news digest #015

Advancements in microbial identification and resistance detection within the veterinary sector

Historically, the MALDI Biotyper® has been employed solely in the positive ion mode for the identification of animal pathogens, food-contaminating organisms, and the confirmation of foodborne pathogens (see also news digests #004 - #010). However, the introduction of the MALDI Biotyper sirius brought the negative ion mode into play, significantly broadening the scope of microbial applications. This expansion encompasses the analysis of lipids, thereby opening up new avenues for innovative applications and the battle against antimicrobial resistance (AMR). One particularly exciting application is the detection of colistin resistance in *E. coli* isolates, a topic of growing importance.

In the veterinary sector, particularly in poultry and pig farming, antibiotics are commonly employed to control gastrointestinal infections in animals as well as to address respiratory or systemic infections. The primary classes of antibiotics used in broilers and turkeys include penicillins, tetracyclines, and polypeptides (including colistin), followed by sulfonamides and others. Antibiotic resistance is a global concern and a defining public health issue of our time. The limited availability of novel antimicrobials and the spreading of multidrug-resistant (MDR) organisms have compelled us to rely on so-called "last-resort antibiotics" for treating MDR Gram-negative bacteria.

For human health, colistin represents a final line of defense against MDR Gram-negative bacteria causing infections. It is a critical option for treating carbapenemase-producing *Enterobacteriaceae* (CPE), which are resistant to a broad range of beta-lactam antibiotics. CPE comprises a group of Gram-negative bacteria that resist carbapenem antibiotics by producing an enzyme called carbapenemase, rendering the drugs ineffective.[1] Typically residing in the animal or human gut, members of the bacterial family *Enterobacteriaceae* have essential roles in the respective microbiome.[2,3,4]

As previously mentioned, the issue of antimicrobial resistance is not limited to humans alone. Colistin is routinely used in the animal sector for livestock, including pig farms, cattle, and poultry, due to its effectiveness in combating infections caused by *Escherichia*, *Pseudomonas*, and *Klebsiella* species. While some bacterial species in the veterinary realm are inherently resistant to colistin (such as *Brucella*, *Francisella tularensis*, *Proteus*, and Gram-negative cocci), veterinary signature bacteria incl. *E. coli*, *Pseudomonas*, and *Klebsiella* remain important for animal health and are potential targets for colistin treatment.

The activity of colistin is based on binding to lipopolysaccharides and phospholipids in the outer cell membrane of Gram-negative bacteria. It competes for divalent cations (Ca^{2+} and Mg^{2+}) on the phosphate groups of membrane lipids, resulting in the disruption of the outer cell membrane, leading to leakage of intracellular contents, and eventual bacterial death.

Whenever there is a new antibiotic entering the market, it is only a question of time before the first resistant bacteria show up in this never ending arms race. As for colistin itself, until 2015, known colistin resistance mechanisms were primarily associated with chromosomal mutations that affected the charge of lipopolysaccharides. The new strains bearing these mutations showed reduced interaction of colistin with the outer cell membrane of Gram-negative bacteria. The first identified plasmid-encoded colistin resistance gene capable of horizontal gene transfer between bacterial strains is mcr-1.[7] It was discovered in 2011 on a pig farm in China where colistin was routinely used, and garnered worldwide attention in November 2015. Subsequently, the first mobile colistin resistance gene, mcr-1, was found in *Escherichia coli* isolates of various origins, initially in China and then in various bacterial species worldwide. To monitor multidrug-resistant strains, several national and international surveillance programs systematically collect data and regularly report trends in many EU countries.

E. coli is a widely distributed commensal bacterium in both humans and animals. Apart from its non-pathogenic presence, *E. coli* can become an opportunistic pathogen and is known to harbor and transmit genetic elements of antimicrobial resistance through various routes. A recent review covering the years from 2016 to 2021 revealed that *E. coli* is the predominant species carrying mcr genes, but plasmid-mediated colistin resistance can also be transferred to other species bearing different antibiotic resistance genes. Consequently, *E. coli* is employed as an indicator bacterium for antimicrobial resistance surveillance programs, including those conducted by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA), and national programs in countries like France and Germany. Across the European Union, numerous efforts have been initiated to limit the spread of colistin- or multidrug-resistant bacteria in animals, primarily based on historical data.

For instance, in a commensal *E. coli* strain collection isolated from livestock in France between 2007 and 2014, the prevalence of mcr-1 occurrence was approximately 6% in turkeys and 2% in broilers, suggesting that mobile colistin resistance was already widespread in farm animals before the discovery of mcr-1. The French national Ecoantibio2 plan, launched in 2017, aimed to reduce colistin exposure by 50% in food-producing animals within five years, from 2015 until 2020. A recent study published in 2023 provided updated prevalence and epidemiological data regarding colistin resistance in poultry production in France. *E. coli* strains originating from broilers and turkeys at slaughterhouses from 2011 to 2020 were investigated. The study reported a decrease in poultry exposure to colistin since 2014, but more than 80% of isolates exhibited multi-drug resistance, with 40% of isolates from turkeys and 44% from broilers displaying co-resistance to the critically important antimicrobial ciprofloxacin.

In Germany, the "German Antibiotic Resistance Strategy" (DART) encompasses action plans, surveillance, and monitoring systems based on random sample surveys. Monitoring veterinary antimicrobial resistance includes programs for commensal pathogens and pathogens known as GERM-Vet. GERM-Vet yearly reports antimicrobial resistance data for various livestock species, including pigs. A recent study in Germany, which involved 6,569 *Escherichia coli* isolates from pigs in Bavaria from 2016 to 2020, revealed trends similar to those observed in France.[5]

Specifically, colistin resistance decreased, enrofloxacin resistance increased, and ceftiofur resistance remained constant. Nevertheless, given the evolving landscape of antibiotic resistance in animal bacteria, continued monitoring of colistin is imperative in the years to come, especially since it ranks among the top five most frequently used antibiotics in the veterinary sector in many countries. The advent of the new generation of MALDI-TOF MS instruments has made such studies much more feasible, particularly due to the focus on lipid A and the enhanced capabilities of the latest Bruker instrument, which includes the negative ion mode for rapid colistin resistance detection.

How can we employ MALDI-TOF MS technology, besides bacterial identification, also for antimicrobial resistance (AMR) research? A study conducted on samples from pigs and farm workers simultaneously demonstrated the utility of the MALDI Biotyper in identifying bacterial species in these samples. A research group characterized colistin-

resistant Enterobacterales isolated from farmers, swine, and hospitalized patients in Thailand between November 2014 and December 2017.[3] Stool samples and rectal swabs from patients, farmers, and swine were cultured on MacConkey agar, and any observed bacterial colonies after 24 hours at 35°C were subcultured for colony purification. The bacterial species were identified using a MALDI Biotyper in positive ion mode, which was the only ion mode available on the older generations of MALDI Biotyper systems. Notably, while MALDI-TOF has primarily been employed for bacterial identification, colistin resistance assessment was conducted through the broth microdilution method. The study determined an overall colistin-resistant Enterobacterales colonization rate of 26.2% (n = 39/149).

The new generation MALDI Biotyper sirius Systems are also equipped with negative ion mode expanding their capabilities to encompass lipidomics research, enabling the identification of bacteria on species level and detection of colistin resistance on a single platform. This innovation positions the MALDI Biotyper as a rapid method in the field of AMR research, alongside traditional broth microdilution.

A pioneering area in MALDI-TOF lipidomics research involves the swift identification of modifications in lipid A that are related to colistin resistance within such resistant bacteria. Leveraging its linear negative ion mode, the MALDI Biotyper sirius, when combined with the MBT Lipid Xtract™ Kit and the MBT HT LipidART Module, can unveil distinct lipid A modifications that are characteristic of colistin-resistant *E. coli* variants. Furthermore, a similar application for *Pseudomonas aeruginosa* has been shown.[6] This approach isn't limited to detecting lipid A alone. A recent study showcased the discovery of molecules targeting bacteria resistant to polymyxins, a class of antibiotics considered as a last resort. The technology can serve as a valuable tool for assay development, enabling the monitoring of purified proteins' inhibition.[8] Given the global threat of antimicrobial-resistant bacteria, innovative solutions are imperative to identify new molecules that could reverse bacterial resistance or target virulence factors. In the mentioned study, a library of 1,200 natural compounds was tested against an *E. coli* strain expressing mcr-1, which is known to modify lipid A by adding phosphoethanolamine (pETN), making the strain resistant to colistin. Identifying compounds that reduce this lipid A modification by mcr-1 is feasible and could be a potential strategy for reversing resistance. The MALDI Biotyper plays a significant role in supporting research in animal health and pharmaceuticals, in addition to its application in routine environmental monitoring or veterinary microbial identification.

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