EBioMedicine 22 (2017) 26–27

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Commentary

The Accidental Orthodoxy of Drs. Mueller and Hinton

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ARTICLE INFO

Article history:
Received 3 July 2017
Accepted 3 July 2017
Available online 4 July 2017

Three-quarters of a century ago, two investigators at Harvard Medical School and School of Public Health, J. Howard Mueller and Jane Hinton, published a succinct research note addressing a vexing problem in the diagnostic laboratory: a stable serum-free bacteriologic media that supported the growth of two otherwise very fastidious bacteria, the meningococcus and the gonococcus (Mueller and Hinton, 1941). So-called "Mueller-Hinton Broth" (MHB), composed of dehydrated beef infusion, hydrolyzed casein and starch, and later tweaked with supplemental cations to enhance *Pseudomonas* recovery, spread through the microbiology world as a reliable means for propagating the vast majority of pathogenic bacteria encountered in clinical practice. Coincident with the dawn of the antibiotic era, the versatility of MHB (and its solid agar derivatives) was a perfect match, allowing a standardized, consistent medium for broth dilution or disk diffusion testing of antibiotic potency, defined most frequently in terms of minimum inhibitory concentration (MIC). In recent decades, MHB has been endorsed by the Clinical and Laboratory Standards Institute (CLSI), the global nonprofit organization that ensures quality in healthcare testing, as the appropriate media for routine bacterial antibiotic susceptibility determination, with updated cutoff standards (CLSI, 2015) for designating resistant (R) and susceptible (S) strains published frequently and lodged in databases maintained by the U.S. Food and Drug Administration (FDA).

Far beyond the modest ambitions of its inventors, MHB has contributed significantly to the practice of medicine, the guidance of public health policy, and the antibiotic drug discovery efforts of academic scientists and the biopharmaceutical industry. Based upon analysis of MIC profiles performed in MHB, innumerable patients have had their antibiotic coverage expanded or narrowed, outbreaks and epidemics of antibiotic-resistant clones have been tracked across the globe, and chemical structures and formulations of new antibiotic agents have been optimized for the clinical market. Enormous success. But as Andy Grove, the noted Hungarian-American business leader and scientific pioneer in semiconductors, once famously warned: "Success breeds complacency. Complacency breeds failure." (Grove, 1996). As well documented, we have arrived at a crisis point in medical history, with the threat of antibiotic resistance capturing the attention of government leaders and international agencies at the highest levels. Complacency cannot be afforded.

Likely never anticipated by Drs. Mueller and Hinton and their contemporaries, patients in many countries including the U.S. today are experiencing serious infections with bacteria resistant to all safe and reliable antibiotics by standard MIC testing. Exemplars among superbugs producing this urgent threat are strains of *Klebsiella pneumoniae* and *Escherichia coli* producing extended-spectrum β-lactamases, membrane modifications or efflux pumps promoting resistance to even "last resort" antibiotics such as carbapenems or colistin (Logan and Weinstein, 2017). Risk factors for acquiring highly antibiotic-resistant infections include prolonged ICU stay, complicated surgery, chronic disease or immunosuppression – but no matter their unique clinical history, these patients share at least two things in common: (1) a bacterial pathogen spreading in their body is producing a life-threatening medical emergency, and (2) they are not made of MHB.

In the current issue of *EBioMedicine*, Ersoy et al. (2017) probe a simple but profound question. What if antibiotic testing were performed not in a classical enriched bacteriologic media such as MHB, but in a testing media designed to better resemble the chemical constitution of normal human body fluids? For example, a common medium used for successful propagation of primary human cells and immortalized cell lines in tissue culture - Dulbecco's Modified Eagle Medium (DMEM), yielded markedly different antibiotic MIC results for many important pathogens when compared to results obtained in MHB. When antibiotics with discordant results were investigated in a mouse model of infection, treatment with an antibiotic predicted to be effective only in the host-mimicking media (azithromycin) was associated with markedly enhanced survival outcomes compared to an antibiotic predicted to be effective only in MHB (co-trimoxazol). Fascinatingly, supplementation of MHB with a single molecule abundant in most tissue culture media, sodium bicarbonate ($NaHCO_3$), markedly improved the accuracy by which MIC testing predicted *in vivo* antibiotic efficacy.

Beyond the potential for neglecting or overrating antibiotic potential under more physiologic ionic and nutrient conditions, strict reliance on MIC testing performed in bacteriologic media such as MHB has another

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2017.05.026.
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http://dx.doi.org/10.1016/j.ebiom.2017.07.002

obvious shortcoming: it is entirely agnostic of the host immune system. The establishment of a deep-seated bacterial infection reflects not only the pathogenic potential of the bacterium, but a simultaneous failure of essential host innate defense mechanisms. The natural evolution of the rational approach showcased by Ersoy et al. (2017) can be to further develop testing media that more accurately model antibiotic action in synergy with additional aspects of the *in vivo* environment including endogenous host defense peptides, serum complement and phagocytic cells. Indeed, synergy of certain antibiotics with cationic host defense peptides such as cathelicidin (LL-37 in humans) appears to explain a remarkable efficacy of azithromycin *vs.* carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Lin et al., 2015) or of nafcillin *vs.* methicillin-resistant *Staphylococcus aureus* (Sakoulas et al., 2014) in mouse infection models, despite negligible activity of either drug in standard MIC testing performed in MHB.

All that said, antibiotic efficacy as predicted by standard MIC testing in MHB, the trusted standard worldwide, remains sufficient to guide successful antibiotic management in the majority of patients with routine bacterial infections, or even severe disease with strains lacking high level resistance phenotypes. However, in cases of high risk patients, unusually virulent strains, or multi-drug resistance, where infection-associated morbidity and mortality are high, why stop there? Exploration of antibiotic action and potential for immunological synergy, against the patient's bacterial isolate in media designed to model the micro-environmental conditions at the site of infection, is the essence of modern personalized medicine, and could yield additional or better treatment options.

Finally, should such thinking extend beyond the clinic to the drug discovery pipeline, entirely new classes of antibiotics might soon be revealed – ones that work at the host-pathogen interface rather than simply killing bacteria (Munguia and Nizet, 2017; Johnson and Abramovitch, 2017). These agents, whether they inhibit a bacterial virulence mechanism, sensitize the bacteria to host innate immunity, or act upon the phagocytic cell to boost its microbicidal activity, will expand our pharmaceutical arsenal against antibiotic-resistant superbugs, an existential threat to the practice of modern medicine.

Disclosure

The author declares no conflicts of interest.

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