* DNA in the filtrate was calculated as the filtrate concentration divided by the feed concentration. ddPCR was performed using QX200 ddPCR Supermix for EvaGreen (BioRad); 10% of the final reaction volume was template. Published primers targeting the *uidA* gene in *E. coli* were used [92] at a final concentration of 500 nM. Cycling conditions consisted of 5.0 min at 95˚C, followed by 40 cycles of 95˚C for 30 s, 60˚C for 30 s, and 72˚C for 30 s, with final dye stabilization steps of 4˚C for 5.0 min followed by 90˚C for 5.0 min.

**pol-aAST validation with clinical isolates**

For pol-aAST validation experiments, *E. coli* and *Enterobacter* spp. isolates were exposed to either 2.0 μg/mL CRO, 0.5 μg/mL ETP, or 1.0 μg/mL MEM. *K. pneumoniae* isolates were exposed to either 2.0 μg/mL CRO, 1.0 μg/mL ETP, or 1.0 μg/mL MEM. Some isolates were run multiple times on different days. If this was the case, the average TTPD<sub>CT</sub> and TTPD<sub>LT</sub> are reported for that isolate. All isolates were exposed to antibiotics for 15 min in 100 μL reaction volumes in 200 μL PCR tube strips. After 15 min of antibiotic exposure, 10 μL of samples were transferred as template to LAMP reaction mix (as described earlier) on ice in technical triplicate. Amplification was immediately started.

**Timed sample-to-answer using contrived urine samples**

Timed sample-to-answer experiments were performed in the same fashion as pol-aAST validation experiments, except with the following modifications. Following initial growth and measurement of OD, isolates were resuspended in fresh, never-frozen, pooled human urine from healthy donors (Lee BioSciences). Additionally, a timer was started as soon as samples were added to the antibiotic exposure conditions. *E. coli* and *K. pneumoniae* isolates were exposed to 0.5 and 1.0 μg/mL ETP (respectively) for 13 min. The duration of 13 min was chosen to ensure that all handling steps could be completed within the first 15 min of the assay. Amplification was performed until all reactions reached a fluorescence value of 1,000 relative fluorescent units (RFU) or greater. Amplification was then stopped, and TTP values were copied into a spreadsheet pre-populated with formulas to automatically output susceptibility calls. The timer was stopped once a susceptibility call had been determined.

**Testing of pol-aAST with clinical samples**

UCLA CML performed urinalysis, confirmation of UTI, pathogen isolation and ID, and subsequent gold-standard AST using broth microdilution. Gold-standard AST results were sent to
Caltech researchers on the same day samples were received. Enterobacteriaceae-positive samples were shipped at ambient temperature to Caltech in BD Vacutainer Plus C&S preservative tubes (Becton Dickinson, Catalog Number 364951) containing a boric acid preservative. The pol-aAST experiments were performed directly on these samples within 3–5 d of their collection at UCLA. Urine samples were first warmed up to 37°C without shaking for 30 min, to approximate temperature of freshly collected urine. Then, 30 μL of urine was diluted into 70 μL of Cation-adjusted MHB (BD) containing 0.1% Tween-20 (Teknova, Hollister, CA) and placed at 37°C with shaking at 750 rpm for 3 min. Samples were then centrifuged at 5,000 rcf for 2 min. The supernatant was removed, and the sample was resuspended in 100 μL of MHB. Samples were then incubated for 30 min at 37°C with 750 rpm shaking. Antibiotic exposure was performed in a final volume of 100 μL, after transfer of 20 μL of incubated sample to 80 μL of the exposure condition: 75 μL of MHB and 5 μL of 20X antibiotic stock solution in NF-H2O for treated aliquots, or 75 μL of MHB with 5 μL of NF-H2O alone for control aliquots. For measurement of ETP susceptibility, the exposure condition contained a final concentration of 1μg/mL of ETP. Aliquots were incubated at 37°C with shaking for 20 min. For measurement of AMP susceptibility, the antibiotic-exposure condition contained a final concentration of 16 μg/mL of AMP, and aliquots were incubated at 37°C with shaking for 45 min. The control and treated aliquots were subjected to a set of dilutions to account for variable bacterial load of the samples and resolution within the working range of the LAMP reaction. Following dilution, 1 μL of the control and treated aliquots was added to each LAMP reaction well. There were 3 technical replicates (3 LAMP reaction wells) for each condition (control and treated). We measured the TTP for the reactions at each dilution, and then selected the dilution that yielded a control TTP value later than 4.7 min. The TTP results from this dilution were used to calculate TTPD<sub>CT</sub> (and determine susceptibility). Samples with a TTPD<sub>CT</sub> > 0.25 min were considered susceptible, while samples with TTPD<sub>CT</sub> ≤ to 0.25 min were considered resistant. The susceptibility determination of the pol-aAST method was then compared to the gold-standard culture results obtained by the UCLA CML to measure assay performance.

**Statistical analysis**

Significance referenced in the text for Fig 2 were calculated using GraphPad Prism 8.0 software from an unpaired, two-tailed t test comparing the averages of 3 replicate Cq values of each control sample to each treated sample. A significance value of 0.02 was used for statistical significance. All percent release values (Fig 3) and TTPD values (Figs 4–6) were calculated using Microsoft Excel. Data were plotted using GraphPad Prism 8.0 software. Thresholds for determining susceptibility in TTPD<sub>CT</sub> and TTPD<sub>LT</sub> plots were set halfway between the lowest S and highest R values for each organism/antibiotic combination. For preliminary tests with clinical samples, we defined a TTPD<sub>CT</sub> of above 0.25 min for a susceptible determination; this value would be further defined in a subsequent larger-scale clinical trial.

**Supporting information**

**S1 Table. Clinical isolates used in this study.** Isolates were obtained from the UCLA CML and the CDC’s Enterobacteriaceae Carbapenem Breakpoint panel. The MIC of each isolate (based on broth microdilution performed by UCLA CML) are provided.

(XLSX)

**S2 Table. Clinical urine samples from patients with UTIs used in this study.** Clinical samples were obtained from the UCLA CML. MICs based on broth microdilution performed by
UCLA CML are provided along with urinalysis results.
(XLSX)

S3 Table. Raw data and calculated error for all pol-aASTs performed using clinical isolates. Cqs and TTPs are provided.
(XLSX)

S4 Table. Raw data and calculated error for all pol-aASTs performed using clinical UTI samples. Cqs and TTPs are provided.
(XLSX)

S5 Table. Raw data for amplification curves shown in Fig 2. Technical triplicate values are provided for control and treated (0.50 μg/mL ETP) samples run using LAMP and PCR. Grey lines in Fig 2 represent standard deviation of the triplicate samples calculated using Graphpad Prism.
(CSV)

S6 Table. Raw data for percentage of DNA release shown in Fig 3. Negative percentage release values were set to zero before averaging. Averages and standard deviations of each isolate/antibiotic combination were calculated using GraphPad Prism.
(XLSX)

S1 Fig. Validation of pol-aAST TTPD_CT method using AMP. E. coli isolates were exposed to 16 μg/mL AMP for 15 min. Threshold was set halfway between the lowest susceptible and highest resistant TTPD_CT value. Data are in S3 Table. R, resistant; S, susceptible.
(TIF)

S1 Text. Detailed statement of author contributions.
(PDF)

Acknowledgments

We thank Sukantha Chandrasekaran, Shelley Miller, Romney Humphries, Marisol Trejo, Catherine Le, and Lyna Chheang at the UCLA Clinical Microbiology Laboratory for providing isolates and clinical urine samples and for discussion of gold-standard practices. We thank Jennifer Dien Bard at the Keck School of Medicine of USC for performing Cepheid Xpert Carba-R tests. We also thank Natasha Shelby for help with writing and editing this manuscript.

Author Contributions

**Conceptualization:** Nathan G. Schoepp, Eric J. Liaw, Emily S. Savela, Rustem F. Ismagilov.

**Data curation:** Nathan G. Schoepp.

**Formal analysis:** Nathan G. Schoepp, Alexander Winnett, Emily S. Savela.

**Funding acquisition:** Nathan G. Schoepp, Alexander Winnett, Rustem F. Ismagilov.

**Investigation:** Nathan G. Schoepp, Eric J. Liaw, Alexander Winnet.

**Methodology:** Nathan G. Schoepp, Eric J. Liaw, Alexander Winnett, Emily S. Savela, Rustem F. Ismagilov.

**Project administration:** Rustem F. Ismagilov.

**Resources:** Omai B. Garner, Rustem F. Ismagilov.
Supervision: Rustem F. Ismagilov.
Validation: Nathan G. Schoepp, Alexander Winnett.
Visualization: Nathan G. Schoepp.
Writing – original draft: Nathan G. Schoepp, Eric J. Liaw.
Writing – review & editing: Nathan G. Schoepp, Alexander Winnett, Emily S. Savela, Omai B. Garner, Rustem F. Ismagilov.

References
1. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, et al. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. Lancet Infect Dis. 2014; 14(8):742–50. https://doi.org/10.1016/S1473-3099(14)70780-7 PMID: 25022435
2. Versporten A, Zarb P, Caniaux I, Gros MF, Drapier N, Miller M, et al. Antimicrobial consumption and resistance in adult hospital inpatients in 53 countries: results of an internet-based global point prevalence survey. Lancet Global Health. 2018; 6(6):e619–e29. https://doi.org/10.1016/S2214-109X(18)30186-4 PMID: 29681513
3. Magill SS, Edwards JR, Beldavs ZG, Dumyati G, Janelle SJ, Kainer MA, et al. Prevalence of antimicrobial use in US acute care hospitals, May-September 2011. JAMA. 2014; 312(14):1438–46. https://doi.org/10.1001/jama.2014.12923 PMID: 25291579
4. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, et al. Epidemiology of Carbapenem-Resistant Enterobacteriaceae Infections: Report from the China CRE Network. Antimicrob Agents Chemother. 2018; 62(2):e01882–17. https://doi.org/10.1128/AAC.01882-17 PMID: 29203488
5. Satlin MJ, Chen L, Patel G, Gomez-Simmonds A, Weston G, Kim AC, et al. Multicenter Clinical and Molecular Epidemiological Analysis of Bacteremia Due to Carbapenem-Resistant Enterobacteriaceae (CRE) in the CRE Epicenter of the United States. Antimicrob Agents Chemother. 2017; 61(4):e02349-16. https://doi.org/10.1128/AAC.02349-16 PMID: 28167547
6. Iovleva A, Doi Y. Carbapenem-Resistant Enterobacteriaceae. Clin Lab Med. 2017; 37(2):303–15. https://doi.org/10.1016/j.cll.2017.01.005 PMID: 28457352
7. WHO. Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics 2017 [December 3, 2018]. Available from: https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/. [cited 3 March 2020].
8. CDC. Antibiotic Resistance Threats in the United States 2013 [December 3, 2018]. Available from: https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. [cited 3 March 2020].
9. Gelband H, Miller-Petrie M, Pant S, Levinson SGJ, Barter D, Laxminarayan AWR. State of the World’s Antibiotics 2015. Available from: https://cddep.org/publications/state_worlds_antibiotics_2015/. [cited 3 March 2020].
10. CDC. Antibiotic/Antimicrobial Resistance: Biggest Threats and Data 2019 [April 2019]. Available from: https://www.cdc.gov/drugresistance/biggest_threats.html. [cited 3 March 2020].
11. Guh AY, Bulens SN, Mu Y, Jacob JT, Reno J, Scott J, et al. Epidemiology of Carbapenem-Resistant Enterobacteriaceae in 7 US Communities, 2012–2013. JAMA. 2015; 314(14):1479–87. https://doi.org/10.1001/jama.2015.12480 PMID: 26436831
12. Cerqueira GC, Earl AM, Ernst CM, Grad YH, Dekker JP, Feldgarden M, et al. Multi-institute analysis of carbapenem resistance reveals remarkable diversity, unexplained mechanisms, and limited clonal outbreaks. Proc Natl Acad Sci U S A. 2017; 114(5):1135–40. https://doi.org/10.1073/pnas.1616248114 PMID: 28096418
13. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Submitted to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol. 2016; 37(11):1288–301. https://doi.org/10.1017/ice.2016.174 PMID: 27573805
14. White House. National Action Plan for Combating Antibiotic-Resistant Bacteria 2015 [December 3, 2018]. Available from: https://obamawhitehouse.archives.gov/sites/default/files/docs/national_action_plan_for_combating_antibiotic-resistant_bacteria.pdf. [cited 3 March 2020].
15. WHO. Global Action Plan on Antimicrobial Resistance 2015 [December 3, 2018]. Available from: https://www.who.int/antimicrobial-resistance/global-action-plan/en/. [cited 3 March 2020].
16. O'Neill J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. Review on Antimicrobial Resistance [Internet]. 2016 12/01/2016. Available from: https://amr-review.org/Publications.html. [cited 3 March 2020].

17. Renner LD, Zan J, Hu L, Martinez M, Resto PJ, Siegel AC, et al. Detection of ESKEAPE Bacterial Pathogens at the Point of Care Using Isothermal DNA-Based Assays in a Portable Degas-Actuated Microfluidic Diagnostic Assay Platform. Appl Environ Microbiol. 2017;83(4). https://doi.org/10.1128/aem.02449-16 PMID: 27986722

18. Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. PLoS ONE. 2011; 6(10):e26047. https://doi.org/10.1371/journal.pone.0026047 PMID: 22039434

19. Gootenberg J, Abdulyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science. 2018; 360(6387):439–44. https://doi.org/10.1126/science.aag1019 PMID: 29449508

20. Phaneuf CR, Mangadu B, Piccini ME, Singh AK, Koh CY. Rapid, Portable, Multiplexed Detection of Bacterial Pathogens Directly from Clinical Sample Matrices. Biosensors. 2016; 6(4):E49. https://doi.org/10.3390/bios6040049 PMID: 27669320

21. Ahn H, Batule BS, Seok Y, Kim MG. Single-Step Recombinase Polymerase Amplification Assay Based on a Paper Chip for Simultaneous Detection of Multiple Foodborne Pathogens. Anal Chem. 2018; 90 (17):10211–6. https://doi.org/10.1021/acs.analchem.8b01309 PMID: 3075080

22. Raja B, Goux HJ, Marapadaga A, Rajagopalan S, Kourouzit K, Willson RC. Development of a panel of recombinase polymerase amplification assays for detection of common bacterial urinary tract infection pathogens. J Appl Microbiol. 2017; 123(2):544–55. https://doi.org/10.1111/jam.13493 PMID: 28510991

23. CLSI. M07-A10; Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria That Grow Aerobically; Approved Standard-Tenth Edition: Clinical and Laboratory Standards Institute; 2015.

24. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis. 2009; 49(11):1749–55. https://doi.org/10.1086/647952 PMID: 19857164

25. Blank S, Daskalakis DC. Neisseria gonorrhoeae—Rising Infection Rates, Dwindling Treatment Options. N Engl J Med. 2018; 379(19):1795–7. https://doi.org/10.1056/NEJMp1812269 PMID: 30403946

26. Piddock LJ. Assess drug-resistance phenotypes, not just genotypes. Nat Microbiol. 2016; 1(8):16120. https://doi.org/10.1038/s41564-016-0003 PMID: 27573119

27. Bard J, Lee F. Why can’t we just use PCR? The role of genotypic versus phenotypic testing for antimicrobial resistance. Clin Microbiol Newsfl. 2018; 40(11):87–95. https://doi.org/10.1016/j.clinmicnews.2018.05.003.

28. van Belkum A, Bachmann TT, Ludke G, Kahlmeier G, Mohess A, et al. Developmental roadmap for antimicrobial susceptibility testing systems. Reviews Nature Microbiology. 2018. https://doi.org/10.1038/s41579-018-0098-9 PMID: 30333569

29. WHO. No time to wait: Securing the future from drug-resistant infections Report to the Secretary-General of the United Nations; 2019. Available from: https://www.who.int/antimicrobial-resistance/interagency-coordination-group/final-report/en/. [cited 3 March 2020].

30. Kostic T, Ellis M, Williams MR, Stedtfeld TM, Kaneene JB, Stedtfeld RD, et al. Thirty-minute screening of antibiotic resistance genes in bacterial isolates with minimal sample preparation in static self-dispensing 64 and 384 assay cards. Appl Microbiol Biotechnol. 2015; 99(18):7711–22. https://doi.org/10.1007/s00253-015-6774-z PMID: 26227406

31. Kalsi S, Vaidi M, Tsaloglug MN, Parry-Jones L, Jacobs A, Watson R, et al. Rapid and sensitive detection of antibiotic resistance on a programmable digital microfluidic platform. Lab Chip. 2015; 15 (14):3065–75. https://doi.org/10.1039/c5lc00462d PMID: 26086197

32. Nakano R, Nakano A, Ishiy U, Ubagai T, Kikuchi-Ueda T, Kikuchi H, et al. Rapid detection of the Klebsiella pneumoniae carbapenemase (KPC) gene by loop-mediated isothermal amplification (LAMP). J Infect Chemother. 2015; 21(3):202–6. https://doi.org/10.1016/j.jiac.2014.11.010 PMID: 25529001

33. Mu X, Nakano R, Nakano A, Ubagai T, Kikuchi-Ueda T, Tansho-Nagakawa S, et al. Loop-mediated isothermal amplification: Rapid and sensitive detection of the antibiotic resistance gene ISAba1-blaOXA-51-like in Acinetobacter baumannii. J Microbiol Methods. 2016; 121:36–40. https://doi.org/10.1016/j.jmimet.2015.12.011 PMID: 26707336

34. Hu C, Kalsi S, Zeimpekis I, Sun K, Ashburn P, Turner C, et al. Ultra-fast electronic detection of antimicrobial resistance genes using isothermal amplification and Thin Film Transistor sensors. Biosens Bioelectron. 2017; 96:281–7. https://doi.org/10.1016/j.bios.2017.05.016 PMID: 28505562
35. Cortegiani A, Russotto V, Graziano G, Geraci D, Saporito L, Cocorullo G, et al. Use of Cepheid Xpert Carba-R(R) for Rapid Detection of Carbapenemase-Producing Bacteria in Abdominal Septic Patients Admitted to Intensive Care Unit. PLoS ONE. 2016; 11(8):e0160643. https://doi.org/10.1371/journal.pone.0160643 PMID: 27490684

36. Biofire. The BioFire FilmArray 2019 [March 8, 2019]. Available from: https://www.biofiredx.com/products/filarray/. [cited 3 March 2020].

37. Shawar R. 510(k) Premarket Notification for K160901 Cepheid Xpert Carba-R Assay. Silver Spring, MD: USA Food and Drug Administration, Department of Health and Human Services; 2016.

38. Lasserre C, De Saint Martin L, Cuzon G, Bogaerts P, Lamar E, Glupczynski Y, et al. Efficient Detection of Carbapenemase Activity in Enterobacteriaceae by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry in Less Than 30 Minutes. J Clin Microbiol. 2015; 53(7):2163–71. https://doi.org/10.1128/JCM.03467-14 PMID: 25926485

39. Bogaerts P, Yunus S, Massart M, Huang TD, Glupczynski Y. Evaluation of the BYG Carba Test, a New Electrochemical Assay for Rapid Laboratory Detection of Carbapenemase-Producing Enterobacteriaceae. J Clin Microbiol. 2016; 54(2):349–58. https://doi.org/10.1128/JCM.02404-15 PMID: 26637378

40. Robinson AM, Medicott NJ, Ussher JE. The rapid detection of cefotaxime-resistant Enterobacteriaceae by HPLC. Future Sci OA. 2016; 2(4):FSO142. https://doi.org/10.4155/fsoa-2016-0042 PMID: 28116124

41. Bernaboe S, Dortet L, Naas T. Evaluation of the beta-CARBA test, a colorimetric test for the rapid detection of carbapenemase activity in Gram-negative bacilli. J Antimicrob Chemother. 2017; 72(6):1646–58. https://doi.org/10.1093/jac/dko061 PMID: 28333363

42. Zhang Y, Lei JE, He Y, Yang J, Wang W, Wasey A, et al. Label-Free Visualization of Carbapenemase Activity in Living Bacteria. Angew Chem Int Ed Engl. 2018; 57(52):17120–4. https://doi.org/10.1002/anie.201810834 PMID: 30378725

43. Santiso R, Tamayo M, Gosalvez J, Bou G, Fernandez Mdel C, Fernandez JL. A rapid in situ procedure for determination of bacterial susceptibility or resistance to antibiotics that inhibit peptidoglycan biosynthesis. BMC Microbiol. 2011; 11:191. https://doi.org/10.1186/1471-2180-11-191 PMID: 21867549

44. Bou G, Otero FM, Santiso R, Tamayo M, Fernandez Mdel C, Tomas M, et al. Fast assessment of resistance to carbapenems and ciprofloxacin of clinical strains of Acinetobacter baumannii. J Clin Microbiol. 2012; 50(11):3609–13. https://doi.org/10.1128/JCM.0175-12 PMID: 22933604

45. Park K, Jeong J, Yi SY, Lee WS, Shin YB. FRET probe-based antibacterial susceptibility testing (F-AST) by detection of bacterial nucleases released by antibiotic-induced lysis. Biosensors Bioelectron. 2019. https://doi.org/10.1016/j.bios.2019.01.033 PMID: 30745284

46. Faria-Ramos I, Espinar MJ, Rocha R, Santos-Antunes J, Rodrigues AG, Canton R, et al. A novel flow cytometric assay for rapid detection of extended-spectrum beta-lactamases. Clin Microbiol Infect. 2013; 19(1):E8–E15. https://doi.org/10.1111/j.1469-0691.2012.03986.x PMID: 23145853

47. Burnham CA, Frobel RA, Herrera ML, Wickes BL. Rapid ertapenem susceptibility testing and Klebsiella pneumoniae carbapenemase phenotype detection in Klebsiella pneumoniae isolates by use of automated microscopy of immobilized live bacterial cells. J Clin Microbiol. 2014; 52(3):982–6. https://doi.org/10.1128/JCM.03255-13 PMID: 24391202

48. Su IH, Ko WC, Shih CH, Yeh FH, Sun YN, Chen JC, et al. A dielectrophoresis system for testing antimicrobial susceptibility of Gram-negative bacteria to beta-lactam antibiotics. Anal Chem. 2017. https://doi.org/10.1021/acs.analchem.7b00220 PMID: 28314101

49. Kang W, Sarkar S, Lin ZS, McKenney S, Konry T. Ultrafast Parallelized Microfluidic Platform for Antimicrobial Susceptibility Testing of Gram Positive and Negative Bacteria. Anal Chem. 2019; 91(9):6242–9. https://doi.org/10.1021/acs.analchem.9b00939 PMID: 30938989

50. Cansizoglu MF, Tamer YT, Farid M, Koh AY, Toprak E. Rapid ultrasensitive detection platform for antimicrobial susceptibility testing. PLoS Biol. 2019; 17(5):e3000291. https://doi.org/10.1371/journal.pbio.3000291 PMID: 31145726

51. Mo M, Yang Y, Zhang F, Jing W, Iriya R, Popovich J, et al. Rapid Antimicrobial Susceptibility Testing of Patient Urine Samples Using Large Volume Free-Solution Light Scattering Microscopy. Anal Chem. 2019; 91(15):10164–71. https://doi.org/10.1021/acs.analchem.9b02174 PMID: 31251566

52. Lange C, Schubert S, Jung J, Kostrzewa M, Sparber K. Quantitative matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid resistance detection. J Clin Microbiol. 2014; 52(12):4155–62. https://doi.org/10.1128/JCM.01872-14 PMID: 25232164

53. Rolain JM, Mallet MN, Fournier PE, Raoult D. Real-time PCR for universal antibiotic susceptibility testing. J Antimicrob Chemother. 2004; 54(2):538–41. https://doi.org/10.1093/jac/dkh324 PMID: 15231761
54. Mach KE, Mohan R, Baron EJ, Shih MC, Gau V, Wong PK, et al. A biosensor platform for rapid antimicrobial susceptibility testing directly from clinical samples. J Urol. 2011; 185(1):148–53. https://doi.org/10.1016/j.juro.2010.09.022 PMID: 21074208

55. Halford C, Gonzalez R, Campuzano S, Hu B, Babbiitt JT, Liu J, et al. Rapid antimicrobial susceptibility testing by sensitive detection of precursor rRNA using a novel electrochemical biosensing platform. Antimicrob Agents Chemother. 2013; 57(2):936–43. https://doi.org/10.1128/AAC.00615-12 PMID: 23229486

56. Mezger A, Guilberg E, Goransson J, Zorzet A, Hethnek D, Tano E, et al. A general method for rapid determination of antibiotic susceptibility and species in bacterial infections. J Clin Microbiol. 2015; 53(2):425–32. https://doi.org/10.1128/JCM.02434-14 PMID: 25411178

57. Barczak AK, Gomez JE, Kaufmann BB, Hinson ER, Cosimi L, Borowsky ML, et al. RNA signatures allow rapid identification of pathogens and antibiotic susceptibilities. Proc Natl Acad Sci U S A. 2012; 109(16):6217–22. https://doi.org/10.1073/pnas.1119540109 PMID: 22474362

58. Bhattacharyya RP, Bandyopadhyay N, Ma P, Son SS, Liu J, He LL, et al. Simultaneous detection of genotype and phenotype enables rapid antibiotic susceptibility determination. Nat Med. 2019; 25(12):1858–64. https://doi.org/10.1038/s41591-019-0650-9 PMID: 31768064

59. Schoepp NG, Khrosheva EM, Schiappi TS, Curtis MS, Humphries RM, Hindler JA, et al. Digital Quantification of DNA Replication and Chromosome Segregation Enables Determination of Antimicrobial Susceptibility after only 15 Minutes of Antibiotic Exposure. Angew Chem Int Ed Engl. 2016; 55(33):9557–61. https://doi.org/10.1002/anie.201602773 PMID: 27357773

60. Schoepp NG, Schiappi TS, Curtis MS, Butkovich SS, Miller S, Humphries RM, et al. Rapid phenotypic-specific phenotypic antibiotic susceptibility testing using digital LAMP quantification in clinical samples. Sci Transl Med. 2017; 9(410). https://doi.org/10.1126/scitranslmed.aad3693 PMID: 28978750

61. Khazaei T, Barlow JT, Schoep NG, Ismagilov RF. RNA markers enable phenotypic test of antibiotic susceptibility in Neisseria gonorrhoeae after 10 minutes of ciprofloxacin exposure. Sci Rep. 2018; 8(1). https://doi.org/10.1038/s41598-018-29707-w PMID: 30072794

62. Ota Y, Furushashi K, Namba T, Yamanaka K, Ishikawa J, Nagura O, et al. A rapid and simple detection method for phenotypic antimicrobial resistance in Escherichia coli by loop-mediated isothermal amplification. J Med Microbiol. 2019. https://doi.org/10.1099/jmm.0.009003 PMID: 30624176

63. Marston HD, Dixon DM, Knisely JM, Palmore TN, Fauci AS. Antimicrobial Resistance. JAMA. 2016; 316(11):1193–204. https://doi.org/10.1001/jama.2016.11764 PMID: 27654605

64. Hicks JM, Haeckel R, Price CP, Lewandrowski K, Wu AHB. Recommendations and opinions for the use of point-of-care testing for hospitals and primary care: summary of a 1999 symposium. Clin Chim Acta. 2001; 303(1–2):1–17. https://doi.org/10.1016/s0009-8881(00)00400-9 PMID: 11163017

65. Valiadi M, Kalsi S, Jones IGF, Turner C, Sutton JM, Morgan H. Simple and rapid sample preparation system for the molecular detection of antibiotic resistant pathogens in human urine. Biomed Microdevices. 2016; 18(1). https://doi.org/10.1007/s10544-016-0331-9 PMID: 26846875

66. CDC. Enterobacteriaceae Carbapenem Breakpoint Panel 2015 [cited 2018 November 20, 2018]. Available from: https://www.cdc.gov/ARIsolateBank/Panel/PanelDetail?ID=7. [cited 3 March 2020].

67. Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM. Phenotypic and molecular characteristics of carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013. J Clin Microbiol. 2014; 52(11):4003–9. https://doi.org/10.1128/JCM.01397-14 PMID: 25210072

68. Cepheid. Xpert Carba-R 2018 [cited 2018 November 26, 2018]. Available from: http://www.cephied.com/us/cephied-solutions/clinical-ivd-tests/healthcare-associated-infections/xpert-carba-r. [cited 3 March 2020].

69. Hawkey PM, Livermore DM. Carbapenem antibiotics for serious infections. BMJ. 2012; 344:e3236. https://doi.org/10.1136/bmj.e3236 PMID: 22654063

70. UCLA Health System. Antimicrobial Susceptibility Summary 2019; 2019. Available from: https://www.asp.mednet.ucla.edu/files/view/AMIC_2017.pdf. [cited 3 March 2020].

71. Savela ES, Schoepp NG, Cooper MM, Rolando JC, Klausner JD, Soge OO, et al. Surfactant-enhanced DNA accessibility to nuclease accelerates phenotypic β-lactam antibiotic susceptibility testing of Neisseria gonorrhoeae. PLoS Biol. 2020; 18(3):e3000651. https://doi.org/10.1371/journal.pbio.3000651

72. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012; 67(11):2640–4. https://doi.org/10.1093/jac/dks261 PMID: 22782487

73. Chang YY, Chuang YC, Siu LK, Wu TL, Lin JC, Lu PL, et al. Clinical features of patients with carbapenem nonsusceptible Klebsiella pneumoniae and Escherichia coli in intensive care units: a nationwide multicenter study in Taiwan. J Microbiol Immunol Infect. 2015; 48(2):219–25. https://doi.org/10.1016/j.jmii.2014.05.010 PMID: 25074627
74. Tamma PD, Goodman KE, Harris AD, Tekle T, Roberts A, Taiwo A, et al. Comparing the Outcomes of Patients With Carbapenemase-Producing and Non-Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae Bacteremia. Clin Infect Dis. 2017; 64(3):257–64. https://doi.org/10.1093/cid/ciw741 PMID: 28013264
75. Rui P, Okeyode T. National Ambulatory Care Survey: 2015 State and National Summary Tables [December 3, 2018]. Available from: https://www.cdc.gov/nchs/data/ahcd/namcs_summary/2015_namcs_web_tables.pdf. [cited 3 March 2020].
76. Braykov NP, Eber MR, Klein EY, Morgan DJ, Laxminarayan R. Trends in resistance to carbapenems and third-generation cephalosporins among clinical isolates of Klebsiella pneumoniae in the United States, 1999–2010. Infect Control Hosp Epidemiol. 2013; 34(3):259–68. https://doi.org/10.1086/669523 PMID: 23388360
77. Musta AC, Riederer K, Shenep S, Chase P, Jose J, Johnson LB, et al. Vancomycin MIC plus heteroresistance and outcome of methicillin-resistant Staphylococcus aureus: trends over 11 years. J Clin Microbiol. 2009; 47(6):1640–4. https://doi.org/10.1128/JCM.01235-08 PMID: 19369444
78. Baltekin O, Boucharin A, Tano E, Andersson DI, Elf J. Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging. Proc Natl Acad Sci U S A. 2017; 114(34):9170–5. https://doi.org/10.1073/pnas.1708558114 PMID: 28790187
79. Choi J, Yoo J, Lee M, Kim EG, Lee JS, Lee S, et al. A rapid antimicrobial susceptibility test based on single-cell morphological analysis. Sci Transl Med. 2014; 6(267):267ra174–267ra174. https://doi.org/10.1126/scitranslmed.3009650 PMID: 25250395
80. Etayash H, Khan MF, Kaur K, Thundat T. Microfluidic cantilever detects bacteria and measures their susceptibility to antibiotics in small confined volumes. Nat Commun. 2016; 7:12947. https://doi.org/10.1038/ncomms12947 PMID: 27698375
81. Longo G, Alonso-Sarduy L, Rio LM, Bizzini A, Trampuz A, Notz J, et al. Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors. Nat Nanotechnol. 2013; 8(7):522–6. https://doi.org/10.1038/nnano.2013.120 PMID: 23812189
82. Pidgeon SE, Pires MM. Vancomycin-Dependent Response in Live Drug-Resistant Bacteria by Metabolic Labeling. Angew Chem Int Ed Engl. 2018; 57(37):11856–66. https://doi.org/10.1002/anie.201704851 PMID: 28570012
83. Stone MRL, Butler MS, Phetsang W, Cooper MA, Blaskovich MAT. Fluorescent Antibiotics: New Research Tools to Fight Antibiotic Resistance. Trends Biotechnol. 2016. https://doi.org/10.1016/j.tibtech.2018.01.004 PMID: 29478675
84. Deshayes S, Xia W, Schmidt NW, Kordbacheh S, Lieng J, Wang J, et al. Designing Hybrid Antibiotic Peptide Conjugates To Cross Bacterial Membranes. Bioconjug Chem. 2017; 28(3):793–804. https://doi.org/10.1021/acs.bioconjchem.6b00725 PMID: 28248495
85. Phillips EA, Moehling TJ, Bhadra S, Ellington AD, Linnes JC. Strand Displacement Probes Combined with Isothermal Nucleic Acid Amplification for Instrument-Free Detection from Complex Samples. Anal Chem. 2018; 90(11):6580–6. https://doi.org/10.1021/acs.analchem.8b00269 PMID: 29667809
86. Cai S, Jung C, Bhadra S, Ellington AD. Phosphorothioated Primers Lead to Loop-Mediated Isothermal Amplification at Low Temperatures. Anal Chem. 2018; 90(14):8290–4. https://doi.org/10.1021/acs.analchem.8b02062 PMID: 29968462
87. Daher RK, Stewart G, Boissinot M, Bergeron MG. Recombinase Polymerase Amplification for Diagnostic Applications. Clin Chem. 2016; 62(7):947–58. https://doi.org/10.1373/clinchem.2015.245829 PMID: 27160000
88. Toley BJ, Covelli I, Belousov Y, Ramachandran S, Kline E, Scarr N, et al. Isothermal strand displacement amplification (iSDA): a rapid and sensitive method of nucleic acid amplification for point-of-care diagnostics. Analyst. 2015; 140(22):7540–9. https://doi.org/10.1039/c5an01632k PMID: 26393240
89. Reid MS, Le XC, Zhang H. Exponential Isothermal Amplification of Nucleic Acids and Assays for Proteins, Cells, Small Molecules, and Enzyme Activities: An EXPAR Example. Angew Chem Int Ed Engl. 2018; 57(37):11856–66. https://doi.org/10.1002/anie.201712217 PMID: 29703405
90. Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, et al. Evaluation of diagnostic tests for infectious diseases: general principles. Nat Rev Microbiol. 2006; 4(9 Suppl):S21–31. https://doi.org/10.1038/nrmicro1525 PMID: 17034069
91. Matsuda K, Tsuji H, Asahara T, Kado Y, Nomoto K. Sensitive quantitative detection of membranal bacteria by rRNA-targeted reverse transcription-PCR. Appl Environ Microbiol. 2007; 73(1):32–9. https://doi.org/10.1128/AEM.01224-06 PMID: 17071791
92. Chem EC, Siefring S, Paar J, Doolittle M, Haugland RA. Comparison of quantitative PCR assays for Escherichia coli targeting ribosomal RNA and single copy genes. Lett Appl Microbiol. 2011; 52(3):298–306. https://doi.org/10.1111/j.1472-765X.2010.03001.x PMID: 21204885