A new resistance combatting strategy

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The global antimicrobial resistance (AMR) crisis is a major threat to human health. In a recently published article, the Antimicrobial Resistance Collaborators estimated that 4.95 million deaths were associated with bacterial AMR in 2019[1]. These numbers are predicted to increase year by year. AMR has changed the way medicine is practiced. For example, infections previously treated with oral antibiotics now require injectable treatment and, because whether an antimicrobial resistant bacterium may be involved is often unknown when therapy is initiated, unnecessarily broad-spectrum antibiotics are oftentimes prescribed, or alternatively, the prescribed regimen may not even treat the infection because of unrecognised underlying resistance. In addition, antibiotics are frequently administered to patients who do not need them because they do not have a bacterial infection.

AMR involves hundreds of microbial species, dozens of antimicrobial agents and a multitude of clinical syndromes (e.g., pneumonia, urinary tract infection, intra-abdominal infection). The WHO's Priority Pathogens List for new antibiotics and the United States Centers for Disease Control and Prevention's "urgent", "serious" and "concerning" threats provide listings, albeit slightly different, of resistant bacteria, but do not comprehensively list all possible species or resistance-types involved. While development of new antibiotics and antimicrobial stewardship are essential to address this evolving situation, better diagnostics and appropriate use thereof are an additional strategy that needs to be better incorporated.

The classic three-step diagnostic paradigm used in clinical medicine (**Figure 1**), taught to medical students and applied by medical professionals throughout the world, involves asking whether a patient's clinical presentation could be due to infection (step 1: based on history, physical examination, initial tests); what the causative pathogen(s) might be (step 2: based on culture, serologic testing, molecular testing for microorganisms); and finally, which treatment should be administered (step 3: based on culture -based antimicrobial susceptibility testing). This classic approach, although intellectually interesting, is at once contributing to the AMR crisis and failing because of it.

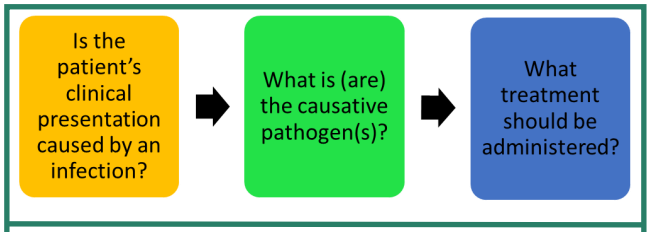

Figure 1. Classic Diagnostic Paradigm

Creative use of technology can help; fortunately, we are in a technology revolution. There have been major advances in the application of proteomics, nucleic acid amplification tests and sequencing-based diagnostics, microbial imaging, microbial metabolomics and advanced host response assessment for infectious diseases in recent years, and point-of-care diagnostics are in the process of transforming where testing can be done (including at non-traditional sites and in the home). In my view, we need to rethink approaches to the challenge of AMR by practicing medicine in a more modern way using better diagnostics to inform antimicrobial therapy.

Modern diagnostic tests can help curb emergence of AMR by informing improved use of antibiotics (a patient and societal benefit), leading to avoidance of unneeded testing and treatment (a patient benefit), decreasing transmission of infectious diseases (a societal benefit) and informing new discoveries and better delivery of healthcare (which will have future benefits). A decade ago, the first large multiplex PCR panel was cleared / approved by the United States Food and Drug Administration (FDA) for testing positive blood culture bottles. Our team executed a randomised controlled clinical trial of this panel showing that its implementation would reduce unneeded use of antibiotics and more quickly get patients with drug-resistant infections on appropriate antibiotic therapy[2]. In this study, results of multiplex panel testing were provided with interpretive comments with therapeutic guidance, an approach recently supported by a recommendation from the Diagnostics Committee of the Antibacterial Resistance Leadership Group[3]. In the multiplex PCR panel study, the only Gram-negative resistance marker included in the panel was blaKPC, for which there were no detections[2]; accordingly, effects on antibiotic use in Gram-negative bacteraemia were minimal. A second randomised controlled study evaluated rapid microbial imaging-based phenotypic susceptibility testing for patients with blood cultures positive for Gram-negative bacilli; in that study, time to first antibiotic modification was faster with the rapid test for all antibiotics and Gram-negative antibiotics, with antibiotic escalation being faster for antimicrobial-resistant infections[4].

The blood culture diagnostics highlighted above (although performed after incubation of blood cultures) illustrate an important pathway forward – that is, detecting microorganisms and immediately defining their clinically relevant antibiotic susceptibility. This has been delivered by tests such as the GeneXpert (Cepheid) MRSA/SA SSTI assay (which detects *Staphylococcus aureus* and methicillin resistance / susceptibility) and MTB/RIF assay (which detects *Mycobacterium tuberculosis* and rifampin resistance /susceptibility). Our group recently described an assay for detection of *Helicobacter pylori* and associated clarithromycin resistance / susceptibility[5], and *Mycoplasma pneumoniae* and associated azithromycin resistance / susceptibility[6]; assays to detect ciprofloxacin resistance / susceptibility in *Neisseria gonorrhoeae* and azithromycin resistance in *Mycoplasma genitalium* are other examples of this approach.

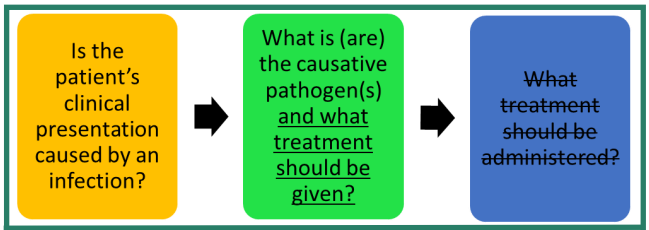

Figure 2. New Diagnostic Paradigm (delivered by improved diagnostic tests and transformation of healthcare). Beyond eliminating a third step, in the future, the first two steps (shown in yellow and green) will likely be consolidated to one.

Beyond nucleic acid amplification-based microbial detection and gene- or mutation-based characterisation of resistance, microbial sequencing directly from clinical specimens is being developed, and can theoretically both detect the infecting organism(s) and characterise resistance / susceptibility to clinically relevant antibiotics. In a case report, for example, *Mycoplasma salivarium* was identified as a cause of periprosthetic joint infection, using shotgun metagenomic sequencing, with simultaneous detection of a mutation associated with macrolide resistance[7]. The possibility of going from microbial sequence data to near-full recapitulation of results of phenotypic susceptibility may be realised in the future, especially with improved understanding of resistance mechanisms and advanced analytics[8-11]. This may in turn facilitate rapid full recapitulation of phenotypic susceptibility testing in a clinically actionable way, directly from clinical specimens[12, 13]. In addition, deep sequencing may allow simultaneous assessment of microorganisms and host response, helping with interpretation of clinical significance of detected microorganisms[14, 15].

Finally, in addition to transforming clinical practice and optimising use of antibiotics, improved diagnostics may deliver new findings, as illustrated by the surprising discoveries of *Borrelia mayonii*[16], *Yersinia rochesterensis*[17], and the cause of hyperammonemia syndrome in lung transplant recipients – *Ureaplasma urealyticum* and *Ureaplasma parvum*[18-20].

In summary, because of improved diagnostic testing, we are positioned to undo the classic (and slow) diagnostic paradigm (**Figure 1**), using diagnostics that detect microorganisms and directly call out ideal therapy in a single step (**Figure 2**), so-called, microbial "theranostics". To move forward, we need continued development of better diagnostics combined with changes in the way healthcare is delivered, facilitated by better diagnostics and necessary to harness their value.

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