## Reply to Lesho and Clifford

To the Editor—Lesho and Clifford offer insightful comments regarding the analytical and molecular approach undertaken in the PRIMERS I and II study [1, 2]. They correctly acknowledge that the "genotype to phenotype" determination made in our analysis was solely based on the presence or absence of *bla* genes included in the 3 platforms used (polymerase chain reaction [PCR]/electrospray ionization mass spectrometry, Molecular Beacons, and Check-Points). We also used the Ion Torrent, a benchtop, next-generation sequencing technology, to sequence PCR amplification products designed to detect a defined set of β-lactamase genes. We concur that the resistance phenotype could have been the result of novel or unknown genes or alleles not included in our screen and that our approach could have missed unknown *bla* genes and non-*bla* gene–mediated mechanisms of resistance. Lesho and Clifford point out that whole-genome sequencing (WGS), if done in a very timely manner, would have provided a comprehensive approach at a reasonable cost. But, at least today, is WGS really a "rapid" molecular diagnostic? Lesho and Clifford's approach has a turnaround time of "about 48 hours," similar to that of phenotypic susceptibility testing, whereas our assays had turnaround times that were less. And WGS may not be available in all medical centers, extending the turnaround time if the test needs to be sent out. In addition, the issue of cost must be considered. Lesho and Clifford's proposed cost is $30–$50 per genome, but for a clinical test, one would need to factor in the cost of DNA preparation, sequencing, sequence analysis, nonbatched testing (especially germane if rapid results are desired), and quality control, all of which have the potential to render true costs higher than proposed. We acknowledge that WGS is a "game changer in infectious diseases" and that the field has yet to realize its true power. We are certain that in the next few years, WGS will be increasingly accessible and tell us about pathophysiology, virulence, and epidemiology in addition to resistance. Results of our study will inform future similar studies evaluating WGS for resistance prediction.

PRIMERS I and II attempted to answer 2 major questions: (1) Can rapid molecular diagnostic (RMD) platforms accurately detect resistance genes, discriminate between resistance and susceptibility, and predict whether an isolate of *Escherichia coli* or *Klebsiella pneumoniae* will be resistant/susceptible to certain β-lactams; and (2) can analytical strategies that are based upon prevalence of resistance in a community be devised to better inform empiric therapy? To this end, 268 isolates were studied and >20 000 data points analyzed. Our results showed that clinicians can have confidence in interpreting these RMDs. There were errors, of course. RMDs are not perfect, nor are clinicians, and strategies to measure that imperfection are being developed.

However, when one examines the data obtained from WGS, could it suffer from the same or even more limitations? How does one interpret single-nucleotide polymorphisms in a variety of genes that modulate resistance and change expression levels of phenotypic resistance? In the exercise in PRIMERS I and II, the genome sizes we would need to analyze are between 4.6 Mb (*E. coli*) and approximately 5.3 Mb (*K. pneumoniae*). Extrapolating from what we have learned from PRIMERS I and II, information obtained from WGS would require even more sophisticated analyses and validation. Applying WGS and the information it yields to a population with a changing prevalence would still need to be carried out. New approaches such as sequence-independent, single-primer amplification (SISPA) combined with next-generation sequencing (SISPA-Seq) are extremely exciting, especially when this platform can distinguish between different allelic variants of the same gene; this was recently applied with success to *Acinetobacter baumannii* [3]. Methods such as SISPA-Seq bring many advantages to the table, including low-cost, rapid DNA preparation and a typical total cost less than one-half that of standard genome sequencing. But the challenge remains: Will these technologies enhance our ability to make better decisions in the clinic and at the bedside? We can't predict this. The trials answering these questions still need to be correctly designed and executed.

Last, we need to be mindful of the questions we are asking. WGS applied to these settings is exploratory, and casts a "wide net." PRIMERS I and II were focused and asked targeted questions ("If I look for the genes reportedly associated with resistance, what can this tell us?"). PRIMERS I and II looked for what a specific set of algorithms could tell us. By its very nature, WGS embraces more complex algorithms that require lots of data and validation.

Molecular diagnostics is rapidly changing infectious diseases. We are "in the eye of the storm" with our new technologies. Clinicians are being introduced to new platforms and asked to embrace their power, but these new tools need to be validated in the clinical setting. PRIMERS I and II were a step forward in this regard and new studies are following (PRIMERS III and IV). Our responsibility in infectious diseases is to navigate these turbulent waters safely, and WGS studies are a perfect candidate for future study in this regard.

### Note

***Potential conflicts of interest.*** B. K. consults for Pfizer and Abbott. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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