Molecular Diagnostics

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NACMID
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Objectives

- Discuss background concepts related to molecular diagnostics
- Provide an overview of molecular diagnostic history
- Highlight 2 specific molecular diagnostic techniques
- Patient case application(s)

INTRODUCTION

Molecular diagnostics

- Using molecular biology to;
  - Expand the scientific knowledge of the natural history of diseases, identify people at risk for specific diseases, and diagnose diseases at the molecular level
  - Uses sequence specific information in macromolecules
    - Peptides/proteins
    - Polysaccharides
    - Polynucleotides/nucleic acids

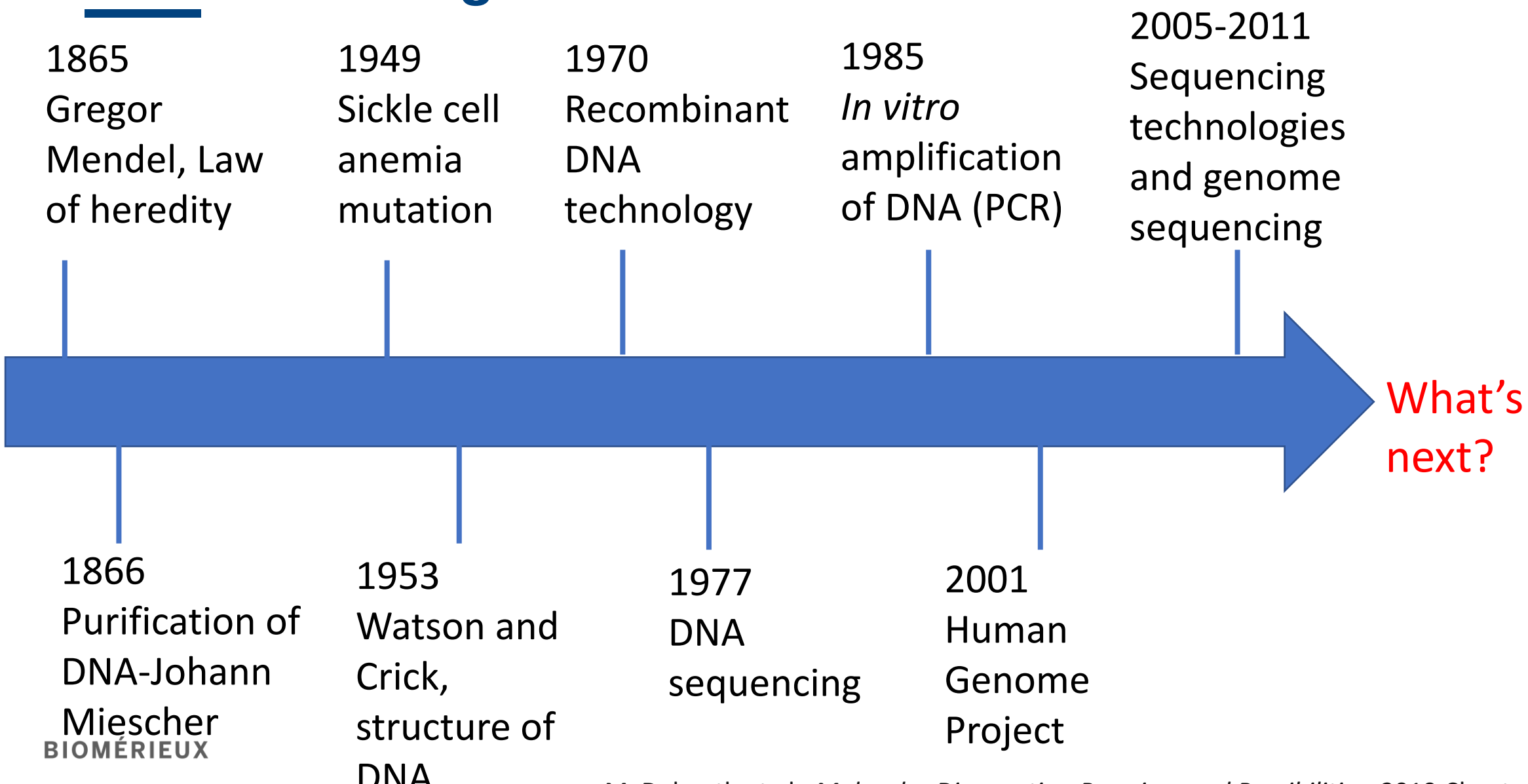
M. Debnath et al., *Molecular Diagnostics: Promises and Possibilities*. 2010;Chapter 1:1-10.

Molecular diagnostics

- Detection of pathogenic mutations in DNA and RNA samples
  - Aid in detection
  - Diagnosis and subclassification
  - Prognosis
  - Monitoring response to therapy
- > 80% of molecular tests performed address infectious disease detection and management

M. Debnath et al., *Molecular Diagnostics: Promises and Possibilities*. 2010;Chapter 1:1-10.

Molecular diagnostics timeline



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M. Debnath et al., *Molecular Diagnostics: Promises and Possibilities*. 2010;Chapter 1:1-10.

Common applications

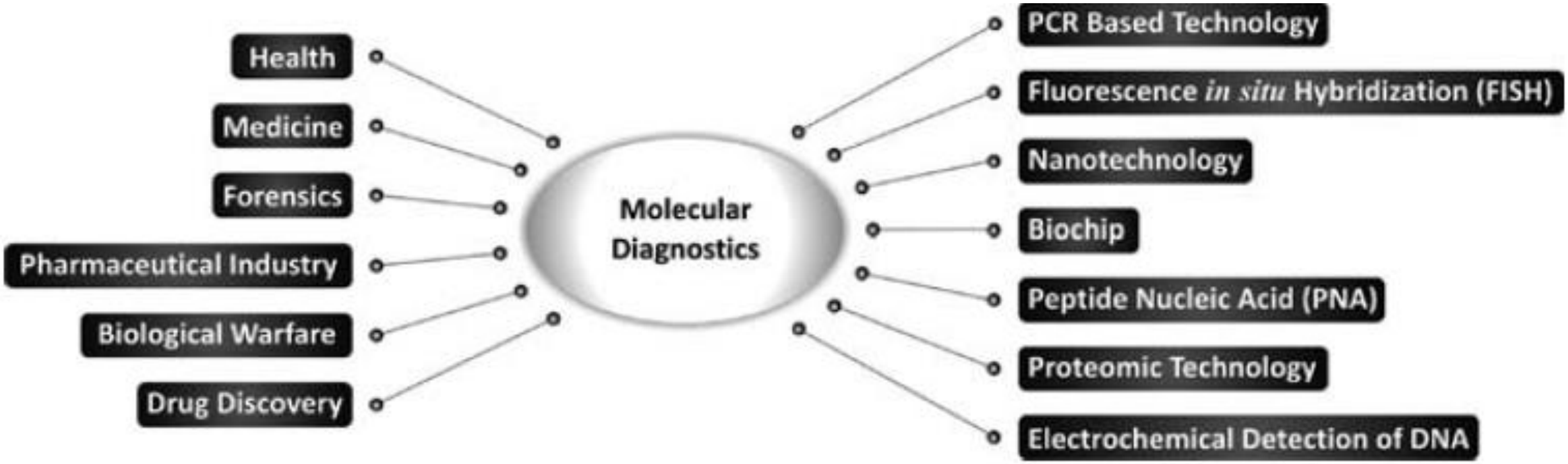
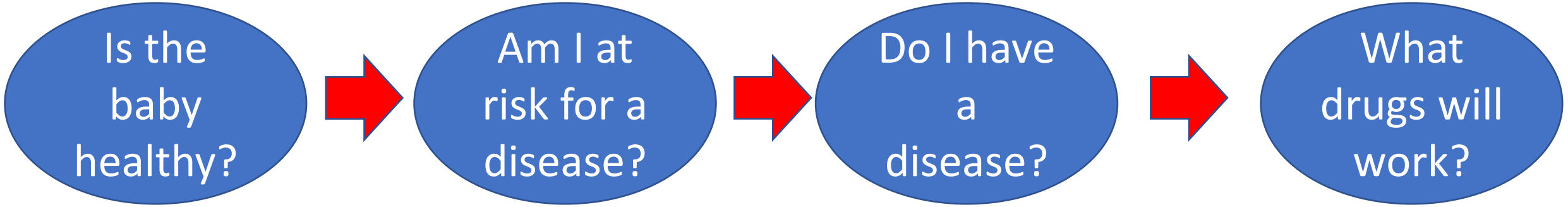


Fig. 1.1 Applications of molecular diagnostics in the field of clinical diagnostics

M. Debnath et al., *Molecular Diagnostics: Promises and Possibilities*. 2010;Chapter 1:1-10.

Common applications

- Molecular diagnostics is > $3 billion worldwide
- Growing at > 20% annually



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M. Debnath et al., *Molecular Diagnostics: Promises and Possibilities*. 2010;Chapter 1:1-10.

TECHNIQUES

Old vs new molecular diagnostics

- Old
  - Grow cells/pathogen then test
  - Problematic, slow, and costly
- New
  - Direct test
  - Immunological
  - DNA/RNA based

M. Debnath et al., *Molecular Diagnostics: Promises and Possibilities*. 2010;Chapter 1:1-10.

Non-amplified probe technology

- Detection done via
  - Chromogenic enzyme
  - Fluorophore
  - Radioisotope
- Adjuvant to culture based methods
- Applied to cultures with 1st growth of an organism

J Clin Micro. 2022;60(10):1-17

Non-amplified probe technology

- Among the 1st molecular *in vitro* diagnostics (IVDs) to be FDA approved
- Does not include nucleic acid amplification
- Usually require *in vitro* microbial growth
- Molecular probes hybridize with microbial nucleic acids
- Molecular probes identify a targeted sequence of the organism

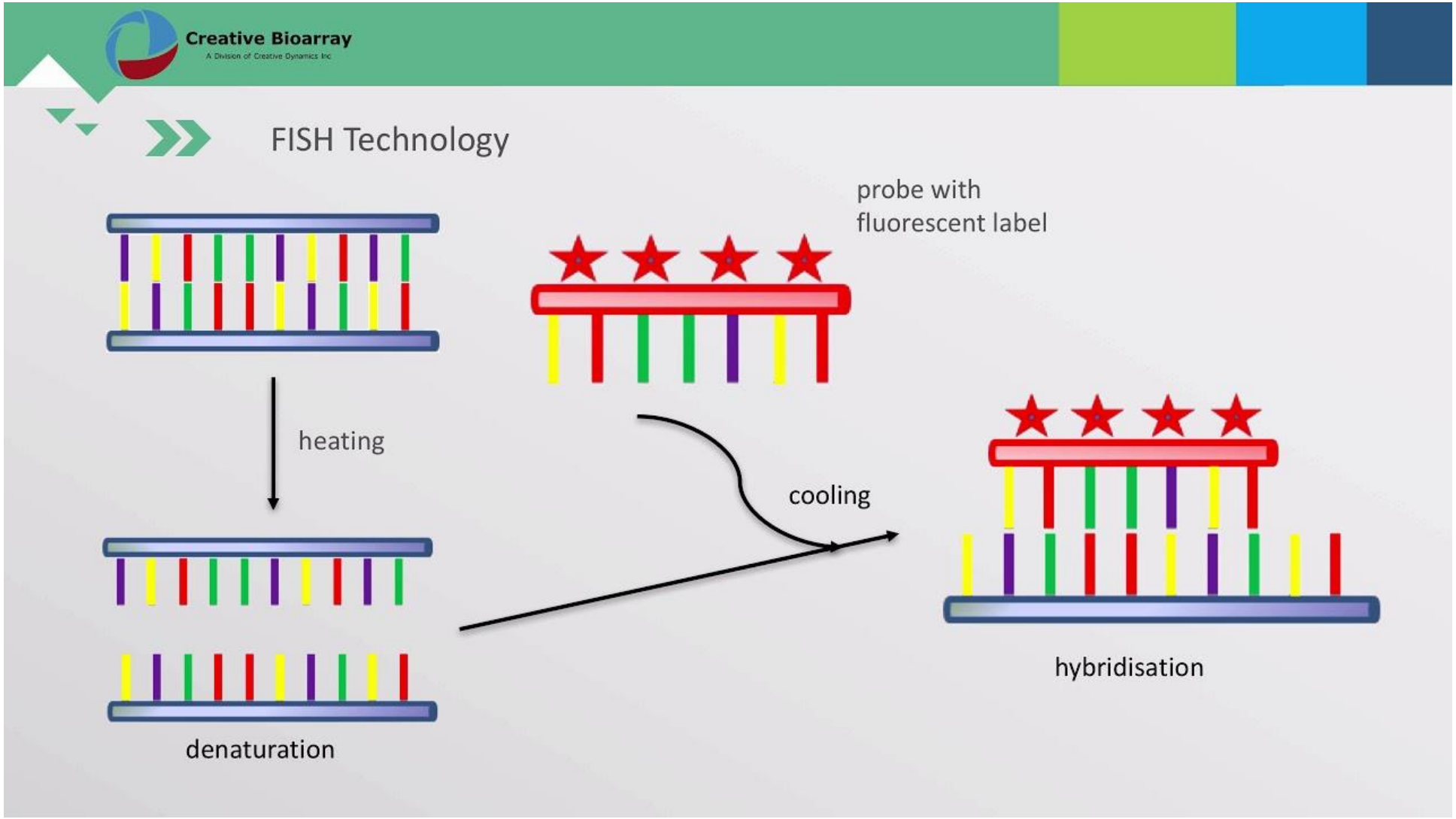
J Clin Micro. 2022;60(10):1-17

Non-amplified probe technology

- Fluorescent *in situ* hybridization (FISH)
  - Popular in early 2000s
  - Individual probes directed against common pathogens causing bacteremia

J Clin Micro. 2022;60(10):1-17

FISH



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Creative Bioarray - YouTube

Nucleic acid amplification

- Advent in 1980s with development of polymerase chain reaction (PCR) by Mullis
- Synthesize double stranded DNA from a single stranded DNA template with a primer
- The primer allows for amplification of a targeted DNA sequence

J Clin Micro. 2022;60(10):1-17

Nucleic acid amplification

- High sensitivity (e.g., single-digit copies of starting template)
- Amplification occurs through multiple heating/cooling temperature cycles
- Each cycle theoretically doubles the amplicons
- Current innovations allow for ~ 40 cycle reactions in ~ 30 minutes.

J Clin Micro. 2022;60(10):1-17

Nucleic acid amplification

- 3 component steps
  - Specimen processing and nucleic acid extraction
  - Target amplification
  - Detection/characterization of amplified product
- Allows for qualitative and quantitative determination

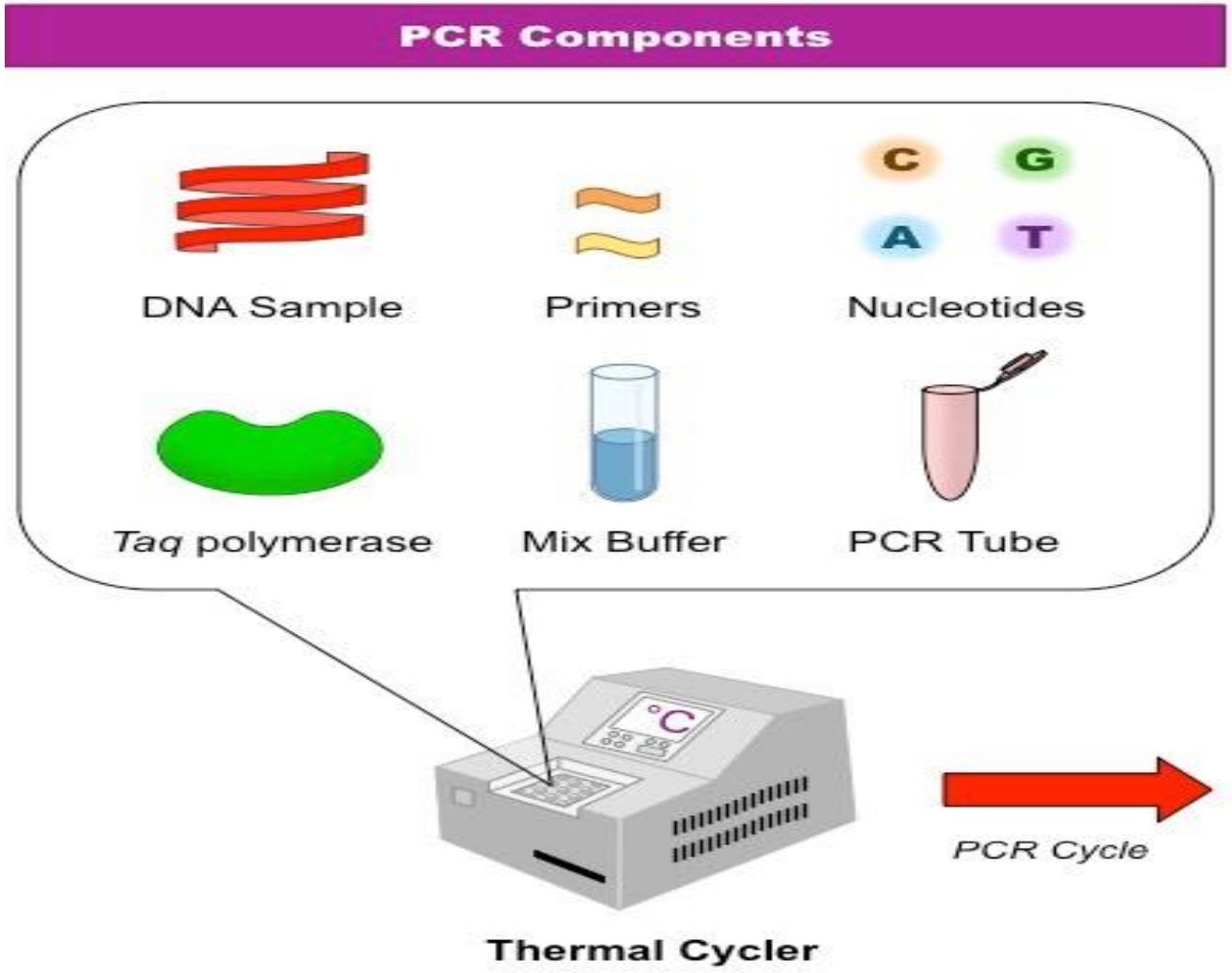
J Clin Micro. 2022;60(10):1-17

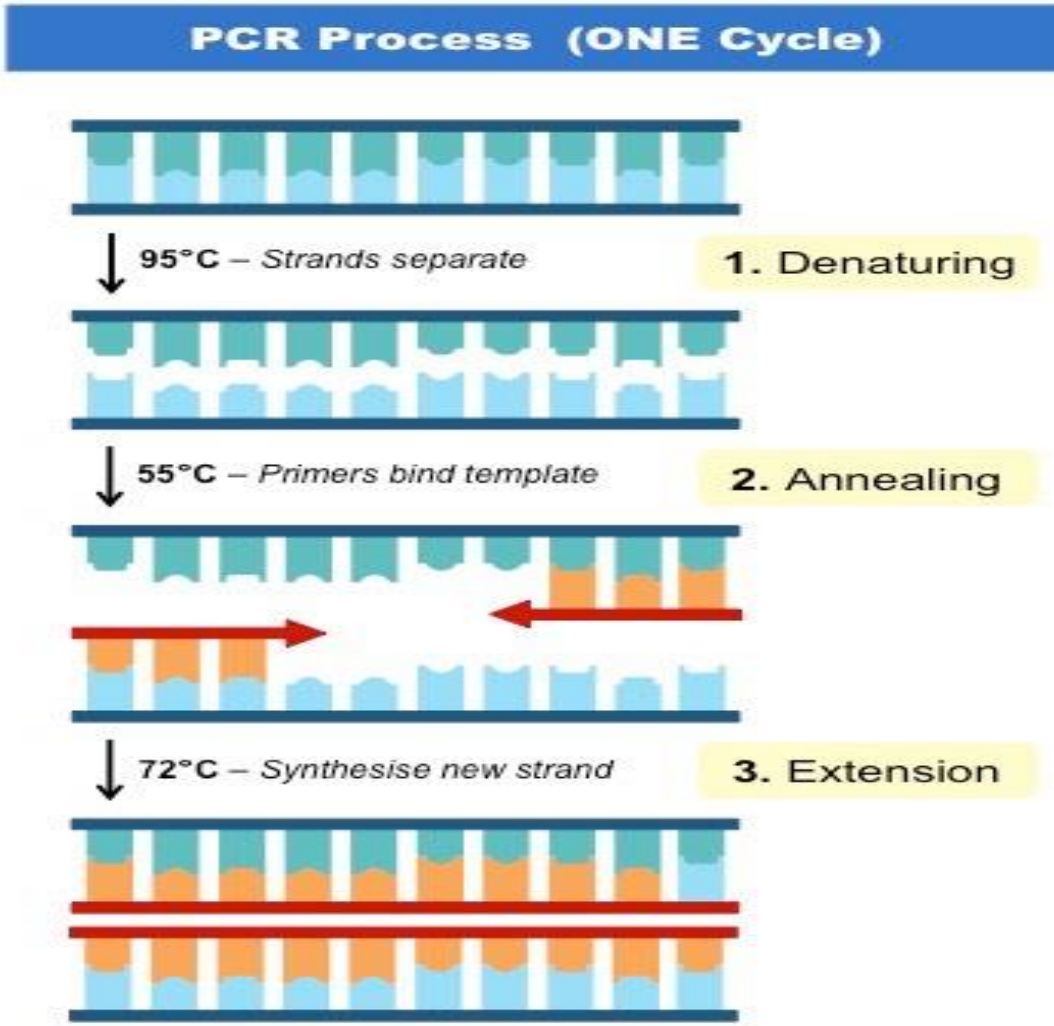
Nucleic acid amplification

- Real time PCR is most commonly application in use
- Amplification occurs through multiple heating/cooling temperature cycles
- Each cycle theoretically doubles the amplicons
- Current innovations allow for ~ 40 cycle reactions in ~ 30 minutes
- Single-plex: single pathogen detection
- Multi-plex: multiple pathogen detection

J Clin Micro. 2022;60(10):1-17; *Medicine* 2009;37(10):535-540

NAAT-PCR





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PCR | BioNinja

Advantages

- Wide range of detection
- Time to detection
- Sensitivity
- Specificity

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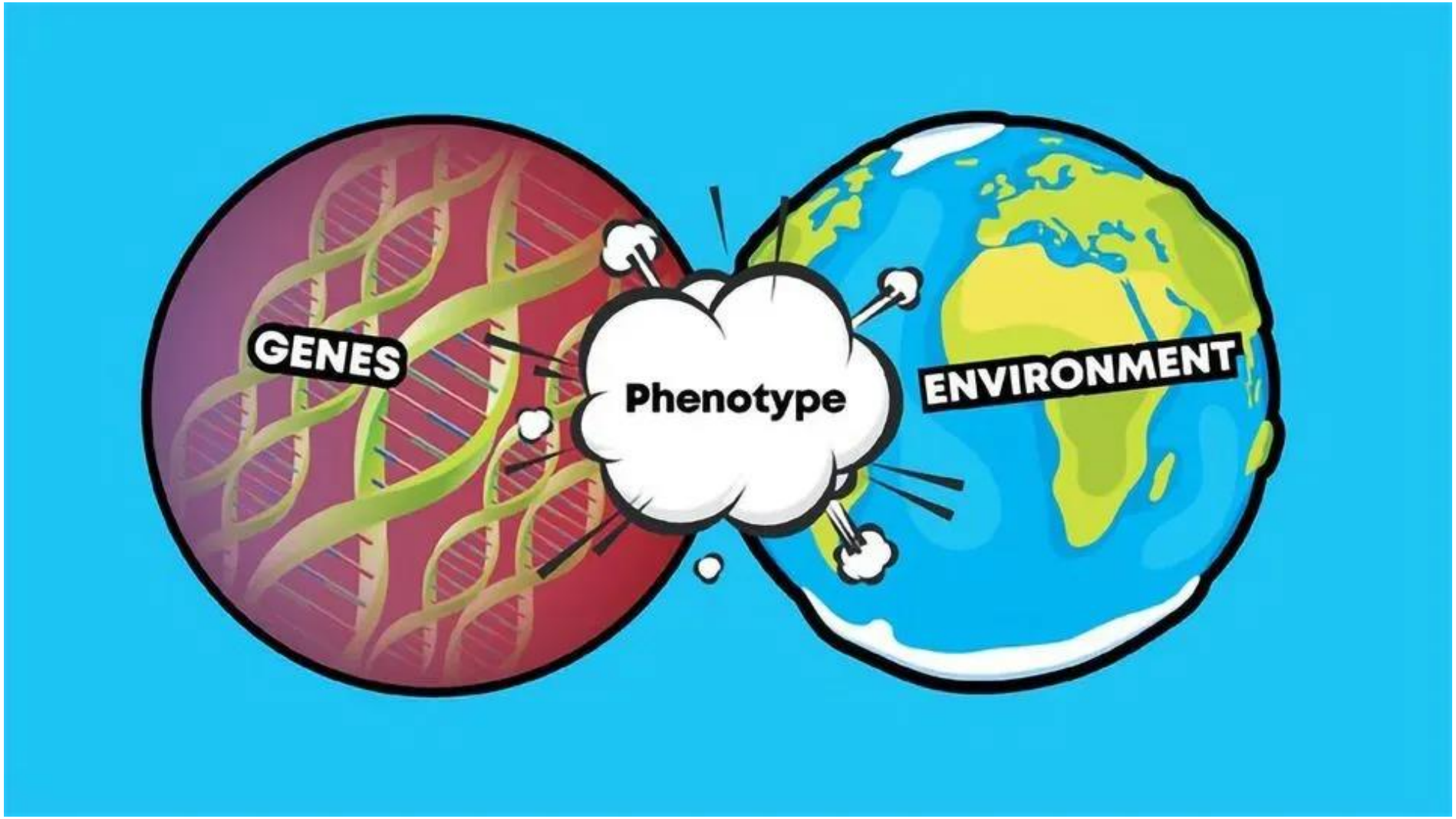
Disadvantages

- False positives - specimen contamination
- False positive/negative - genotype vs. phenotype
- False negative - off panel target
- Cost
- Rigor of technique and QC

J Clin Micro. 2022;60(10):1-17; *Medicine* 2009;37(10):535-540

Genotype vs phenotype



Genotype vs Phenotype: Examples and Definitions | Technology Networks

Genotype

- The genetic constitution of an individual organism
- Unique sequence of DNA
- Molecular diagnostics test for genes
- Presence of a particular gene does not equate to presence of a disease
- Presence of a particular gene does not equate to pres

Merriam-Webster.com Dictionary, Merriam-Webster, Accessed 12 Sep. 2023

Phenotype

- The observable traits/characteristics of an organism
- Based on genotype and the genotypes interaction with the environment
- Detectable expression of a genotype (e.g., clinical presentation)

Merriam-Webster.com Dictionary, Merriam-Webster, Accessed 12 Sep. 2023

Resistance gene detection

Gene	Gram-positive	Gram-negative
*mec*A/C	+	
*mec*A/C and MREJ	+	
*van*A/B	+	
CTX-M		+
KPC		+
NDM		+

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J Clin Micro 2019;57(2):e01597; *BMC Infectious Diseases* 2022;22:794

CASE 1

Case 1

- 4 year old patient short bowel syndrome, colostomy, GJ tube, BPD
- Central line for chronic TPN and hydration management
- Presentation
  - Temperature 102 ^{o}F
  - Blood pressure 68/40 mmHg
  - Respiratory rate 25 breathes/minute
  - Heart rate 110 beats/minute
  - Capillary refill time 4 seconds

Pharmacotherapy 2018;38(12):e87-e91

Case 1

- Day 1 management
  - Isotonic fluids 60-100 mL/kg
  - Vasoactives
  - Blood cultures
  - Vancomycin 15 mg/kg/dose IV q6h
  - Cefepime 50 mg/kg/dose IV q12h then q6h

Pharmacotherapy 2018;38(12):e87-e91

Case 1

- Day 2 management
  - Isotonic fluids
  - Vasoactives
  - Repeat blood cultures
  - Meropenem 40 mg/kg/dose IV q8h
  - Blood culture from day 1 → gram-negative rods
  - Multi-plex nucleic acid blood culture test → KPC producing *Klebsiella pneumoniae*

Pharmacotherapy 2018;38(12):e87-e91

Case 1

- Day 2 management
  - Regimen changed to meropenem/vaborbactam 40 mg/kg/dose of the meropenem component IV q6h infused over 3 hours

Pharmacotherapy 2018;38(12):e87-e91

Case 1

Pharmacokinetics of the Meropenem Component of Meropenem-Vaborbactam in the Treatment of KPC-Producing *Klebsiella pneumoniae* Bloodstream Infection in a Pediatric Patient

Alexandra M. Hanretty,[1] Ishminder Kaur,[1,2] Alan T. Evangelista,[1] Wayne S. Moore II,[3] Adela Enache,[4] Arun Chopra,[2,5,6] and Jeffrey J. Cies[1,2,3]*

[1]St. Christopher's Hospital for Children, Philadelphia, Pennsylvania; [2]Drexel University College of Medicine, Philadelphia, Pennsylvania; [3]The Center for Pediatric Pharmacotherapy, Pottstown, Pennsylvania; [4]Atlantic Diagnostic Laboratories, Bensalem, Pennsylvania; [5]NYU Langone Medical Center, New York, New York; [6]NYU School of Medicine, New York, New York

Pharmacotherapy 2018;38(12):e87-e91

Case 1

Drug	MIC
Amikacin	S
Gentamicin	S
Tetracycline	S
Tigecycline	S
Tobramycin	S
Ceftazidime/avibactam	S
Colistin	S
Meropenem/vaborbactam	S

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Pharmacotherapy 2018;38(12):e87-e91

CASE 2

Case 2

- Methicillin resistance in *staphylococci* is mediated by penicillin binding protein 2a (PBP 2a)
- Encoded by *mecA* on mobile staphylococcal cassette chromosome *mec* (SCC*mec*) element
- MRSA is a *S. aureus* isolate that displays phenotypic resistance to beta-lactams

Antimicrob Agents and Chemother 2011;55(8):3765-3773

Case 2

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2011, p. 3765–3773 Vol. 55, No. 8
0066-4804/11/$12.00 doi:10.1128/AAC.00187-11

Detection of Staphylococcal Cassette Chromosome *mec* Type XI Carrying Highly Divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* Genes in Human Clinical Isolates of Clonal Complex 130 Methicillin-Resistant *Staphylococcus aureus*▽†

Anna C. Shore,[1] Emily C. Deasy,[1] Peter Slickers,[2] Grainne Brennan,[3] Brian O'Connell,[3,4] Stefan Monecke,[5] Ralf Ehricht,[2] and David C. Coleman[1]*

Antimicrob Agents and Chemother 2011;55(8):3765-3773

Case 2

- Two *S. aureus* isolates from an Irish hospital
- GeneXpert real time PCR assay
  - Identified as methicillin susceptible *S. aureus*
- Phenotypically PBP 2a (+) → methicillin resistant *S. aureus*
- Lacked *mecA* by conventional PCR and DNA microarray screening
- Whole genome sequencing
  - SCC*mec* type XI
  - Bovine source
  - Not identified with commercially available PCR technologies

Antimicrob Agents and Chemother 2011;55(8):3765-3773

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P I O N E E R I N G D I A G N O S T I C S