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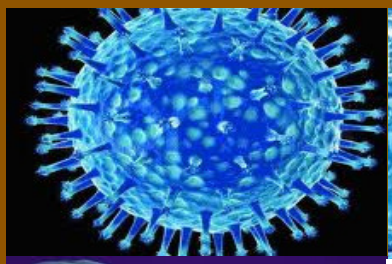

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Review article Open Access

Microbial Forensics: A Powerful Tool for Pursuing Bioterrorism Perpetrators and the Need for an International Database

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Abstract

The threat of bioterrorism has attracted a great deal of attention after the nightmare of September 11, 2001 and the subsequent days when letters containing anthrax spores attacked the USA. The urgent need for source-tracing of the anthrax spores promoted the rapid birth of microbial forensics, which includes the reliable identification of molecular variation (biomarkers) between related microbial strains for inferring the origin of a particular isolate. The critical step in the development of microbial forensics is to establish a comprehensive database for microbial source tracing. In addition, the development of a series of standardized protocols for this new discipline is essential as has been shown by the human forensics community. Genetic variations within *Yersinia pestis, Bacillus anthracis* and *Vibrio cholera* are given as examples for illustrating their use for source tracing. It is certainly easier for scientists within a single country to collaborate for establishing a genetic database for a given pathogen, but such a limited regional database will restrict the ability to track, or even exclude, international perpetrators. To overcome these limitations, an international collaboration mechanism needs to be developed by coordinating scientific, political and regulatory aspects.

The Real Threat of Bioterrorism

Bioterrorism has long been recognized as a threat to human beings, animals and agriculture [1]. The world was awakened with a real case following September 11th 2001 in the U.S.A. with the anthrax-letter attacks. Bioterrorism is the deliberate use of living organisms or their byproducts (toxins) to inflict harm to susceptible humans, animals, plants, the environment or material by individuals, state-sponsored groups, insurgent/rebel, Doomsday/cult-type group, nonaligned terrorists, splinter groups, or lone offenders, in order to cause societal panic or menace societal safety and stability. The motives behind bioterrorism can be political, ideological, religious, or criminal, and sometimes only for personal reprisal. It is different from biological warfare, which is also the use of living organisms or their byproducts (toxins) to inflict harm, but it has been used by armies for military purposes [2]. Both bioterrorism and biological warfare can have devastating effects on all kinds of biodiversity, including humans, animals, plants, and other life. They can also be directed against resources, for example water or food supplies [3] and, in modern applications, against natural and manufactured materials [4]. Other expressions, including biocrime (biological crime), bioattack (biological attack), biological incidence, biothreat (biological threat) and biowar (biological war) have also been used to describe bioterrorism or biological warfare.

Bioterrorism or biological warfare is not a new concept because history offers several, if not many, examples of the intentional use of biological agents or diseased cadavers as weapons for inflicting harm upon enemies. We can trace examples back to the 14th century's siege of Kaffa (now in Ukraine) for the earliest recorded biological war, which occurred between the Tatars and Kaffa. The Tatar forces catapulted plague corpses into the besieged city for spreading this disease. A plague outbreak was documented in Kaffa and it has been postulated that the fleeing citizens (as well as rats) of Kaffa who escaped via ship to various Mediterranean ports aided in the development of the second plague pandemic in the mid-1300s [5]. Another example of using disease as a biological weapon was the distribution of blankets or handkerchiefs used by the British smallpox victims as gifts to the hostile Native Americans by British troops in the 18th century [6]. Although there was indeed an outbreak of smallpox among these Native American tribes, it is difficult to know if these outbreaks were due to the British troop's activities or a natural consequence of susceptibility. Biological warfare has occurred more recently during World War I and II and was supported by the biological weapons research programs of German, American, British, USSR and Japanese governments for military use [6]. Particularly notable was the Japanese Army's notorious Unit 731, whose military medical doctors and specialists, under the command and direction of Lt. Gen. Ishii Shirō carried out diabolical experiments in Pingfan, Harbin, China on humans for the purpose of developing effective biological warfare weapons [7].

Modern examples of biological weapon and bioterrorism activity include: incidents in former USSR's Biopreparat [8,9]; Iraq's biological weapons program [8]; *Salmonella* contamination of the salad bars in The Dalles, Oregon, by the Rajneeshee commune for religious and political purposes [10]; anthrax hoaxes in the late 1990s in the United States [11]; bioterrorist attacks and development of botulinum toxin, anthrax, cholera, and Q fever for bioterrorist use in Japan by Aum Shinrikyo [12]; assassination of two Bulgarian traitors, Vladimir Kostov and Georgi Markov, by ricin ammunition in a small bullet shot by a modified umbrella [6]; anthrax spore letter attack in the United State in 2001 [13]; ricin bioterrorism attack in U.K. and several other documented bioterrorism hoaxes and non-succeed bioterrorist attacks [14].

The knowledge needed for developing biological weapons is accessible to individuals through the open literature and the Internet. Many bioterrorism-associated pathogens exist in host animals or vectors in their natural foci; bulk production of biological agents is cheap, with

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delivery and release not difficult to accomplish. The availability of information and ease of operation makes bioterrorism a method that could be quickly applied to many different nefarious purposes.

With advances of biotechnology, it is feared that bacteria and viruses could be modified in various ways to turn them into more powerful bioweapons [15]. The traditional biological agents, *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Brucella* spp., etc., can be engineered with new features, such as antibiotic resistance, vaccine-avoidance, decontaminant insensitive, environmentally stable and many others. Some have even speculated that advanced biotechnologies could be used by bioterrorists for creating engineered agents that target specific racial groups, a stealth virus that could incorporate itself into the DNA of the target population, and engineered virus with genes that control human behaviors and affect whole populations. These advanced technologies have been demonstrated through the creation of a novel virus by recombinating IL4 gene into mousepox virus [16], and the *in vitro* recreation of the 1918 Spanish flu virus [17] and polio virus [18]. Scientists in different fields that are paying attention to bioterrorism and biotechnology advances are increasingly worried about the misuse of biotechnology[4,15,19-22].

Microbial Forensics

Microorganisms have been used as means to perpetrate criminal acts, most recently highlighted by the terrorist attack on the U.S.A. using anthrax spore-containing letters in the fall of 2001. There are a number of issues for us to answer: such as the first responders' safety and safety of the public, suspected site investigation, sample collection, sample transfer, pathogen's isolation and identification, and the source-tracing of the bioagent. Source-tracing of a pathogen is of paramount importance for supporting criminal investigations and prosecuting the perpetrator(s) [23]. A number of questions needed to be addressed during the anthrax attacks about the relationship and the cause of cases in the four states, the relationship and origin of the anthrax bacteria isolated in different states, and the identity of the domestic or international perpetrator who released the pathogen [23]. All these questions can be answered within the purview of microbial forensics, which focuses on the individual or group who perpetrated the threat. During a bioterrorism criminal investigation, microbial forensics will employ traditional investigative methodologies, established molecular techniques, and newer advanced methods that still may be just under development for the identification and source-tracing of the pathogen [23]. As early as 1995, Dr. Randal S. Murch suggested that the investigation and solution of bioterrorism events would rely heavily on forensic science [24]. The laboratory analyses used for microbial forensics may include molecular sequencing, microbial cultures, biochemistry, electron microscopy, crystallography, and mass spectrometry. These analyses go well beyond those used for medical diagnoses and epidemiologic investigations [25].

The Federal Bureau of Investigation (FBI) of the United States created the Scientific Working Group on Microbial Genetics and Forensics, assembled from leading experts in the relevant fields, U.S. government agencies, professional organizations, and nonprofit and academic institutions, in order to develop, establish, and validate analytical methods, protocols and informatics, standards, training and certification programs, quality assurance programs, and research priorities for enhancing the capabilities of microbial forensics. The National Bioforensics Analysis Center was established recently in the United States by the Department of Homeland Security and it will be operated in partnership with the FBI for providing a central facility to conduct analysis of evidentiary material.

The task for microbial forensics includes sample identification, collection, handling and preservation, analytical method selection, casework analysis, and result's interpretation, validation and quality assurance. Hence, the new discipline of microbial forensics is a conglomeration of an array of well-established fields, such as microbial genomics, phylogenetics, forensic information, bioinformatics, computer science, and traditional microbiology and epidemiology. Microbial forensics will be most effective if there is sufficient basic scientific information concerning genetics, evolution, physiology and ecology of the biological agents. Molecular techniques have been used for years to trace outbreaks of microbial diseases, a practice called molecular epidemiology. PulseNet USA was established in 1996 by the United States Center for Disease Control for molecular surveillance of foodborne infections [26]. It has been successfully used for detection, investigation and control of numerous outbreaks caused by Shiga toxin-producing *Escherichia coli* O157:H7, *Salmonella enterica, Listeria monocytogenes, Shigella* spp., and *Campylobacter*. The PulseNet network is now being replicated in different ways in Canada, Europe, the Asia Pacific region, and Latin America [27]. These independent networks work together in PulseNet International allowing public health officials and laboratorians to share molecular epidemiologic information in real-time and enabling rapid recognition and investigation of multi-national foodborne disease outbreaks [27]. It represents a good example on how to establish a platform to be used as a primary tool in preparedness and response to bioterrorism. The types of genetic markers and features that can impact statistical inferences of microbial forensic evidence include: single nucleotide polymorphisms (SNPs), repetitive sequences, insertions and deletions, mobile elements, pathogenicity islands, virulence and resistance genes, housekeeping genes, structural genes, whole genome sequences, asexual and sexual reproduction, horizontal gene transfer, conjugation, transduction, lysogeny, gene conversion, recombination, gene duplication, rearrangements, and mutational hotspots [28]. Nucleic acid based typing technologies include multilocus sequence typing (MLST) [29], Multilocus variable-number tandem-repeat analysis (MLVA) [30], clustered regularly interspaced short palindromic repeats (CRISPR) [31], whole genome sequencing [32], and microarrays [33-39]. Some of these techniques have been used successfully for tracking *B. anthracis* used in the "white powder letter" biocrimes in 2001 [32] and release of aerosolized *B. anthracis* spores over Kameido, Japan by the Aum Shinrikyo cult [40]. Other potential molecular markers used for microbial forensics include protein and chemical markers of lipid polysaccharide and sugar etc. The stable isotope ratios of a seized pathogen culture could potentially reveal information about the environment in which the agent was produced [41-45]. It has been employed for predicting the delta(18)O and deltaD values of spores produced in nutritionally identical media and local water sources for five different locations around the United States [44]. No matter which techniques are used, a basic reference database for these markers is of paramount importance. Next, we will show the development of a genetic polymorphism database for *Yersinia pestis* and an example of tracking *B. anthracis* used in the 2001 anthrax-letter biocrime in the United States by different techniques.

Genetic Polymorphism Database for Source-Tracing of Bioterrorism-Associated Agents

As mentioned above, different techniques can be employed for establishing basic reference databases for microbial forensics analysis. Genetic, protein and chemical markers have been investigated for *Yersinia pestis*, the causative agent of bubonic and pneumonic plagues. It is thought to be one of the most dangerous bacterial pathogens in

the world. Credible estimates of the number of people killed by this bacterium during the course of history approach 200 million. Plague is maintained in natural disease foci in wild rodents through transmission between rodents by their flea ectoparasites. The striking lethality is a consequence of vector-borne transmission peculiarities for this pathogen in nature as infected fleas have to leave their dead hosts in the search of a blood meal and, thus, carry the infection to a new victim, new host. *Y. pseudotuberculosis* is believed to be the ancestor of *Y. pestis* [46-48]. Their evolutionary divergence occurred 2,600-28,000 years ago [46]. This speciation and further intraspecific modifications resulted in the wide spectrum of *Y. pestis* intraspecies groups (biovars, subspecies, ecotypes, plasmidovars, genotypes, etc.) differing in their host range and lethality. Currently, the microevolution of *Y. pestis* accommodates the adaptation of this pathogen to more than 200 species of wild rodents inhabiting natural plague foci on all of the continents except for Australia and Antarctica. Plague transmission is mediated by at least 80 different species of fleas. Studying *Y. pestis* microevolution may help solve basic medical microbiological questions such as the origin and evolution of animal and human infectious diseases, as well as, improve the methods of microbial forensics, agent identification, geno- and genomotyping of *Y. pestis*.

In the past few years, and owing in part to the availability of whole genome sequence data from many bacterial species including different strains from the same species, a high number of polymorphism sources, and consequently typing methods, have emerged. For *Yersinia pestis* these include: MLST [49], SNPs [50], MLVA [51,52], different region (DFR) typing [53] and analysis [31]. These new methods (SNPs, MLVA, DFR and CRISPRs) are likely to replace the previous pattern-comparison methods (such as insertion sequence (IS) typing by southern blotting, ribotyping or pulsed-field gel electrophoresis) which are more expensive and not fully appropriate to the creation of international databases [54-58]. As sequencing technologies advance, bacterial whole genome sequence analysis is becoming relatively easy for even conventional laboratories [59,60]. Using genome-wide variations of a pathogen for source tracing analysis will become popular because it will give us a comprehensive view of genetic variation from a specific pathogen and, hence, relatively definitive conclusions.

SNPs

Due to the close evolutionary relationship between *Y. pestis* and *Y. pseudotuberculosis*, they cannot be differentiated by MLST [49]. However, 76 conservative sSNPs within 3,250 orthologous CDSs (coding sequences) were revealed by comparative analysis of three whole genomes of *Y. pestis*, representing different biovars of this pathogen [50]. A three-branched phylogenetic tree was inferred by using SNP variations and a novel genetic typing scheme was proposed. By using whole genome sequence analysis, more SNPs have been uncovered for genetic typing, which is more discriminating [46]. By using SNP information, the etiology for the Black Death was successfully traced, confirming multiple importation events of the plague to Europe and that the ancient agent was the ancestor of modern *Y. pestis* biovars Orientalis and Medievalis [61]. By using whole genome sequencing and SNP information, *Y. pestis* from a specific region could be differentiated [62,63]. In our laboratory we have sequenced more than 100 strains of *Y. pestis* from different natural plague foci in China and more than 2000 new SNPs were found and used to create a very finely resolved phylogenetic tree (data not published), which will help us to design a more discriminative genotyping method to distinguish *Y. pestis* isolates.

MLVA

The genomic polymorphism associated with tandem repeats has been instrumental in mammalian genetics for the con¬struction of genetic maps and still is the basis of DNA fin¬gerprinting in forensic applications [64,65]. Tandem repeats are usually classified as satellites sequences (spanning megabases of DNA and associated with heterochromatin), minisatel¬lites (repeat units in the range 6-100 bp and spanning hun¬dreds of basepairs) or microsatellites (repeat units in the range 1-5 bp and spanning a few tens of nucleotides).

Recent studies show that tandem repeat polymorphisms of mini and mi¬crosatellites are likely to be a highly significant source of very informative markers for the identification of patho¬genic bacteria, even for recently emerged, highly monomorphic species [66-73]. Tandem repeats prob¬ably contribute to a pathogen's adaptation to its host, and they also account for bacterial phenotypic var¬iations depending on their genomic location. For example, if a tandem repeat is lo¬cated within the regulatory region of a gene it can play an on/off switching role in gene expression at the transcriptional level [74]. Similarly, if they are located within coding regions with a repeat unit length of three bases they may cause antigen variation for that bacterium; but if the repeat unit length is not a mul¬tiple of three, they can induce a truncated translation product.

VNTR sequences are common in the *Y. pestis* genome and occur frequently in gene coding regions. They are present at an average of 2.18 arrays per 10 kbp and are distributed evenly throughout the genome and the two virulence plasmids, pCD1 and pMT1 [75]. Tandem-repeat typing may prove to be a powerful complement to the existing phylogenetic tools for *Y. pestis*. Adair et al. [76] identified a tetranucleotide repeat sequence, (CAAA)N, in the genome of Yersinia pestis, and demonstrated that this region has nine alleles and great diversity (calculated as 1 minus the sum of the squared allele frequencies) (diversity value = 0.82) within a set of 35 diverse *Y. pestis* strains.

Multiple-locus VNTR analysis (MLVA) has proved to be capable of both distinguishing closely related strains and successfully classifying more distant relationships. Pourcel et al. [52] and Klevytska et al. [75] examined representative strains of *Y. pestis* by using 25 and 42 VNTR loci, respectively, and vast differences in diversity were observed among these loci. Pourcel et al. [52] grouped 180 *Y. pestis* into 61 different genotypes with the three biovars distributed correctly into the three main branches. They showed the heterogeneity of biovar Medievalis and proposed that 7 selected VNTR markers would be sufficient for quick characterization of a new strain. Compared to other genotyping methods, MLVA is easily standardized for establishing databases. The fact that *Y. pestis* is one of the most dangerous bioterrorism agents prevents the international exchange of bacterial strains and limits collaborative research; which creates a barrier for better understanding plague ecology and global evolution. Web-based comparison and identification of VNTR genotypes of *Y. pestis* capitalizes upon standardized typing systems and minimizes the impact of international transfer barriers.

Le Flèche et al. [74] created a database (http://minisatellites.u-psud.fr) of tandem repeats for pathogenic bacteria based on publicly available bacterial genomes. Its wide applicability was illustrated by the characterization of minisatellites from two important human pathogens, *Y. pestis* and *Bacillus anthracis*. They found that Y .pestis contains 64 minisatellites with repeat units at least 9 bp long where the unit is repeated at least 7 times.

Denoeud et al. [77] then presented an Internet-based resource to help develop and perform tandem repeats based bacterial strain typing. These tools are also accessible through the above Web link. There are four parts to the web page: 1) The "Tandem Repeats Database" enables the identification of tandem repeats across entire genomes. 2) The "Strain Comparison Page" identifies tandem repeats differing between different genome sequences from the same species. 3) The "Blast in the Tandem Repeats Database" facilitates the search for a known tandem repeat and the prediction of PCR product sizes using primers containing the tandem repeat. 4) The "Bacterial Genotyping Page" is a service for strain identification at the subspecies level.

MLVA has been used by American scientists to establish a database for more than 1000 *Y. pestis* strains [78]. The 43 markers used are relatively shorter VNTRs while the Europe scientists employed relatively longer repeats for database establishment [52]. We also employed these markers for successfully tracing the source of a primary pneumonic plague outbreak in 2009 [79]. If an internationally accepted database is developed, the method employed needs to be standardized.

DFR

Bacteria can adapt themselves to a new niche by gene loss and/or acquisition. These events can be analyzed by using whole genome sequencing or subtractive hybridization, but they are too expensive and laborious to be applied to a large number of bacterial strains. Whole genome-based DNA microarray is an ideal technique for analyzing genomic fragment differences among large numbers of bacterial strains. Zhou et al. employed a whole genome DNA microarray, comprising 4005 ORFs (genes) from *Y. pestis* 91001 and CO92, to screen the differences between 43 strains of *Y. pestis* and 7 strains of *Y. pseudotuberculosis* [53]. They found twenty-two DFRs among these strains. Next, one or more genes were chosen from each DFR to represent the genomic difference. PCR amplification of the selected genes was performed on more than 900 isolates of *Y. pestis*, isolated from different natural plague foci and different years, to screen for the distribution of DFRs in these strains. The DFR profiles of these isolates could be assigned into fourteen genomovar. The DFRs represent the dynamic regions of the *Y. pestis* genome in natural populations; suggesting their features of acquisition or deletion have a role in the adaptive evolution of *Y. pestis*. The parallel microevolution of the *Y. pestis* genome in natural populations was examined and revealed evidence of genome content flux through the loss/acquisition of plasmids and chromosomal segments.

The microevolution of the genomovars is perfectly consistent with the expansion of plague foci [53,80]. Each of the genomovars is confined to a specific geographic region, namely a plague focus or part of a focus with a unique set of environments, reservoirs and vectors. Most of the geographic regions with different primary reservoirs have their own unique genomovars [80]. Sometimes, there is more than one genomovar in a single focus and within a single primary reservoir, but each of the genomovars corresponds to a unique natural environment and primary vector(s). Therefore, DFR could be used to roughly locate the origin of an unknown *Y. pestis*, but more discriminative differentiation of its strains needs the help of other molecular methods such as MLVA or SNPs [81].

CRISPRs

CRISPRs are well-defined structures and present in many bacteria and in most archaea, sometimes in multiple copies per genome. The CRISPR structure itself is usually surrounded by CRISPR-associated genes (*cas* genes). New spacer sequences are not synthesized *de novo*, but are copied from existing DNA sequences [31]. The vast majority of known spacers lack any similarity to currently available sequences. However, when similarities exist, they most often correspond to short portions of mobile elements such as phages. These observations have led to the suggestion that CRISPRs were a defense-mechanism against genetic invasions [31]. Simple evolution rules have been proposed for CRISPRs which open the way to phylogenetic investigations: (1) new spacers are acquired in a polarized way from one extremity adjacent to the leader sequence which acts as a transcription promoter; (2) losses may occur randomly along the array; (3) the probability of acquisition of the same spacers independently is extremely low. The analysis of CRISPRs have already played an important role in investigating the epidemiology of the major human pathogen, *Mycobacterium tuberculosis* (the corresponding typing method is called "spoligotyping"). A database containing the typing information from thirty thousand isolates has been built [82]. Although this represents only a very small fraction of TB isolates worldwide, the database is by far the largest existing typing database for a bacterial pathogen. One reason is that the method is sufficiently robust and easy to run at a reasonable cost, such that many laboratories could produce the data and easily share it. Secondly, the resulting data is useful for phylogenetic analysis and has enabled definition of large strain families. A third reason was the relatively simple status of the *M. tuberculosis* CRISPR locus. The locus is apparently inactive; it does not acquire new spacers, so that a fixed and limited set of relevant spacers could be defined to produce "spoligotyping membranes". The availability of a similar approach for *Y. pestis* would be of use for at least two reasons. First, it would allow for the large-scale screening of *Yersinia pseudotuberculosis* strains to identify *Y. pestis*'s closest neighbors, as well as providing a systematic and routine typing approach for current *Y. pestis* collections and new isolates. Secondly, CRISPRs represent a potentially very interesting tool for the investigation of ancient DNA (aDNA). The nature of the early plague pandemics is still controversial, and one reason for this may be the lack of appropriate genetic targets for *Y. pestis* aDNA investigation. *Y. pestis* CRISPR loci are still active31 and able to acquire new spacers (in contrast with the *M. tuberculosis* CRISPRs). Therefore, it is necessary to list the repertoire of existing CRISPR spacers. If this repertoire eventually turns out to be very large, as in some bacteria, then the development of such an assay will necessitate the use of DNA chips, which are able to deal with a larger number of spacers compared to the current spoligotyping assay format. The first repertoire of CRISPR spacers was deduced from *Y. pestis* isolates that only represent a small part of the global and historical genetic diversity for *Y. pestis* [31]. Less than 40 spacers were identified, in the three *Y. pestis* CRISPR loci, suggesting that perhaps the repertoire within a larger collection might remain tractable.

Whole genome sequencing analysis

Whole genome sequencing has been widely used for *Y. pestis* and used for tracing the source of a laboratory acquired plague [83]. With whole genome sequences, we can identify different kinds of genetic variations, including SNPs, gene rearrangement, gene loss and gain, VNTRs and CRISPRs for a comprehensive understanding of bacterial genome diversity and for a fine source-tracing analysis. When these genome-wide variations are used for typing purpose, it is refered to WGST (Whole Genome Sequence Typing). With the rapid advances of next generation sequencing technologies, we can obtain genome sequences from tens or hundreds of bacterial strains in a single species, providing us an opportunity to build a database of genome diversity for source-tracing analysis. As shown above, the different genetic variation

types can be input into databases and then coupled to source-tracing software, rendering a rapid and accurate tracking of the target pathogen.

Other methods

Other methods, including fatty acids analysis and bacterial mass fingerprinting (BMF), are potentially useful for strain discrimination. Song et al. evaluated the possibility of utilizing Cellular Fatty Acids (CFA) information for typing *Y. pestis* [84]. They used 58 strains representing different ecotypes isolated in different regions of China. Major CFAs of the tested strains are 16:0, cyclo 17:0, 3OH-14:0, ω7c 16:1 and monounsaturated 18 carbon acids, which account for more than 70% of the total CFAs. Dendrogram construction of the tested strains reveals a great homogeneity among these *Y. pestis* isolates, which suggests that CFA analysis cannot serve as a powerful typing tool for *Y. pestis* strain differentiation. All the experimental *Y. pestis* strains were mis-identified as *Y. pseudotuberculosis* by the Sherlock system. Later, Tan et al. found that these two pathogens could be differentiated by using ratios of certain CFA components as chemical markers [85]. Additionally, Liu et al. developed a universal sample preparation method for BMFanalysis and has demonstrated the discriminatory power of BMF [86]. BMF was obtained by MALDI-TOF MS and it has been shown to be potential for inexpensive and rapid typing of MRSA in infection control [87,88]. MALDI-TOF MS was also successfully employed to identify *Legionella* spp. with easy operation and high efficiency [89].

Tracking of *Bacillus Anthracis* used in Biocrime of 2001 in the United States

After it was determined that the anthrax cases following September 11 were bioterrorist attacks, the FBI used every investigative technique available to investigate the criminal perpetrator(s). The FBI employed all possible strategies including: round-the-clock surveillances, eavesdropping, searches and hundreds of polygraphs. Altogether, agents conducted 5,000 interviews and served more than 1,700 grand jury subpoenas, which involved 40 of the FBI's 56 field offices, as well as many of its 44 overseas legal attaches. The Bureau established 112 separate databases to store information about the case, and even offered a reward of $2.5 million for information leading to a conviction. In the end, it was the scientific evidence, including genome sequencing, VNTR analysis and analysis of the extrasporular material in the envelopes, that provided the final results that the *B. anthracis* attack strain was the Ames strain and "homemade in USA" [90]. Comparison of the whole-genome sequence of *Bacillus anthracis* isolated from a victim of the bioterrorist anthrax attack with a reference revealed 60 new markers that included single nucleotide polymorphisms (SNPs), inserted or deleted sequences, and tandem repeats [32]. Further molecular subtyping of *Bacillus anthracis* strains during the 2001 bioterrorism-associated outbreak demonstrated that all isolates were MLVA genotype 62, the same as the Ames strain used in laboratories [13]. Sequence analysis of the protective antigen gene (*pagA*) from 42 representative outbreak isolates determined that they all had a *pagA* sequence indistinguishable from the Ames strain (PA genotype I) [13]. Recently, a systematic microbiological analysis of the spores from 2001 letters revealed morphological mutants that are not commonly seen in the environment. The morphological mutant analysis was a novel approach for forensics that combined systematic microbiological analysis with whole-genome sequencing and comparative genomics [91].

Source-Tracing of *Vibrio Cholera* Outbreak in Haiti after Earthquake In 2010

On January 12, 2010 there was a 7.0 magnitude earthquake in the capital of Haiti, Port-au-Prince, and surrounding areas. Some 10 months later there was a devastating outbreak of cholera [92]. Its origin could not be confirmed, but origin of the disease was rumored to be a battalion of Nepalese soldiers serving as United Nations peacekeepers [93,94]. Though the use of WGST, the Haiti outbreak strains were confirmed to be closely linked to Nepalese strains, with only one or two base-pair differences, providing sound evidence that the Haiti outbreak of cholera was caused by a *Vibrio cholera* strain from Nepal [95]. The importance of this source-tracing analysis is that it shows the rapid global transmission of infectious disease and the power of molecular biological techniques in tracking the agent of infectious disease. This work also strengthens the need for a genetic diversity database based on whole genome sequencing analysis and argues for the sharing of data for all known pathogens though international collaboration. It is only through international data sharing and databases that source-tracing of pathogens will be truly effective.

Database-Building and Perspectives

The new discipline of microbial forensics represents a powerful approach for tracking pathogens associated with bioterrorism or natural disease's outbreaks. As mentioned above, the key factor for successful development of microbial forensics is database-building for different biomarkers and all available global strains. Given that the transfer of bioterrorism-associated pathogens and their byproducts, even DNAs is frequently restricted or even prohibited by law, it makes database-building for international sharing extremely difficult. In other words, development of such a database is not only a technical challenge for the scientific community, but also a political challenge for the international community. How we use our wisdom to bridge this gap is paramount for the successful development of international microbial forensics. A country-limited database will not be as useful for the investigation of international bioterrorism and international spread of infectious diseases as a global joint effort. Communication between scientists and politicians from different nations, and collaborations between scientists from different countries, will be essential for developing internationally accepted techniques for standardized biomarkers and also critical for establishment of such a database. For example, there are two well-developed MLVA techniques for *Y. pestis*, but scientists from the U.S.A. employed smaller repeats markers [78], and those from Europe larger ones [52], making these data mostly incompatible. Without the collaboration of scientists from Russia and China, where the most diversified strains of *Y. pestis* are distributed [48,96,97], the MLVA databases of *Y. pestis* will not be comprehensive. Therefore, an authoritative database should be built based on the consensus reached by scientists in the field. WGST [95] is now the most applicable technique for such database-building and finely selected MLVA loci could be the second technique of choice because it is relatively easy to standardize [40,79]. Although PFGE has been successfully used for tracing the source of food-borne pathogens, its discriminative power is relatively low and its operation is tedious, rendering this technique unsuitable for forensic database-building. However, the experiences with this technique for database-building should be taken as an example for microbial forensics [26,98-101]. Other kinds of methods, including MLST, DFR and CRISPR analysis, could also be used for a specific case investigation.

In conclusion, development of microbial forensics is critical to the pursuit of biocriminals, and international collaborations and

political decisions will play key roles in successful progress of this new discipline.

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References

1. Wilson TM, Gregg DA, King DJ, Noah DL, Perkins LE, et al. (2001) Agroterrorism, biological crimes, and biowarfare targeting animal agriculture. The clinical, pathologic, diagnostic, and epidemiologic features of some important animal diseases. Clin Lab Med 21: 549-591.
2. Hilleman MR (2002) Overview: cause and prevention in biowarfare and bioterrorism. Vaccine 20: 3055-3067.
3. Spake A (2001) Food fright. Terrorism spotlights the risks in the food supply. US News World Rep 131: 48-50.
4. Atlas RM, Dando M (2006) The dual-use dilemma for the life sciences: perspectives, conundrums, and global solutions. Biosecur Bioterror 4: 276-286.
5. Klietmann WF, Ruoff KL (2001) Bioterrorism: implications for the clinical microbiologist. Clin Microbiol Rev 14: 364-381.
6. Noah DL, Huebner KD, Darling RG, Waeckerle JF (2002) The history and threat of biological warfare and terrorism. Emerg Med Clin North Am 20: 255-271.
7. Drea E, Bradsher G, Hanyok R, Lide J, Petersen M, Yang D (2007) Researching Japanese War Crimes Records: an introductory essay. Nazi War Crimes and Japanese Imperial Government Records Interagency Working Group ISBN: 1-880875-28-4.
8. Davis CJ (1999) Nuclear blindness: an overview of the biological weapons programs of the former Soviet Union and Iraq. Emerg Infect Dis 5: 509-512.
9. Shoham D, Wolfson Z (2004) The Russian biological weapons program: vanished or disappeared? Crit Rev Microbiol 30: 241-261.
10. Torok TJ, Tauxe RV, Wise RP, Livengood JR, Sokolow R, et al. (1997) A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. JAMA 278: 389-395.
11. Moran GJ (1999) Update on emerging infections from the Centers for Disease Control and Prevention: bioterrorism alleging use of anthrax and interim guidelines for management-United States, 1998. Ann Emerg Med 34: 229-232.
12. Olson KB (1999) Aum Shinrikyo: once and future threat? Emerg Infect Dis 5: 513-516.
13. Hoffmaster AR, Fitzgerald CC, Ribot E, Mayer LW, Popovic T (2002) Molecular subtyping of *Bacillus anthracis* and the 2001 bioterrorism-associated anthrax outbreak, United States. Emerg Infect Dis 8: 1111-1116.
14. Budowle B, Murch R, Chakraborty R (2005) Microbial forensics: the next forensic challenge. Int J Legal Med 119: 317–330.
15. Spier RE (2003) Bioterrorism and the biotechnologist. Vaccine 21: 582-585.
16. Jackson RJ, Ramsay AJ, Christensen CD, Beaton S, Hall DF, et al. (2001) Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. J Virol 75: 1205-1210.
17. Reid AH, Fanning TG, Janczewski TA, Taubenberger JK (2000) Characterization of the 1918 “Spanish” influenza virus neuraminidase gene. Proc Natl Acad Sci U S A 97: 6785-6790.
18. Cello J, Paul AV, Wimmer E (2002) Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. Science 297: 1016-1018.
19. Atlas RM (2003) Bioterrorism and biodefence research: changing the focus of microbiology. Nat Rev Microbiol 1: 70-74.
20. Service RF (2006) Biosecurity. Synthetic biologists debate policing themselves. Science 312: 1116.
21. Coupland R, Leins KR (2005) Science and prohibited weapons. Science 308: 1841.
22. Check E (2005) Synthetic biologists face up to security issues. Nature 436: 894-895.
23. Keim P (2003) Microbial Forensics: A Scientific Assessment. A Report from American Academy of Microbiology.
24. Murch RS (2003) Microbial Forensics: Building a National Capacity to Investigate Bioterrorism. Biosecur Bioterror 1: 117-122.
25. Schutzer SE, Budowle B, Atlas RM (2005) Biocrimes, Microbial Forensics, and the Physician. PLoS Med 2: e337.
26. Gerner-Smidt P, Hise K, Kincaid J, Hunter S, Rolando S, et al. (2006) PulseNet USA: a five-year update. Foodborne Pathog Dis 3: 9-19.
27. Swaminathan B, Gerner-Smidt P, Ng LK, Lukinmaa S, Kam KM, et al. (2006) Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. Foodborne Pathog Dis 3: 36-50.
28. Budowle B, Johnson MD, Fraser CM, Leighton TJ, Murch RS, et al. (2005) Genetic analysis and attribution of microbial forensics evidence. Crit Rev Microbiol 31: 233-254.
29. Schouls LM, Reulen S, Duim B, Wagenaar JA, Willems RJ, et al. (2003) Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. J Clin Microbiol 41: 15-26.
30. Marsh JW, O’Leary MM, Shutt KA, Pasculle AW, Johnson S, et al. (2006) Multilocus variable-number tandem-repeat analysis for investigation of *Clostridium difficile* transmission in Hospitals. J Clin Microbiol 44: 2558-2566.
31. Pourcel C, Salvignol G, Vergnaud G (2005) CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology 151: 653-663.
32. Read TD, Salzberg SL, Pop M, Shumway M, Umayam L, et al. (2002) Comparative genome sequencing for discovery of novel polymorphisms in *Bacillus anthracis*. Science 296: 2028-2033.
33. Cebula TA, Jackson SA, Brown EW, Goswami B, LeClerc JE (2005) Chips and SNPs, bugs and thugs: a molecular sleuthing perspective. J Food Prot 68: 1271-1284.
34. Chandler DP, Alferov O, Chernov B, Daly DS, Golova J, et al. (2006) Diagnostic oligonucleotide microarray fingerprinting of Bacillus isolates. J Clin Microbiol 44: 244-250.
35. Sergeev N, Distler M, Courtney S, Al-Khaldi SF, Volokhov D, et al. (2004) Multipathogen oligonucleotide microarray for environmental and biodefense applications. Biosens Bioelectron 20: 684-698.
36. Wilson WJ, Strout CL, DeSantis TZ, Stilwell JL, Carrano AV, et al. (2002) Sequence-specific identification of 18 pathogenic microorganisms using microarray technology. Mol Cell Probes 16: 119-127.
37. Tuanyok A, Tom M, Dunbar J, Woods DE (2006) Genome-wide expression analysis of *Burkholderia pseudomallei* infection in a hamster model of acute melioidosis. Infect Immun 74: 5465-5476.
38. Willse A, Straub TM, Wunschel SC, Small JA, Call DR, et al. (2004) Quantitative oligonucleotide microarray fingerprinting of *Salmonella enterica* isolates. Nucleic Acids Res 32: 1848-1856.
39. Cebula TA, Brown EW, Jackson SA, Mammel MK, Mukherjee A, et al. (2005) Molecular applications for identifying microbial pathogens in the post-9/11 era. Expert Rev Mol Diagn 5: 431-445.
40. Keim P, Smith KL, Keys C, Takahashi H, Kurata T, et al. (2001) Molecular investigation of the Aum Shinrikyo anthrax release in Kameido, Japan. J Clin Microbiol 39: 4566-4567.
41. Kreuzer-Martin HW, Chesson LA, Lott MJ, Dorigan JV, Ehleringer JR (2004) Stable isotope ratios as a tool in microbial forensics--Part 1. Microbial isotopic composition as a function of growth medium. J Forensic Sci 49: 954-960.
42. Kreuzer-Martin HW, Chesson LA, Lott MJ, Dorigan JV, Ehleringer JR (2004) Stable isotope ratios as a tool in microbial forensics--Part 2. Isotopic variation among different growth media as a tool for sourcing origins of bacterial cells or spores. J Forensic Sci 49: 961-967.
43. Kreuzer-Martin HW, Chesson LA, Lott MJ, Ehleringer JR (2005) Stable isotope

ratios as a tool in microbial forensics--part 3. Effect of culturing on agar-containing growth media. J Forensic Sci 50: 1372-1379.

44. Kreuzer-Martin HW, Lott MJ, Dorigan J, Ehleringer JR (2003) Microbe forensics: oxygen and hydrogen stable isotope ratios in Bacillus subtilis cells and spores. Proc Natl Acad Sci U S A 100: 815-819.

45. Fang J, Lovanh N, Alvarez PJ (2004) The use of isotopic and lipid analysis techniques linking toluene degradation to specific microorganisms: applications and limitations. Water Res 38: 2529-2536.

46. Morelli G, Song Y, Mazzoni CJ, Eppinger M, Roumagnac P, et al. (2010) *Yersinia pestis* genome sequencing identifies patterns of global phylogenetic diversity. Nat Genet 42: 1140-1143.

47. Song Y, Tong Z, Wang J, Wang L, Guo Z, et al. (2004) Complete genome sequence of *Yersinia pestis* strain 91001, an isolate avirulent to humans. DNA Res 11: 179-197.

48. Zhou D, Han Y, Song Y, Huang P, Yang R (2004) Comparative and evolutionary genomics of *Yersinia pestis*. Microbes Infect 6: 1226-1234.

49. Achtman M, Zurth K, Morelli G, Torrea G, Guiyoule A, et al. (1999) *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A 96: 14043-14048.

50. Achtman M, Morelli G, Zhu P, Wirth T, Diehl I, et al. (2004) Microevolution and history of the plague bacillus, *Yersinia pestis*. Proc Natl Acad Sci USA 101: 17837-17842.

51. Achtman M (2004) Population structure of pathogenic bacteria revisited. Int J Med Microbiol 294: 67-73.

52. Pourcel C, Andre-Mazeaud F, Neubauer H, Ramisse F, Vergnaud G (2004) Tandem repeats analysis for the high resolution phylogenetic analysis of *Yersinia pestis*. BMC Microbiol 4: 22.

53. Zhou D, Han Y, Song Y, Tong Z, Wang J, et al. (2004) DNA microarray analysis of genome dynamics in *Yersinia pestis*: insights into bacterial genome microevolution and niche adaptation. J Bacteriol 186: 5138-5146.

54. Huang XZ, Chu MC, Engelthaler DM, Lindler LE (2002) Genotyping of a homogeneous group of *Yersinia pestis* strains isolated in the United States. J Clin Microbiol 40: 1164-1173.

55. Guiyoule A, Rasoamanana B, Buchrieser C, Michel P, Chanteau S, et al. (1997) Recent emergence of new variants of *Yersinia pestis* in Madagascar. J Clin Microbiol 35: 2826-2833.

56. Voskressenskaya E, Leclercq A, Tseneva G, Carniel E (2005) Evaluation of ribotyping as a tool for molecular typing of *Yersinia pseudotuberculosis* strains of worldwide origin. J Clin Microbiol 43: 6155-6160.

57. Odaert M, Berche P, Simonet M (1996) Molecular typing of *Yersinia pseudotuberculosis* by using an IS200-like element. J Clin Microbiol 34: 2231-2235.

58. Grif K, Dierich MP, Much P, Hofer E, Allerberger F (2003) Identifying and subtyping species of dangerous pathogens by automated ribotyping. Diagn Microbiol Infect Dis 47: 313-320.

59. Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, et al. (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet 12: 499-510.

60. Brockhurst MA, Colegrave N, Rozen DE (2011) Next-generation sequencing as a tool to study microbial evolution. Mol Ecol 20: 972-980.

61. Haensch S, Bianucci R, Signoli M, Rajerison M, Schultz M, et al. (2010) Distinct clones of *Yersinia pestis* caused the black death. PLoS Pathog 6: e1001134.

62. Vogler AJ, Driebe EM, Lee J, Auerbach RK, Allender CJ, et al. (2008) Assays for the rapid and specific identification of North American *Yersinia pestis* and the common laboratory strain CO92. Biotechniques 44: 201, 3-4, 7.

63. Touchman JW, Wagner DM, Hao J, Mastrian SD, Shah MK, et al. (2007) A North American *Yersinia pestis* draft genome sequence: SNPs and phylogenetic analysis. PLoS One 2: e220.

64. Pepinski W, Janica J, Aleksandrowicz-Bukin M, Skawronska M, Koc-Zorawska, et al. (2004) Population genetics of 10 short tandem repeat (STR) loci in a population sample of the ethnic group of Polish Tatars living in the Podlasie area (Northeastern Poland). Folia Morphol (Warsz) 63: 249-252.

65. Kondopoulou H, Kouvatsi A, Triantaphyllidis C (2001) Forensic evaluation of 10 STRs and two minisatellite loci in the Greek population. Forensic Sci Int 124: 228-230.

66. Parra A, Fernandez-Llario P, Tato A, Larrasa J, Garcia A, et al. (2003) Epidemiology of *Mycobacterium bovis* infections of pigs and wild boars using a molecular approach. Vet Microbiol 97: 123-133.

67. Banu S, Gordon SV, Palmer S, Islam MR, Ahmed S, et al. (2004) Genotypic analysis of *Mycobacterium tuberculosis* in Bangladesh and prevalence of the Beijing strain. J Clin Microbiol 42: 674-682.

68. Crawford JT (2003) Genotyping in contact investigations: a CDC perspective. Int J Tuberc Lung Dis 7: S453-457.

69. Sabat A, Krzyszton-Russjan J, Strzalka W, Filipek R, Kosowska K, et al. (2003) New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. J Clin Microbiol 41: 1801-1804.

70. Savine E, Warren RM, van der Spuy GD, Beyers N, van Helden PD, et al. (2002) Stability of variable-number tandem repeats of mycobacterial interspersed repetitive units from 12 loci in serial isolates of *Mycobacterium tuberculosis*. J Clin Microbiol 40: 4561-4566.

71. Kim W, Hong YP, Yoo JH, Lee WB, Choi CS, et al. (2002) Genetic relationships of *Bacillus anthracis* and closely related species based on variable-number tandem repeat analysis and BOX-PCR genomic fingerprinting. FEMS Microbiol Lett 207: 21-27.

72. Chanchaem W, Palittapongarnpim P (2002) A variable number of tandem repeats result in polymorphic alpha -isopropylmalate synthase in *Mycobacterium tuberculosis*. Tuberculosis (Edinb) 82: 1-6.

73. van Belkum A, Scherer S, van Leeuwen W, Willemse D, van Alphen L, et al. (1997) Variable number of tandem repeats in clinical strains of *Haemophilus influenzae*. Infect Immun 65(12): 5017-5027.

74. Le Fleche P, Hauck Y, Onteniente L, Prieur A, Denoeud F, et al. (2001) A tandem repeats database for bacterial genomes: application to the genotyping of *Yersinia pestis* and *Bacillus anthracis*. BMC Microbiol 1: 2.

75. Klevytska AM, Price LB, Schupp JM, Worsham PL, Wong J, et al. (2001) Identification and characterization of variable-number tandem repeats in the *Yersinia pestis* genome. J Clin Microbiol 39: 3179-3185.

76. Adair DM, Worsham PL, Hill KK, Klevytska AM, Jackson PJ, et al. (2000) Diversity in a variable-number tandem repeat from *Yersinia pestis*. J Clin Microbiol 38: 1516-1519.

77. Denoeud F, Vergnaud G (2004) Identification of polymorphic tandem repeats by direct comparison of genome sequence from different bacterial strains: a web-based resource. BMC Bioinformatics 5: 4.

78. Lowell JL, Zhansarina A, Yockey B, Meka-Mechenko T, Stybayeva G, et al. (2007) Phenotypic and molecular characterizations of *Yersinia pestis* isolates from Kazakhstan and adjacent regions. Microbiology 153: 169-177.

79. Wang H, Cui Y, Wang Z, Wang X, Guo Z, et al. (2011) A dog-associated primary pneumonic plague in Qinghai Province, China. Clin Infect Dis 52: 185-190.

80. Li Y, Dai E, Cui Y, Li M, Zhang Y, et al. (2008) Different region analysis for genotyping *Yersinia pestis* isolates from China. PLoS One 3: e2166.

81. Li Y, Cui Y, Hauck Y, Platonov ME, Dai E, et al. (2009) Genotyping and phylogenetic analysis of *Yersinia pestis* by MLVA: insights into the worldwide expansion of Central Asia plague foci. PLoS One 4: e6000.

82. Brudey K, Driscoll JR, Rigouts L, Prodinger WM, Gori A, et al. (2006) *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol 6: 23.

83. (2011) Fatal laboratory-acquired infection with an attenuated *Yersinia pestis* Strain--Chicago, Illinois, 2009. MMWR Morb Mortal Wkly Rep 60: 201-205.

84. Song Y, Guo Z, Zhang M, Yang R, Zhao M, Cong X, et al. (2002) Cellular Fatty acids analysis of *Yersinia pestis* strains in China. Zhonghua Wei Sheng Wu Yu Mian Yi Xue Za Zhi.

85. Tan Y, Wu M, Liu H, Dong X, Guo Z, et al. (2010) Cellular fatty acids as chemical markers for differentiation of *Yersinia pestis* and *Yersinia pseudotuberculosis*. Lett Appl Microbiol 50: 104-111.

86. Liu H, Du Z, Wang J, Yang R (2007) A universal sample preparation method for

characterization of bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl Environ Microbiol 73: 1899-1907.

87. Wolters M, Rohde H, Maier T, Belmar-Campos C, Franke G, et al. (2011) MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant *Staphylococcus aureus* lineages. Int J Med Microbiol 301: 64-68.

88. Du Z, Yang R, Guo Z, Song Y, Wang J (2002) Identification of *Staphylococcus aureus* and determination of its methicillin resistance by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal Chem 74: 5487-5491.

89. Gaia V, Casati S, Tonolla M (2011) Rapid identification of Legionella spp. by MALDI-TOF MS based protein mass fingerprinting. Syst Appl Microbiol 34: 40-44.

90. Gary Matsumoto (2003) Anthrax Powder: State of the Art?. Science 302: 1492-1497.

91. Rasko DA, Worsham PL, Abshire TG, Stanley ST, Bannan JD, et al. (2011) *Bacillus anthracis* comparative genome analysis in support of the Amerithrax investigation. Proc Natl Acad Sci U S A 108: 5027-5032.

92. Walton DA, Ivers LC (2011) Responding to cholera in post-earthquake Haiti. N Engl J Med 364: 3-5.

93. Enserink M (2011) Haiti's cholera outbreak. Cholera linked to U.N. forces, but questions remain. Science 332: 776-777.

94. Enserink M (2011) Epidemiology. Despite sensitivities, scientists seek to solve Haiti's cholera riddle. Science 331: 388-389.

95. Hendriksen RS, Price LB, Schupp JM, Gillece JD, Kaas RS, et al. (2011) Population Genetics of Vibrio cholerae from Nepal in 2010: evidence on the origin of the Haitian outbreak. MBio 2: e00157-11.

96. Anisimov AP, Lindler LE, Pier GB (2004) Intraspecific diversity of *Yersinia pestis*. Clin Microbiol Rev 17: 434-464.

97. Zhou D, Han Y, Yang R (2006) Molecular and physiological insights into plague transmission, virulence and etiology. Microbes Infect 8: 273-284.

98. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, et al. (2006) Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, Salmonella, and Shigella for PulseNet. Foodborne Pathog Dis 3: 59-67.

99. Hunter SB, Vauterin P, Lambert-Fair MA, Van Duyne MS, Kubota K, et al. (2005) Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. J Clin Microbiol 43: 1045-1050.

100. Graves LM, Swaminathan B (2001) PulseNet standardized protocol for subtyping Listeria monocytogenes by macrorestriction and pulsed-field gel electrophoresis. Int J Food Microbiol 65: 55-62.

101. Gerner-Smidt P, Scheutz F (2006) Standardized pulsed-field gel electrophoresis of Shiga toxin-producing *Escherichia coli*: the PulseNet Europe Feasibility Study. Foodborne Pathog Dis 3: 74-80.

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