Next-generation sequencing to investigate antimicrobial resistance

Fleres, Giuseppe

DOI:
10.33612/diss.250884559

Chapter 1

General introduction & Scope of the thesis

General introduction

Antimicrobial resistance (AMR) is a leading cause of death around the world with, according to the most comprehensive global estimates to date, 4.95 million deaths associated with bacterial AMR in 2019[1]. The emergence of antimicrobial resistance (AMR) and rising numbers of infections caused by antimicrobial-resistant bacteria represent a global threat to public health and agriculture[2].

Considering that only a few antimicrobial drugs are currently in development[3], it is crucial to investigate and monitor the emergence of new resistance mechanisms and the dissemination of resistance determinants. The large majority of antimicrobials are used in both humans and animals, and its overuse represents the main driver of resistance[4]. Due to pollution caused by contaminated sewage, pharmaceutical industry waste etc., reservoirs of resistance exist in every place where antimicrobials are used, including several different ecological niches (humans and nosocomial environments, as well as animal farms and aquaculture environments, water and soil) and reducing amount of antimicrobial pollution is key to preserve the effectiveness of antimicrobial treatments[5,6].

AMR develops by numerous mechanisms such as point mutations in target genes (e.g., gyrase A), duplication or overexpression of genes (e.g., efflux pumps), as well as the acquisition of new genes through horizontal gene transfer (HGT)[7]. In fact, microorganism exposed to antimicrobial selective pressure enhance their fitness by acquiring and expressing ARGs and subsequently share them with other bacteria. The mobilization of antimicrobial resistance genes (ARGs) between bacterial communities and pathogens has been reported, particularly in animal and human microbiomes[8]. Currently, last-resort ARGs encoding for extended-spectrum beta-lactamases, carbapenemases and mobile colistin resistance genes[9] have become globally ubiquitous in Gram-negative pathogens, limiting treatment options and causing the emergence of untreatable bacterial infections.

Given the complexity of the AMR problem, it is necessary to look at it from different disciplines and to frame it within the One-Health approach. One-Health is defined as "an integrated, unifying approach that aims to sustainably balance and optimize the

health of people, animals and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent"[10].

Antimicrobial Resistance in the Nosocomial Environment

Hospitals are major ecological niches of antimicrobial resistant AMR, where the antibiotic pressure, severely ill patients, a very susceptible population, and the constant "traffic" of potentially pathogenic microorganisms help the dissemination and emergence of ARB. A One-Health perspective is key to understand the emergence and spread of antimicrobial resistance in the nosocomial environment, providing practical ways to incorporate environmental and animal contact considerations into patient care. Hospitals can act as multiplier of ARB, where both patients and healthcare workers can become colonized and/or infected with ARB that will be then introduced in the community after hospital discharge, resulting in a cyclic dissemination loop[11,12,13].

The first pathogen documented as spreading through the hospital environment was Methicillin-resistant *Staphylococcus aureus* (MRSA), however, infection prevention measures are contributing to its decline in recent years[11,14]. This pathogen is important in a One-Health perspective, as some strains and other multidrug-resistant staphylococci are associated with animals and livestock especially[15]. While fifteen years ago MRSA and other Gram-positive pathogens, such as vancomycin-resistant enterococci (VRE), were the most common nosocomial pathogens, more recently the attention has shifted on Gram-negative pathogens, including multi-drug (MDR) and extensively-drug (XDR) resistant Enterobacterales[16]. The rapid increase in Gram-negative resistant pathogens is due to the emergence and rapid global spread of resistance determinants related to third-generation cephalosporins, carbapenems and, more recently, colistin that are associated with mobile genetic elements (i.e. plasmids and transposons) easily spreading through bacterial populations[17,18].

Bacterial contamination of the healthcare environment is responsible for up to 25% of healthcare-associated infections (HAIs) and an additional 20-40% of cases can be related to colonized healthcare workers[19]. HAIs are acquired by patients while receiving care and occur in 7 and 10 out of every 100 hospitalized patients in high-income countries and low- and middle-income countries respectively[20]. Many studies have demonstrated cross-contamination of clinically relevant bacteria between the hospital environment, healthcare workers' hands and patients[21,22,23]. In clinical settings, humid compartments (e.g., sinks, showers, toilet) and specifically water should be considered of particular relevance due to the frequent events of exposure, including drinking, sanitation, patient bathing, water birth pools, cleaning and reprocessing of medical devices[24]. Several studies using culture-based methods have shown that the hospital water environment, including tap-water, faucets, sink surfaces and wastewater drainage systems, serve as reservoirs for nosocomial pathogens that can cause outbreaks and HAIs[5,26,27]. Outbreaks associated with the hospital water environment and caused by pathogens resistant to last-resort antimicrobials, such as carbapenem-resistant microorganisms (CRMO), have been increasingly reported, with *P. aeruginosa* and *Acinetobacter baumannii* being the most frequent microorganisms and distributed across all water reservoirs[28-30].

Antimicrobial Resistance in Livestock

Antimicrobial resistance driven by antibiotic overuse and bacterial evolution is a global threat, not only in healthcare settings but also in agriculture. While antimicrobial usage in humans is mostly related to clinical infectious diseases treatment and prophylactic, in food animals these drugs have also been used for economic reasons as growth promoters. This controversial practice is an important contributor to the development and spread of AMR because low-dose drugs are administered to entire groups of animals, usually long-term treatments, creating optimal conditions for the selection and spread of resistant bacteria[31,32]. This practice has been banned in Europe and phased out in some other countries, including USA and Canada. However, the use of antibiotics as grow promoters is still allowed in many

other countries, often low- and middle-income[33]. Two-thirds of overall antibiotic usage is destined for animal production, with a recent global estimate of 200,000 tons by 2030[34]. Global usage of antimicrobials in food animal production was estimated at around 63 tons in 2010 and projected to rise by 67% by 2030 due to the growing number of animals raised for food production and a larger proportion of animals to be raised in intensive farming systems[35,36].

The World Health Organization (WHO) listed glycopeptides, fluoroquinolones, third-fourth- and fifth-generation cephalosporins, macrolides and polymyxins as "highest priority critically important" antibiotics in human health[37]. While penicillin, macrolides, and fluoroquinolones are the most frequently used for human medicine, tetracyclines, penicillin, and sulfonamides are the most commonly use drugs in animals[38]. The rise of colistin resistance in recent years serves as a good example of how antimicrobial resistance can be aggravated by overuse of antimicrobials in animal farms. In fact, the emergence and dissemination of colistin resistance and mobile colistin resistance genes (*mcr*) has almost certainly been intensified by the use of colistin on Chinese and Southeast Asia farms[39]. Colistin (polymyxin B) has been used in both humans and animals for decades, however, its systemic administration causes nephrotoxicity and for this reason its use in human health has been paused. Only recently, administration of colistin has been increased, being considered a last-resort antibiotic for treating serious infections caused by MDR Gram-negative bacteria. Since 2015, when the first *mcr* gene was reported[9], the efficacy of this antibiotic has been challenged by the emergence and global spread of mobile colistin resistance (*mcr*) determinants, which threaten human, animal and environmental health.

Next-Generation Sequencing Technologies

The first generation of sequencing platforms was introduced in 1977 with Sanger sequencing, however, only at the end of the 90s this technology was implemented in clinical microbiology [40]. Read- length and low throughput were the most important limitation of this technology. In the mid- and late-2000s, the introduction of next-generation sequencing (NGS), or 2nd generation, platforms provided technical advances, such as higher throughputs, speed and cost saving that favored the use of

NGS in the microbiology field[41-43]. Short-read sequencing (SRS) platforms of Illumina (San Diego, CA) and Thermofisher (Waltham, MA) are the most used, with Illumina dominating the sequencing market. A limitation of SRS platforms is that the read lengths are often shorter than the repetitive regions that flank ARGs, resulting in highly fragmented assemblies and can lead to challenges in characterizing the genetic environment[44]. Long-reads sequencing (LRS), or 3rd generation, platforms were commercially released in 2011 (Oxford Nanopore Technologies, ONT) and 2014 (Pacific Biosciences, PacBio), and since then have become suitable for an increasing number of applications. LRS platforms generate 30- to 50-kb reads on average but suffer from low read accuracy and higher per-read costs compared to those of short-read platforms. More recently, improvements in ONT technology and its latest chemistry enable >99% raw read accuracy at high data yields and can generate near-finished bacterial genomes without short-read or reference polishing[45]. ONT platforms provide LRS that span most repetitive regions, resulting in more complete and less fragmented assemblies in complex microbial communities[46]. In addition, MinION (ONT) devices are portable and its usefulness has been reported for field surveillance and real-time outbreak investigations[46,47].

a. Culture dependent approach: whole-genome sequencing. Microbial whole-genome sequencing (WGS) determines the entire genomic sequence of the microorganism and thus allows characterization of pathogens, including taxonomical classification, epidemiological typing, prediction of resistance and virulence. Given a bacterial strain isolated by culture, WGS generates multiple reads that can be assembled into contigs based on overlapping regions (*de novo* assembly), and/or mapped to previously published reference genomes (reference-based assembly), allowing the comparison between bacterial strains[48]. Advances in high-throughput NGS technologies and bioinformatic analysis have significantly improved the capacity to perform low-cost, efficient whole-genome sequencing (WGS), and has made it a feasible tool for infection control and prevention as well as a diagnostic and outbreak management tool. WGS of bacteria was first implemented in public health research practice during the cholera epidemic in Haiti in 2010[49] the international outbreak of

Escherichia coli O104:H4[50], and *Acinetobacter baumannii* hospital outbreak[51]. Compared to standard microbiological methods, WGS has several advantages regarding strain characterization, including higher degree of resolution in outbreak investigation, improved turnaround time and identification of new pathogens and resistance mechanisms, in both clinical and public health microbiology[52-56].

b. Culture independent approach: amplicon-based and shotgun metagenomics.

Although culture methods are routinely used, they are laborious, time-consuming and may underestimate the level of microbial pathogens, especially fastidious or non-culturable microorganisms. Recently, advances in NGS technologies have allowed to study entire metagenomes. Shotgun metagenomics sequencing (SMg) allows untargeted sequencing of nucleic acids directly from a given sample. It can detect and characterize bacteria, viruses, fungi, and parasites using a single assay[57].

Metagenomics sequencing has increased our knowledge of the microbiome and AMR diversity not only in human healthcare, but also in animal settings[58,59]. The untargeted nature of shotgun metagenomic sequencing (SMg) enables the detection of fastidious, anaerobic, and non-pathogenic commensal bacteria, which could be a reservoir for ARGs, but are rarely investigated using conventional culture-based methods. Animals in particular are in close contact with the environment, where unknown and uncharacterized ARG variants have been reported[60]. As a result, SMg could be a promising tool for detecting and characterizing novel ARG variants and could increase our understanding of AMR transmission[8]. Metagenomic sequencing have been used in several recent studies and have shown that read mapping approach describes AMR abundance in bacterial communities more accurately than commonly used methods using selected indicator organisms[61].

Despite SMg technology becoming more broadly available and accessible to the scientific community, mostly due to low-cost NGS platforms and the rising number of user-friendly bioinformatics tools being developed this approach still faces many challenges, such as limited sensitivity and lack of standardization[62,63].

Scope of the thesis

The application of novel approaches such as 2nd and 3rd next-generation sequencing technologies in a One-health perspective is key to investigate the complex issue of antimicrobial resistance and to prevent its further global spread. This thesis aims to implement NGS technologies for the surveillance of antimicrobial resistant pathogens and to track the dissemination of resistance determinants in human healthcare and animal farms, two different built-environments.

Part I of this thesis highlights the advantages of applying second and third next generation sequencing technologies to investigate the presence and dissemination of ARB and antimicrobial resistance determinants in human healthcare and nosocomial environments.

Chapter 2 and 3 show the potential of the culture-based approach and WGS to detect and characterize potentially pathogenic bacterial strains in the hospital humid environment and investigate if those strains are responsible for HAIs or colonization. In **Chapter 2** we demonstrate the possibility of applying short- and long-read WGS to characterize environmental *Legionella* spp. strains and investigate bacterial molecular epidemiology in the nosocomial environment. Classical culture, combined with WGS, allows the identification of a unique clone of *Legionella anisa* in several dental chair units in the same hospital dental ward, revealing tap-water contamination in direct contact with patients and the usefulness of WGS to investigate bacterial molecular epidemiology. In **Chapter 3** we demonstrated the possibility of applying a culture-based approach and WGS to investigate the presence of colistin-resistant Gram-negative bacteria in sink and shower drains of high-risk hospital units, throughout the 5 months of the sampling period. Monitoring the hospital water-related environment for multidrug resistant bacteria is an essential tool to identify reservoirs of relevant pathogens and synchronized screening in hospitalized patients would provide insights into transmission routes to design appropriate control measures and prevent further dissemination.

Currently, our understanding of the drinking-water microbiome is predominately obtained mainly by culture-based methods and is specific cases by 16S amplicon

sequencing. In **Chapter 4** we highlight the potential of applying shot-gun metagenomics (SMg) to monitor the bacterial community and resistome profiles of tap-water samples collected from high-risk hospital units. In addition, we use a culture-dependent approach to investigate the presence of: ARB resistant to last-resort antibiotics, such as Carbapenem-resistant (CRE) and Colistin-Resistant Enterobacterales (ColRE), in tap-water and hospital wastewater drains (sinks and showers). **Chapter 5** we show the potential of combining short- and long-read SMg to detect and characterize a novel *mcr* gene variant in hospital tap water.

Part II of this thesis highlights the potential of applying culture-independent next-generation sequencing approaches to investigate antimicrobial resistance dissemination in livestock. Oral Fluid (OF) sampling is a non-invasive procedure that enables to monitor farms at the herd level. In **Chapter 6**, we apply a combination of oral fluid (OF) sampling and untargeted SMg to detect and characterize antimicrobial resistance genes, heavy metal resistance genes and clinically relevant pathogens. In **Chapter 7** we characterized an unusually small plasmid carrying a mobile colistin resistance gene in a pig OF sample, showing the potential of combining SRS and LRS to obtain highly accurate and circular plasmid contigs without the need for culturing.

References

1. Murray, C. J. *et al.* Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* S0140673621027240 (2022) doi:10.1016/S0140-6736(21)02724-0.

2. Authority, E. F. S. & European Centre for Disease Prevention and Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. *EFSA J.* **15**, e04694 (2017).

3. Brown, E. D. & Wright, G. D. Antibacterial drug discovery in the resistance era. *Nature* **529**, 336–343 (2016).

4. Aarestrup, F. M., Wegener, H. C. & Collignon, P. Resistance in bacteria of the food chain: epidemiology and control strategies. *Expert Rev. Anti Infect. Ther.* **6**, 733–750 (2008).

5. Marti, E., Variatza, E. & Balcazar, J. L. The role of aquatic ecosystems as reservoirs of antibiotic resistance. *Trends Microbiol.* **22**, 36–41 (2014).

6. Huijbers, P. M. C. *et al.* Role of the Environment in the Transmission of Antimicrobial Resistance to Humans: A Review. *Environ. Sci. Technol.* **49**, 11993–12004 (2015).

7. Boolchandani, M., D'Souza, A. W. & Dantas, G. Sequencing-based methods and resources to study antimicrobial resistance. *Nat. Rev. Genet.* **20**, 356–370 (2019).

8. Hu, Y. *et al.* The Bacterial Mobile Resistome Transfer Network Connecting the Animal and Human Microbiomes. *Appl. Environ. Microbiol.* **82**, 6672–6681 (2016).

9. Liu, Y.-Y. *et al.* Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* **16**, 161–168 (2016).

10. Mackenzie, J. S. & Jeggo, M. The One Health Approach—Why Is It So Important? *Trop. Med. Infect. Dis.* **4**, 88 (2019).

11. Dulon, M., Peters, C., Schablon, A. & Nienhaus, A. MRSA carriage among healthcare workers in non-outbreak settings in Europe and the United States: a systematic review. *BMC Infect. Dis.* **14**, 363 (2014).

12. Xue, Y. & Gyi, A. A. Predictive Risk Factors for Methicillin-Resistant Staphylococcus aureus (MRSA) Colonisation among Adults in Acute Care Settings: A Systematic Review. *JBI Libr. Syst. Rev.* **10**, 3487–3560 (2012).

13. Patel, P. K., Mantey, J. & Mody, L. Patient Hand Colonization With MDROs Is Associated with Environmental Contamination in Post-Acute Care. *Infect. Control Hosp. Epidemiol.* **38**, 1110–1113 (2017).

14. Evans, M. E., Kralovic, S. M., Simbartl, L. A., Jain, R. & Roselle, G. A. Eight years of decreased methicillin-resistant Staphylococcus aureus health care-associated infections associated with a Veterans Affairs prevention initiative. *Am. J. Infect. Control* **45**, 13–16 (2017).

15. Price, L. B. *et al.* Staphylococcus aureus CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock. *mBio* **3**, e00305-11 (2012).

16. McEwen, S. A. & Collignon, P. J. Antimicrobial Resistance: a One Health Perspective. *Microbiol. Spectr.* **6**, 6.2.10 (2018).

17. Kumarasamy, K. K. *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* **10**, 597–602 (2010).

18. D'Andrea, M. M., Arena, F., Pallecchi, L. & Rossolini, G. M. CTX-M-type β-lactamases: A successful story of antibiotic resistance. *Int. J. Med. Microbiol.* **303**, 305–317 (2013).

19. Costa, D. M. *et al.* Biofilm contamination of high-touched surfaces in intensive care units: epidemiology and potential impacts. *Lett. Appl. Microbiol.* **68**, 269–276 (2019).

20. World Health Organization. *Report on the burden of endemic health care-associated infection worldwide*. (World Health Organization, 2011).

21. Price, J. R. *et al.* Transmission of Staphylococcus aureus between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. *Lancet Infect. Dis.* **17**, 207–214 (2017).

22. Tofteland, S., Naseer, U., Lislevand, J. H., Sundsfjord, A. & Samuelsen, Ø. A Long-Term Low-Frequency Hospital Outbreak of KPC-Producing Klebsiella pneumoniae Involving Intergenus Plasmid Diffusion and a Persisting Environmental Reservoir. *PLOS ONE* **8**, e59015 (2013).

23. Roux, D. *et al.* Contaminated sinks in intensive care units: an underestimated source of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the patient environment. *J. Hosp. Infect.* **85**, 106–111 (2013).

24. Ferranti, G., Marchesi, I., Favale, M., Borella, P. & Bargellini, A. 2014. Aetiology, source and prevention of waterborne healthcare-associated infections: a review. *J. Med. Microbiol.* **63**, 1247–1259.

25. Decker, B. K. & Palmore, T. N. The Role of Water in Healthcare-Associated Infections. *Curr. Opin. Infect. Dis.* **26**, 345–351 (2013).

26. Yiek, W.-K. *et al.* Outbreaks of healthcare-associated infections linked to water-containing hospital equipment: a literature review. *Antimicrob. Resist. Infect. Control* **10**, 77 (2021).

27. Kanamori, H., Weber, D. J. & Rutala, W. A. Healthcare Outbreaks Associated With a Water Reservoir and Infection Prevention Strategies. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **62**, 1423–1435 (2016).

28. Kizny Gordon, A. E. *et al.* The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections—A Systematic Review of the Literature. *Clin. Infect. Dis.* **64**, 1435–1444 (2017).

29. Kinsey, C. B. *et al.* Pseudomonas aeruginosa Outbreak in a Neonatal Intensive Care Unit Attributed to Hospital Tap Water. *Infect. Control Hosp. Epidemiol.* **38**, 801–808 (2017).

30. Chia, P. Y. *et al.* The role of hospital environment in transmissions of multidrug-resistant gram-negative organisms. *Antimicrob. Resist. Infect. Control* **9**, 29 (2020).

31. Wall, B. A. *et al. Drivers, dynamics and epidemiology of antimicrobial resistance in animal production*. http://www.fao.org/3/a-i6209e.pdf (2016).

32. Aidara-Kane, A. *et al.* World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals. *Antimicrob. Resist. Infect. Control* **7**, 7 (2018).

33. Hernando-Amado, S., Coque, T. M., Baquero, F. & Martínez, J. L. Defining and combating antibiotic resistance from One Health and Global Health perspectives. *Nat. Microbiol.* **4**, 1432–1442 (2019).

34. Van Boeckel, T. P. *et al.* Reducing antimicrobial use in food animals. *Science* **357**, 1350–1352 (2017).

35. Van Boeckel, T. P. *et al.* Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci.* **112**, 5649–5654 (2015).

36. European Centre for Disease Prevention and Control (ECDC), Authority (EFSA), E. F. S. & Agency (EMA), E. M. Third joint inter-agency report on integrated analysis of consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals in the EU/EEA. *EFSA J.* **19**, e06712 (2021).

37. Critically important antimicrobials for human medicine : 6th revision. https://www.who.int/publications-detail-redirect/9789241515528.

38. Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans | Elsevier Enhanced Reader. https://reader.elsevier.com/reader/sd/pii/S2590053620301099?token=1F69CB21258292C957104065305A69oF90F4498598F7743FE1DB615A7826CFED1415408BBAEC0833F89500C221F48C0E&originRegion=us-east-1&originCreation=20220612005621 doi:10.1016/j.bsheal.2020.09.004.

39. Walsh, T. R. & Wu, Y. China bans colistin as a feed additive for animals. *Lancet Infect. Dis.* **16**, 1102–1103 (2016).

40. Sanger, F., Nicklen, S. & Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* **74**, 5463–5467 (1977).

41. Slatko, B. E., Gardner, A. F. & Ausubel, F. M. Overview of Next-Generation Sequencing Technologies. *Curr. Protoc. Mol. Biol.* **122**, e59 (2018).

42. Besser, J., Carleton, H. A., Gerner-Smidt, P., Lindsey, R. L. & Trees, E. Next-generation sequencing technologies and their application to the study and control of bacterial infections. *Clin. Microbiol. Infect.* **24**, 335–341 (2018).

43. Taboada, E. N., Graham, M. R., Carriço, J. A. & Van Domselaar, G. Food Safety in the Age of Next Generation Sequencing, Bioinformatics, and Open Data Access. *Front. Microbiol.* **8**, (2017).

44. Petersen, L. M., Martin, I. W., Moschetti, W. E., Kershaw, C. M. & Tsongalis, G. J. Third-Generation Sequencing in the Clinical Laboratory: Exploring the Advantages and Challenges of Nanopore Sequencing. *J. Clin. Microbiol.* **58**, e01315-19 (2019).

45. Sereika, M. *et al.* Oxford Nanopore R10.4 long-read sequencing enables the generation of near-finished bacterial genomes from pure cultures and metagenomes without short-read or reference polishing. *Nat. Methods* **19**, 823–826 (2022).

46. Rambo-Martin, B. L. *et al.* Influenza A Virus Field Surveillance at a Swine-Human Interface. *mSphere* **5**, e00822-19 (2020).

47. Hoenen, T. *et al.* Nanopore Sequencing as a Rapidly Deployable Ebola Outbreak Tool. *Emerg. Infect. Dis.* **22**, 331–334 (2016).

48. Abdelbary, M. M. H., Basset, P., Blanc, D. S. & Feil, E. J. 24 - The Evolution and Dynamics of Methicillin-Resistant Staphylococcus aureus. in *Genetics and Evolution of Infectious Diseases (Second Edition)* (ed. Tibayrenc, M.) 553–572 (Elsevier, 2017). doi:10.1016/B978-0-12-799942-5.00024-X.

49. Barzilay, E. J. *et al.* Cholera Surveillance during the Haiti Epidemic — The First 2 Years. *N. Engl. J. Med.* **368**, 599–609 (2013).

50. Mellmann, A. *et al.* Prospective Genomic Characterization of the German Enterohemorrhagic Escherichia coli O104:H4 Outbreak by Rapid Next Generation Sequencing Technology. *PLOS ONE* **6**, e22751 (2011).

51. Lewis, T. *et al.* High-throughput whole-genome sequencing to dissect the epidemiology of Acinetobacter baumannii isolates from a hospital outbreak. *J. Hosp. Infect.* **75**, 37–41 (2010).

52. Schürch, A. C. & van Schaik, W. Challenges and opportunities for whole-genome sequencing–based surveillance of antibiotic resistance. *Ann. N. Y. Acad. Sci.* **1388**, 108–120 (2017).

53. Moran-Gilad, J. *et al.* Proficiency testing for bacterial whole genome sequencing: an end-user survey of current capabilities, requirements and priorities. *BMC Infect. Dis.* **15**, 174 (2015).

54. Cimmino, T., Le Page, S., Raoult, D. & Rolain, J.-M. Contemporary challenges and opportunities in the diagnosis and outbreak detection of multidrug-resistant infectious disease. *Expert Rev. Mol. Diagn.* **16**, 1163–1175 (2016).

55. Nadon, C. *et al.* PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. *Eurosurveillance* **22**, 30544 (2017).

56. Kwong, J. C., Mccallum, N., Sintchenko, V. & Howden, B. P. Whole genome sequencing in clinical and public health microbiology. *Pathology (Phila.)* **47**, 199–210 (2015).

57. Couto, N. *et al.* Critical steps in clinical shotgun metagenomics for the concomitant detection and typing of microbial pathogens. *Sci. Rep.* **8**, 13767 (2018).

58. Kwok, K. T. T., Nieuwenhuijse, D. F., Phan, M. V. T. & Koopmans, M. P. G. Virus Metagenomics in Farm Animals: A Systematic Review. *Viruses* **12**, 107 (2020).

59. Schuele, L., Cassidy, H., Peker, N., Rossen, J. W. A. & Couto, N. Future potential of metagenomics in microbiology laboratories. *Expert Rev. Mol. Diagn.* **21**, 1273–1285 (2021).

60. Berglund, F. *et al.* Identification and reconstruction of novel antibiotic resistance genes from metagenomes. *Microbiome* **7**, 52 (2019).

61. Munk, P. *et al.* Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. *Nat. Microbiol.* **3**, 898–908 (2018).

62. Greninger, A. L. The challenge of diagnostic metagenomics. *Expert Rev. Mol. Diagn.* **18**, 605–615 (2018).

63. Chiu, C. Y. & Miller, S. A. Clinical metagenomics. *Nat. Rev. Genet.* **20**, 341–355 (2019).

Part I.
Antimicrobial Resistance in Human Healthcare and Nosocomial Environment