GEOSPATIAL SPREAD OF ANTIMICROBIAL RESISTANCE, BACTERIAL AND FUNGAL THREATS TO COVID-19 SURVIVAL, AND POINT-OF-CARE SOLUTIONS

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RUNNING TITLE: ***POC Solutions for 21st Century Threats***

ABSTRACT

CONTEXT. Point-of-care testing (POCT) is *inherently spatial*, that is, performed where needed, and *intrinsically temporal*, because it accelerates decision making. POCT efficiency and effectiveness have the potential to facilitate antimicrobial resistance (AMR) detection, decrease risks of co-infections for critically ill COVID-19 patients, and improve the cost-effectiveness of healthcare.

OBJECTIVES. To assess AMR identification using POCT, describe the United States AMR Diagnostic Challenge, and improve global standards of care for infectious diseases.

DATA SOURCES. PubMed, WWW, and other sources were searched for papers focusing on AMR and POCT. EndNote X9.1 (Clarivate Analytics) consolidated abstracts, URLs, and PDFs representing ~500 articles assessed for relevance. Panelist insights at Tri•Con 2020 in San Francisco and finalist POC technologies competing for a US $20,000,000 AMR prize are summarized.

CONCLUSIONS. Co-infections represent high risks for COVID-19 patients. POCT potentially will help target specific pathogens, refine choices for antimicrobial drugs, and prevent excess morbidity and mortality. POC assays that identify patterns of pathogen resistance can help tell us how infected individuals spread AMR, where geospatial hotspots are located, when delays cause death, and how to deploy preventative resources. Shared AMR data "clouds" could help reduce critical care burden during pandemics and optimize therapeutic options, similar to use of antibiograms in individual hospitals. Multidisciplinary healthcare personnel should learn the principles and practice of POCT, so they can meet needs with rapid diagnostic testing. The stakes are high. AMR is projected to cause millions of deaths annually and cumulative financial loses in the trillions by 2050.

INTRODUCTION

OBJECTIVES

The objectives of this article are to assess identification of antimicrobial resistance (AMR) using point-of-care testing (POCT) and to glean future directions from progress of the AMR Diagnostic Challenge promoted by the National Institutes of Health (NIH) and the Biomedical Advanced Research and Development Agency (BARDA).[1]

The AMR Diagnostic challenge seeks to reduce the use of antibiotic regimens that generate "superbugs,"[2] for which new drugs are sorely lacking, and to improve global standards of therapeutic care for infectious diseases by diminishing AMR threats to survival. These threats include co-infections in Coronavirus infectious disease 2019 (COVID-19) patients who do not respond to antimicrobials.

ANTIMICROBIAL RESISTANCE DIAGNOSTIC CHALLENGE

Rationale — In the United States, antibiotic-resistant bacteria cause at least 2.8 million infections and more than 35,000 deaths each year.[3] Bacterial, fungal, and viral co-infections contribute to COVID-19 morbidity and mortality. Rapid POC solutions that determine AMR in human samples will help combat the development and spread of drug-resistant bacteria.[1]

Table 1 presents the five technologies and finalists for the $20 million Diagnostic Challenge prize, the largest ever offered by the United States government. Competitors are creating innovative and novel diagnostics that identify and characterize antibiotic resistant bacteria and/or distinguish between viral and bacterial infections to reduce unnecessary use of antibiotics, a major cause of antibiotic resistance.

Vision — The AMR Challenge is a joint effort between NIH and the Health and Human Services Office of the Assistant Secretary for Preparedness and Response (ASPR) in support of

the National Action Plan for Combating Antibiotic Resistant Bacteria.[4] NIH's National Institute of Allergy and Infectious Diseases (NIAID) and ASPR's BARDA are each contributing $10 million to the prize.

The vision is that with real-time detection, healthcare providers will be able to identify infecting pathogens and resistance factors quickly, perhaps in as little as one hour, rather than days, and use the knowledge to tailor treatment for each individual patient. In one study of critically ill COVID-19 patients, 50% who died had a dangerous secondary infection.[5]

The Diagnostic Challenge supports the POCT theme of the House Energy and Commerce Committee and the Senate Homeland Security and Government Affairs Committee letter dated June 9, 2015, and sent to the Comptroller General of the US Governmental Accountability Office.[6] Congress wants to improve the cost-effectiveness of American healthcare by helping providers to rapidly target antimicrobial therapy for improved patient outcomes.

The NIH-BARDA Challenge differs from "DISARM" (Developing an Innovative Strategy for AMR), a legislative initiative to fund targeted antibiotics in hospitals,[7] and from the "AMR Challenge," a long-term program administered by the Centers for Disease Control and Prevention (CDC) to accelerate the fight against AMR.[8] This effort has recruited more than 350 organizations across the globe committed to slow AMR.

Timeline — The deadline for submission of Step 1 concepts was January 9, 2017. Semifinalists each received $50,000. Step 2 was open to semifinalists and others who wished to enter the competition anew. Finalists were announced in December, 2018, and each received $100,000 to develop prototypes. Now, the competition is in the third and final Step 3 with planned culmination July 31, 2020.[1] The remaining prize pool is about $19,000,000.

Winners must have tested *in vitro* diagnostic prototypes using two independent Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories to assess the performance of their assays compared to the performance claims for Food and Drug Administration (FDA)-approved assays. Two of the Challenge finalists recently presented their research in a panel moderated by the author at the Tri•Con Cambridge Healthtech Symposium held in San Francisco, March 3rd, just prior to lockdown and safe spacing (social distancing) orders in that city.

Obviously, not all five finalists can win, but the United States and other countries, especially settings in limited-resource countries will benefit because of the anticipated success of numerous new inventions spinning off from all the three steps and some emerging successfully from the commercial pipeline. Please see the Challenge "FAQ" webpage for additional details of Step 3 and the process of evaluating the technologies.[9]

NOMENCLATURE

GEOSPATIAL SCIENCE

Geospatial science identifies and leverages the power of location data.[10] Location data embody a geographic dimension. Location intelligence is the process of turning geographic data into insights for decision making. POCT is pivotal to quick decision making, triage, and quarantine. A spatial care path is the most efficient route taken by the patient when receiving definitive care in a small-world network. A geospatial care path adds geographic and topographic coordinates, physical sites, and quantitative metrics to the healthcare small-world network.

HOTSPOTS

A "hotspot" is a topographic area or region of unusual danger to personal or public health. People in the community may be at extreme risk during an outbreak of a highly infectious disease that spreads quickly, as we are witnessing with the COVID-19 pandemic. Additionally, dangerous situations, such as civil strife and war, belligerent political attitudes, and hacking of computers used in vaccine research and development, may complicate the control of hotspots, rendering them even more difficult to address medically, quell socially, and stop quickly.

POINT-OF-CARE TESTING

Point-of-care testing, defined as diagnostic testing at or near the site of patient care, is *inherently spatial*, that is, performed at points of need, and also *intrinsically temporal*, because it produces fast actionable results. This definition does not depend on the size or format of the handheld, portable, or transportable instrument, test module (e.g., for a smartphone), or assay design. POCT encompasses near-patient testing (NPT), rapid diagnostic tests (RDT, e.g., lateral flow), disposable test strips, and *in situ*, *ex vivo*, *in vivo,* and *on vivo* monitoring (e.g., pulse oximeters, wearables, and remote temperature monitoring).

The "Cape Cod" group codified this definition,[11] which first appeared in standard dictionaries of the English language years ago. The Point-of-care Testing Center for Teaching and Research (POCT•CTR) wrote the original Wikipedia article.[12] Historical terms include alternate site testing, testing outside the clinical laboratory, point-of-need testing, rapid diagnostic test, and others, now mostly abandoned in favor of the simplified concept above that professionals, laypersons, and politicians alike recognize, especially now during the pandemic when POC strategies have moved to the front line of medical rapid response in communities throughout the world.

CORONAVIRUS

Disease nomenclature enables analysis of prevention, spread, transmissibility, severity, and treatment. Virus names based on genetic structure facilitate development of diagnostic tests, vaccines, and medicines. SARS-CoV-2 is the virus, and COVID-19, the disease it causes. The virus name reflects genetic relationship with the Coronavirus responsible for the SARS outbreak in 2003. However, the two viruses differ in symptoms, signs, transmission, and severity. For example, the estimated mortality rates are 10% for SARS and 0.25-3% for COVID-19, while COVID-19 is transmitted more easily due to higher viral load in the nose and throat shortly after symptoms develop.

The World Health Organization (WHO) is responsible for human disease preparedness, response, and nomenclature in the International Classification of Diseases (ICD). China called the outbreak, "Novel Coronavirus Pneumonia," based on its primary clinical manifestation diagnosed initially by chest X-ray and CT scan (e.g., multifocal pneumonia), since neither reliable nor accurate antigen or antibody testing was available in Wuhan, the original epicenter.

METHODS

RESEARCH SCOPE

This article assesses the importance of AMR and its association with POCT. Numerous sources identified through the old and newly designed versions of PubMed dealt with the general areas of AMR. Papers assessed totaled ~500. Chronological and relevance searches were limited to rapid response and POCT in relation to COVID-19 and co-infections.

Only those publications explicitly discussing or integrating POCT or closely related mobile technologies, and conceptually relevant geospatial AMR concepts, obtained in a separate search, are tabulated here. Biothreat agents (e.g., *Bacillus anthracis* and *Yersinia pestis*) and

COVID-19 virus co-infections were excluded.

DATA SOURCES

PubMed, the World-wide Web, and other timely sources were gathered and assessed, along with key updates, papers, chapters, government documents, maps, flowcharts, schematics, and geospatial concepts. EndNote X9.1 (Clarivate Analytics, https://clarivate.com/) consolidated literature entries and automatically retrieved papers as URLs and PDFs placed in groups. The Centers for Disease Control and Prevention (CDC) open access report, *Antibiotic Resistance Threats in the United States, 2019*,[3] provided relevant AMR data and illustrations.

CONFOUNDING RISKS

The rapidly growing COVID-19 literature was analyzed for infectious disease risk factors. Geospatial science articles were assessed previously for geospatial relevance to POCT and closely related mobile technologies.[13] Molecular diagnostics for highly infectious threats can be found in a recent book chapter[14] and comprehensive analysis published elsewhere.[15,16] Point-of-care strategies for defeating the COVID-19 pandemic were summarized in a recent open access paper accepted by this journal and posted early release online April 13, 2020.[17]

THREATS, THEIR IMPACT, AND POCT

Table 2 summarizes the recent outcomes of the so-called "superbugs" that threaten to kill untold numbers of people in the next thirty years, because the current antibiotic armamentarium will become increasingly ineffective and irrelevant to survival.[3,18] **Table 3**, drawn from the CDC scheme of priorities[3] categorizes the resistant organisms as urgent, serious, concerning, and emerging. The CDC criteria used to assign threat levels comprise clinical impact, economic impact (when available), incidence, 10-year projection of incidence (new infections over the next

10 years), transmissibility (how easily a germ spreads or causes infections), availability of effective antibiotics, and barriers to prevention.

Experts say the antibiotic era has already come to a close. The facts presented here support that view and document the shocking extent of the problem. The Center for Disease Dynamics, Economics, and Policy generated interactive AMR maps and bar graphs that illustrate the geospatial distribution of AMR.[19] **Table 4** presents the economic impact and lives lost from the top ten threats identified by the CDC.[3] It is easy to understand the motivation of the Congressional Committees when they singled out AMR as one of the worst enemies to public welfare in the United States. The solution recommended is rapid multiplex POCT.

Now that rapid diagnostic testing has risen to the forefront of strategies for fighting the COVID-19 pandemic,[17] several experienced academic laboratories and commercial entrepreneurs should be able to more easily generate POC solutions for AMR. Government and private support, such as the new so-called "Manhattan Project for COVID-19,"[20] target and promote the most promising of the technologies, and also consider co-infections, which extend intensive care unit (ICU) stays, saturate critical care resources, and lead to excess mortality.

POINT-OF-CARE SOLUTIONS

Table 5 summarizes published POC strategies and concepts[21-54] underlying rapid response testing, detection of AMR, and susceptibility assessment. A multitude of technical approaches and creative POC solutions have been introduced by academic and commercial inventors,[21-54] including novel innovations for determining phenotypic antibiotic susceptibility, distinguishing bacterial from viral infections, and differentiating ordinary influenza from Coronavirus infectious disease-2019 (COVID-19).[17] Gonzalez et al.[32] published a very positive

assessment of rapid diagnostic testing for children, including group A *Streptococcus*, respiratory viruses, and syndromic multiplex respiratory panels. **Table 5** concludes with a synopsis of the Symposium and AMR panel discussion held in San Francisco in March 2020.[53,54]

Point-of-care solutions comprise small portable testing platforms, smartphone modules, molecular diagnostics, biosensors, mass measurements, single cell biometric analysis, and several others.[21-54] Variety seems to be the most prominent and creative characteristic of work completed to date. With a few exceptions, such as MRSA (methicillin-resistant *Staphylococcus aureus*),[50] tuberculosis (discussed later), and importantly, pediatric infectious diseases,[32] widespread commercial reduction to practice (building beyond conception) in practical and environmentally robust formats that one might find implemented for onsite diagnosis in clinics, emergency rooms, and hospitals has yet to fully appear. Nonetheless, some reports, such as Hubner et al.[24] point to the cost-effectiveness of POC AMR detection.

Table 6 lists POC projects, concepts, and solutions for ARM testing intended for sexually transmitted diseases (STD),[55-72] while **Tables 7** covers tuberculosis (TB)[73-81] and **Table 8**, urinary tract infections (UTI).[82-96] It is no surprise that progress is substantial for STDs[55-72] in view of societal impact, reasonable for TB[73-81] considering the deployment of relatively rapid Cepheid instrument and other solutions badly needed in Africa, and promising for UTIs and associated phenotypic antibiotic susceptibility testing, [82-96] because of the relatively straightforward urine sample matrix and ease of analysis compared to whole blood and other specimen types.

Regarding STDs, Fingerhuth *et al.*[60] arrived at a very interesting and perhaps unique conclusion about POCT — "POC with high sensitivity to detect AMR can keep gonorrhea

treatable longer than culture or NAAT (nucleic acid amplification tests). POC tests without reliable resistance detection should not be introduced, because they can accelerate the spread of antibiotic-resistant gonorrhoea." In a limited resource setting, Verwijs et al.[70] found targeted POCT improved performance of WISH (a health interview for women) and excelled relative to WHO-recommended syndromic algorithms.

Shafiee et al.[57] and Duarte et al.[59] addressed POCT for HIV and concluded that more POC CD4(+) T lymphocyte count, viral load, and resistance testing is necessary to offset the increasing infectiveness of antiretroviral regimens. Rhee et al.[56] observed that in the context of a public health approach to antiviral drugs for HIV, a reliable POC genotypic resistance test could identify which patients should receive standard first-line therapy and which should receive protease-inhibitor-containing regimens.

Some **Table 6** entries directly or indirectly reflect past or ongoing work of NIH-BARDA finalists (see **Table 1**). They include the projects of Tsalik et al.,[31,37] Affinity Biosensors,[47] and Klaris Diagnostics[49] in **Table 5**; multiplex solid-phase melt curve analysis for POC detection of HIV-1 by Clutter at al.[66] in **Table 6**; and uropathogen identification and phenotypic antimicrobial susceptibility testing by the Gau group (Altobelli et al.,[87] Chen et al.,[88,89] Liu et al.,[90] Lu et al.,[91] Mach et al.,[92] and Pan et al.[93]) in **Table 8.** Of course, the majority of the Diagnostic Challenge predated COVID-19 and even earlier, Cooke et al.[97] predicted the need for POCT for antibacterial use in respiratory tract infections.

Details published may not correspond to the methods or sophistication of finalists' emerging technologies (see **Table 1**). While the author was on the NIH-BARDA Step 1 and Step 2 review panels, competing inventions are strictly confidential, and only publically accessible

information is provided here. One must wait until the prize is awarded to see the exciting POC solutions that the Diagnostic Challenge inspired!

Meanwhile, Yang and Rothman[98] have explained the uses, limitations, and future applications of rapid diagnostics for infectious diseases in acute care settings. The POCT•CTR and six other chapter author teams in *Global Point of Care*[99] cover POCT for infectious diseases in disasters, emergencies, and public health resilience. Hays et al.[38] propose "mix and match" (see **Table 5**) designed to encourage the implementation of rapid infectious disease and AMR POCT in transnational medical environments for use in the fight against increasing antimicrobial resistance. Several authors recognize the importance of stewardship and surveillance, which go hand-in-hand with AMR POCT.

GEOSPATIAL SPREAD OF ANTIMICROBIAL RESISTANCE

The speed of worldwide dissemination of SARS-CoV-2, lack of preparation for such a crisis, and absence of planning for adequate diagnostic testing[17] has taught us that the same mistakes should not be made with AMR. While AMR is evolving on an apparently slower and less perceptible, but inexorable timetable, the problem has spread in patches and in nationally confluent regions across the globe, as the reader can see from the interactive maps cited earlier.[19] The Diagnostic Challenge is stimulating awareness, research, and development of rapid POC diagnosis. Examples of the macro- and microspatial geographic chases that have begun follow.

Figure 1 illustrates the global geospatial distribution of *Candida auris*.[3] The CDC ranks this fungus as an "urgent" threat (the gravest category), because a) it is multidrug-resistant, with some strains resistant to all three available classes of antifungals, b) it can cause significant outbreaks in healthcare facilities, c) some common healthcare disinfectants are less effective at

eliminating it, and d) it can be carried on patients' skin without causing infection, allowing spread to others.[3] In fact, a remarkable feature is the way it has become a global problem, because of its ability to spread easily between people, including patients in long-term care facilities, similar to the sites of highly vulnerable patients hit severely by COVID-19.

C. auris represents an example of global macrospatial translocation (see **Figure 1**) of a threat with almost no therapeutic defense. A recent review[100] of POCT for *Candida* species concluded, "Despite considerable advances for candidiasis detection, the development of simple, compact and portable POC diagnostics (including lab on a chip devices) for rapid and precise testing that automatically performs cell lysis, nucleic acid extraction, purification, and detection still remains a challenge." On the other hand, there is a promising nano-gold immunodiagnostic assay for rapid on-site detection of invasive *Aspergillosis*, even in resource-limited settings,[101] important because of the unusual geospatial distribution and antifungal pressure producing local hotspots of azole resistance[102] that disseminate from farm and field through healthcare small-world networks.

The spread of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* represents an example of community microspatial translocation (**Figure 2**). The CDC ranks this threat as serious, justified by adverse outcomes—197,400 hospitalized in one year, 9,100 deaths, and $1.2 billion in healthcare cost.[3] Community-associated infections constitute 47% of the total, while community onset with recent healthcare exposure, long-term care facility onset, and hospital onset are 34%, 14%, and 5%, respectively. Fortunately, there is at least some progress in POC assays for antibiotic sensitivity for this superbug. See Huang et al.[23] regarding rapid electrochemical detection, and Lee et al.[26] for a microfluidic device in **Table 5**.

While it now is somewhat dated, the 2015 WHO report, "Worldwide country situation analysis: response to antimicrobial resistance,"[103] provides additional insight into geospatial dissemination of AMR, progress and plans to stop it, and means of surveillance for bacteria, as well as TB, malaria, influenza, and HIV. One serious problem identified was the widespread practice of broad-spectrum antimicrobial administration without prescription, which is rampant geographically in the Americas, Southeast Asia, and the Western Pacific. Geospatial care paths in these regions may lack microbiology or sensitivity testing capabilities.

The report also stated (p. 14): "Only one of the African countries that responded reported having a national plan, whereas having a comprehensive, funded national plan is one of the best ways to control antimicrobial resistance." Other limited-resource regions had the same deficiency. Additionally (p. 37), "…in many (countries) poor laboratory capacity, infrastructure and data management prevented effective surveillance." "The sale of antimicrobial medicines without prescription was widespread, and many countries lacked standard treatment guidelines for healthcare workers." There was no strategic plan for POC AMR testing.

In 2017, the WHO identified pathogens for which new effective antibiotics are badly needed.[104] The three deemed most critical were carbapenem-resistant *Acinetobacter baumanii*, 3rd generation cephalosporin-resistant *Enterobacteriacea*e (CRE), and *Pseudomonas aeruginosa*. In 2019, the CDC categorized the first two as urgent and the third, serious.[3] *Acinetobacter* threatens the microspatial environment of hospitalized patients, because it contaminates facility surfaces and shared medical equipment. Patients who require devices (e.g., catheters) and those taking long courses of antibiotics are most at risk for CRE infections. *Pseudomonas* was associated with a hotspot in Mexico where in 2018 twenty surgery patients subsequently brought the pathogen to several states in the U.S. Unfortunately, POC methods for these pathogens have

yet to be fully explored and should be integrated into worldwide surveillance and efforts to enhance awareness of AMR.[105]

Of the 21 threats on the CDC list (see **Table 3**), additional AMR papers (not associated with POCT) that addressed geospatial distribution included analysis of *S. pneumoniae* serotypes in the United states,[106] *Salmonella* hotspots in the Democratic Republic of the Congo,[107] and febrile illness in Asia.[108] The authors of the Asia study summed up the problem nicely: "More investment in developing accurate and affordable diagnostic tests for rural Asia and their independent evaluation are needed. Enhanced AMR surveillance and openly accessible databases of geography-specific AMR data would inform policy on empirical and specific therapy. More investment in innovative strategies facilitating infectious disease surveillance in remote rural communities would be an important component of poverty reduction and improving public health."[108]

CO-INFECTIONS AND AMR RISK FOR COVID-19 PATIENTS

Table 9 summarizes co-infections in COVID-19 patients. Wuhan investigators noted that the antibiotic use rate of 49-100% intended for infections was greater in COVID-19 patients than the reported incidence of infections.[5] In their experience, infection control protocols aimed to prevent the transmission and cross infection of SARS-CoV-2 among patients, and not necessarily to prevent bacterial or fungal secondary infection. They observed secondary infection in 15% of COVID-19 patients overall and 1% of survivors, but notably, 50% of non-survivors.[5,109]

In the Wuhan patient population studied by Zhou et al.,[109] 100% (54/54) of non-survivors had sepsis, and 70% had septic shock. Others had cardiovascular risk factors. The median time from start of illness to death or discharge was 18.5 days in non-survivors and 22.0 days in

survivors, roughly matching the duration of viral shedding. Co-infections increase the burden on ICU resources, while prolonged viral shedding increases risks to both clinical and laboratory staff. Co-infections also included other viruses.

One can hypothesize that bacterial, fungal, and viral co-infections contribute substantially to adverse outcomes in COVID-19 patients, and next, predict that POC solutions, such as those in **Tables 5-8**, would benefit not only patients, but also personnel, biosafety, and workflow. According to Chinese investigators (see **Table 9**), conventional microbiological work-up of specimens from COVID-19 patients incurs significant risk of aerosols and contact transmission for healthcare providers, for whom personal protective equipment has been in short supply.

In Wuhan and elsewhere in China, these and other considerations initially led to omission of pathogen workup in the laboratory.[5,109] Additionally, convention workup of fungal infections, such as *Candida auris,* typically is prolonged, possibly longer to result than the documented median ICU stay. *C. auris* is a common hospital-acquired fungal infection in ICUs and one of the most resistant pathogens.[110] Workup of *Candida* species and their resistance spectrum challenges even the most sophisticated technologies.[111] Besides pulmonary co-infections and ventilator-acquired infections, heart failure, hypertension, acute cardiac injury, and diabetes add to COVID-19 patient risk.[5,109]

Radiological findings in COVID-19 patients comprise multifocal consolidative opacities, septal thickening, and development of a "crazy paving pattern," with the greatest severity of CT findings visible around day 10 after symptom onset.[112] This builds a strong case for research, invention, and design of POC technologies that provide rapid multiplex pathogen and AMR testing to include the spectrum of bacterial and fungal pulmonary threats to COVID-19 patients

documented in **Table 9**. If necessary for personnel safety, this testing could be performed in isolation laboratories, similar to the ones we designed for Ebola virus disease.[14,15,113]

CONCLUSIONS AND FUTURE VISION

The COVID-19 pandemic is proving, like Ebola epidemics in Africa,[15,16] that POC strategies are essential to deal with real-time crises.[17] Subtle AMR pathogens are progressively knocking out therapeutic options. Editorials and expert opinions summarized in **Table 10** warn of incipient acceleration of AMR, because of the widespread use of broad-spectrum antimicrobials for critically ill patients hospitalized during the COVID-19 pandemic.

Evolving knowledge of critically ill COVID-19 patients likely will reveal compounding challenges, that is, details of the deadly synergy of SARS-CoV-2 cytokine storm + bacteria/fungus/virus co-infections (see **Table 9**) + resistance to antimicrobial and antifungal drugs. One can envision using multiplex POCT (**Figure 3**) for rapid diagnoses that decrease ICU burden, fatality rates, and excess mortality. Judging from progress to date, exciting advances in POC technologies will help navigate 21st Century diagnostic challenges and save lives.

POCT has proven potential to target specific pathogens, refine choices for antimicrobials, and avoid indiscriminate use of antibiotic drugs. Point-of-care assays that identify patterns of pathogen resistance tell us how infected individuals spread AMR, where geospatial hotspots are located, and when to deploy preventative resources, especially in limited-resource countries that lack adequate microbiology laboratory resources.

Co-infections present high risks for COVID-19 patients and complicate ICU stays. Shared AMR geospatial “clouds” could help diminish critical care burden during pandemics and

optimize therapeutic options, similar to use of antibiograms in individual hospitals. Multidisciplinary healthcare personnel should learn the principles and practice of POCT, so they can meet these diagnostic testing needs.

If the most dire forecasts prove true, AMR could become the "newdemic"[114] of the 21st Century, signifying a medical crisis out of control, especially in crowded and heavily populated metropolises like New York City, and requiring concerted national and international effort to avoid large medical, economic, and social loses. On the other hand, if POC solutions are developed in time and applied across the economic strata of resource-rich and resource-poor settings and countries, AMR spread might be curtailed.

Primary care physicians write the majority of antibiotic prescriptions. Typically, they feel under pressure to generate them, but lack the tools necessary for rapid differential diagnosis. This dilemma calls for POC strategies that quickly differentiate viral from bacterial causes of fever. If bacterial, then diagnostic tests should immediately identify the pathogen and its AMR profile. Adequate evidence and timely decision making call for multiplex, but affordable POCT. The tables highlight novel POC approaches for UTIs, STDs, and TB. Progress is substantial, but the POC solutions should include phenotypic susceptibility testing where appropriate. Fortunately, Diagnostic Challenge finalists address some of these key issues (see **Table 1**).

Point-of-care instruments often serve as default testing methods in limited-resource community hospitals. Environmental conditions may not be well controlled where diagnostic testing is performed in tiny laboratories, ERs, clinics, and primary care clinics in tropical and harsh settings. Point-of-care diagnostics, reagents, and QC supplies must be environmentally robust, certified for the conditions encountered, and monitored. Environmental stresses, such as

high or low temperature or humidity can cause both false negative and false positive POC test results.[115-119] Environmental stress research is pivotal to national security and to successfully defeating AMR, as well as the COVID-19 pandemic.

POC inventions (see **Tables 5-8**), novel molecular diagnostics,[120] biomarker prediction platforms,[121] and other interesting innovations can bridge resource gaps in limited-resource nations and facilitate physician knowledge of targeted antibiotic therapy, community AMR, and COVID-19 hotspots. The stakes are high. AMR is projected to cause millions of deaths annually and cumulative financial loses in the trillions by 2050.[18,164] These human and economic penalties will sap national budgets, shift more spending to the healthcare sector, elevate inequities if not uniformly distributed, and determine the fate of future civic and public health leaders.

Pharmaceutical companies formed a $1 billion AMR Action Fund for new antibiotics, invested $164 million in early pipeline development, and gathered $500 million for a university consortium.[164] The Disarm Act of 2019 and Pasteur Act of 2020 increase reimbursement.[164] The potential for huge economic losses from AMR organisms justify substantial investments, realistically, multiples of the $20,000,000 Diagnostic Challenge prize. However, the prize is a good starting point to motivate creative diagnostic technologies and badly needed POC geospatial solutions. We will not be free from new threats, as illustrated by the discovery of drug resistant *Neiserria meningitides* now spreading in the United States.[165]

DISCLAIMER

Devices must comply with jurisdictional regulations in specific countries, operator use limitations based on patient conditions, federal and state legal statutes, hospital accreditation requirements, and emergency decrees. Not all POC devices presented are FDA cleared for use in

the United States. FDA polices are in flux. Please check with manufacturers for the current status of diagnostics and POC tests within the relevant national domain of use.

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TABLES

Table 1. Finalist Projects in the NIH/BARDA Antimicrobial Resistance Point-of-Care Testing Grand Challenge

Principal Investigator	Affiliation	Title and Description of Invention
Ken Babcock	Affinity Biosensors	**Ultra-Rapid Infection Confirmation and Phenotypical AST by Microbe Mass Measurement** The test employs a novel microfluidic sensor that weighs individual microbes at high throughput and rapidly detects bacterial growth and response to a panel of the most advanced antibiotics and in key cases, can do so even faster than the microbes an multiply. The test targets blood stream infection and sepsis, acute UTI, and bacterial meningitis, and has been shown to successfully diagnose the most pernicious "superbugs," i.e., those that are resistant to multiple advanced antibiotics and notoriously difficult to diagnose in rapid timeframes. It assesses growth phenotypically and will not be defeated as microbes evolve new resistance mechanisms. It can be extended to additional sources of infection and new antibiotics as developed.
Kyle Fieleke	Klaris Diagnostics	**Single Cell Biometric Analysis for Rapid ID/AST** Conventional antibiotic susceptibility testing requires laboratory processing time that often exceeds 48 hours. Clinicians must treat empirically, often utilizing broad spectrum antibiotics while they await culture results. This triggers a cycle wherein empiric therapy regimens must periodically escalate to next-line antibiotics to account for increasing prevalence of resistance. Rapid ID/AST tests can help thwart this cycle by dramatically shortening the empiric treatment time window. Earlier therapeutic optimization will maintain patient outcomes while also helping to preserve the efficacy of our most potent antibiotics. Additional details appear later in paper.
Vincent Gau	GeneFluidics	**Fully Automated Pathogen ID and AST Directly from Blood and Urine** A compact rapid pathogen ID and AST can address both the unnecessary use and overuse of antibiotics, effectively reducing antibiotic microbial resistance. The goal is to deliver a molecular diagnostic platform that is capable of rapid diagnosis of common bacterial infections in as short as 30 min and profiling their antibiotic resistance in as short as 90 min. UtiMax, a rapid UTI ID/AST test, seeks FDA *de novo* clearance. BsiMax can process whole-blood samples for bloodstream infections with a LOD < 4 CFU/mL. Both UtiMax and BsiMax can be performed by our robotic liquid handling systems, with associated reagent kits and sensor chips. The two can quantify the unique species-specific nucleic acid sequence associated with each target pathogen without using PCR and conduct AST without need of obtaining a clinical isolate or positive blood (urine) culture sample.
Gary Schoolnik	Visby Medical	**Patient-side, Disposable, Molecular PCR Diagnostic Device for *Neisseria gonorrhea* and Drug Resistance Markers**

	(dba Click Diagnostics)	Through miniaturization, optimization, and cost reduction of PCR technology, the team created a fast, inexpensive, single-use (disposable) molecular diagnostic device that integrates and automates sample processing, PCR amplification, and amplicon detection into a simple, easy-to-use test. As the device does not require a separate instrument, it enables moving molecular testing from laboratories and large healthcare facilities to POC locations, such as clinics, retail pharmacies, and the home. Click's first product for detecting the sexually transmitted infections *N. gonorrhoeae, C. trachomatis* and *T. vaginalis* is currently in a combined 510(k)/CLIA-waiver clinical study. A Neisseria gonorrhoeae ciprofloxacin drug-susceptibility test is being added.
Ephraim Tsalik	Predigen	**Host Gene Expression to Classify Viral and Bacterial Infection Using Rapid Multiplex PCR** An alternative diagnostic strategy is to focus on the host's response. The patient responds to bacterial and viral causes of acute respiratory illness in distinct and stereotypic ways. Based on that principle, we offer an innovative solution focusing on the patient's response to infection. By combining machine learning analyses with system-wide gene expression measurements, we have identified host response patterns that distinguish bacterial, viral, and non-infectious etiologies. Supported by the NIH-sponsored Antibacterial Resistance Leadership Group (ARLG), we embarked on a diagnostic development pathway called RADICAL (Rapid Diagnostics in Categorizing Acute Lung Infection), which has validated these host response signatures and supported a collaboration with BioFire Diagnostics. As a result, we have developed a 45-min, sample-to-answer test that uses host gene expression to distinguish bacterial from viral infection.

Abbreviations
AST, antibiotic susceptibility testing; BARDA, Biomedical Advanced Research and Development Authority; CLIA, Clinical Laboratory Improvement Amendments; FDA, Food and Drug Administration; ID, identification; LOD, limit of detection; NIH, National Institutes of Health; PI, Principal Investigator; POC, point-of-care; and PCR, polymerase chain reaction.

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Table 2. The Impact of Worldwide Ubiquitous Antimicrobial Resistant Superbugs

Focus	Impact[3,18]
United States	In the U. S., currently ~2.9 million antibiotic-resistant infections per year
Impact	> 35,000 deaths/year with an additional 12,800 deaths from *C. difficile*
Forecast	By 2050, global deaths/year are projected to be10 million, more than cancer
Pandemics	Like the COVID-19, global travel spreads antibiotic resistance easily
Superbugs	Wanton antibiotic use in animals generates superbugs in food
AMR Threats	Now 5 urgent, 11 serious, 2 new, and a watch list of 3 threats (see **Table 3**)
POC Detection	Early detection will have positive public health and economic outcomes

Abbreviations
AMR, antimicrobial resistant; POC, point-of-care.

Version 3.2
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Table 3. Urgent, Serious, Concerning, and Emerging Antimicrobial Resistant Threats

Priority of Threat[3,19]	**Resistant Organisms**
Urgent (5)	Carbapenem-resistant *Acinetobacter* [interactive AMR map available — see below]
	Candida auris (C. auris)
	Clostridioides difficile (C. difficile)
	Carbapenem-resistant Enterobacteriaceae (CRE) [see AMR map]
	Drug-resistant *Neisseria gonorrhoeae (N. gonorrhoeae)*
Serious (11)	Drug-resistant *Campylobacter*
	Drug-resistant *Candida*
	Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae [see AMR map]
	Vancomycin-resistant *Enterococci* (VRE) [see AMR map]
	Multidrug-resistant *Pseudomonas aeruginosa (P. aeruginosa)* [see AMR map]
	Drug-resistant nontyphoidal *Salmonella*
	Drug-resistant *Salmonella* serotype Typhi [see AMR map]
	Drug-resistant *Shigella*
	Methicillin-resistant *Staphylococcus aureus* (MRSA) [AMR map]
	Drug-resistant *Streptococcus pneumoniae (S. pneumoniae)* [see AMR map]
	Drug-resistant Tuberculosis
Concerning (2)	Erythromycin-resistant group A *Streptococcus*
	Clindamycin-resistant group B *Streptococcus*
Emerging (3)	Azole-resistant *Aspergillus fumigatus (A. fumigatus)*
	Drug-resistant *Mycoplasma genitalium (M. genitalium)*
	Drug-resistant *Bordetella pertussis (B. pertussis)*
Critical new addition to AMR pathogens	Ciprofloxacin-resistant, beta-lactamase-producing *Neiserria meningitides* (*N. meningitides*) serogroup Y reported by CDC in the United States[165]
Geospatial Sites — Interactive World Maps, Trends, and Bar Graphs	Interactive world maps, trends, and bar graphs of AMR are available at source 2. Select the class of antibiotic and then observe which countries have resistant pathogens and learn relative percentages of invasive isolates. Interactive tools also are available for *Escherichia coli* and *Klebsiella pneumonia*, pathogens not categorized by the CDC.

Abbreviations

AMR, antimicrobial resistance; and CDC, Centers for Disease Prevention and Control.

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Table 4. Top 10 Antimicrobial Resistance Threats and Levels by Number of Cases

Pathogen[a]	Threat Level[b]	Number of cases[c]
Drug-resistant *Streptococcus pneumoniae*	Serious	900,000
Drug-resistant *Neisseria gonorrhoeae*	URGENT	550,000
Drug-resistant *Camplylobacter*	Serious	448,400
Methicillin-resistant *Staphylococcus aureus (MRSA)*	Serious	323,700
Clostridiodes difficile	URGENT	223,900
Drug-resistant nontyphoidal *Salmonella*	Serious	212,500
Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriacea*	Serious	197,400
Drug-resistant *Shigella*	Serious	77,000
Vancomycin-resistant *Enterococci* (VRE)	Serious	54,500
Drug-resistant *Candida*	Serious	34,800

Footnotes

a. Source: See reference **3**.
b. URGENT reflects a range of 323 to 550,000 cases. Urgent represents a higher threat level than serious. The range for the serious threat level is 4,100 to 900,000 cases.
c. Data are the most recent year reported by the Centers for Disease Control and Prevention.

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Table 5. Point-of-Care Strategies and Concepts for Antimicrobial Resistance and Antibiotic Susceptibility Testing

AUTHOR, JO. YR., REF.[21-54]	PATHOGEN/ APPLICATION	TITLE SYNOPSIS
Butler CC[21] *Scand J Prim Health Care* 2008	Distinguishing viral from bacterial infection	**General practitioners' perceptions of introducing near-patient testing for common infections into routine primary care: a qualitative study** Several indicated that tests would be useful only for a limited number of patients and they were concerned by time pressures, apparatus maintenance and quality control, cost, and possible objections from patients, especially children.
Kadlec MW[22] *J Lab Auto* 2014	Antimicrobial susceptibility testing by cell phone	**A cell phone-based microphotometric system for rapid antimicrobial susceptibility testing** The authors determine antimicrobial resistance phenotypically. The growth of pathogens in microwell arrays is detected under different antibiotic conditions. Antibiotics can also be precoated in the microwell array to simplify the assay protocol toward point-of-care applications. A low-cost cell phone-based microphotometric system was developed for detecting the bacterial growth in the microwell array.
Huang JM[23] *Anal Chem* 2015	*Enterobacter-iacae resistance*	**Rapid electrochemical detection of New Delhi metallo-beta-lactamase genes to enable point-of-care testing of carbapenem-resistant *Enterobacteriaceae*** The authors report the successful development of an electrochemical biosensor to detect bla(NDM), the gene encoding the emerging New Delhi metallo-beta-lactamase, using label-free electrochemical impedance spectroscopy.

Hubner C[24] *Antimicrob Resist Infect Control* 2015	MRSA hospital screening	**Impact of different diagnostic technologies for MRSA admission screening in hospitals – a decision tree analysis** Results showed rapid test methods (POC or PCR) were always cost-minimizing in comparison to culture methods. Risk-based POC screening showed the highest mean cost savings with 14.98 euro per admission in comparison to no screening. Rapid universal screening methods became favorable at high MRSA prevalence. Early detection of MRSA by rapid POC or PCR technologies and consistent implementation of appropriate hygienic measures lead to high economic efficiency of MRSA management.
Campbell J[25] *Biomed Microdevices* 2016	Antibiotic susceptibility testing	**Microfluidic advances in phenotypic antibiotic susceptibility testing** The field of microfluidics promises several advantages over existing macro-scale methods, including: faster assays, increased multiplexing, smaller volumes, increased portability for potential point-of-care use, higher sensitivity, and rapid detection methods.
Lee WB[26] *Biosens Bioelectron* 2017	Microfluidic antimicrobial susceptibility testing	**A microfluidic device for antimicrobial susceptibility testing based on a broth dilution method** Wild-type (ATCC 29212) and vancomycin-resistant *Enterococcus* cells were incubated at 5 different vancomycin concentrations on-chip, and the sample injection, transport, and mixing processes occurred within 5 reaction chambers and 3 reagent chambers via the chip's automatic dispensation and dilution functions within 9 minutes.
Li Y[27] *SLAS Technol* 2017	Antibiotic susceptibility testing	**Emerging microtechnologies and automated systems for rapid bacterial identification and antibiotic susceptibility testing** The authors summarize and analyze representative emerging micro- and nanotechnologies, as well as automated systems for bacterial ID/AST, including both phenotypic (e.g., microfluidic-based bacterial culture, and digital imaging of single cells) and molecular (e.g., multiplex PCR, hybridization probes, nanoparticles, synthetic biology tools, mass spectrometry, and sequencing technologies) methods. They also discuss representative POC systems that integrate sample processing, fluid handling, and detection for rapid bacterial ID/AST.
Safavieh M[28] *ACS Appl Mater Interfaces* 2017	Whole blood biosensor for antibiotic susceptibility testing	**Rapid real-time antimicrobial susceptibility testing with electrical sensing on plastic microchips with printed electrodes** The authors report a biosensor for rapid (<90 min), real time, and label-free bacteria isolation from whole blood and antibiotic susceptibility testing. Target bacteria are captured on flexible plastic-based microchips with printed electrodes using antibodies (30 min), and its electrical response is monitored in the presence and absence of antibiotics over an hour of incubation time. We evaluated the microchip with *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) as clinical models with ampicillin, ciprofloxacin, erythromycin, daptomycin, gentamicin, and methicillin antibiotics. The technology presented here has the potential to provide precise and rapid bacteria screening and guidance in clinical therapies by identifying the correct antibiotics for pathogens.
Schroder UC[29] J *Biophotonics* 2017	Microbial susceptibility	**On-chip spectroscopic assessment of microbial susceptibility to antibiotics within 3.5 hours** Ciprofloxacin resistant *E. coli* were differentiated from sensitive *E. coli* with high accuracy within roughly 3 hrs total analysis time paving the way for future POC devices. Spectral changes leading to the discrimination between sensitive and resistant bacteria are in excellent agreement with expected metabolic changes in the bacteria due to the mode of action of the drug.
Tamura S[30] *J Microbiol Methods* 2017	LAMP detection of resistance	**Development of a highly resolved loop-mediated isothermal amplification method to detect the N526K ftsI mutation of beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae*** The amplification refractory mutation system-SNP LAMP method is a simple and rapid method for SNP-genotyping of a clinical isolate as POCT technology suitable for resource-limited situations and clinical settings due to its simplicity and convenience.
Tsalik EL[31] **[NIH]** *Clin Infect Dis* 2017	Overview of infectious disease diagnostics and resistance mechanisms	**Advancing diagnostics to address antibacterial resistance: The Diagnostics and Devices Committee of the Antibacterial Resistance Leadership Group** Diagnostics must contend with scores of potential pathogens, dozens of clinical syndromes, emerging pathogens, rapid evolution of existing pathogens, and their associated resistance mechanisms. Therefore, the Antibacterial Resistance Leadership Group has identified diagnostics as 1 of 4 major areas of emphasis, and

		provides an overview highlighting where innovation in study design, content, and execution is advancing the field of infectious diseases diagnostics.
Gonzalez MD[32] *Infect Dis Clin N Am* 2018	Rapid diagnostic assays, syndromic multiplex panels	**New developments in rapid diagnostic testing for children** New diagnostics assays for Group A *Streptococcus*, influenza, and respiratory syncytial virus combine rapid turnaround time with high sensitivity and specificity. Multiplex syndromic panels test for a broad range of pathogens associated with a single clinical presentation. Molecular infectious disease testing is now being developed in easy-to-use platforms, which are available at the point of care.
Leonard H[33] *ACS Sens* 2018	Antimicrobial resistance design factors	**Recent advances in the race to design a rapid diagnostic test for antimicrobial resistance** AST techniques rely on the prediction of antibiotic resistance via extracted bacterial DNA content, while phenotypic determinations typically track physiological changes in cells and/or populations exposed to antibiotics. Regardless of the method used for AST, factors such as cost, scalability, and assay time need to be weighed into their design.
Li B[34] *Anal Bioanal Chem* 2018	Isothermal detection of plamid-mediated resistance	**An enzyme-free homogenous electrochemical assay for sensitive detection of the plasmid-mediated colistin resistance gene mcr-1** Antibiotic resistance associated with the mcr-1 gene of Gram-negative bacteria, which confers resistance to drugs of last resort and has the potential to spread via plasmids, is a pressing issue facing global health today. This enzyme-free, isothermal platform is a rapid, portable, disposable, and sensitive method for detection of plasmid-mediated colistin resistance.
Mitsakakis K[35] *Future Mcobiol* 2018	POC resistance targets	**Challenges in identifying antibiotic resistance targets for point-of-care diagnostics in general practice** Current 'gold standard' antibiotic resistance detection strategies tend to be slow, taking up to 48 h to obtain a result, although the implementation of point-of-care testing by general practitioners could help achieve the goal of targeted antibiotic prescribing practices. However, deciding on which antibiotic resistances to include in a POC diagnostic is not a trivial task.
Roisin S[36] *Eur J Clin Microbiol Infect Dis* 2018	Syndromic respiratory pathogen and resistance detection	**Prospective evaluation of a high multiplexing real-time polymerase chain reaction array for the rapid identification and characterization of bacteria causative of nosocomial pneumonia from clinical specimens: a proof-of-concept study** The authors evaluated the VAPChip "Rapid-Array-PCR-technology," which targets 13 respiratory pathogens and 24 beta-lactam resistance genes directly on respiratory clinical specimens simultaneously. This system seems promising, but extraction needs to be automated. This point-of-care automated platform permits a syndromic approach, the future challenge in the management of infectious diseases.
Tsalik EL[37] **[NIH]** *Ann Rev Med* 2018	Overview of molecular diagnostics for antibacterial resistance	**New molecular diagnostic approaches to bacterial infections and antibacterial resistance** Recent advances have given rise to host- and pathogen-centered diagnostic approaches. Most focus on pathogen detection and characterization. Host-focused diagnostics have recently emerged and are based on detecting the activation of biological pathways that are highly specific to the type of infecting pathogen (e.g., viral, bacterial, protozoan, fungal). Although progress is encouraging, it is unlikely that any single diagnostic platform will fully address needs for fast actionable data.
Hays FP[38] *Eur J Clin Microbiol Infect Dis* 2019	"Mix-and-match" for POCT for infectious diseases and resistance	**The successful uptake and sustainability of rapid infectious disease and antimicrobial resistance point-of-care testing requires a complex 'mix-and-match' implementation package** Mix-and-match is designed to encourage implementation of rapid infectious disease and antimicrobial resistance POCT in transnational medical environments for use in the fight against increasing antimicrobial resistance and is defined as a mixture of recommendations individually chosen to best match the needs of healthcare providers, technology innovators, and the general public, whilst helping to ensure the sustainability of rapid infectious disease and antimicrobial resistance testing.
Inglis TJ[39] *J Med Microbiol* 2019	Advocating for same-day antimicrobial resistance testing results in sepsis	**Rapid antimicrobial susceptibility tests for sepsis; the road ahead** Early adopters in well-equipped teaching centres in close proximity to large clinical laboratories are likely to be early beneficiaries of rapid AST. Simplified and lower-cost technology is needed to support poorly resourced hospitals in developing countries, with their higher burden of AMR. For a specific, same-day diagnosis underpinned by definitive AST results, advocate more effectively for the clinical

		benefits of bacterial detection and susceptibility testing at critical decision points in the sepsis management.
Kang W[40] *Anal Chem* 2019	Phenotypic drop-based MICs for POCT	**Ultrafast parallelized microfluidic platform for antimicrobial susceptibility testing of Gram positive and negative bacteria** Six concentrations of bactericidal and bacteriostatic antibiotics (oxacillin and tetracycline, respectively) were tested to determine the MIC of strains as well as the heterogeneity in growth profiles of bacteria at single cell resolution. Those MICs from phenotypic analysis in droplets matched the MICs obtained from broth microdilution method for all strains. Advantages of the proposed droplet-based AST include rapid drug sensitivity response, morphological analysis, and heterogeneity in antibiotic-resistance profile. It is an excellent alternative to standard phenotypic AST with potential applications in clinical diagnostics and POCT.
Li H[41] *SLAS Technol* 2019	Limiting the spread of drug-resistant bacteria	**Rapid single-cell microbiological analysis: Toward precision management of infections and dysbiosis** Need for new antibiotics has outpaced the development of new classes of antibiotics (only two new classes of antibiotics have reached the market in the last 20 years), in large part due to prohibitive cost and historically poor return on investment to develop new antibiotics. Consequently, clinicians have limited treatment options, particularly in the neediest patients. To tackle this major global health issue, the authors are developing novel technological approaches for rapid definitive clinical microbiological analysis. These technologies will improve the clinical management of bacterial infections and reduce the improper use of antibiotics in current practice, hopefully limiting the spread of drug-resistant organisms.
Malpartida-Cardenaas K[42] *Biosens Bioelectron* 2019	*P. falciparum* (Malaria) resistance	**Quantitative and rapid Plasmodium falciparum malaria diagnosis and artemisinin-resistance detection using a CMOS Lab-on-Chip platform** The authors describe quantitative fully-electronic detection of *Plasmodium falciparum* using a Lab-on-Chip platform and demonstrate on-chip detection of C580Y, the most prevalent single-nucleotide polymorphism associated to artemisinin-resistant malaria. Real-time non-optical DNA sensing is facilitated using Ion-Sensitive Field-Effect Transistors, fabricated in unmodified complementary metal-oxide-semiconductor technology, coupled with loop-mediated isothermal amplification. They cite potential for a fully portable malaria diagnostic used as POCT.
Parkes-Ratanshi R[43] *Int J STD AIDS* 2019	Survey in Uganda — barriers to use of POCT	**Point-of-care diagnostics: needs of African health care workers and their role combating global antimicrobial resistance** Of 555 participants answering the survey (7.3% response rate), 62% completed, 91% were from Uganda and 50.3% were male. The most commonly-used POCTs were pregnancy tests (74%), urine dipstick (71%), syphilis rapid test (66%), and Gram stain (41%). The majority (74%) practiced syndromic diagnosis for sexually transmitted infections/HIV. Lack of availability of POCTs, increased patient wait time, and lack of training were the leading barriers for POCT use. Increasing POCT availability and training could improve uptake of POCTs for sexually transmitted infections in Africa, decrease syndromic management, and possibly impact antimicrobial resistance.
Shi Y[44] *Talanta* 2019	Test strip for *S. aureus* antibiotic resistance	**Antibiotic-affinity chromatographic test strip for quantitative analysis and antibiotic resistance testing of *Staphylococcus aureus*** Results for penicillin, daptomycin, gentamicin, cefoxitin and clindamycin against *S. aureus* showed agreement with those of traditional broth dilution method. Procedures for bacterial analysis and resistance require 20 and 110 min, respectively. The antibiotic-affinity chromatographic test strip showed promise in POCT because of its portability and rapidity.
Shin D[45] *Ann Rev Anal Chem (Palo Alto CA)* 2019	Rapid pathogen identification and susceptibility testing	**Emerging analytical techniques for rapid pathogen identification and susceptibility testing** Progress in microfluidics and nucleic acid amplification is pushing the boundaries of timescale for diagnosing bacterial infections. An integrative perspective is needed to understand the significance of these developments. The authors examine the scope of new developments in assay technologies grouped by key enabling domains of research.
Song D[46] *Molecules* 2019	Slide imaging susceptibility testing	**Whole slide imaging for high-throughput sensing antibiotic resistance at single-bacterium level and its application to rapid antibiotic susceptibility testing** The authors claim their method prevails over other imaging-based AST approaches in allowing rapid and accurate determination of antibiotic susceptibility for

		phenotypically heterogeneous samples, in which the number of antibiotic resistant cells was negligible compared to that of the susceptible cells. Hence, the method shows promise for both rapid AST and POCT of complex clinical bacteria isolates.
Affinity Biosensors[47] **[NIH] [Babcock K]** 2020	Resonant mass measurement for antibiotic sensitivity	***Ultra-rapid infection confirmation and phenotypical AST by microbe mass measurement*** LifeScale counts and measures the mass of individual microbes as they pass through the sensor of the instrument with an accuracy of better than 1% while at the same time keeping track of the volume of sample consumed, and is well suited to measure the growth and response of microbial populations to external stimuli, such as antibiotics. The authors target Gram negative rods from positive blood culture and phenotypic antibiotic sensitivity testing.
He PJW[48] *Biosens Bioelectron* 2020	*E. coli*	**Laser-patterned paper-based sensors for rapid point-of-care detection and antibiotic-resistance testing of bacterial infections** The authors designed laser-patterned paper-based devices for detection and susceptibility testing of Escherichia coli, via a simple visually observable color change. The results indicate suitability for timely identification of bacterial infections at the POC and usefulness in providing a beneficial pathway for accurate antibiotic prescribing and a novel route to tackling the global challenge of AMR.
Klaris Diagnostics[49] **[NIH] [Fielekel K]** 2020	Digital culture, pathogen identification, and antibiotic susceptibility testing	**Single Cell Biometric Analysis for Rapid ID/AST** Pattern's Digital Culture technology delivers pathogen identification (ID) and antibiotic susceptibility test (AST) results within 4 hours. Single-cell analysis shortens test time by measuring the live response of each pathogenic cell directly, bypassing time-consuming culture. Digital Culture technology enables testing directly from non- sterile specimens. Artificial intelligence and deep learning enable recasting of assay development as a software problem. Deep learning deciphers how living cells respond and produces millions of measurements per run. Phenotypic pattern recognition covers every resistance mechanism: known, unknown, existing, emerging, simple, complex, genetic, or epigenetic. [web page promotion]
Mizusawa M[50] *Expert Rev Anti Infect Ther* 2020	MRSA rapid identification and susceptibility testing	**Novel strategies for rapid identification and susceptibility testing of MRSA** Currently there are many diagnostic options for the detection of MRSA in surveillance and clinical samples. These are highly accurate and have resulted in improvements in targeted management and reduction in hospital and ICU length of stay for both MSSA and MRSA. Impact on mortality has been variable. Promising novel technologies will not only accurately identify pathogens and detect their resistance markers but will allow discovery of virulence determinants that might further affect patient management.
Opalski AS[51] *Micromachines (Basel)* 2020	Antibiotic susceptibility testing	**Combinatorial antimicrobial susceptibility testing enabled by non-contact printing** The authors demonstrate the utility of non-contact printing to fabricate the "mAST," an easy-to-operate, microwell-based microfluidic device for combinatorial antibiotic susceptibility testing in a POC format. Wells are prefilled with antibiotics in any desired concentration and combination by non-contact printing (spotting).
Wang R[52] *Anal Chem* 2020	Antibiotic susceptibility testing	**cAST: Capillary-based platform for real-time phenotypic antimicrobial susceptibility testing** Antimicrobial resistance affects nearly 2 million people a year in the US alone and places an estimated $20 billion burden on the healthcare system. Cast delivers an expedited time-to-readout by means of optical assessment of bacteria incubated in a small capillary with a resazurin dye within 4-8 hours.
Kost GJ[53,54] Tri•Con Symposium San Francisco 2020	NIH-BARDA Grand Challenge Panel — Summary of POC AMR progress to date	**Geospatial "hot spots" in need of rapid point-of-care diagnostics for highly infectious threats and antimicrobial resistance**[53] The speaker developed a framework for deploying novel POC technologies that detect antimicrobial resistance. Hot spots occur across world locations no longer limited geospatially. We can integrate geoscience tools and POCT to quickly, directly, and efficiently detect microbial and viral threats. Spatial patterns of resistance will allow us to target therapy cost-effectively. **Addressing antimicrobial resistance through public-private partnerships and the NIH-BARDA Grand Challenge**[54] Antimicrobial resistance represents a growing public health concern, leading to BARDA and the NIH working with private companies to develop novel diagnostics. The NIH-BARDA Grand Challenge has charged participants with developing innovative and novel rapid diagnostic tests to identify resistant bacteria or to distinguish between viral and bacterial infections to reduce over- prescription of

		antibiotics. Two of the five finalists presented their work and the challenges their technologies address, as well as a company collaborating directly with BARDA to advance and commercialize their assay.

Abbreviations

AMR, antimicrobial resistance; AST, antibiotic susceptibility testing; BARDA, Biomedical Advanced Research and Development Authority; ID, identification; LAMP, loop-mediated isothermal amplification; MRSA, methicillin-resistant *Staphylococcus aureus*; NIH, National Institutes of Health; POC, point-of-care; POCT, point-of-care testing; and SNP, single-nucleotide polymorphism.

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Table 6. Point-of-Care Diagnostics and Strategies for Antimicrobial Resistance and Antibiotic Susceptibility Testing — Sexually Transmitted Diseases

AUTHOR, JO. YR., REF.[55-72]	PATHOGEN/ APPLICATION	TITLE SYNOPSIS
Sadiq ST[55] *Sex Transm Infect* 2010	"Gonococcal epidemiology"	**Point-of-care antibiotic susceptibility testing for gonorrhoea: improving therapeutic options and sparing the use of cephalosporin's** With the emergence of rapid diagnostic technologies for STIs and other infections, POC antimicrobial susceptibility testing might soon be ready for gonococcal epidemiology, depending on: current knowledge of the molecular basis of gonococcal resistance; availability of molecular diagnostic platforms supporting detection of multiple molecular resistance markers; and validation of these assays.
Rhee SY[56] *PloS* ONE 2015	HIV POC genotype resistance	**HIV-1 drug resistance mutations: Potential applications for point-of-care genotypic resistance testing** A POC genotypic resistance test is likely to involve the use of allele-specific point mutation assays for detecting drug-resistance mutations (DRMs). This study proposes that two major nucleoside reverse transcriptase inhibitor (NRTI)-associated DRMs (M184V and K65R) and four major NNRTI-associated DRMs (K103N, Y181C, G190A, and V106M) would be the most useful for POC genotypic resistance testing.
Shafiee H[57] *Ann Rev Med* 2015	Review of POC HIV testing technologies	**Emerging technologies for point-of-care management of HIV infection** Diagnostics is critical for HIV prevention, screening and disease staging, and monitoring antiretroviral therapy. Available diagnostic assays, polymerase chain reaction, enzyme-linked immunosorbent assay, and western blot, are complex, expensive, and time consuming and ill suited for use in low- and middle-income countries. Therefore, innovative, inexpensive, disposable, and rapid POC diagnostic technologies are urgently needed.
Dona V[58] *Expert Rev Mol Diagn* 2017	Antimicrobial resistance in *Neisseria gonorrhoeae*	**Recent advances in the development and use of molecular tests to predict antimicrobial resistance in *Neisseria gonorrhoeae*** The choice of molecular assay needs to consider the assay target, quality controls, sample types, limitations intrinsic to molecular technologies, and specific to the chosen methodology, and the intended use of the test. Improved molecular- and particularly genome-sequencing-based methods will supplement AMR testing for surveillance purposes, and translate into POC tests that will lead to personalized treatments, while sparing the last available empiric treatment option (ceftriaxone). However, genetic AMR prediction will never completely replace phenotypic AMR testing, which detects also AMR due to unknown AMR determinants.
Duarte HA[59] *J Infect Dis* 2017	Analysis of drug resistance status for HIV therapy	**Current status of point-of-care testing for human immunodeficiency virus drug resistance** Given that HIV drug resistance mutations have historically developed to antiviral regimens, resistance will likely continue to diminish the long-term success of therapy. Accessible testing can enable an evidence-based approach to medical care. With further development, POCT will improve clinical outcomes.
Fingerhuth SM[60] *BMC Medicine* 2017	Mathematical modeling	**Detection of antibiotic resistance is essential for gonorrhea point-of-care testing: a mathematical modelling study**

		POCT with high sensitivity to detect antibiotic resistance can keep gonorrhoea treatable longer than culture or nucleic acid amplification testing. POCT without reliable detection should not be introduced, because they can accelerate the spread of antibiotic-resistant gonorrhea.
Sadiq ST[61] *Sex Transm Infect* 2017	Combined diagnostics and antimicrobial resistance testing for sexually transmitted illnesses	**Rapid accurate point-of-care tests combining diagnostics and antimicrobial resistance prediction for *Neisseria gonorrhoeae* and *Mycoplasma genitalium*** Spread of *N. gonorrhoeae* and *M. genitalium* is compounded by nucleic acid amplification techniques for diagnosis, resulting in reduced phenotypic AMR testing for *N. gonorrhoeae* and absence or suboptimal AMR surveillance for guiding treatment of both STIs. Combined rapid POC diagnostic and AMR testing will combat STI burden and AMR by enabling diagnosis and treatment at the first healthcare visit, potentially reducing selection pressure on recommended antimicrobials, reducing transmission of resistant strains, and providing means for AMR surveillance.
Tuite AR[62] *J Infect Dis* 2017	Math modeling of POC susceptibility testing effect on Gonorrhea resistance	**Impact of rapid susceptibility testing and antibiotic selection strategy on the emergence and spread of antibiotic resistance in gonorrhea** Continued empiric treatment without POC testing was projected to result in >5% of isolates being resistant to combination therapy within 15 years. Use of either POC test in 10% of identified cases delayed this by 5 years. Rapid diagnostics reporting antibiotic susceptibility may extend the usefulness of existing antibiotics for gonorrhea treatment, but ongoing monitoring of resistance patterns will be critical.
Turner KM[63] *BMJ Open* 2017	*N. gonorrhoeae* resistance test	**Analysis of the potential for point-of-care test to enable individualised treatment of infections caused by antimicrobial-resistant and susceptible strains of *Neisseria gonorrhoeae*: a modelling study** An estimated 33,431 ceftriaxone treatments are administered annually and 792 gonococcal infections remain untreated due to loss to follow-up in trhe UK. The use of an AMR POCT for ciprofloxacin could reduce these ceftriaxone treatments by 66%, and for an AMR POCT for penicillin by 79%. POCT could extend the useful life of dual ceftriaxone and azithromycin therapy, thus pushing back the time when gonorrhoea may become untreatable.
Khazaei T[64] *Sci Rep* 2018	RNAS markers for phenotypic antibiotic susceptibility testing of *N. gonorrhoeae*	**RNA markers enable phenotypic test of antibiotic susceptibility in *Neisseria gonorrhoeae* after 10 minutes of ciprofloxacin exposure** Using two markers (porB and rpmB) determined the antibiotic susceptibility and resistance of 49 clinical isolates after 10 min exposure to ciprofloxacin. RNA signatures can build rapid AST devices for *N. gonorrhoeae* at the point-of-care, which is critical for disease management, surveillance, and antibiotic stewardship efforts.
Thakur SD[65] *J Antimicrob Chemother* 2018	Need for susceptibility testing of *N. gonoprrhaeae* to use older antibiotics	**High levels of susceptibility to new and older antibiotics in *Neisseria gonorrhoeae* isolates from Saskatchewan (2003-15): time to consider point-of-care or molecular testing for precision treatment?** Gonorrhoea in Saskatchewan is primarily (>95%) diagnosed by nucleic acid amplification testing, which does not permit antimicrobial susceptibility testing. Molecular testing, or POC tests, to evaluate antimicrobial susceptibility, would enhance knowledge of true levels of resistance for older but still effective antibiotics.
Clutter DS[66] **[Schoolnik] [NIH]** *J Mol Diagn* 2019	HIV-1 drug resistance	**Multiplex solid-phase melt curve analysis for the point-of-care detection of HIV-1 drug resistance** A POC HIV-1 genotypic resistance assay that could be performed during a clinic visit would enable informed treatment decisions for patients starting therapy or experiencing therapeutic failure. Multiplexed solid-phase melt curve analysis is a promising approach for developing POC assays to distinguish between different codons in genetically variable regions surrounding HIV-1 drug resistance mutations.
Fernandez-Huerta M[67] *Sex Transm Infect* 2019	Screening for *M. genitalium* macrolide resistance among asymptomatic people	**Prevalence of *Mycoplasma genitalium* and macrolide resistance among asymptomatic people visiting a point of care service for rapid STI screening: a cross-sectional study** The research provides further data regarding the prevalence of MG and macrolide resistance among asymptomatic individuals. It also identifies higher risk subpopulations which might be targets for MG screening. There is insufficient data to justify MG testing among asymptomatic individuals and current STI guidelines should be followed until evidence shows the cost and effectiveness of screening.

Fuller SS[68] *PLoS ONE* 2019	Antimicrobial for STDs—UK POCT expectations patient survey	**A qualitative study of UK patient opinions and expectations for implementation of point of care tests for sexually transmitted infections and antimicrobial resistance** Our data suggest patients may accept new POCT pathways if given information on changes prior to attending services and to consider implementing POCTs among patients who are anxious about their infection and/or who are experiencing symptoms.
Melendez JH[69] *Pathogens* 2019	Targeted diagnosis for Ciprofloxacin susceptibility	**Can Ciprofloxacin be used for precision treatment of gonorrhea in public STD clinics? Assessment of Ciprofloxacin susceptibility and an opportunity for point-of-care testing** Participants >35 years were 2.35 x more likely to have a gyrA mutant NG infection than younger participants ($p < 0.001$). Race, sexual orientation, symptomology, or co-infection the HIV or syphilis were not associated with a particular NG gyrA genotype. Resistance to ciprofloxacin in Baltimore is lower than other regions and indicates that ciprofloxacin may be appropriate for targeted treatment. Point-of-care tests for NG diagnosis and susceptibility testing are urgently needed to identify individuals who can be treated with this targeted approach.
Verwijs[70] Lancet Infect Dis 2019	Targeted POCT for STDs in Rwanda	**Targeted point-of-care testing compared with syndromic management of urogenital infections in women (WISH): a cross-sectional screening and diagnostic accuracy study** Point-of-care testing for urogenital infections might improve case-finding and infection management and is feasible in resource-poor settings. Point-of-care tests should be further developed, including those targeting multiple conditions. Additional studies in other populations, including populations with low prevalence of sexually transmitted and urogenital infections, are warranted.
Wadsworth CB[71] *Antimicrob Agents Chemother* 2019	RNA transcripts for a rapid POC diagnostic for antimicrobial susceptibility testing	**Impact of species diversity on the design of RNA-based diagnostics for antibiotic resistance in *Neisseria gonorrhoeae*** Quantitative assessment of antibiotic-responsive RNA transcripts holds promise for a rapid POC diagnostic for antimicrobial susceptibility testing. Assays aim to distinguish susceptible and resistant isolates by transcriptional differences upon drug exposure. An overlooked dimension of designing these tests is that the genetic diversity within a species may yield differential transcriptional regulation independent of resistance phenotype. The authors use a phylogenetically diverse panel of *Neisseria gonorrhoeae* and transcriptome profiling coupled with reverse transcription-quantitative PCR to test this hypothesis, to identify azithromycin responsive transcripts and evaluate their potential diagnostic value, and to evaluate previously reported diagnostic markers for ciprofloxacin resistance (porB and rpmB). Results suggest that RNA signatures as a diagnostic tool are promising for future POC diagnostics.
Sweeney EL[72] *J Clin Microbiol* 2020	*M. genitalium* & macrolide resistance	**Evaluation of the ResistancePlus MG FleXible cartridge for near-point-of-care testing of *Mycoplasma genitalium* and associated macrolide resistance mutations** The authors evaluate a cartridge-based system for POCT and macrolide resistance.

Abbreviations

AMR, antimicrobial resistance; AST, antibiotic susceptibility testing; POC, point-of-care; and STI, sexually transmitted illnesses.

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Table 7. Point-of-Care Diagnostics and Strategies for Antimicrobial Resistance and Antibiotic Susceptibility Testing — Tuberculosis

AUTHOR, JO. YR., REF.[73-81]	PATHOGEN/ APPLICATION	TITLE SYNOPSIS
Banada PP[73] *J Clin Microbiol* 2010	Tuberculosis diagnosis in POC settings	**Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings** The Xpert MTB/RIF assay has POC potential, but biohazard containment must be studied. The authors compared bioaerosols generated by the Xpert assay to acid-fast

		bacillus microscope slide smear preparation. Results suggested that benchtop use of the Xpert MTB/RIF assay limits infection risk to the user.
Page AL[74] *Int J Tuberc Lung Dis* 2015	Tuberculosis RIF resistance	**Routine use of Xpert MTB/RIF in areas with different prevalences of HIV and drug-resistant tuberculosis** Despite the additional numbers of cases detected by Xpert compared to microscopy, large proportions of patients are still started on treatment empirically in routine practice. Patient and specimen flow should be optimized to reduce delays in treatment initiation. Simple, non-sputum-based point-of-care tests with high sensitivity are needed to improve TB diagnosis and management.
Pimkina E[75] Respir Med 2015	Tuberculosis RIF resistance	**The Xpert MTB/RIF assay in routine diagnosis of pulmonary tuberculosis: A multicentre study in Lithuania** Results demonstrate very good performance of the Xpert MTB/RIF assay for the detection of TB and RIF resistance on primary respiratory specimens, and provide strong evidence that implementation of the assay in high drug-resistance settings may improve and facilitate TB diagnosis.
Chakravorty S[76] *J Clin Microbiol* 2017	Extremely drug resistant tuberculosis	**Detection of Isoniazid-, Fluoroquinolone-, Amikacin-, and Kanamycin-resistant Tuberculosis in an automated, multiplexed 10-Color assay suitable for point-of-care use** The assay enables testing for XDR-TB in point-of-care settings, identifying highly drug-resistant TB more quickly and simply than currently available methods.
Chakravorty S[77] *mBio* 2017	Rifampin resistant tuberculosis	**The New Xpert MTB/RIF Ultra: Improving detection of Mycobacterium tuberculosis and resistance to Rifampin in an assay suitable for point-of-care testing** Testing on clinical sputum samples, Ultra versus Xpert, resulted in an overall sensitivity of 87.5% (95% confidence interval [CI], 82.1, 91.7) versus 81.0% (95% CI, 74.9, 86.2) and a sensitivity on sputum smear-negative samples of 78.9% (95% CI, 70.0, 86.1) versus 66.1% (95% CI, 56.4, 74.9). Both tests had a specificity of 98.7% (95% CI, 93.0, 100), and both had comparable accuracies for detection of RIF-R.
Floridia M[78] *Clin Infect Dis* 2017	POC case finding for TB & Rifampicin resistance	**Tuberculosis case finding with combined rapid point-of-care assays (Xpert MTB/RIF and Determine TB LAM) in HIV-positive individuals starting antiretroviral therapy in Mozambique** The prevalence of tuberculosis among Mozambican HIV-positive patients starting antiretroviral therapy was 10%, with limited rifampicin resistance. Combined POC tests increased case finding, with a short time to treatment.
Xie YL[79] N *Eng J Med* 2017	Molecular susceptibility test for TB drugs	**Evaluation of a rapid molecular drug-susceptibility test for Tuberculosis** The assay accurately detected M. tuberculosis mutations associated with resistance to isoniazid, fluoroquinolones, and aminoglycosides and holds promise as a rapid POC test to guide therapeutic decisions for patients with tuberculosis.
Ramachandran R[80] *Expert Rev Anti-Infect Ther* 2018	Molecular diagnostics for resistant TB	**Rapid molecular diagnostics for multi-drug resistant tuberculosis in India** There is a desperate need for India to adopt modern, rapid, molecular tools with POC tests being currently evaluated. New molecular diagnostic tests appear to be cost effective and also help in detecting missing cases. There is enough evidence to support the scaling up of these new tools in India.
Phelan JE[81] *Genome Med* 2019	Portable genome sequencing to facilitate TB resistance detection	**Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs** The new version of TBProfiler can rapidly and accurately predict anti-TB drug resistance profiles across large numbers of samples with whole genome sequencing (WGS) data. The computing architecture allows for the ability to modify the core bioinformatic pipelines and outputs, including the analysis of WGS data sourced from portable technologies. TBProfiler has the potential to be integrated into the point of care and WGS diagnostic environments, including in resource-poor settings.

Abbreviations

CI, confidence interval; HIV, human immunodeficiency syndrome; MTB, *Mycobacterium tuberculosis*; POC, point-of-care; RIF, rifampicin; TB, tuberculosis; and XDR-TB, extensively resistant tuberculosis.

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Table 8. Point-of-Care Diagnostics and Strategies for Antimicrobial Resistance and Antibiotic Susceptibility Testing — Urinary Tract Infections

AUTHOR, JO. YR., REF.[82-96]	PATHOGEN/ APPLICATION	TITLE SYNOPSIS
Blom M[82] *Scand J Infect Dis* 2002	Susceptibility testing of UTI in primary care	**Validation of FLEXICULT SSI-urinary Kit for use in the primary health care setting** The kit is designed as an ordinary Petri dish divided into 6 compartments: 1 large one for quantitative analysis and 5 smaller ones for susceptibility testing. Although identification of the isolates is not a feature of this kit, it is suitable for point-of-care diagnosis and for susceptibility testing of uncomplicated UTIs in the primary health care setting.
Bates J[83] *BMC Fam Pract* 2014	UTIs and antibiotic resistance	**POCT for urinary tract infection in primary care: protocol for a randomized controlled trial of the clinical and cost effectiveness of FLEXICULT informed management of uncomplicated UTI in primary care** This POCT could benefit individual sufferers and provide evidence for health care authorities to develop evidence based policies to combat the spread and impact of the unprecedented rise of infections caused by antibiotic resistant bacteria in Europe.
Holm A[84] *BMC Fam Pract* 2015	UTIs susceptibility testing in primary care	**Point of care susceptibility testing in primary care – does it lead to a more appropriate prescription of antibiotics in patients with uncomplicated urinary tract infections? Protocol for a randomized controlled trial** The results may provide important evidence to recommend POCT culture and susceptibility testing in all patients with suspected uncomplicated UTI. This could become an additional strategy to fight antibiotic resistance.
Tchesnokova V[85] *Open Forum Infect Dis* 2016	Antimicrobial susceptibility testing from urine specimens	**A novel 7-single nucleotide polymorphism-based clonotyping test allows rapid prediction of antimicrobial susceptibility of extraintestinal *Escherichia coli* directly from urine specimens** The authors developed a rapid clonotyping method for extraintestinal *E. coli* based on detection of the presence or absence of 7 single nucleotide polymorphisms (SNPs) within 2 genes (fumC and fimH). 7-SNP-based typing of *E. coli* can be used for both epidemiological studies and clinical diagnostics, which could greatly improve the empirical selection of antimicrobial therapy.
Baltekin O[86] *Proc Natl Acad Sci* 2017	Urinary antibiotic susceptibility testing	**Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging** Bacterial cells directly from samples with low bacterial counts [10(4) cfu/mL] were captured using a custom-designed microfluidic chip that monitors individual growth rates using microscopy. By averaging the growth rate response to an antibiotic over many individual cells, the detection time matches the biological response time of the bacteria, and for antibiotic susceptibility testing, from loading of sample to diagnostic readout, was less than 30 min, which allows the development of a POC test that can guide correct treatment of UTI.
Gau Group — Altobelli,[87] **Chen,**[88,89] **Liu,**[90] **Lu,**[91] **Mach,**[92] **& Pan**[93] 2010-17	Uropathogen identification and phenotypic antimicrobial susceptibility testing	**Integrated biosensor assay for rapid uropathogen identification and phenotypic antimicrobial susceptibility testing (and other references)** An integrated biosensor platform achieved microbiological results including MIC comparable to standard culture in a significantly shorter assay time. Assay automation rapid molecular diagnosis of UTI. Clinical translation of this device has the potential to significantly expedite and improve treatment of UTIs.
Holm A[94] *BMJ Open* 2017	Urinary tract POC susceptibility testing	**Effect of point-of-care susceptibility testing in general practice on appropriate prescription of antibiotics for patients with uncomplicated urinary tract infection: a diagnostic randomised controlled trial** Adding POC susceptibility testing to POC culture did not improve antibiotic prescribing for patients with suspected uncomplicated UTI in general practice. Susceptibility testing should be reserved for patients at high risk of resistance and complications.
Markowitz MA[95] *Diagn Microbiol Infect Dis* 2019	Antimicrobial stewardship for UTI	**Rapid diagnostic testing in the management of urinary tract infection: Potentials and limitations** Rapid diagnostics increased the postponement of antimicrobial therapy pending AST results from 16% to 38% and 5% to 54% in 2 vignettes and reduced the use of ineffective antibiotics from 41% to 0% and 69% to 0%. Rapid diagnostics increased the use of narrow spectrum agents, indicating its potential to revitalize physician responsibility in antimicrobial stewardship.

Toosky MN[96] *J Med Microbiol* 2020	Antibiotic susceptibility testing for UTIs	**A rapid, point-of-care antibiotic susceptibility test for urinary tract infections** A rapid POC phenotypic AST device reports susceptibility information within 2 hours. By reducing the timeframe for susceptibility testing from 2-3 days to 2 hours, POC phenotypic AST can provide critical information to clinicians prior to the administration of antibiotic therapy.

Abbreviations
AST, antibiotic susceptibility testing; POC, point-of-care; and UTI, urinary tract infection.

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Table 9. COVID-19 Co-infections, Secondary Infections, and Nosocomial Infections

Clinical Challenge	Type of Infection	Research Paper, Evidence, and/or Observations
Bacteria		
A. baumannii (resistant), *K. pneumoniae*, & *A. flavus—1 case*; *C. glabrata*—1 case; and *C. albicans—3 cases*	Bacterial and fungal co-infections	Most patients were given antibiotic treatment; 25 (25%) patients were treated with a single antibiotic and 45 (45%) patients were given combination therapy. The antibiotics used generally covered common pathogens and some atypical pathogens; when secondary bacterial infection occurred, medication was administered according to the results of bacterial culture and drug sensitivity. Antibiotics used were cephalosporins, quinolones, carbapenems, tigecycline against MRSA, and linezolid. Antifungal drugs also were used.[122]
Hemophilus parainfluenzae, Moraxella catarrhalis	Co-infections	A severe case with co-infection of SARS-CoV-2 and respiratory pathogens.[123]
Klebseilla pneumonia, aerogenes	Dual bacterial co-infection	First COVID-19 mortality case in Taiwan with bacterial co-infection by national surveillance of critically ill patients with influenza-negative pneumonia.[124]
Pneumoccocus	Co-infection	Unexpected SARS-CoV-2 co-infection despite double RT-PCT negativity in a patient with lymphoma and concomitant pneumococcal pneumonia.[125]
Group A *Streptococcus*	Co-infection	The importance of Novel Coronavirus Disease (COVID-19) and co-infection with other respiratory pathogens.[126]
MRSA, *Streptococcus pneumoniae, Pseudomonas, Aspergillosis, Legionella,* mycoplasma	Secondary bacterial infections with possible multi-resistant organisms	Recognition and management of respiratory co-infection and secondary bacterial pneumonia in patients with COVID-19.[127] Secondary bacterial pneumonia can follow the initial phase of viral respiratory infection or occur during the recovery phase. No obvious pattern of guidelines exist for viral co-infection, combined viral and bacterial pneumonia, or secondary bacterial pneumonia.
Streptococcus pneumonia most common, followed by *Klebsiella pneum*onia and *Haemophilus influenzae*	Co-infections — bacteria and *Aspergillus* and *Candida* in severe cases	Co-infection with respiratory pathogens among COVID-2019 cases.[128] The highest and lowest rates of co-infections were found in patients aged 15-44 and below 15, respectively. Most co-infections occurred within 1-4 days of onset of COVID-19 disease. Viral co-infections, fungal co-infections & bacterial-fungal co-infections were highest in severe COVID-19 cases (*Aspergillus, Candida, Cryptococcus neoformans, Mucor, and Histoplasma capsulatum fungal co-infections*).
Chlamydia pneumonia,	Infections discovered at the	Listed as presenting characteristics without further comment on impact or treatment: 4.8% *Chlamydia pneumonia*, 2.4% *Mycoplasma pneumonia*.[129]

Mycoplasma pneumoniae	time of admission	
Bacteria and fungus infections	Nosocomial infection, co-infections in more severely affected patients	Nosocomial infection during hospitalization occurred in 8.6% (19/221) of COVID-19 patients, and in 16.4% (9/55) of those with severe status. Pathogenic analyses of co-infections in severely affected patients revealed bacteria (25.5%) and fungus (10.9%), and for ICU deaths, 55.6% (5/9) and 44.4% (4/9), respectively.[130]
New pathogen, not present on admission, bacteremia, and/or superimposed sepsis	Secondary infection risk factors for mortality	Secondary infection was diagnosed when patients showed symptoms or signs of pneumonia or bacteremia and a positive culture of a new pathogen was obtained from lower respiratory tract specimens or blood samples. Half of non-survivors experienced a secondary infection, ventilator-associated pneumonia occurred in 31% of those on mechanical ventilation, secondary infection occurred in 28% of all patients., and >50% developed sepsis.[109] Fatal superimposed sepsis in a healthy Coronavirus patient warrants checking procalcitonin levels on admission to rule out on-going bacterial infection.[131]
New pathogen, empiric antibiotic treatment incurs risk of AMR	Secondary infection	10% (4/41) of COVID-19 patients had secondary infections. Secondary infections were higher (31%, 4/13) in non-survivors. All 41 patients (100%) received empirical antibiotic treatment.[132]
Various	Primary bacterial co-infections	Primary respiratory bacterial co-infections in patients with COVID-19.[133]
Ventilator-acquired nosocomial flora	Ventilator-associated pneumonia (VAP)	COVID-19: An alert to ventilator-associated bacterial pneumonia.[134] Higher risks of ventilator-associated bacterial pneumonia in COVID-19 inpatients occur when VAP is not identified early, empiric therapy is delayed, or its adjustment in accordance with microbiological results is not performed. Avoid VAP in COVID-19 patients, especially due to the peculiar immunological profile observed in severe cases.
Antibiotic use rate and safety of microbiology work-up of negative bacilli, *Candida*, and Aspergillosis	Secondary and co-infections in need of targeted therapy to avoid development of AMR	In critically ill COVID-19 patients, the antibiotic use rate of 94% (49/52 patients)[135] was much higher that the 17% rate of secondary or co-infections (9/52).[5] A practical diagnostic process is necessary for microbiology laboratory work-up of bacterial and fungal infections in COVID-19 patients for biosafety and personnel protection that reduces staff risk.
Fungus		
Aspergillus	Associated infection	COVID-19 associated with pulmonary *Aspergillosis.*[136]
Aspergillus	Fungal co-infection — 71-100% received antibacterials	Bacterial and fungal infections among patients with SARS-CoV-2 pneumonia[137] Invasive pulmonary *Aspergillosis* seems to be an increasingly observed complication in critically ill patients with SARS-CoV-2 infection.
Aspergillus fumagatus	Azole-resistant co-infection leading to fatal outcome	Azole-resistant COVID-19-associated pulmonary *Aspergillosis* in an immunocompetent host: A case report[138] The case underscores the importance of early diagnosis and the need for resistance surveillance.
Respiratory		
Respiratory pathogens, including *mycoplasma pneumonia* and various bacteria	Co-infections in adults — screening for co-infections and targeted	Rates of co-infection between SARS-CoV-2 and other respiratory pathogens;[139] Co-infections of SARS-CoV-2 with multiple common respiratory pathogens;[140] Co-infection with SARS-CoV-2 and other respiratory pathogens in COVID-19 patients in Guangzhou, China;[141] Clinical characteristics of 25 death cases with COVID-19: a retrospective review of medical records in a single medical center,

	treatment necessary	Wuhan, China;[142] Critically ill patients with COVID-19 in Hong Kong: a multicentre retrospective observational cohort study.[143]
Myocplasma pneumoniae in children	Co-infection in children — general	Co-infection and other clinical characteristics of COVID-19 in children.[144] Of 34 (45.95%) patients who had nucleic acid testing for common respiratory pathogens, 19 (51.35%) showed co-infection with pathogens other than SARS-CoV-2, including 16 with *mycoplasma pneumonia.*
Tuberculosis		
20 of 24 TB patients who had COVID-19	Co-infection	Clinical characteristics of COVID-19 and active tuberculosis co-infection in an Italian reference hospital.[145] The impact of COVID-19 on active TB appears to be clinically manageable with proper care.
49 COVID-19 patients from 8 countries	Concurrent, co-infection, or previous TB (timing argued in ref. **147**)	Active tuberculosis, sequelae and COVID-19 co-infection: first cohort of 49 cases.[146] Of 49 patients, 26 (53.0%) had TB before COVID-19, 14 (28.5%) had COVID-19 first, and 9 (18.3%) had both diseases diagnosed within the same week (4 on the same day). However, TB is more prevalent, and therefore, likely to have preceded COVID-19 infection.[147]
Severe COVID-19 pneumonia in patients with pre-existing TB	Math model	COVID-19 and tuberculosis: A mathematical model based forecasting in Delhi, India[148] Patients with latent TB infection and TB disease have increased risk of the SARS-CoV-2 infection and predisposition towards developing severe COVID-19 pneumonia. TB treatment centres and hospitals need to be prepared for early diagnosis and management of severe COVID-19 in TB patients.
Patients in middle and low income countries	Co-infection	Anticipating the impact of the COVID-19 pandemic on TB patients and TB control programmes.[149] TB ranks as the leading cause of death from a single infectious disease globally. Thee may be grave consequences for TB patients in middle and low-income countries.

Abbreviations

AMR, antimicrobial resistance; COVID-19, Coronavirus infections disease 2019; ICU, intensive care unity; MRSA, methicillin-resistant *Staphylococcus aureus*; SARS-CoV-2, severe acute respiratory syndrome-Coronavirus 2; and TB, tuberculosis.

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Table 10. Expert Opinions Regarding the Global Acceleration of Antimicrobial Resistance

Source	Focus	Conclusions and Questions
Editorial, *Nature Microbiology*[150]	AMR	Before to SARS-CoV-2, AMR already demanded urgent global action. In the midst of a pandemic, understanding the pathogenesis of SARS-CoV-2 infection and the potential for bacterial co-infections, is imperative. Efforts to prioritize antibiotic stewardship around the world must be redoubled.
Queen's Univ. Belfast, UK[151]	SARS-CoV-2, bacterial co-infections, AMR: a deadly trio in COVID-19	Respiratory viral infections are well known to predispose patients to bacterial co-infections and superinfections. There is limited reference to these in COVID-19. What is the impact of co-infections and AMR?
University of Pittsburg, PA[152]	Superinfection and COVID-19 (nosocomial	An appreciable minority of patients with severe COVID-19 will develop superinfections, most commonly pneumonia due to nosocomial bacteria and *Aspergillosis*. Broad-spectrum antimicrobial use is likely to be widespread as directed and empiric therapy.

	bacteria & *Aspergillosis*)	Stewardship will have a crucial role in limiting unnecessary antimicrobial use and AMR.
Biodiscovery Institute, Univ. of Nottingham, UK[153]	AMR	How will the impact of AMR compare against the recent rapid devastation of the COVID-19 pandemic, and how can we channel some of the good things that come from it to help us combat AMR speedily and definitively?
Feature, *British Medical Journal*[154]	Accelerating AMR	The global threat of antimicrobial resistant bacterial and other superbugs is worsening as many patients admitted to hospital with COVID-19 receive antibiotics to keep secondary bacterial infections in check.
Univ. Southampton, UK[155]	AMR as a global problem	Current antimicrobial medicines are no longer effective for common infections. AMR is a global public health crisis that is universal, affecting anyone and everyone, at any age in any country. Investigating how herbal medicine can be integrated into western medicine will be important to elucidate.
Cardiff Univ., UK[156]	Targeted hygiene in the home and everyday life	With the recent global SARS-CoV-2 pandemic, everyday hygiene measures can play an important role in containing the threat from infectious microorganisms. Achieving a reduction of AMR strains in health care settings requires a mirrored reduction in the community.
Opinion, *Frontiers in Microbiology*[157]	AMR	Measures are being implemented to try to slow the spread of COVID-19. AMR has been cited as the most significant threat to global health and economy. Efforts should be made to understand potential confounding effects.
McMaster Univ., Hamilton, Canada[158]	COVID-19 & AMR: Parallel health emergencies	The COVID-19 pandemic helps to illustrate the potential long-term impact of AMR, which is less acute but not less crucial. There is a push to resort to existing antimicrobials in critically ill COVID-19 patients in the absence of specific treatments.
Comment, Imperial College London[159]	Antimicrobial use, drug-resistant infections and COVID-19	COVID-19 may have a complex impact on AMR. Coordinated strategies at the individual, healthcare and policy levels are urgently required to inform necessary actions to reduce the potential longer-term impact on AMR and on access to effective antimicrobials.
Imperial College, London[160]	Long-term impact of COVID-19	Infection control and antimicrobial stewardship may have to be relaxed to focus on controlling this pandemic, but the global threat of AMR should not be overlooked.
Imperial College, London[161]	COVID-19 antimicrobial prescribing	Despite frequent prescription of broad-spectrum empirical antimicrobials in patients with coronavirus associated respiratory infections, there is a paucity of data to support the association with respiratory bacterial/fungal co-infection. Generation of prospective evidence to support development of antimicrobial policy and appropriate stewardship interventions specific for the COVID-19 pandemic are urgently required.
Australian National University[162]	Global AMR	The use of antibiotics in the COVID-19 pandemic will inevitable exacerbate AMR, and could ultimately lead to more deaths and morbidity as an unintended consequence.
Federal University of Grande Dourados, Brazil[163]	Secondary infections and multi-resistant microorganisms	Actions aimed at reducing mortality in patients with COVID-19 should take into account the worsening of the patient's clinical condition due to secondary and multi-resistant microorganisms. Epidemiological studies should promote quality evidence about antimicrobial intervention effectiveness, especially in the ICU.

Abbreviations

AMR, antimicrobial resistance; COVID-19, Coronavirus infections disease 2019; ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome-Coronavirus 2; and UK, United Kingdom.

FIGURES

Figure 1. Unique Geospatial Spread of *Candida auris* Resistance to United States Hotspots

Figure 2. Extended Spectrum beta-Lactamase (ESBL)-producing *Enterobacteriaceae* Hotspots within US Communities

Figure 3. Multiplex Diagnostics for Antimicrobial Resistance