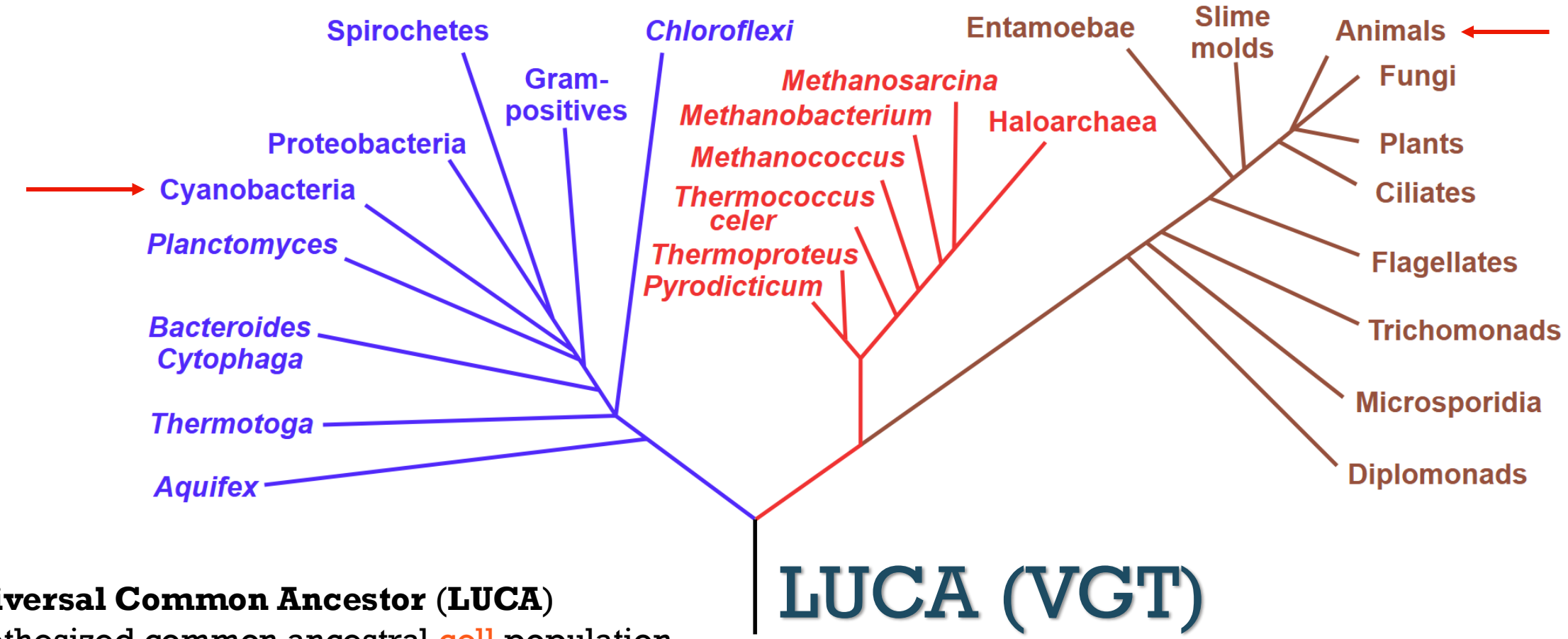
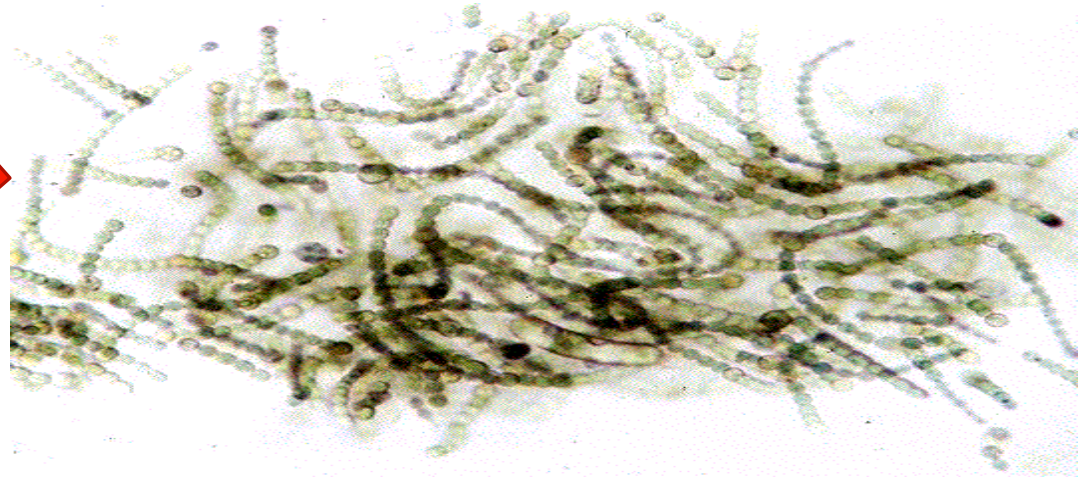
Le infezioni correlate all'assistenza (ICA) e il ruolo del laboratorio di microbiologia

Massimo Clementi
Professore di Microbiologia e Virologia
Professore Emerito, Università Vita-Salute San Raffaele

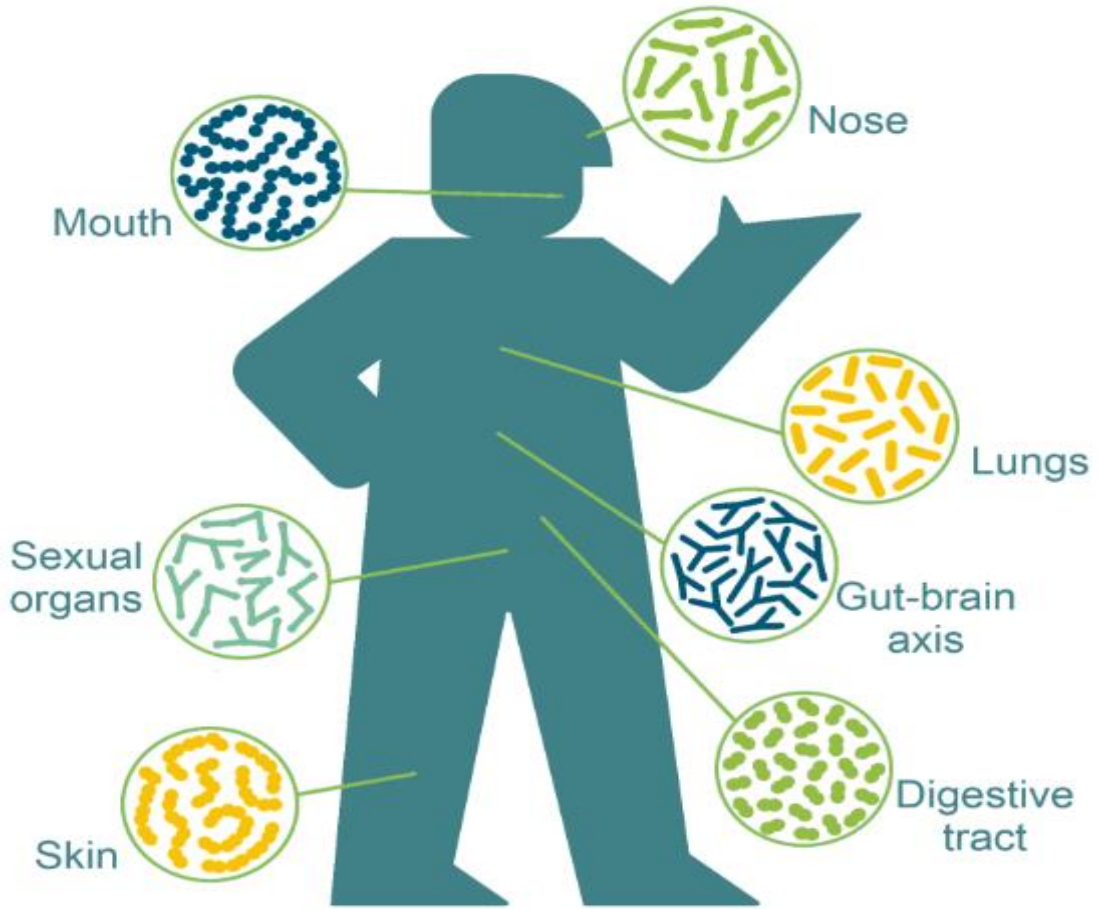


Last Universal Common Ancestor (**LUCA**)
the hypothesized common ancestral cell population
from which all subsequent life forms descend (OGT?)

Cyanobacteria are aquatic and photosynthetic. The oxygen atmosphere that we depend on was generated by numerous cyanobacteria photosynthesizing during the Archaean and Proterozoic Era. Before that time, the atmosphere had a very different chemistry, unsuitable for life as we know it today.



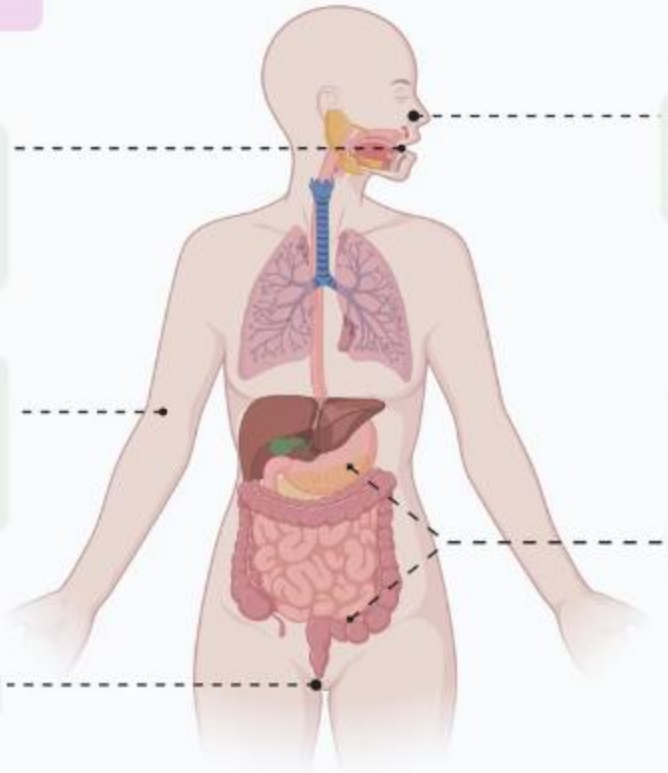
The oldest known fossils are cyanobacteria from rocks of western Australia, dated 3.5 billion years old. This may be somewhat surprising, since the oldest *rocks* are only a little older: 3.8 billion years old!



The Human Microbiome

Distribution data of human microbiome obtained from the Human Microbiome Project

Microbial distribution



Mouth (26%)
Firmicutes
Proteobacteria
Bacteroidetes
Actinobacteria
Fusobacteria

Skin (21%)
Actinobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Proteobacteria

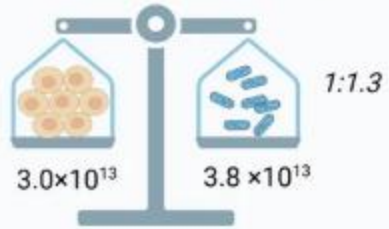
Vagina(9%)
Lactobacilli

Airways (14%)
Actinobacteria
Firmicutes
Proteobacteria
Bacteroidetes

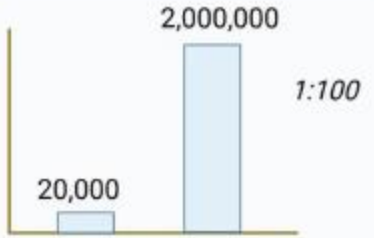
GI tract (29%)
Actinobacteria
Bacteroidetes
Firmicutes
Lactobacillae
Streptococci
Enterobacteria

Human bacterial weight

cell vs bacterial number



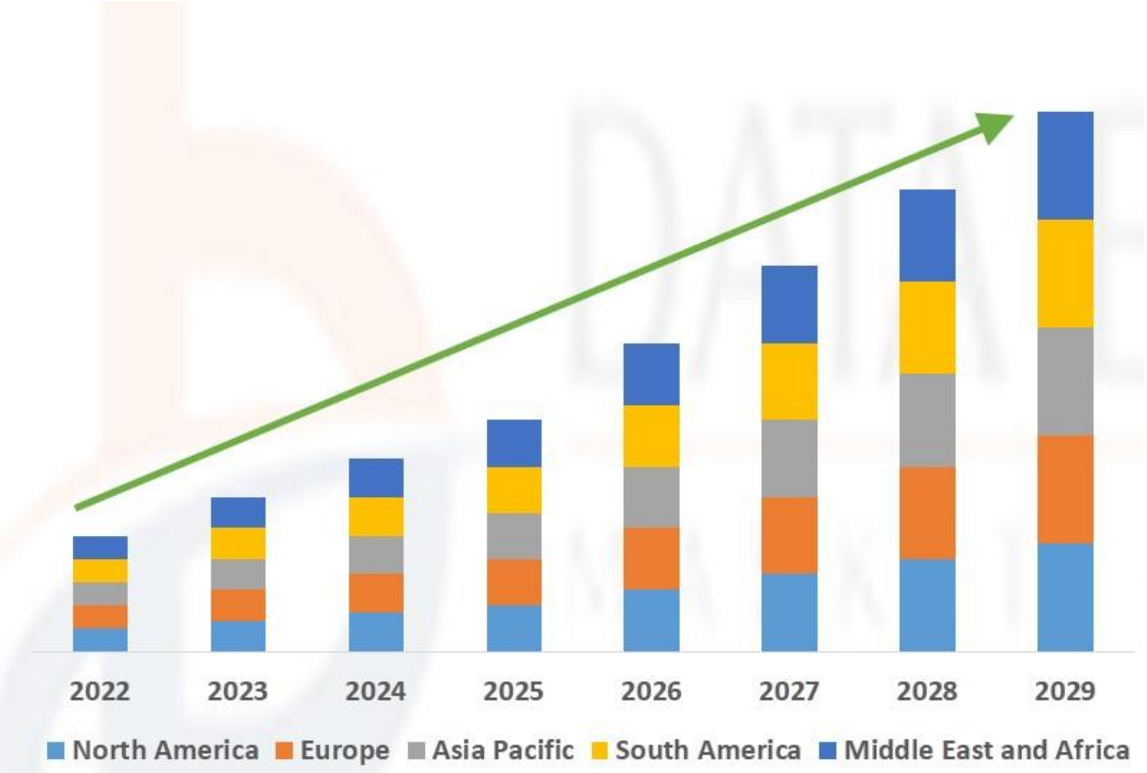
Human genes vs bacterial genes



Magnitude bound for bacterial numbers

healthcare-associated infections

Global healthcare-associated infections



Source: Data Bridge Market Research Market Analysis Study 2022

Risk Factors

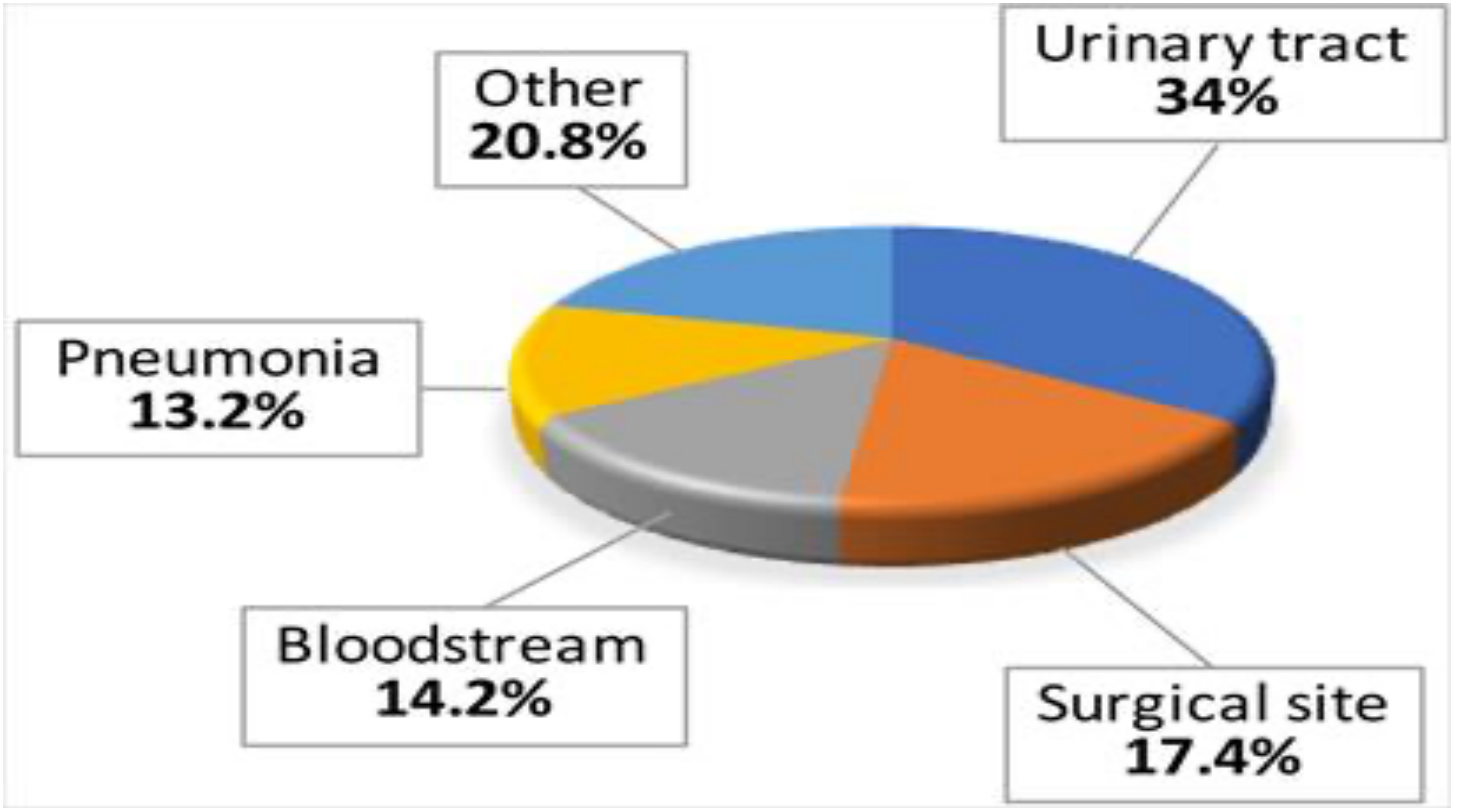
- Immunocompromisation
- Invasive medical procedures including catheterization
- Prolonged use of antimicrobials
- Prolonged hospitalization
- Use of contaminated medical devices
- Contact with other patients and career medical personals
- Chronic diseases
- Age

Relative distribution of nosocomial infections

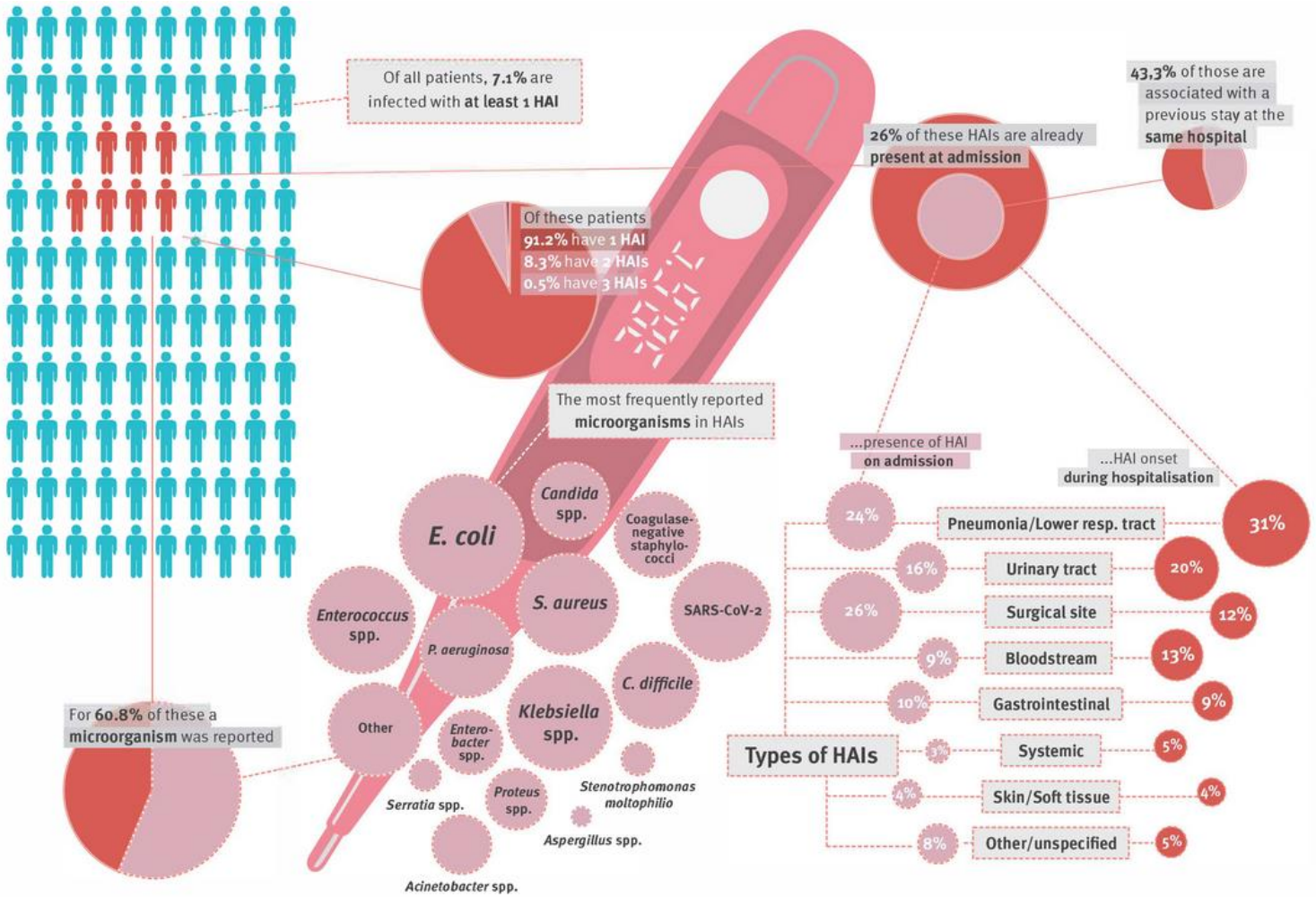
Table 14.5 Principal Sites of Nosocomial Infections

Type of Infection	Comment
Urinary tract infections	Most common, usually accounts for about 40% of all nosocomial infections. Typically related to urinary catheterization.
Surgical site infections	Ranks second in infection incidence (about 20%). An estimated 5–12% of all surgical patients develop postoperative infections; the percentage can reach 30% for certain surgeries, such as colon surgery and amputations.
Lower respiratory infections	Nosocomial pneumonias account for about 15% and have high mortality rates (13–55%). Most of these pneumonias are related to respiratory devices that aid breathing or administer medications.
Cutaneous infections	Cutaneous infections account for about 8% of nosocomial infections. Newborns have a high rate of susceptibility to skin and eye infections.
Bacteremia, caused primarily by intravenous catheterizations	Bacteremias account for about 6% of nosocomial infections. Intravenous catheterization is implicated in nosocomial infections of the bloodstream, particularly infections caused by bacteria and fungi.
Other	All other infection sites account for about 11% of nosocomial infections.

Microbiology Notes



Healthcare-associated infections in European hospitals 2022-2023

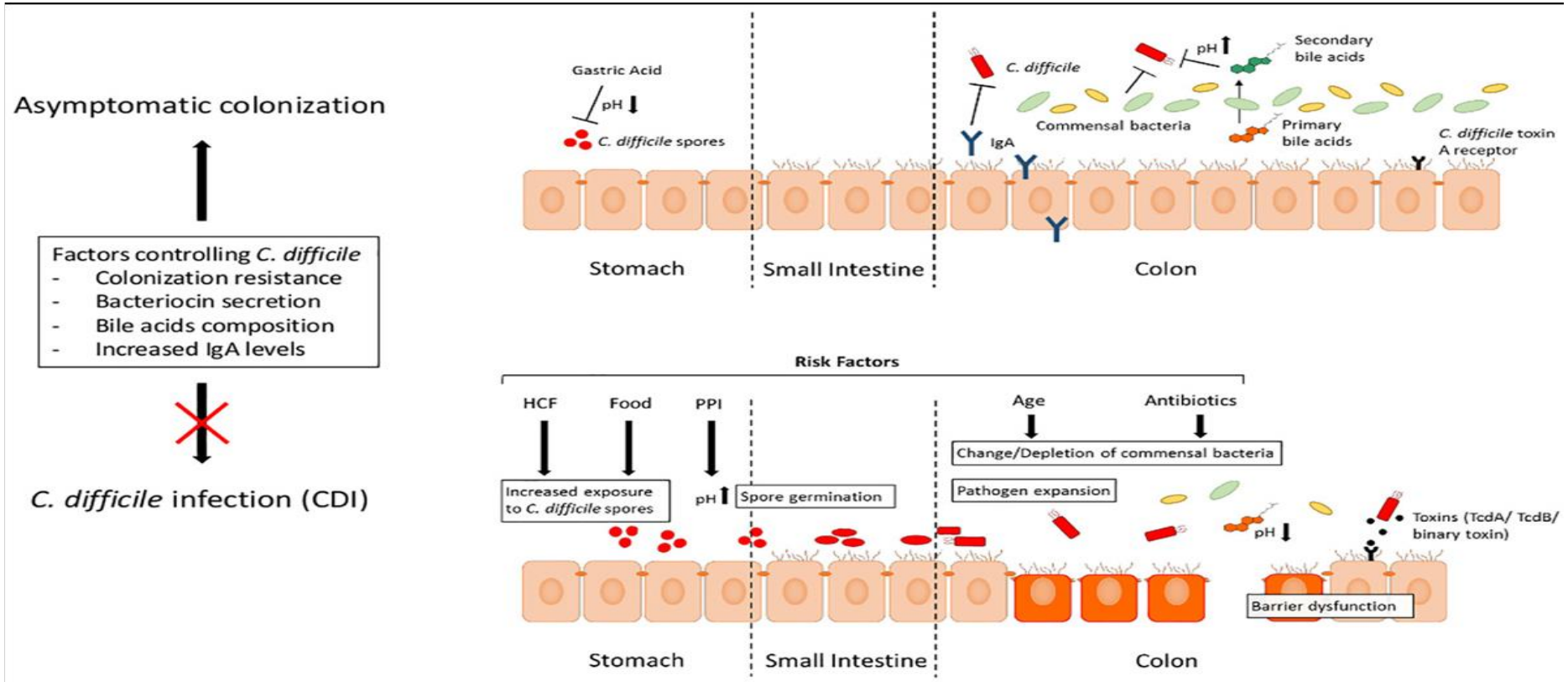


Common Pathogens Responsible for Nosocomial Infections (1)

Bacterial Pathogens

Gram-Positive Bacteria	Gram-Negative Bacteria
Staphylococcus aureus (Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Staphylococcus aureus* (VRSA)) Coagulase Negative *Staphylococcus* (CONS) *Enterococcus spp.* (Vancomycin Resistant Enterococci (VRE)) *Streptococcus spp.* *Clostridium difficile*	Enterobacterales (*E. coli*, *Klebsiella spp.*, *Proteus mirabilis*, *Enterobacter spp.*, *Salmonella spp.*, etc.) *Pseudomonas aeruginosa* *Acinetobacter baumannii* *Haemophilus influenza*

Clostridioides difficile (formerly *Clostridium difficile*)
From Colonization to Infection



Common Pathogens Responsible for Nosocomial Infections (2)

Candida spp. (*Candida albicans*, fluconazole resistant *C. krusei*, *C. glabrata*)

Aspergillus spp. (A. fumigatus, A. flavus)

Mucorales (Mucor spp.)

Fusarium spp.

Pneumocystis jirovecii

Scedosporium spp.

Malassezia spp.

Acremonium spp.

Candida auris
[Candidozyma auris]

Fungal Pathogens

Fungi account for most of the HCAIs after bacteria. They mainly infect severely immunocompromised patients, patients with severe granulocytopenia, and ventilated patients.

Candida auris *[Candidozyma auris]*: **an emerging pathogen**

Why is *Candida auris* a problem?

It causes serious infections. *C. auris* can cause bloodstream infections and even death, particularly in hospital and nursing home patients with serious medical problems. More than 1 in 3 patients with invasive *C. auris* infection (for example, an infection that affects the blood, heart, or brain) die.

It's often resistant to medicines. Antifungal medicines commonly used to treat *Candida* infections often don't work for *Candida auris*. Some *C. auris* infections have been resistant to all three types of antifungal medicines.

It's becoming more common. Although *C. auris* was just discovered in 2009, it has spread quickly and caused infections in more than a dozen countries.

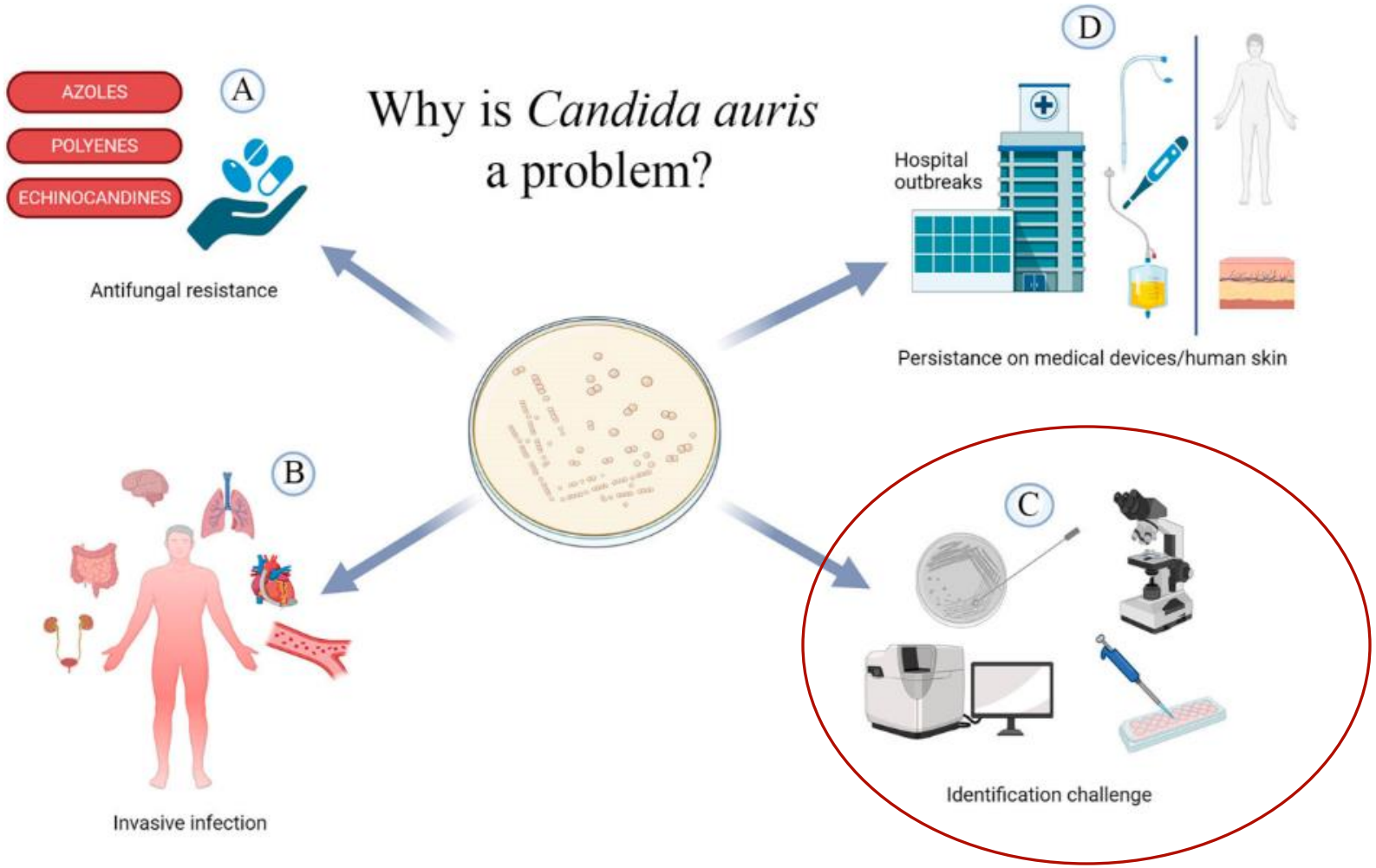
It's difficult to identify. *C. auris* can be misidentified as other types of fungi unless specialized laboratory technology is used. This misidentification might lead to a patient getting the wrong treatment.

It can spread in hospitals and nursing homes. *C. auris* has caused outbreaks in healthcare facilities and can spread through contact with affected patients and contaminated surfaces or equipment. Good hand hygiene and cleaning in healthcare facilities is important because *C. auris* can live on surfaces for several weeks.

Potential Misidentifications of *C auris*, Based on Identification Method

Identification Method	Organism *C auris* Can Be Misidentified As:
Vitek 2 YST	*Candida haemulonii* *Candida duobushaemulonii*
API 20C	*Rhodotorula glutinis* *Candida sake*
API ID 32C	*Candida intermedia* *Candida sake* *Saccharomyces kluyveri*
BD Phoenix yeast identification system	*Candida haemulonii* *Candida catenulata*
MicroScan	*Candida albicans* *Candida famata* *Candida guilliermondii* *Candida lusitaniae* *Candida parapsilosis* *Candida tropicalis*
RapID Yeast Plus	*Candida parapsilosis*

Candida auris species identification	Laboratories that identify to species level/respondents (%)
Onsite	
MALDI	24/51 (47%)
VITEK	2/51 (4%)
API	0/51 (0%)
Chromogenic Agar	0/51 (0%)
PCR/Sequencing	1/51 (2%)
Other	1/51 (2%)
Reference Laboratory Referral	23/51 (45%)
Species Confirmation	Laboratories that refer to a Reference Laboratory or mycology centre for confirmation/respondents (%)
No	7/25 (28%)
Yes	18/25 (72%)


Why is *Candida auris* a problem?
AZOLES
POLYENES
ECHINOCANDINES
A
Antifungal resistance
B
Invasive infection
C
Identification challenge
D
Hospital outbreaks
Persistance on medical devices/human skin

Common Pathogens Responsible for Nosocomial Infections (3)

Viral Pathogens

Several viruses are responsible for a minor portion of nosocomial infections.

Noroviruses

Rotaviruses

Influenza viruses

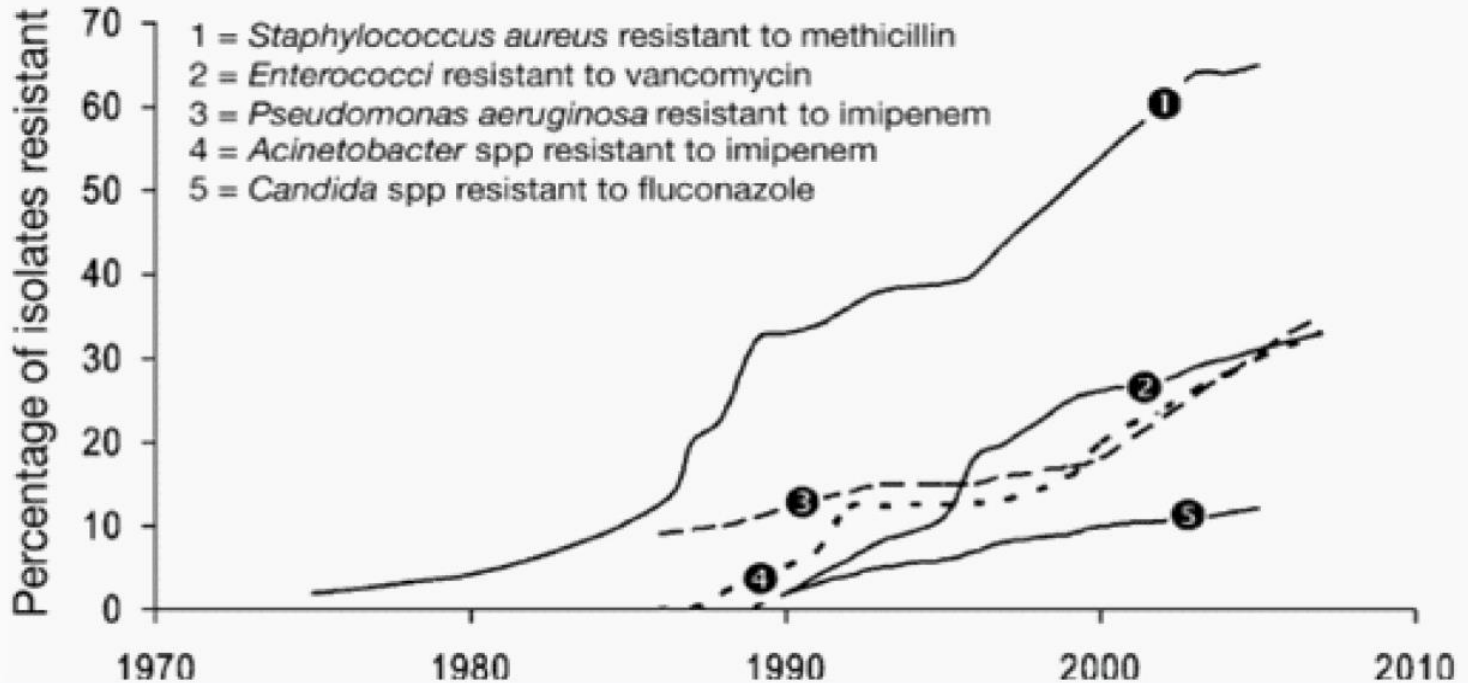
Respiratory syncytial virus

Herpes Simplex Virus

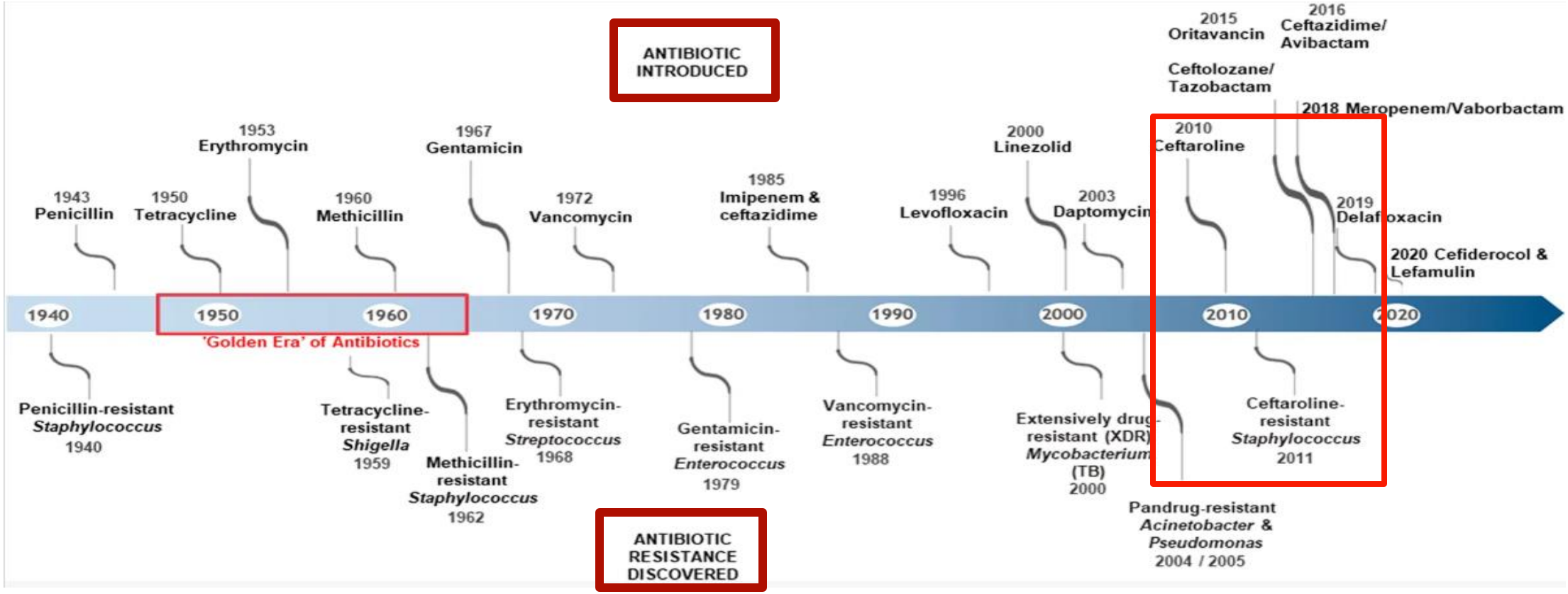
Hepatitis B and C virus

ANTIMICROBIAL RESISTANCE

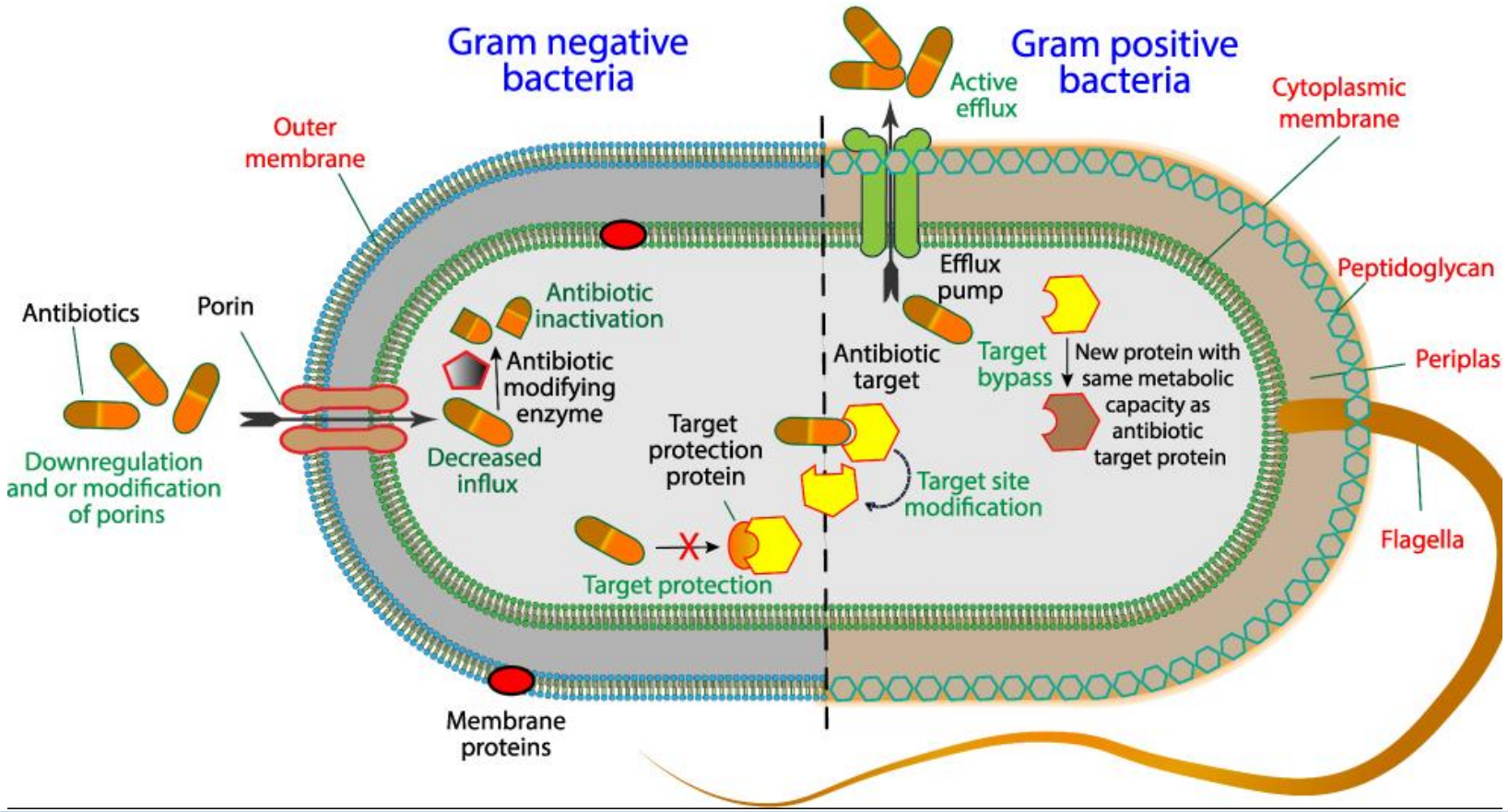
Antimicrobial Resistance for Selected Pathogens over Time



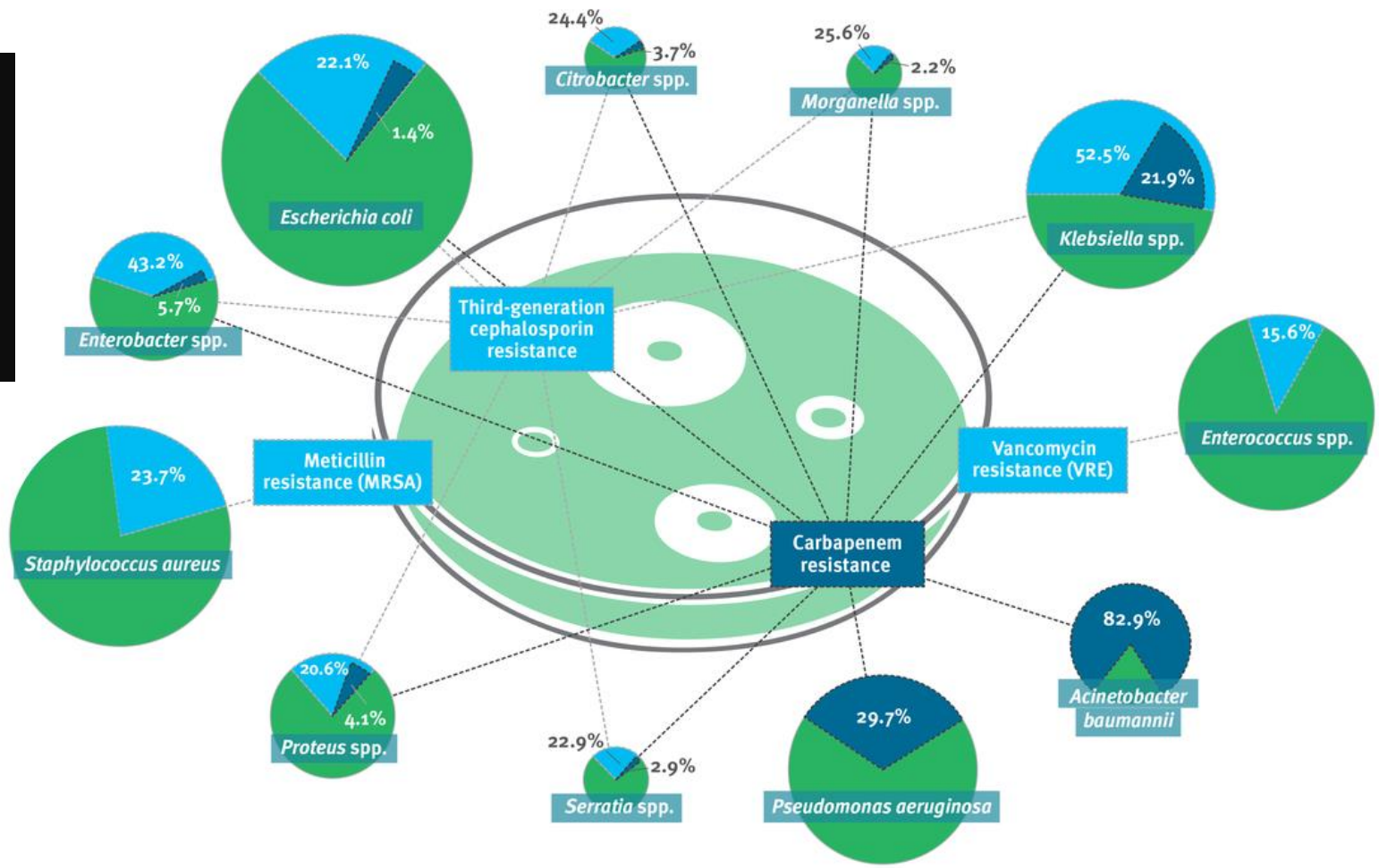
Timeline showing some of the key antibiotic discoveries and reports of the emergence of antibiotic resistance strains



Overview of different molecular mechanisms of MDR in bacteria.



Antimicrobial resistance of microorganisms reported in healthcare-associated infections (HAIs)



Società Italiana di
Medicina Veterinaria Preventiva

Consultazione pubblica sulla riduzione dell'impiego della colistina in allevamento

Data Comunicato: 7 Giugno 2016

L'Agenzia europea per i medicinali (EMA) ha lanciato una consultazione pubblica sul parere del Gruppo di esperti sull'antibiotico resistenza (AMEG), già approvato dal Comitato per i medicinali per uso veterinario (CVMP) e dal Comitato per i medicinali per uso umano (CHMP), che propone di ridurre al minimo le vendite di colistina ad uso veterinario e limitarne l'impiego negli animali solo come ultima risorsa.

E' stata la Commissione Ue a richiedere all'Ema l'aggiornamento del parere del 2013, dopo il primo caso umano registrato negli Stati Uniti, di batterio resistente a tutti gli antibiotici conosciuti, anche la colistina. La resistenza è dovuta al **gene Mcr-1**, già **identificato in Cina a novembre 2015**, potenzialmente trasferibile da batterio a batterio, anche tra specie diverse, e quindi con alto potenziale di diffusione.

L'Ema ha quindi chiesto all'AMEG un nuovo parere sull'importanza della colistina per la salute umana e animale, sull'impatto della resistenza alla colistina, la disponibilità di trattamenti alternativi. Al gruppo è stato inoltre richiesto di proporre adeguate misure per la gestione del rischio.

La Colistina viene usata da 50 anni sia per la salute umana che per la salute animale. In medicina umana è l'ultima risorsa cui si ricorre per trattare le persone che hanno infezioni batteriche resistenti ad altri antibiotici.

In medicina veterinaria viene utilizzata per trattare le infezioni da *Enterobacteriaceae* negli animali d'allevamento come suini, avicoli, bovini, ovini, caprini e conigli. Il suo consumo è in aumento in parte anche a causa dello sviluppo di resistenza ad altre classi di antibiotici. **Oggi è uno dei 5 antibiotici più usati per gli animali in Ue (6,1% sul totale), dopo le tetracicline (36.7%), penicilline (24.5%), sulfamidici (9.6%), e macrolidi (7.4%).**

TYPING OF BACTERIAL ISOLATES

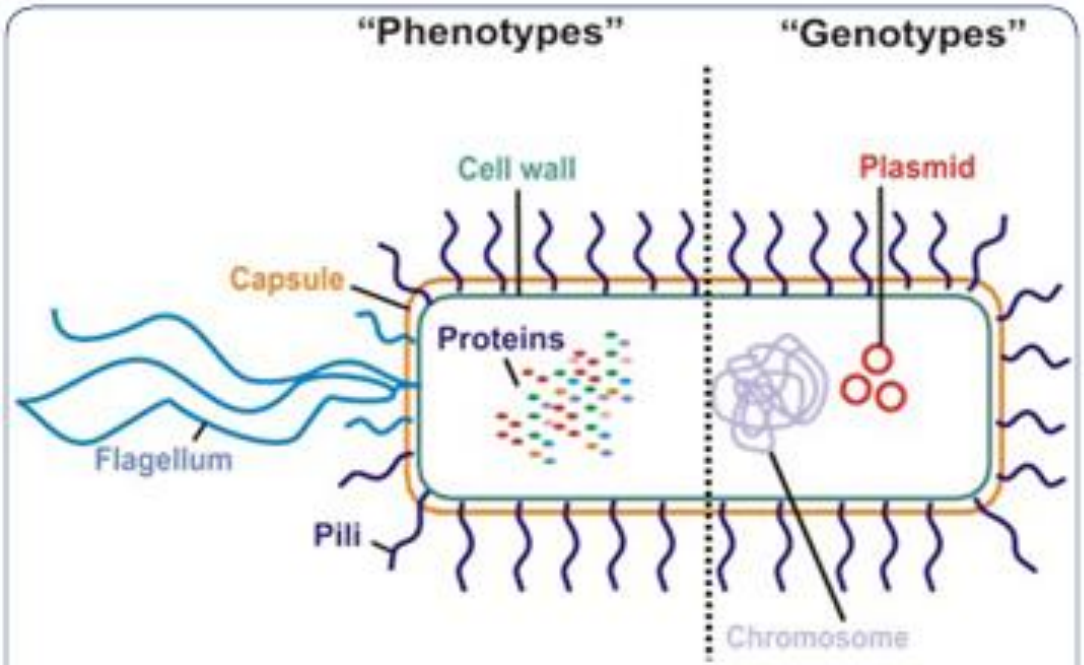
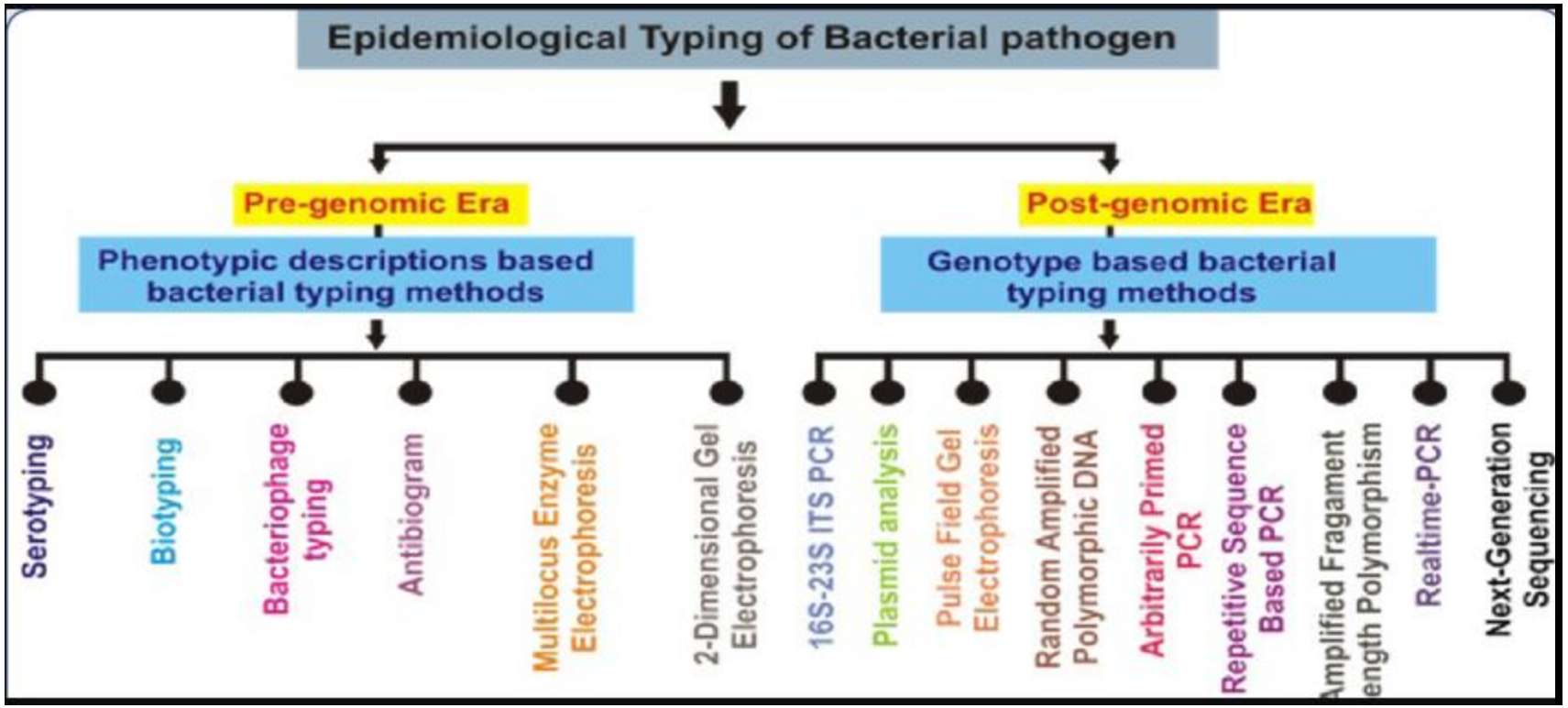


Fig. 1: Phenotype- Genotype distinction within bacterial structure.

'Phenotype'- actual observed properties such as bacterial cell wall, capsule, flagellum, pili, proteins; 'Genotype' – full hereditary information of bacteria (chromosome and plasmid)


Epidemiological Typing of Bacterial pathogen
Pre-genomic Era
Post-genomic Era
Phenotypic descriptions based bacterial typing methods
Genotype based bacterial typing methods
Serotyping
Biotyping
Bacteriophage typing
Antibiogram
Multilocus Enzyme Electrophoresis
2-Dimensional Gel Electrophoresis
16S-23S ITS PCR
Plasmid analysis
Pulse Field Gel Electrophoresis
Random Amplified Polymorphic DNA
Arbitrarily Primed PCR
Repetitive Sequence Based PCR
Amplified Fragament ength Polymorphism
Realtime-PCR
Next-Generation Sequencing

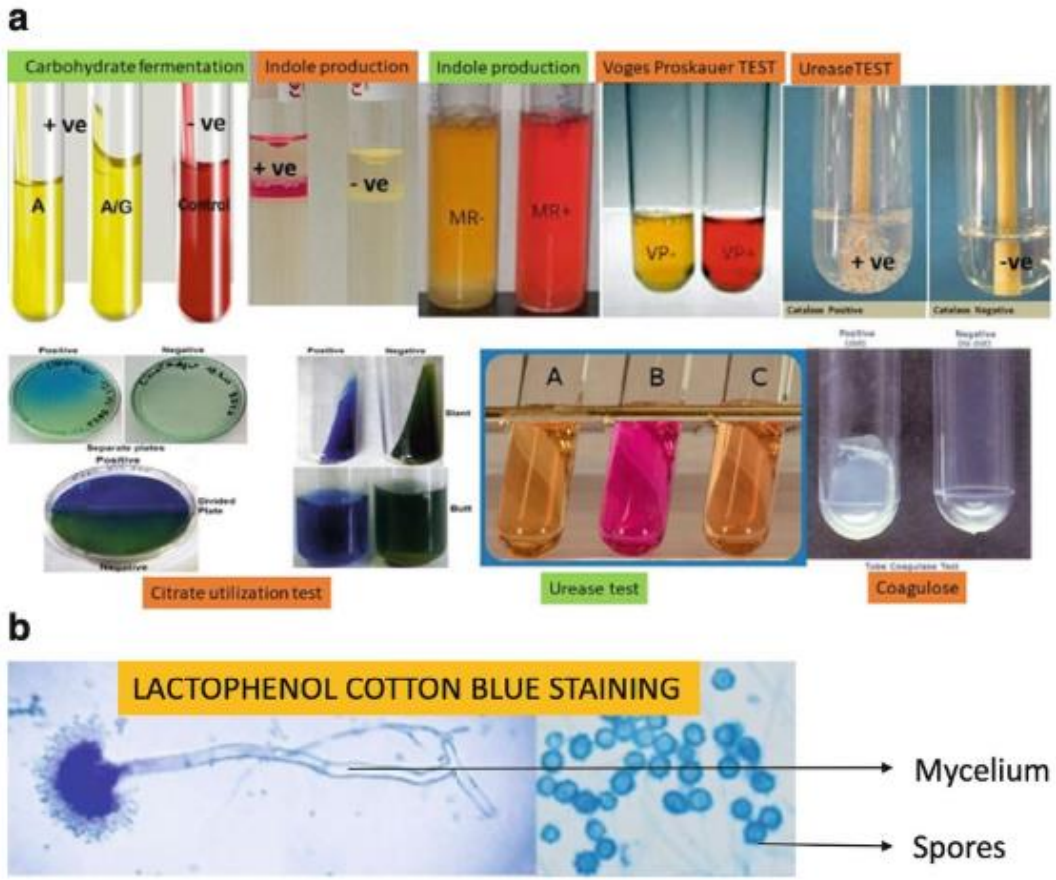
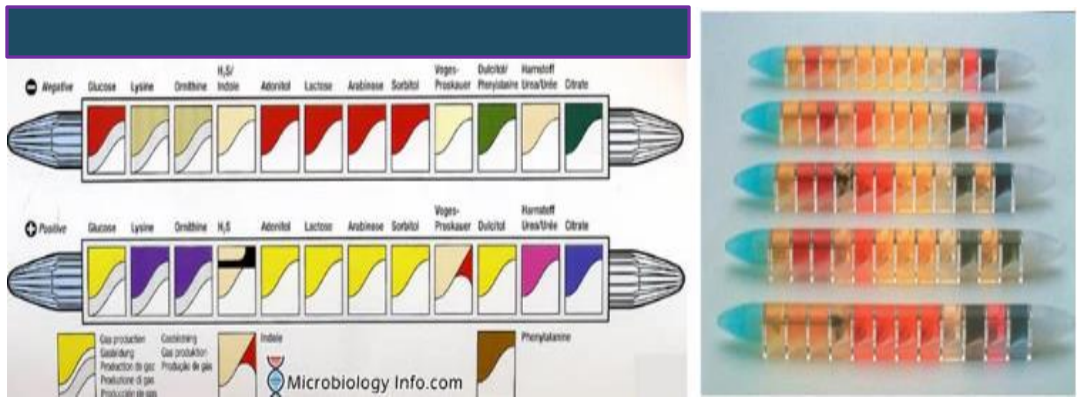


Fig. 8.2 (**a**) Various biochemical tests performed for bacterial identification. (**b**) Lactophenol cotton blue stained fungal mycelium and spores



Main characteristics used for the identification and diagnostics of pathogenic bacteria

PHENOTYPIC CHARACTERISTICS

CHEMOTAXONOMIC COMPOSITION

e.g.,
Fatty acid profiling
Carbohydrate profiing
Ubiquinone profiling
Polar lipid profiling
Cell wall composition analysis

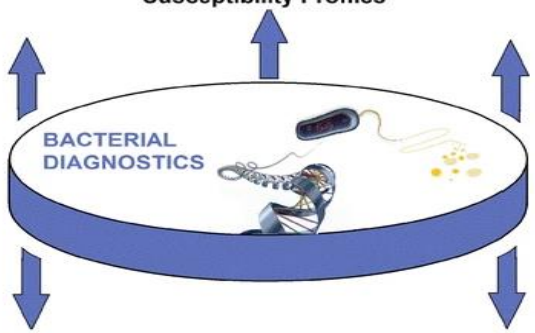
EXPRESSED FEATURES

e.g.,
Morphological typing
Physiological typing
Enzymological typing
Serological typing
Phage and bacteriocin typing

Susceptibility Profiles

PROTEINS

e.g.,
Ribotyping
Enzyme profiling
Cellular and call protein profiling



DNA

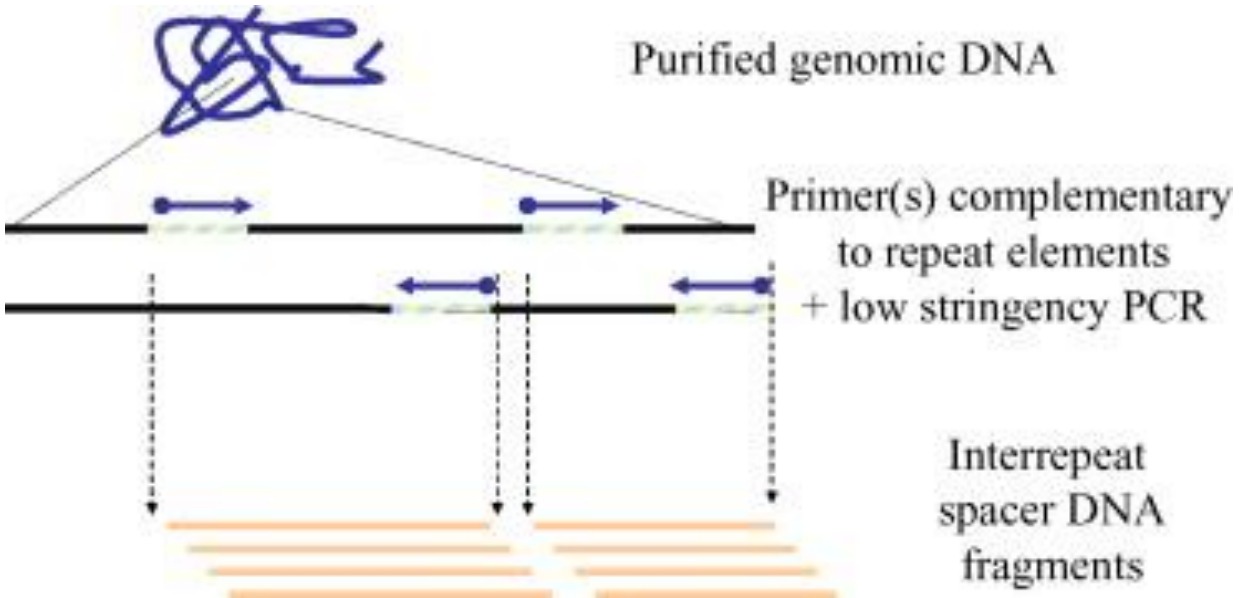
e.g.,
GC% composition
Genome size
DNA:DNA hybridization volume
Restriction (RFLP, PFGE) patterns
DNA fingerprints (ARDRA, AP-PCR, AFLP)
MLST and SNP typing
Whole genome sequences

RNA

e.g.,
mRNA expression profiling
Low molecular weight RNA profiling

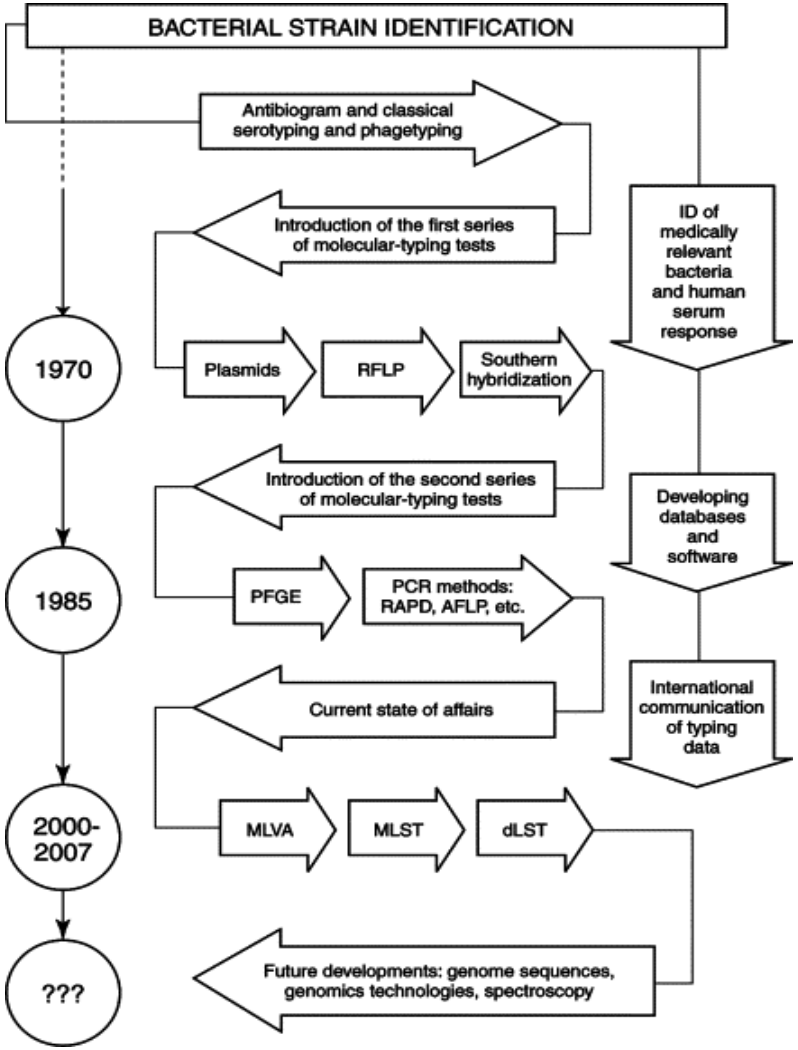
GENOTYPIC CHARACTERISTICS

Broad-range typing systems, such as PFGE, AFLP and sequencing are most suited for hospital epidemiology, as many different bacterial and fungal pathogens are implicated in epidemics.



Molecular methods

Multi-Locus Variable Number of Tandem Repeat Analysis
Multi-Locus Sequence Typing
Bacterial Whole-Genome Sequencing
DNA arrays



Bacterial Whole-Genome Sequencing (WGS)

WGS data processing pipeline would facilitate WGS application to the precision genomic surveillance of HAI. In addition, the results from such a WGS-based analysis would be useful for the precision laboratory diagnosis of infectious microorganisms.

Overview of Sequencing Workflows

a. Whole Genome Sequencing

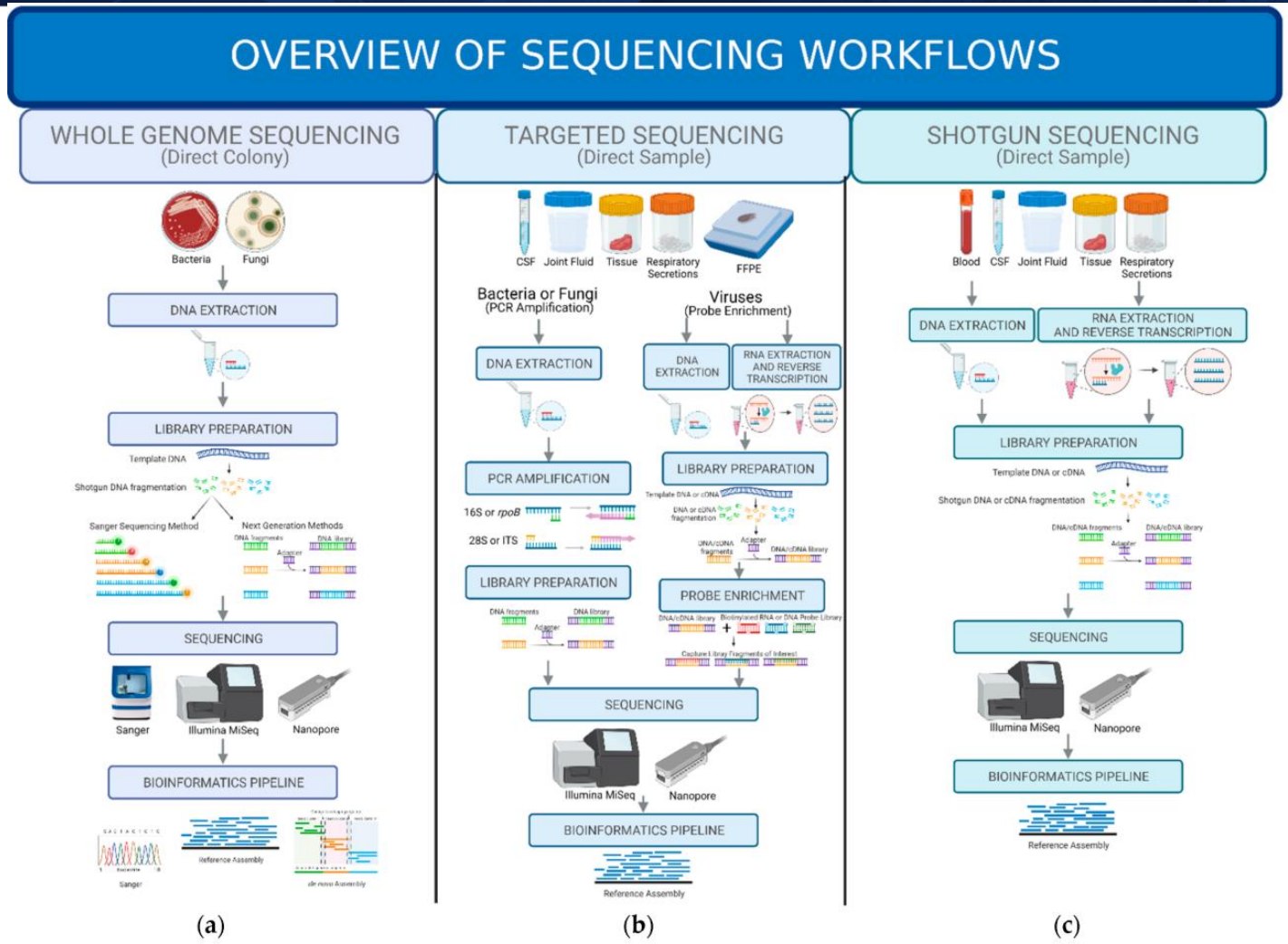
(colony)

b. Targeted Sequencing

(target gene)

c. Shotgun Sequencing

(clinical sample)



Shotgun Sequencing

•**Frammentazione casuale:** L'intero DNA viene frantumato in migliaia o milioni di piccoli frammenti di dimensioni variabili.

•**Sequenziamento dei frammenti:** Ciascuno di questi frammenti viene sequenziato individualmente. Questa fase è possibile grazie alle moderne tecnologie di sequenziamento ad alta capacità (NGS).

•**Assemblaggio al computer:** I frammenti sequenziati, chiamati "reads", vengono poi allineati e assemblati al computer in modo da ricostruire l'ordine originale delle sequenze e, quindi, l'intero genoma e rivelando anche le loro funzioni metaboliche.

Il Sequenziamento Shotgun permette di:

1. Identificare tutte le specie presenti, incluse quelle raramente rappresentate.

2. Rilevare geni specifici associati a funzioni biologiche come il metabolismo o la resistenza agli antibiotici.

3. Esplorare le interazioni microbiche e il ruolo del microbiota nell'ambiente o nel corpo umano.

4. Il sequenziamento Shotgun del microbiota offre una visione unica della diversità microbica e delle sue funzioni, aprendo nuove strade nella ricerca scientifica e nell'applicazione pratica.

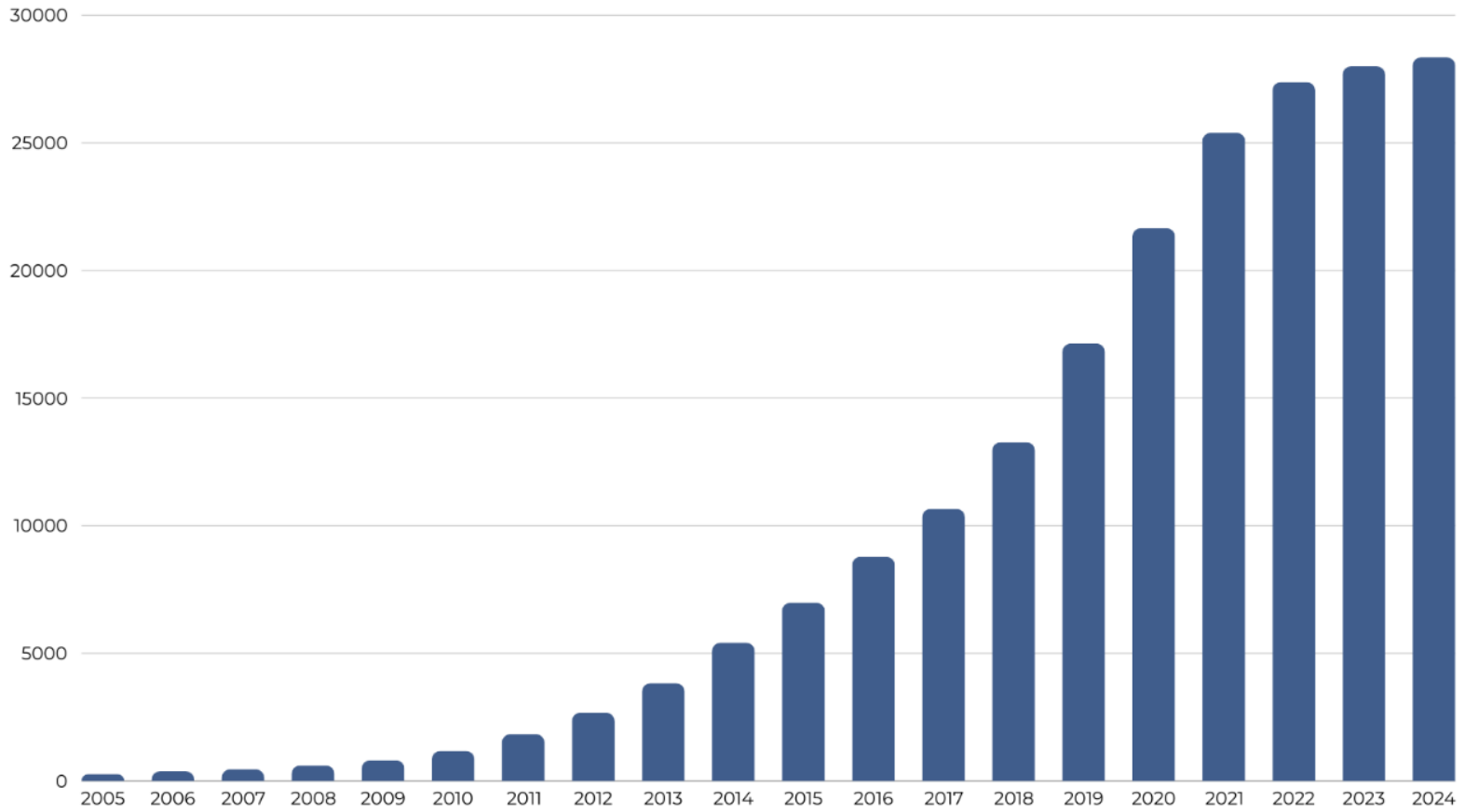
Importance of Metagenomics

The traditional pure culture method in microbiology isolates individual species and studies their responses to specific chemicals in controlled environments. This method limits our understanding of microbial behavior within complex communities.

Metagenomics addresses these limitations of traditional microbiology by allowing the study of microbial communities directly in their natural habitats without the need for culturing individual species.

SCIENTIFIC PUBLICATIONS PER YEAR ON MICROBIOME TOPIC

Search query on Pubmed: microbiome OR microbiota / 2024 extrapolated from already published papers



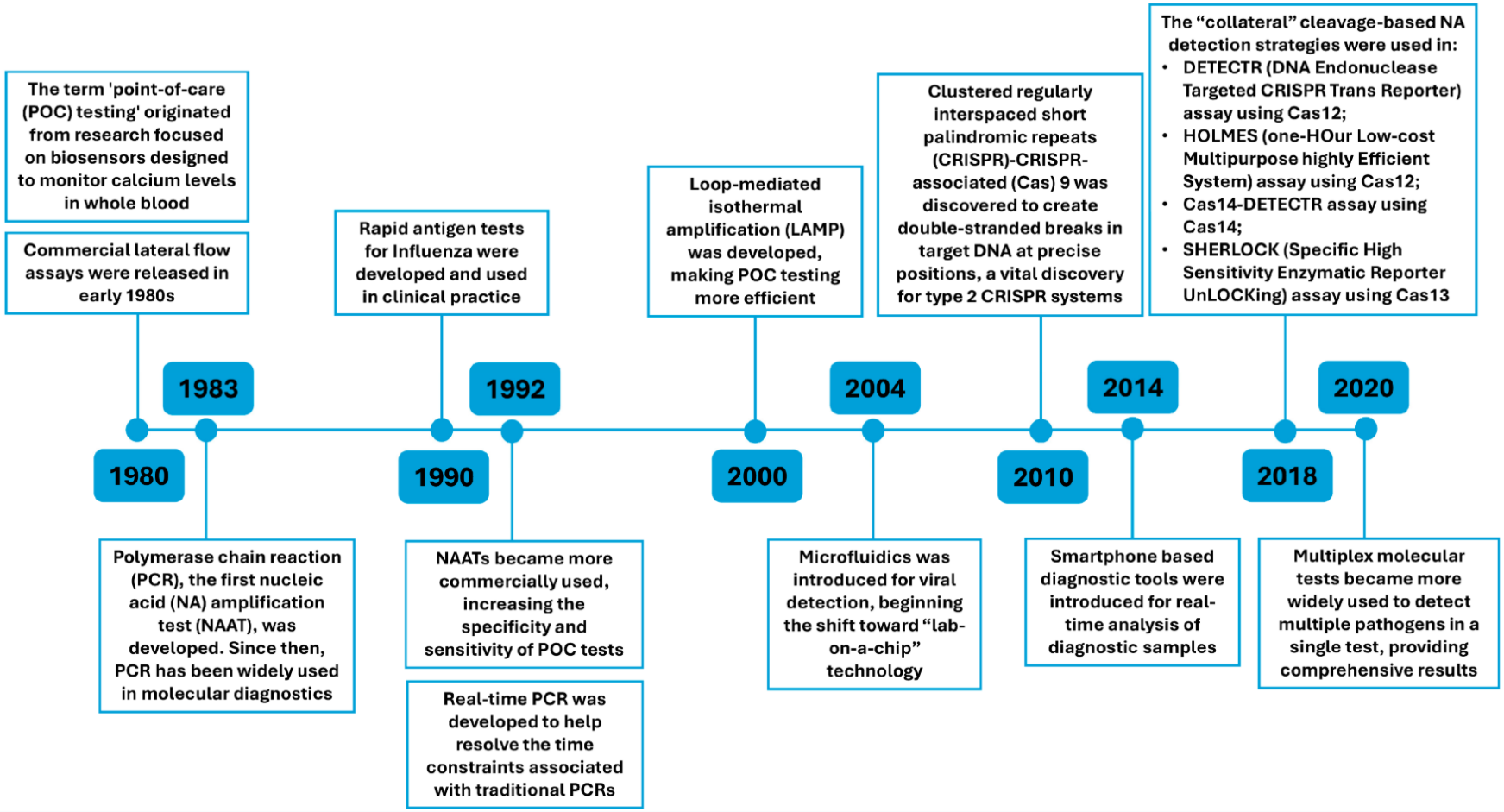
Microbiological diagnostic technologies are evolving in two divergent directions:

(1) concentration of specialised testing in large laboratories with high-throughput automated instruments and expert laboratory personnel

(2) Increased availability of portable, point-of-care testing

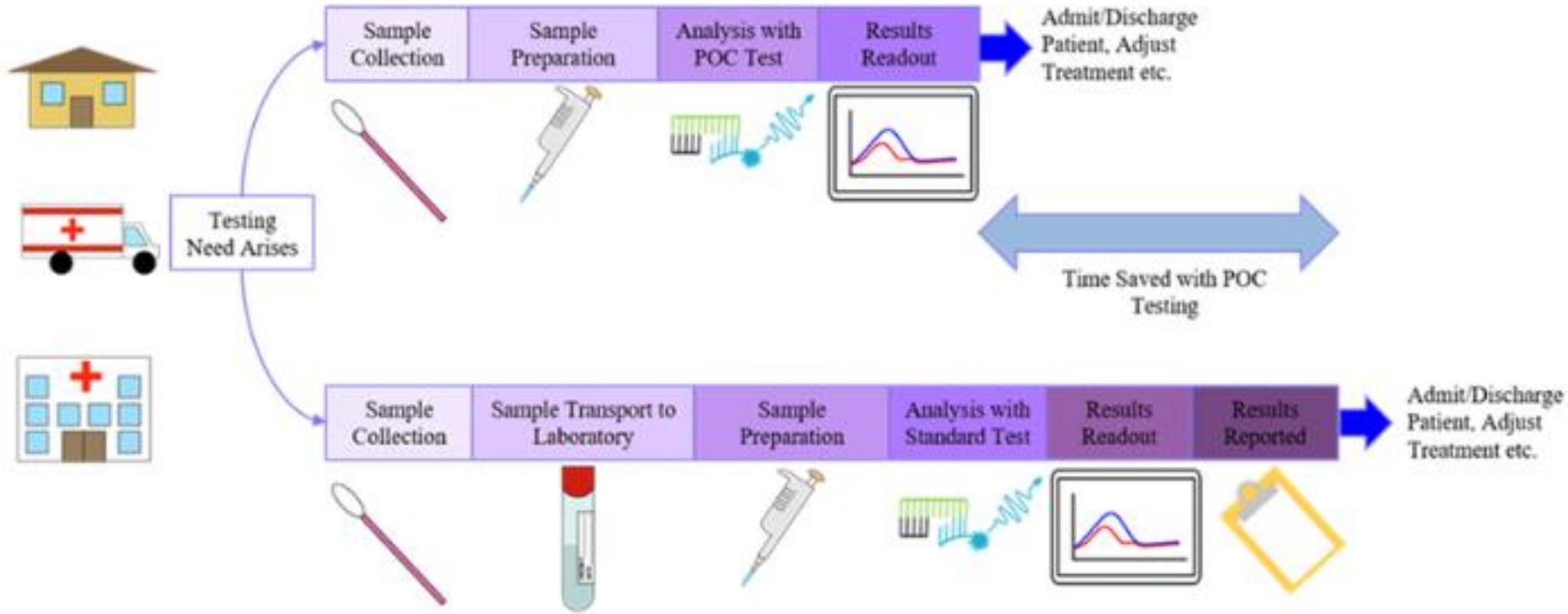
Point of care testing

Timeline of various point-of-care (POC) technologies.

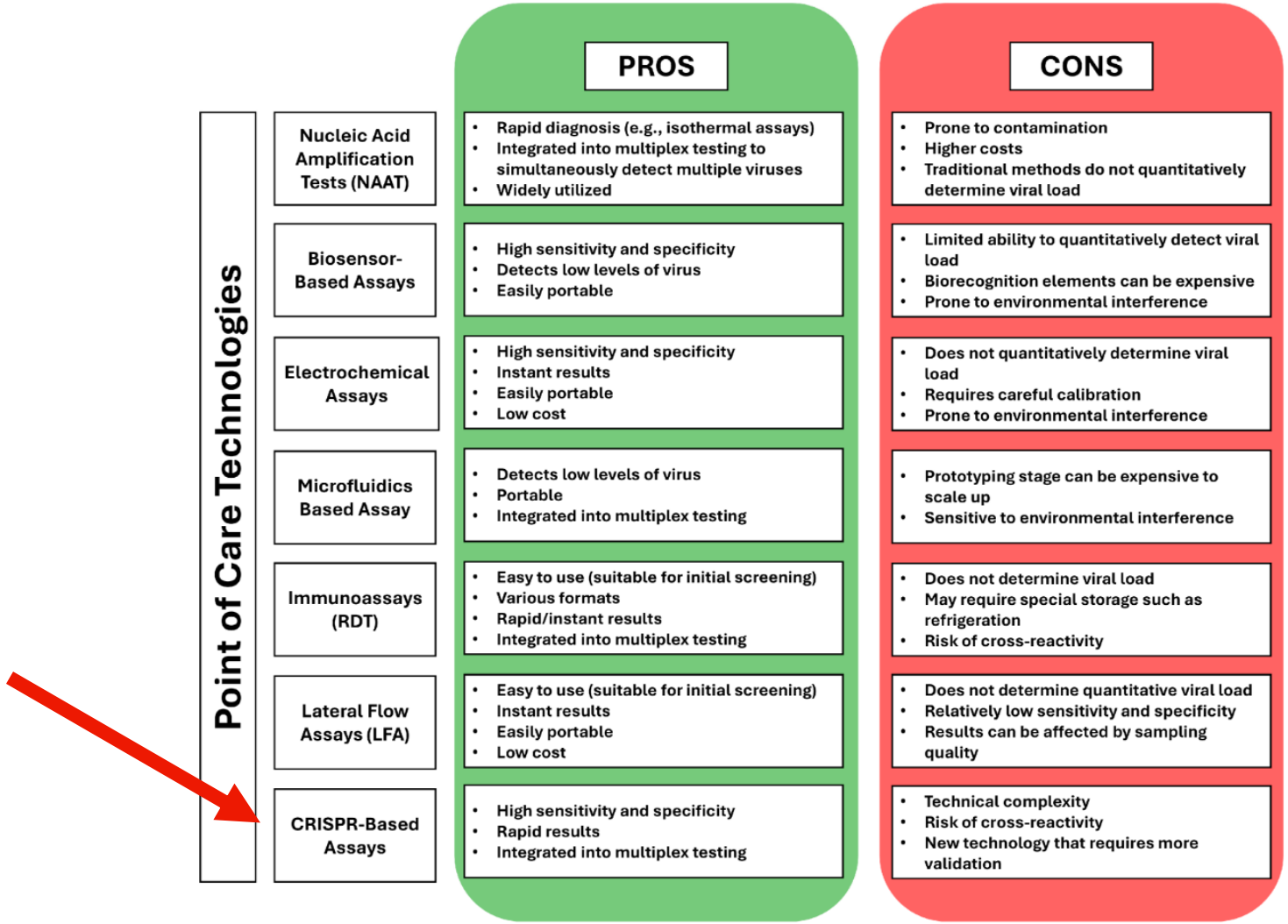


Point-of-care testing (POCT) for infectious diseases represents a set of technologies that can lead to the rapid detection of such diseases which can influence the way patients are treated.

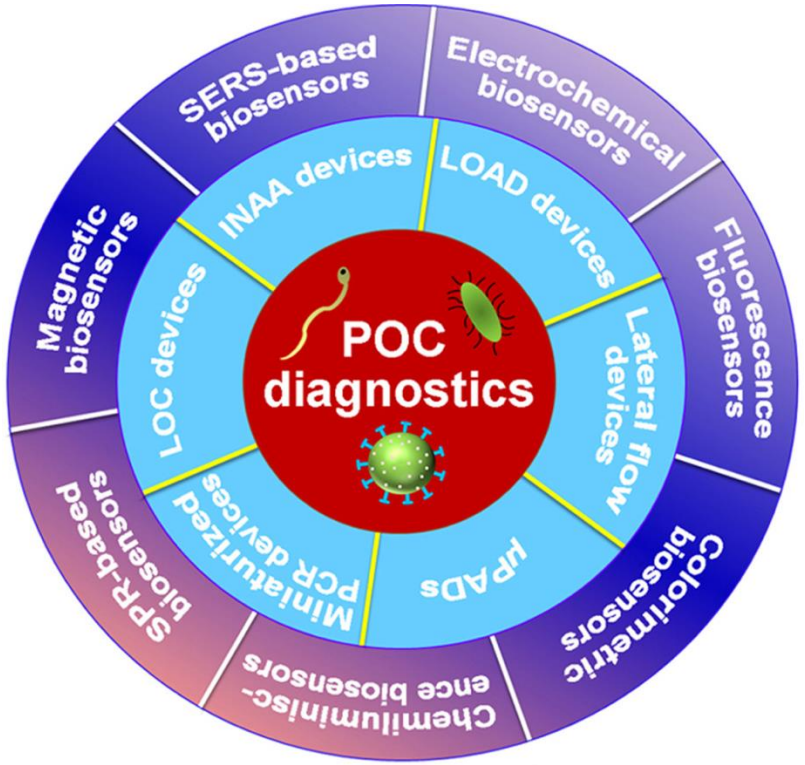
The ability to rapidly diagnose infectious diseases is critical, not only for the appropriate and timely treatment of infected patients, but also for infectious disease surveillance, the detection of outbreaks and controlling the rapid spread of infectious diseases nationally and internationally.



Comparison of the workflows for diagnostic methods in point-of-care (POC) settings (top) and standard centralized laboratory settings (bottom).


Point of Care Technologies
PROS
CONS
Nucleic Acid Amplification Tests (NAAT)
Rapid diagnosis (e.g., isothermal assays)
Integrated into multiplex testing to simultaneously detect multiple viruses
Widely utilized
Prone to contamination
Higher costs
Traditional methods do not quantitatively determine viral load
Biosensor-Based Assays
High sensitivity and specificity
Detects low levels of virus
Easily portable
Limited ability to quantitatively detect viral load
Biorecognition elements can be expensive
Prone to environmental interference
Electrochemical Assays
High sensitivity and specificity
Instant results
Easily portable
Low cost
Does not quantitatively determine viral load
Requires careful calibration
Prone to environmental interference
Microfluidics Based Assay
Detects low levels of virus
Portable
Integrated into multiplex testing
Prototyping stage can be expensive to scale up
Sensitive to environmental interference
Immunoassays (RDT)
Easy to use (suitable for initial screening)
Various formats
Rapid/instant results
Integrated into multiplex testing
Does not determine viral load
May require special storage such as refrigeration
Risk of cross-reactivity
Lateral Flow Assays (LFA)
Easy to use (suitable for initial screening)
Instant results
Easily portable
Low cost
Does not determine quantitative viral load
Relatively low sensitivity and specificity
Results can be affected by sampling quality
CRISPR-Based Assays
High sensitivity and specificity
Rapid results
Integrated into multiplex testing
Technical complexity
Risk of cross-reactivity
New technology that requires more validation

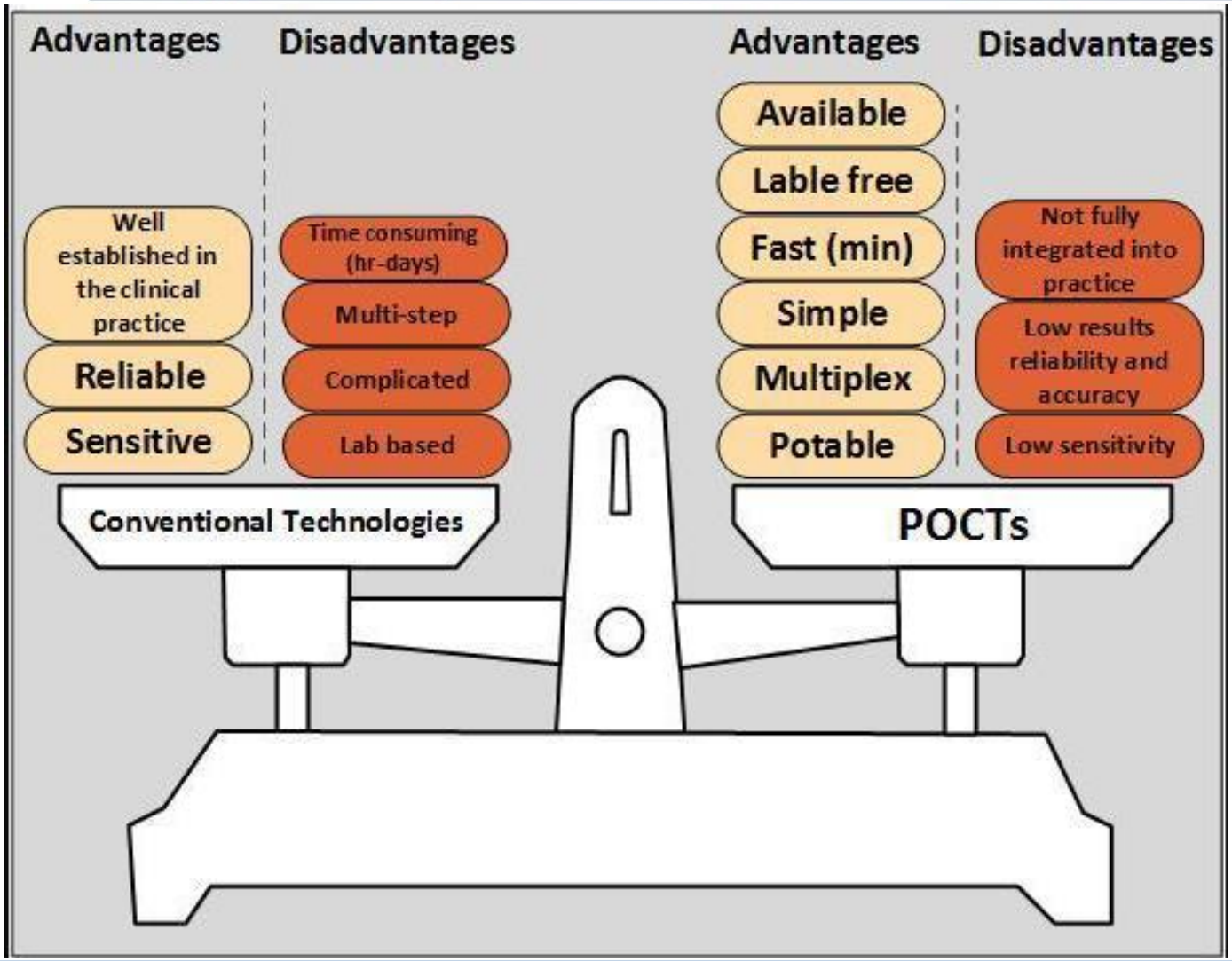
Point-of-care diagnostics for infectious diseases



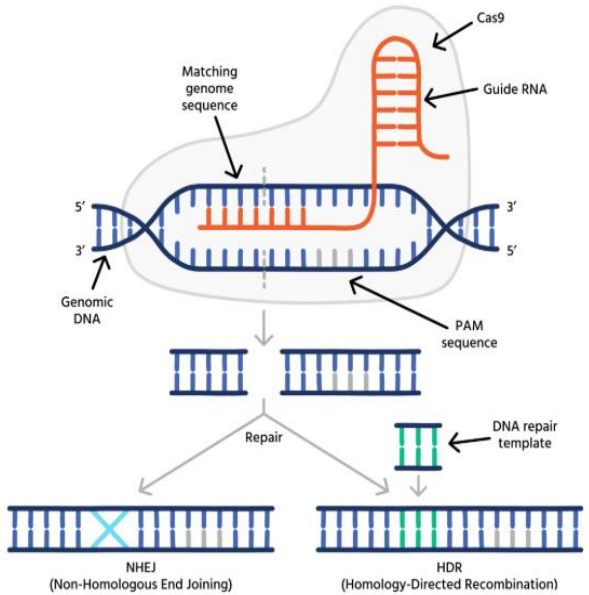
Rapid diagnosis: POC tests can quickly identify infections from samples like blood or saliva, allowing treatment to begin much faster than with traditional lab-based tests.
Improved decision-making: For example, a novel infection score for neonates incorporates a POC CRP test to provide rapid, bedside decision-making for suspected HAIs.
Targeted treatment: POC diagnostics can determine if a specific infection is present, allowing for more targeted and effective antibiotic use, which can help combat antibiotic resistance.
Infection surveillance: Wearable POC devices are being developed for continuous infection monitoring, which could provide early warnings for outbreaks or individual infections.

Challenges with POC in this context

- **Clinical validation:** Many POC platforms need more large-scale clinical validation before they are widely adopted for infectious diseases.
- **Accuracy and calibration:** Challenges can include calibration drift, batch-to-batch variability, and difficulty with complex samples.
- **Costs**.


Advantages
Disadvantages
Advantages
Disadvantages
Well established in the clinical practice
Reliable
Sensitive
Time consuming (hr-days)
Multi-step
Complicated
Lab based
Available
Lable free
Fast (min)
Simple
Multiplex
Potable
Not fully integrated into practice
Low results reliability and accuracy
Low sensitivity
Conventional Technologies
POCTs

Future assays



Schematic overview of CRISPR-Cas9 gene editing. In the absence of a repair template, cells will repair the break via error-prone non-homologous end joining (NHEJ), leading to functional gene disruption (gene/protein knock-out). Alternatively, a repair template can be used to introduce specific sequence changes via homologous-directed repair (HDR).

CRISPR (acronimo di *Clustered Regularly Interspaced Short Palindromic Repeats*) è una rivoluzionaria **tecnologia di ingegneria genetica** che permette di modificare il DNA di un organismo con estrema precisione.

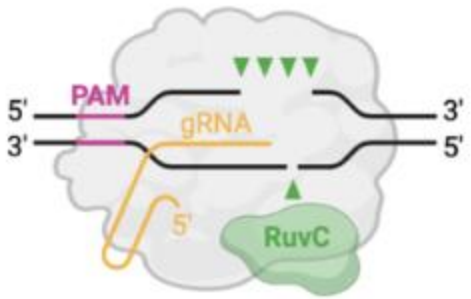
Come funziona il sistema CRISPR-Cas9?

- **RNA guida**: Una molecola di RNA che funziona come una "mappa" per guidare il sistema CRISPR-Cas9 nel punto esatto del DNA da modificare.

- **Proteina Cas9**: Una proteina che agisce come "forbici molecolari", capace di tagliare la doppia elica del DNA

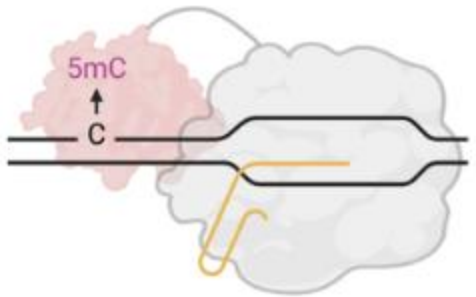
- **Modifica**: Dopo che il sistema ha localizzato e tagliato il DNA, la cellula attiva i suoi meccanismi naturali per riparare il taglio.

- **A seconda dell'obiettivo**, si può lasciare che la riparazione avvenga in modo imperfetto (per disattivare un gene) o fornire un modello di DNA corretto (per sostituire una sequenza).



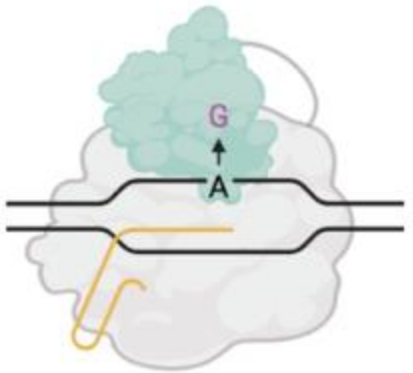
Nuclease

Ultracompact versions of CRISPR that make double stranded breaks to inactivate genes



Epigenetic Editing

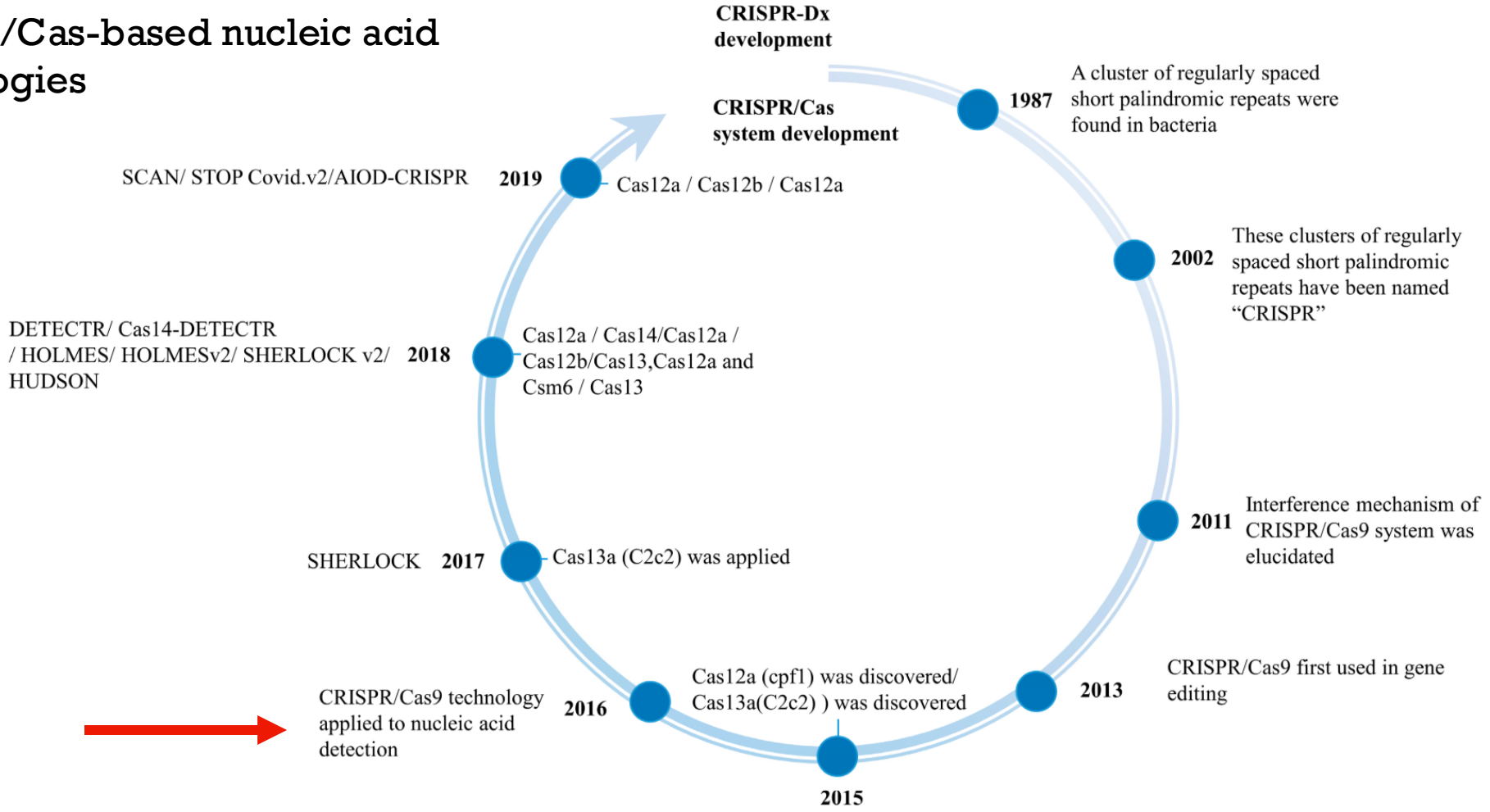
A platform technology that uses epigenetic modifications to silence genes



Base Editing

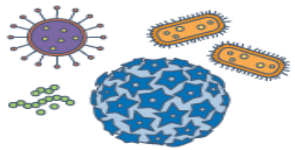
A platform technology that makes a single-letter change to convert A to G or C to T

History of CRISPR/Cas-based nucleic acid detection technologies

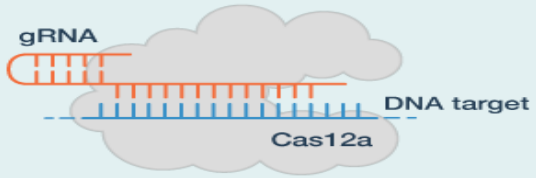


Detecting DNA

DETECTR, a DNA-detection system based on CRISPR biology, can spot specific DNA snippets in human samples, and could help identify infections, cancer, and more.

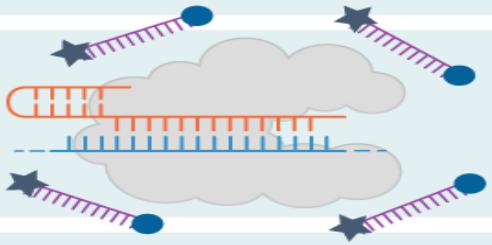


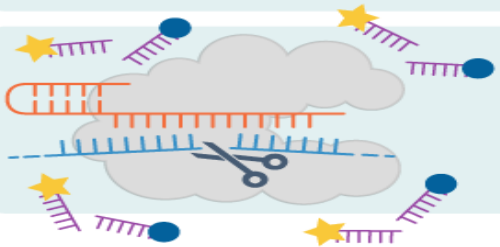
The system relies on a DNA-shredding enzyme called Cas12a and a glowing molecular signal to let researchers know if viral DNA, for example, has been spotted.



Researchers design guide RNAs (gRNA) that help Cas12a find specific DNA targets.

To detect whether the DNA target has been found, researchers use a molecule that can glow (star), but only when separated from a suppressor molecule (circle).

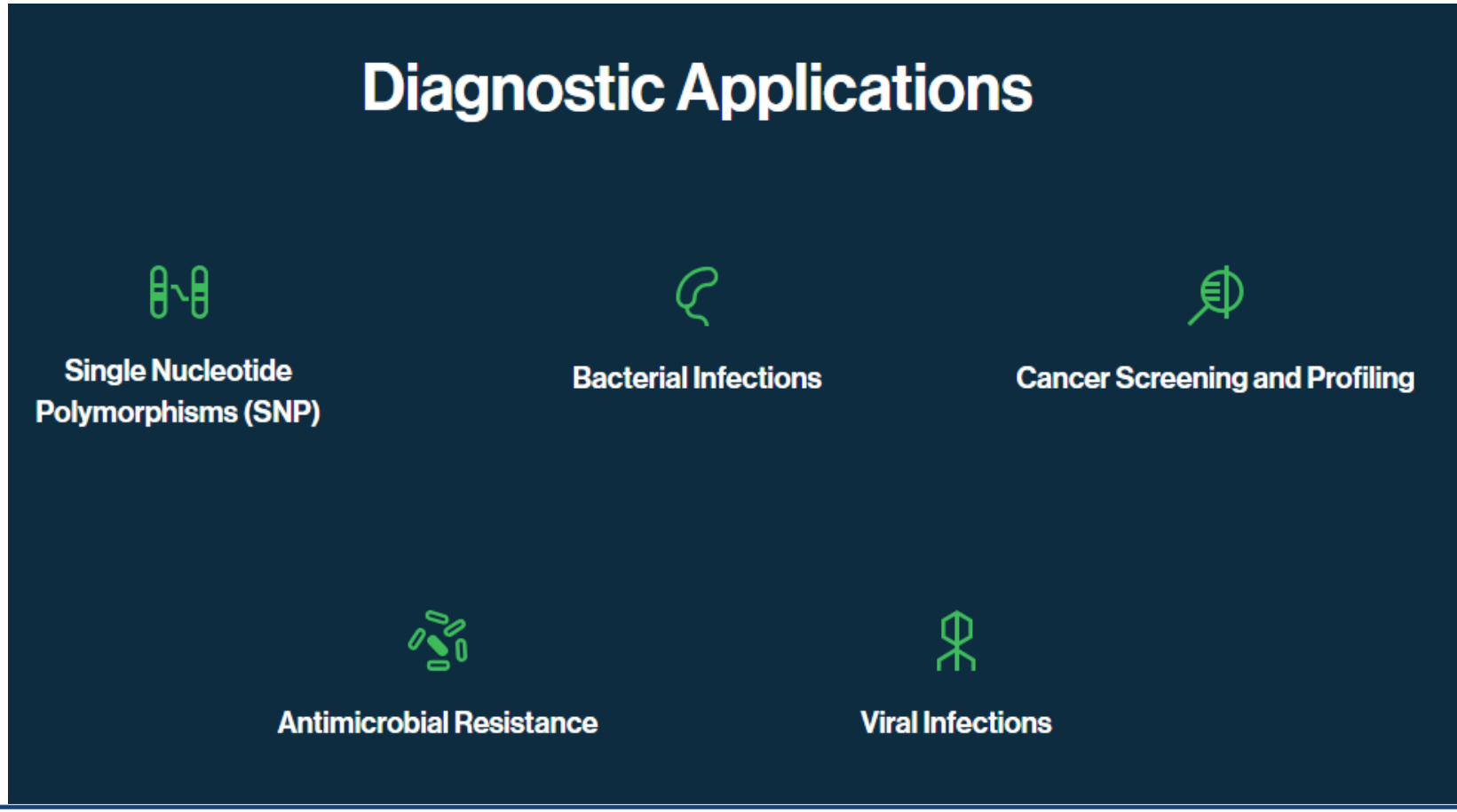




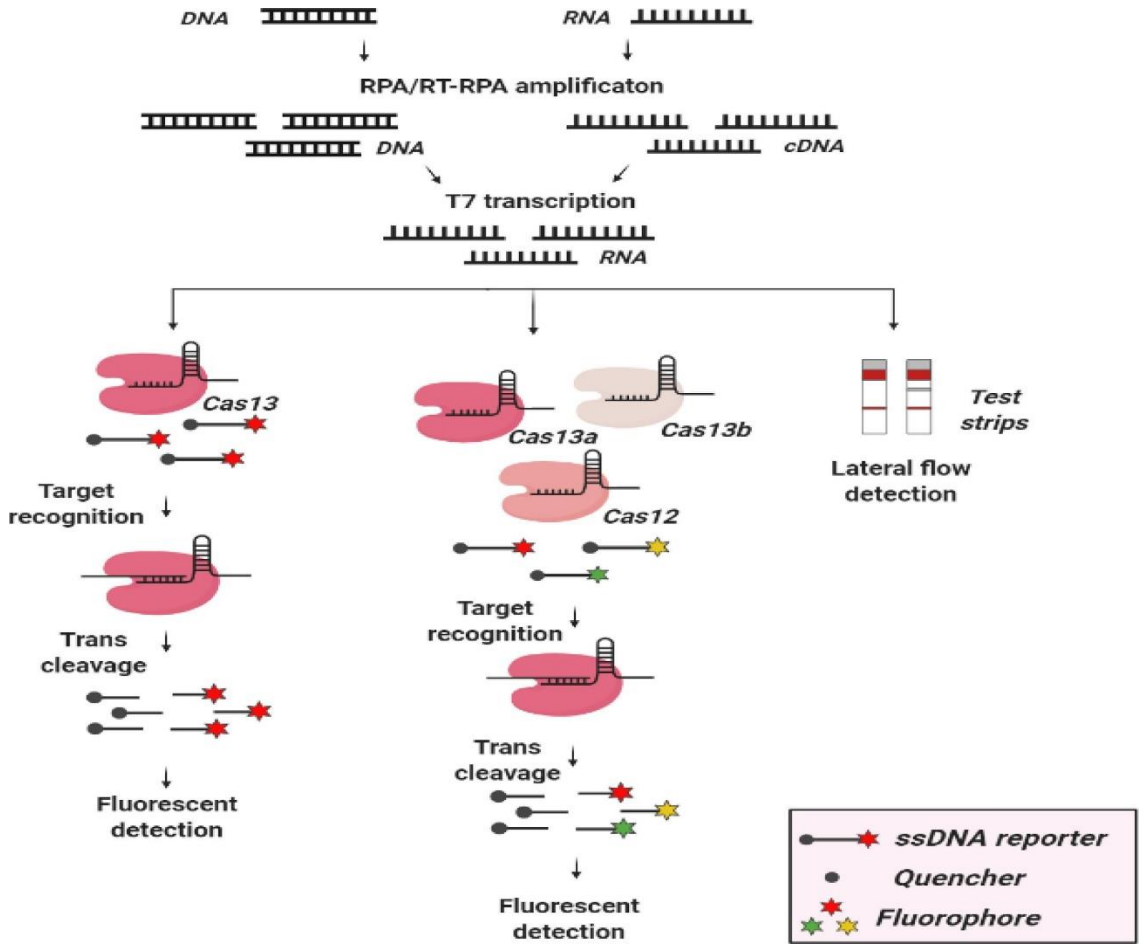
Cas12a slices its DNA target, and then cuts any single-stranded DNA nearby. This frees the suppressor from the glowing molecule, letting it shine. That signal tells researchers that Cas12a has found its DNA target.

hhmi

CRISPR-Cas systems

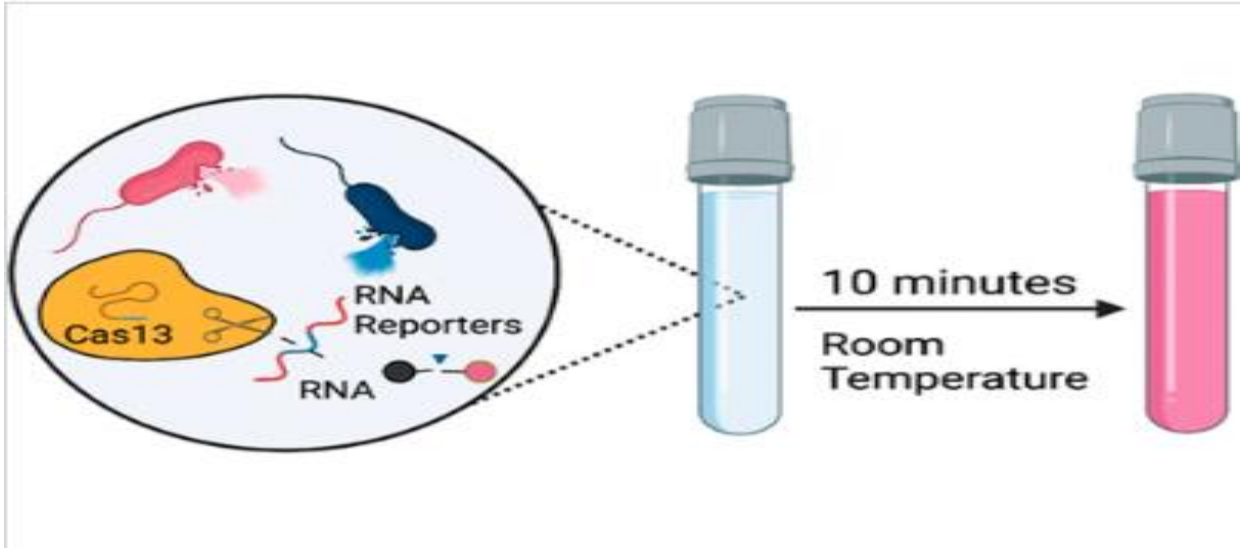


Schematics of CRISPR-Cas diagnostic platforms SHERLOCK/v2.



To conclude, using CRISPR-based methods to detect and qualitatively analyze infectious pathogens is a new reality in the field of molecular diagnostics. Developing new CRISPR tools and platforms for molecular diagnosis promises to reshape health care and improve epidemiological management on a global level.

CRISPR-based diagnostic platforms are inexpensive, simple, and do not require the use of special instrumentation, suggesting they could simplfy access to disease diagnostics.



GRAZIE PER L'ATTENZIONE