# Metabolomic profiling enables the rapid detection of antimicrobial resistant (AMR) human pathogenic bacteria

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## Abstract

Statement of the Problem: Rapid diagnosis of AMR strains of human pathogenic bacteria enables informed decisions regarding therapeutic options and may be critical to the effectiveness of clinical treatment. Techniques such as Polymerase Chain Reaction (PCR), Microbial Culturing, and/or Enzyme-Linked Immunosorbent Assays (ELISA) are well established, however can be time consuming, laborious, and costly. The purpose of this study was to develop a really rapid detection method for the identification of AMR strains of pathogenic bacteria, using Yersinia pestis (the causative agent of the plague) as a model organism. Methodology & Theoretical Orientation: Microbial volatile organic compounds (mVOCs) are a family of structurally diverse, microbial-derived metabolites, generally related by their volatility at room temperature. Here, we employed headspace solid phase microextraction (hSPME), including gas chromatography (GC), for the extraction and analysis of mVOCs emanating from bacterial cultures of untamed type and kanamycin resistant strains of Yersinia pestis. To ensure broad chemical diversity within the derived mVOC profiles, while still enabling a rapid analysis time, we employed a way mentioned as simultaneous multi-headspace SPME (simulti-hSPME). Findings: Using simulti-hSPME with diverse sorbent types, we generated mVOC profiles that serve as metabolomic fingerprints that readily differentiate wild type (kanamycin sensitive) and kanamycin resistant strains of Yersinia pestis. The complete analysis can be completed within 15 minutes. Conclusion & Significance: Rapid diagnosis of AMR strains of human bacterial pathogens is crucial for effective therapeutic intervention. Our mVOC metabolomics profiling approach quickly and effectively differentiates wild type (kanamycin sensitive) and kanamycin resistant strains of Yersinia pestis. Application of this method to other bacteria and other sorts of AMR is ongoing and holds promise as an efficient clinical diagnostic procedure.

Antimicrobial resistance is one among the foremost worrying threats to humankind with extremely high healthcare costs associated. The current technologies utilized in clinical microbiology to spot the bacterial agent and profile antimicrobial susceptibility are time-consuming and regularly expensive. As a result, physicians prescribe empirical antimicrobial therapies. This scenario is often the cause of therapeutic failures, causing higher mortality rates and healthcare costs, as well as the emergence and spread of antibiotic resistant bacteria. As such, new technologies for rapid identification of the pathogen and antimicrobial susceptibility testing are needed. This review summarizes the present technologies, and therefore the promising emerging and future alternatives for the identification and profiling of antimicrobial resistance bacterial agents, which are expected to revolutionize the field of clinical diagnostics.

By discovering penicillin in 1928, Sir Alexander Fleming triggered the beginning of the modern era of antibiotics, which revolutionized medicine and society, saved lives, and increased the life expectancy to what we know today. The remarkable effectiveness of antibiotics led to the euphoria mistaken belief that all infectious diseases could be successfully controlled with antibiotics. However, during the past few decades, the imprudent and excessive use (underuse, overuse, and misuse) of antibiotics regrettably led to the rapid emergence and propagation of bacterial strains resistant to virtually all therapeutically useful antibiotics.[1] The increasing frequency of infections by antimicrobial-resistant bacteria is due to their capacity to recurrently develop new mechanisms of resistance. The lack of alternative treatments results in longer hospital stays, delayed recovery, long-term disability, and an increase in public healthcare costs. In the USA, the estimated healthcare cost associated to antimicrobial resistance (AMR) was $55 billion per year in 2013, and 2 million people were sick every year due to antibiotic-resistant infections, with over 23 000 deaths as a result.[2] In Europe, the 2009 report from European Centre for Disease Prevention and control (ECDC) and European Medicines Agency (EMEA)[3] estimated overall societal costs over 1.5 billion € per year, with over 900 million € in hospital costs. In the EU, about 25 000 patients died due to multidrug-resistant (MDR) bacteria infections.[3,4] It should be noted that the emergence and spread of AMR bacteria are prevalent in both healthcare and community

settings, typically known as healthcare-associated infections and community-acquired infections. The World Health Organization (WHO) recently published a priority list of antibiotic-resistant pathogens. Gram-negative carbapenem-resistant Acinetobacter baumannii, carbapenem-resistant Pseudomonas aeruginosa and carbapenem-resistant and third-generation cephalosporin-resistant Enterobacteriaceae are at the top of this list, classified as critical priority agents. In the high-priority list, gram-positive bacteria for which there are treatment options likely to be successful, were included, namely the methicillin-resistant, vancomycin-intermediate and -resistant Staphylococcus aureus and the vancomycin-resistant Enterococcus faecium.[5] The list does not include Mycobacterium tuberculosis, as it is a globally established priority, urgently needing innovative treatments, and already targeted by several dedicated programs. The WHO[6] also suggested that global research should focus on the development of new diagnostic and therapeutic tools.[7]

The isolation of pathogens from clinical samples still occurs through culture methods, using agar-based media (nutritive, differential, and/or selective). Some clinical laboratories use chromogenic agar-media harboring chromogenic or fluorogenic substrates that are hydrolyzed in the presence of specific enzymes. Several tests are then performed to address genus identification, namely microscopy cell staining, colony morphology, and rapid biochemical tests. To perform identification at the species level, the more common methods are phenotypically based, such as manual (e.g., Api bioMérieux) and automated biochemical tests, which exploit the differences in protein expression within genus (or also between genera), providing a characteristic protein expression fingerprint with a relatively high degree of certainty.[8] For example, the OmniLog ID system (Biolog) is a rapid method for the phenotypic identification of bacteria and fungi, through their ability to oxidize different carbon sources. Here, each well of the card contains one of 94 different carbon compounds and a tetrazolium-redox dye, used as a flag to indicate if the microorganism tested has or not utilized the carbon compound, providing a “metabolic fingerprint” of the microorganism.[9] Although useful and easy to operate, agar-based media and biochemical tests are not completely specific and occasionally fail or provide presumptive identification (percentage of possibilities). Therefore, further confirmation of species identity is often required. Different approaches, not culture-based, either current and emergent can be used, some of these are able to provide both identification and antimicrobial-susceptibility data simultaneously.

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