Vol 5 | Issue 1 | Pages 142-145

Annals of Public Health Reports
ISSN: 2642-4924

Review Article

DOI: 10.36959/856/510

Emerging Infectious Diseases at the Clinic-Clinical Lab-Research Lab Interface

*Nicole Anastasio, Evangeline Green, Samuel Pettit and Meghan May**

Department of Biomedical Sciences, College of Osteopathic Medicine, University of New England, Biddeford, Maine, USA

Abstract

Emerging and re-emerging infectious diseases have been a constant companion of medicine since its onset. In this commentary, we will discuss the multiple manifestations of disease emergence, mechanisms by which novel pathogens or strains emerge, and the practical implications for physicians as they apply to diagnostic, treatment, and control strategies. Finally, we will highlight the ways in which connections between the clinic, the clinical laboratory and the research laboratory lead to major discoveries in emerging diseases, with special emphasis on COVID-19.

Introduction

Perhaps more than any other area of medicine, instances of infectious disease emergence have a relatively short thread connecting physicians to biomedical scientists, with the line running directly between the clinical laboratory and the research laboratory. Cases of emerging or re-emerging infectious diseases are frequently unexpected, and often characterized by an urgent need for control measures and clinical interventions against the backdrop of a paucity of information about the basic biology of the pathogen. Emerging disease outbreaks can stem from the introduction of novel infectious agents into the human population, from the sudden appearance of new strains with altered clinical presentations, or from the evolution of emergent lineages in response to selective pressures applied by clinical interventions. In this piece, we will discuss each of these aspects of emerging infectious diseases, highlighting the connection between the clinic and laboratory, with special emphasis on the emergence of COVID-19.

Emergent Strains and Lineages of Known Pathogens

Established pathogens can emerge with novel clinical presentations, in previously unaffected populations, with altered transmission dynamics, or in populations where disease had previously been controlled. The drivers of this type of emergence varies from pathogen to pathogen and widely by circumstance, but the most common ways are by acquisition of new virulence factor genes, by adaptation to a population's immunity, by random genetic drift resulting in a new virulence phenotype, or by prolonged exposure to antimicrobials. By understanding the unique origins of emerging pathogens, we can then begin exploring what drives their evolution and adaptability to cause various disease outbreaks, with the ultimate goal of forecasting their next move.

The emergence of more virulent bacterial strains can stem from acquisition of new genes, most notably toxin genes. The classic historical example of this is the emergence of *Yersinia pestis*, the causative agent of plague. We now know that *Y. pestis* was not a novel species, but rather an emergent lineage of *Yersinia pseudotuberculosis* that had acquired a small number of new genes conferring grave disease consequences [1]. A more recent example featured a case empirically presenting to a clinician as cutaneous anthrax. The isolate obtained by the local clinical laboratory was identified not as *Bacillus anthracis* but as *Bacillus cereus*, a bacterial species that is typically associated with food borne illness and periodontal disease. In order to understand how a mild, enteric pathogen was causing clinical anthrax, further biological characterizations were needed from research laboratories. Genetic evaluation of this isolate performed by the Centers for Disease Control and Prevention and the Florida Department of Health established that this isolate had acquired the capacity to make the *B. anthracis* capsule and toxins lethal factor (Lef) and edema factor (Cya) [2]. Retrospective sequencing

***Corresponding author:** Dr. Meghan May, Department of Biomedical Sciences, College of Osteopathic Medicine, University of New England, 11 Hills Beach Road, Biddeford, ME 04005, USA

Accepted: January 25, 2021

Published online: January 27, 2021

Citation: Anastasio N, Green E, Pettit S, et al. (2021) Emerging Infectious Diseases at the Clinic-Clinical Lab-Research Lab Interface. Ann Public Health Reports 5(1):142-145

analysis indicated that this isolate was highly genetically homogenous to other *B. cereus* isolates from anthrax-like cases featuring severe pneumonia or eschars [3-5]. Molecular genetic research utilizing these unrelated cases identified emergent lineages of *B. cereus* (now termed *Bacillus cereus* biovar *anthracis*) with potential for severe and fatal disease. This finding can be communicated back to the clinicians for improved surveillance, and more importantly, consideration of non-anthracis anthrax on a differential diagnosis for these severely ill patients.

Novel clinical presentations, such as that of the mosquito-borne flavivirus Zika virus (ZIKV), have similarly triggered further research on known pathogens. The ZIKV outbreaks in the Western Hemisphere and Southeast Asia appeared to challenge the traditional disease model of African strains in terms of methods of host infection, consequent clinical presentation (i.e., in neurological and fetal tissue) and sequelae [6]. A lack of understanding of ZIKV biology made this change both mysterious and unanticipated, and also left physicians and scientists without specific interventions for patients. Since the Western Hemisphere Zika epidemic in 2015, enormous strides have been made by research laboratories toward: a) Identifying the genetic changes resulting in enhanced disease; b) Identifying stable and mutable residues to improve diagnostic detection; and c) Design and development of a candidate vaccine. These advances in pathophysiological understanding and translational research were made possible by close collaboration with clinical laboratories collecting meticulously curated isolates, who are only able to provide these due to thorough evaluations and complete, non-empirical workups by clinicians. The translational research advances that led to improved diagnostic tests, possible treatments, and a candidate vaccine then feed back into the clinical laboratory and ultimately the clinic, wherein they improve outcomes for patients. While this is always the goal of the biomedical research-clinic paradigm, it is often a long, multi-decade process. It is therefore notable that this feedback loop completed within less than five years for ZIKV, and after a small number of cases of *B. cereus* biovar *anthracis*.

Impacts of Emergent Strains or Novel Species on Current Treatment, Detection, and Prevention Strategies

The emergence of novel pathogens and new strains/lineages of known pathogens has the potential to affect current detection, treatment, and prevention strategies. While the failure of a diagnostic test to identify a novel pathogen is predictable, the failure of validated tests to positively identify emergent strains of known pathogens is less appreciated. During the most recent influenza A pandemic (H1N1; 2009), the sudden appearance of off-season cases was not immediately attributed to influenza activity because the rapid diagnostic test was rarely positive. Cases that were definitively diagnosed were done so by positive identification of influenza virus RNA, and the emergence of a novel pandemic strain was confirmed by sequencing. The concordance between RNA detection and rapid diagnostic tests based on antigen detection was 11% when H1N1 pdm 09 initially emerged, meaning that 89% of infected patients tested negative [7]. Upon recognizing that a new pandemic strain had emerged, diagnostic tests and vaccine preparations were adjusted accordingly as soon as they feasibly could be. However, the early failure of the standard diagnostic strategy highlights the very real issue of interpretation of negative results when it comes to emergent strains and novel pathogens. Another related issue - that of a similar disease caused by a novel species -was exemplified by *Borrelia mayonii,* a newly described species of bacteria causing Lyme disease. The diagnostic tests used to confirm Lyme borreliosis are notoriously problematic for a variety of reasons, including within-species and between-species diversity. Diagnostic methods for suspected Lyme disease patients utilizing real-time PCR is routinely performed by the clinical and research arms of the Mayo Clinic, and it was via this method that *B. mayonii* was discovered. The first cases were described after six individuals with suspected Lyme disease produced non-negative, yet abnormal results: PCR detection, but with an atypical melting point [8]. Although these particular patients tested positive using molecular techniques, it is not clear if current diagnostic tests adopted and validated by most clinical laboratories will be able to detect infection with this new species. This is notably problematic because *B. mayonii* infections have been found to have a slightly different presentation of Lyme disease than that caused by *Borrelia burgdorferi*. Although it can cause fever, headache, rash and neck pain in early infection and arthritis later, it is also associated with nausea, vomiting, and a more diffuse rash (as opposed to the classic bullseye rash) [8]. This difference in presentation could mean that it is also possible that individuals with Lyme borreliosis caused by *B. mayonii* are being screened less frequently, and that they may be producing false-negative results. In these two examples, the discovery of the novel strain and novel pathogen (respectively) occurred because research-oriented clinical laboratories pursued extensive testing in order to make a definitive diagnosis, as opposed to stopping at the standard practice.

Failure to identify novel pathogens or emergent strains is not the only diagnostic challenge highlighted by collaborations between the clinical lab and the research lab. Antimicrobial susceptibility testing (AST) is now routine following the widespread emergence of antibiotic resistant organisms. Any lethal intervention is going to exert profound selective pressure on infectious agents to evolve a mechanism to evade or escape that clinical intervention. As a result it is no longer unusual to find patients with both hospital-acquired and community-acquired infections that are resistant to numerous classes of antimicrobials. Resistance can come in the form of having lost or substantially altered the drug target, or having acquired a new gene that either destroys the drug or pumps it back outside the pathogen's cell body. Perhaps one of the most known examples of bacterial resistance to treatment is seen with methicillin-resistant *Staphylococcus aureus* (MRSA). Prior to 1940 infection with *S. aureus* often proved deadly, with mortality rate of over 80%. In 1940, with the introduction of the β-lactam antibiotic penicillin G, the mortality rate improved substantially. However, by 1942 there was evidence of resistance by an enzymatic activity that destroyed the drug, termed penicillinase/β-lactamase. By 1959

methicillin, a penicillinase-resistant β-lactamdrug, was used to treat *S. aureus* infection. Unfortunately, the first reports of resistant isolates were cultured in mid-late 1960. While there are treatments for MRSA, the rise of vancomycin-resistant *Staphylococcus aureus* (VRSA) in the past few decades has created more challenges. All VRSA strains thus far have arisen from an MRSA strain, indicating that these emergent lineages are highly resistance to multiple antibiotic classes. Parallel outcomes involving numerous pathogens and multiple drug classes have been described, causing the World Health Organization to name antimicrobial resistance one of the most urgent health challenges of our time [9].

Early and aggressive treatment of drug-resistant organisms is critical both to individual patient care and to public health at large, as removal of these strains from circulation to the extent possible is ideal. This is why performing AST is now the norm rather than the exception. However, current AST techniques themselves are not without limitations. Molecular detection of known antibiotic resistance genes or drug target alleles is a reliable method, although it allows for the possibility of a drug-resistant organism with an as-yet-unidentified resistance-conferring genotype to go undetected. AST thus often relies on detection of drug resistance phenotypically, which we now understand must be caveated by interpreting the results under laboratory conditions. Our laboratory conducted a study in collaboration with a clinical laboratory initially focused on an isolate of an opportunistic pathogen, *Francisella philomiragia.* Due to its relative obscurity and fastidious growth, AST was performed under both ambient air conditions and 5% CO_2. The disparity between conditions was substantial, with the isolate demonstrating resistance to β-lactams when grown at 5% CO_2 (notably, this is a physiologically relevant level of CO_2 for some body sites such as the respiratory tract). This was due to an induction of β-lactamase activity only under these conditions. The β-lactamase gene whose expression was induced by CO_2 was *tem1*, a gene found in many hospital- and community-derived isolates of numerous bacteria [10]. Antibiotic resistance activity stemming from this gene would not be detected by standard phenotypic AST methods, and treatment failures could ensue. These findings also highlight the potential for a virtuous cycle between clinical labs and research labs, wherein a research lab can provide mechanisms and context for clinical observations, and alternative approaches can then be made that result in improved outcomes for patients.

Novel Pathogen Discovery

Medical history is punctuated with the introductions of new diseases caused by organisms that were unknown at the time of initial emergence. Identifying and learning about each of these organisms is a pioneering opportunity to expand the reach of medical treatment. In 1976, a cluster of acute hemorrhagic fever cases occurred in the town of Bumba, Zaire (now Democratic Republic of Congo), and all were connected to a single index case [11]. When Peter Piot learned that it was not Marburg virus he was looking at that had killed hundreds of Africans, but something never before seen, he described his emotion as one of "excitement" and "of being very privileged, that this was a moment of discovery." [12] The novel pathogen is now known as Zaire Ebola virus (EBOV), and has caused eleven additional epidemics of Ebola virus disease in Central and West Africa. A few years later in 1981, the U.S. Centers for Disease Control and Prevention formed a task force to monitor an outbreak of unusual cases of opportunistic infections affecting patients who had no known reason to be immunocompromised [13]. This novel disease, eventually named acquired immune deficiency syndrome (AIDS) [14,15], had spread to pandemic proportions before the transmission dynamics and the novel pathogen had been elucidated. During the early years of investigation, three research groups working in cooperation with clinicians treating patients, independently found a novel virus in AIDS patients [16-18]. The viruses these groups found ended up being one and the same, now known as the human immunodeficiency virus (HIV) [19]. Again, a novel organism introduced a new set of medical complications for humankind.

In the early morning hours of December 31, the ProMED listserv issued an alert and a request for information (RFI) in response to a cluster of acute respiratory distress syndrome (ARDS) cases in Wuhan, China. The initial information came in the form of guidance for hospitals issued by the Chinese Center for Disease Control, and a clinician shared the guidance with a reporter in Beijing. In the days that followed, speculation mounted in the international community of research and clinical scientists that the ARDS cases were caused by a novel virus. When global attention demanded that samples from patients in intensive care be shared with international researchers, researchers at the Wuhan Institute for Virology produced an isolate of a novel virus. The genome of the novel virus was rapidly sequenced made publicly available to researchers worldwide on January 9, 2020 via GISAID. By this time, probable cases were appearing in neighboring provinces. Global spread ensued shortly thereafter, and clinicians' need for a diagnostic test that could be performed in local clinical laboratories or at the point of care became urgent. Research laboratories that could design molecular diagnostic tests mobilized immediately, and worked in turn with clinical laboratory colleagues to refine and validate the methods. These testing strategies were then deployed to the clinic for use on patients within weeks, and used to facilitate contact tracing and infection control. On a parallel tract, bioinformatics analysis by research laboratories based on the sequences derived by clinical laboratories informed the design of multiple subunit or recombinant vaccines, several of which are now in late-phase clinical trials. It is notable that the pattern of interaction between the clinic, the clinical laboratory, and the research laboratory is consistent across these stories, but the timeframe in which the cycle completes has varied dramatically as biotechnology and information processing has improved.

While the pathologies associated with EBOV, HIV, and SARS-CoV-2 infection have been particularly impactful in the world, they are certainly not alone among new agents to affect humankind. Other novel organisms are identified on an ongoing basis. Some of these "new" organisms have been lurking unrecognized with us throughout our existence,

whereas others are more recent introductions from wildlife reservoirs or creations of evolution. A frequent underlying feature of many stories of novel pathogen discovery is that an isolate is collected and purified, and interactions between the clinical laboratory and research laboratories then occur. Research laboratories, be they located at universities, non-profit institutes, or government agencies, have the capacity to identify agents for which no validated diagnostic test exists, and are unconstrained by workflow efficiency or billing and reimbursement concerns. The emergence and future evolution of novel pathogens will continue to affect humankind, and the more we are able to learn about them through ongoing study and discovery facilitated by strong ties between clinical laboratories and research laboratories, the better equipped we will be to manage the potential complications they impose on our health.

Conclusion

The study of emerging and re-emerging infectious diseases requires a rapid response and coordination between the clinic, the clinical laboratory, and research laboratories. These disease outbreaks can arise in many different ways and can present disparate challenges to our diagnostic, treatment, and prevention strategies. Continuous communication between physicians and clinical laboratories to ensure cultivation of isolates from idiopathic infections, and clinical laboratories and research laboratories to characterize novel pathogens or unusual strains, is critical to the successful development of interventions and control measures to improve patient care and health outcomes.

Acknowledgements

NA, EG, and SP were supported by fellowships awarded by the Peter Morgane Research Fellowship Program. COVID-19 work at the University of New England is supported by a grant from the Kahn Family Foundation (MM).

Authors Contributions

All authors contributed equally to this manuscript.

References

1. Raoult D (2014) Editorial: Emerging clones of bacterial epidemics in the genomic area. Clin Microbiol Infect 20: 371-372.
2. Marston CK, Ibrahim H, Lee P, et al. (2016) Anthrax toxin-expressing bacillus cereus isolated from an anthrax-like eschar. PLoS One 11: e0156987.
3. Hoffmaster AR, Ravel J, Rasko DA, et al. (2004) Identification of anthrax toxin genes in a Bacillus cereus associated with an illness resembling inhalation anthrax. Proc Natl Acad Sci U S A 101: 8449-8454.
4. Hoffmaster AR, Hill KK, Gee JE, et al. (2006) Characterization of Bacillus cereus isolates associated with fatal pneumonias: Strains are closely related to Bacillus anthracis and harbor B. anthracis virulence genes. J Clin Microbiol 44: 3352-3360.
5. Wright AM, Beres SB, Consamus EN, et al. (2011) Rapidly progressive, fatal, inhalation anthrax-like infection in a human: Case report, pathogen genome sequencing, pathology, and coordinated response. Arch Pathol Lab Med 135: 1447-1459.
6. May M, Relich RF (2016) A comprehensive systems biology approach to studying Zika virus. PLoS One 11: e0161355.
7. Drexler JF, Helmer A, Kirberg H, et al. (2009) Poor clinical sensitivity of rapid antigen test for influenza A pandemic (H1N1) 2009 virus. Emerg Infect Dis 15: 1662-1664.
8. Pritt BS, Mead PS, Johnson DKH, et al. (2016) Identification of a novel pathogenic Borrelia species causing Lyme borreliosis with unusually high spirochaetaemia: A descriptive study. Lancet Infect Dis 16: 556-564.
9. (2016) United Nations meeting on antimicrobial resistance. Bull World Health Organ 94: 638-639.
10. Mullen N, Raposo H, Gudis P, et al. (2017) Induction of β-Lactamase activity and decreased β-Lactam susceptibility by CO_2 in clinical bacterial isolates. mSphere 2: e00266-17.
11. (1978) Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ 56: 271-293.
12. Brown R (2014) The virus detective who discovered Ebola. BBC News.
13. Basavapathruni A, Anderson KS (2007) Reverse transcription of the HIV-1 pandemic. FASEB J 21: 3795-3808.
14. Cohen J (2006) HIV/AIDS: Latin America & Caribbean. HAITI: Making headway under hellacious circumstances. Science 313: 470-473.
15. Centers for Disease Control (CDC) (1982) Update on acquired immune deficiency syndrome (AIDS)--United States. MMWR Morb Mortal Wkly Rep 31: 507-508, 513-514.
16. Barré-Sinoussi F, Chermann JC, Rey F, et al. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220: 868-871.
17. Gallo RC, Sarin PS, Gelmann EP, et al. (1983) Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science 220: 865-867.
18. Hoffman AD, Banapour B, Levy JA (1985) Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. Virology 147: 326-335.
19. Wiley CA, Schrier RD, Nelson JA, et al. (1986) Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. Proc Natl Acad Sci USA 83: 7089-7093.

DOI: 10.36959/856/510