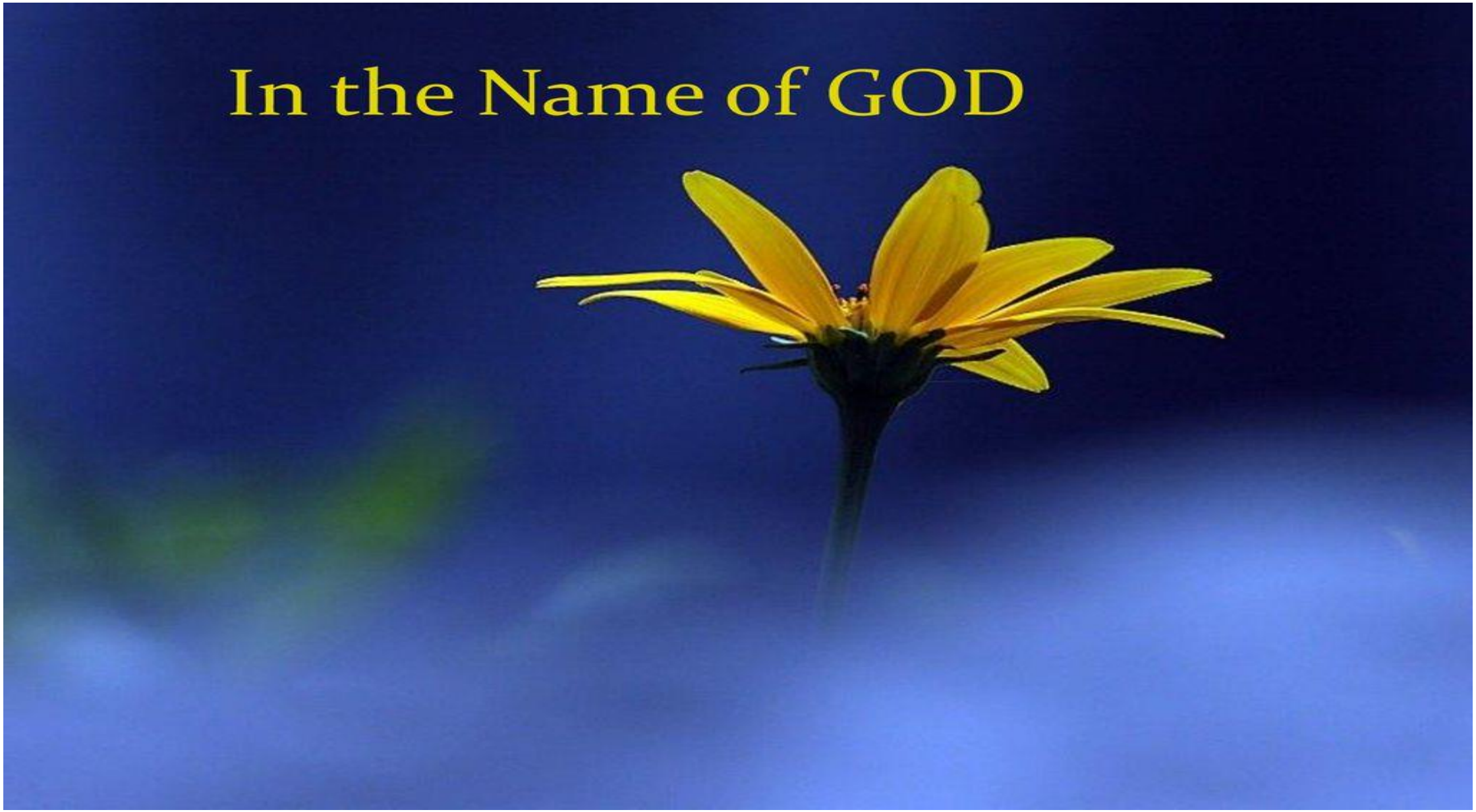
In the Name of GOD

گروه میکروب شناسی علوم پزشکی اصفهان

A General Method for Rapid Determination of Antibiotic Susceptibility and Species in Bacterial Infections

presenter

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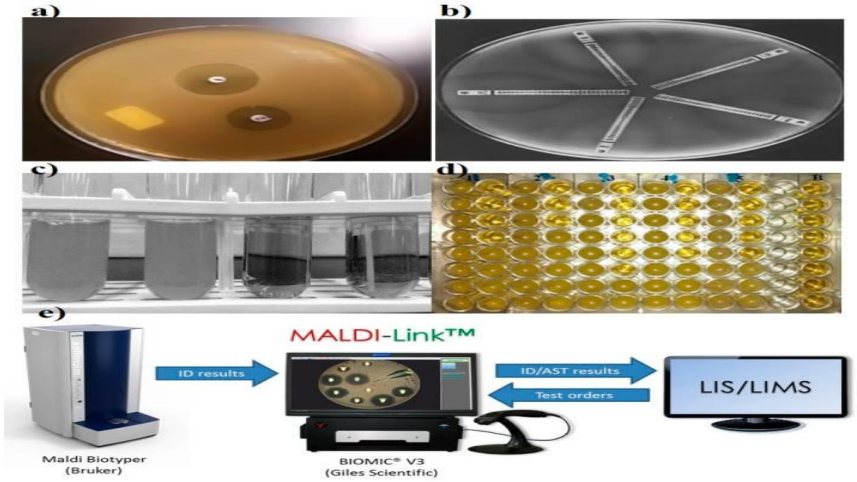
INTRODUCTION

- Antimicrobial Susceptibility Testing (AST) – A procedure used to determine which antibiotics a specific organism or group of organisms are susceptible to.

- To ensure correct antibiotic treatment and reduce the unnecessary use of antibiotics, there is an urgent need for new rapid methods for species identification and determination of antibiotic susceptibility in infectious pathogenic bacteria.



INTRODUCTION

- The new hybridization/PCR-based methods are generally faster and more sensitive than are the classical phenotypic methods.
- phenotypic methods detect the realization of susceptibility (no growth in the presence of antibiotic).

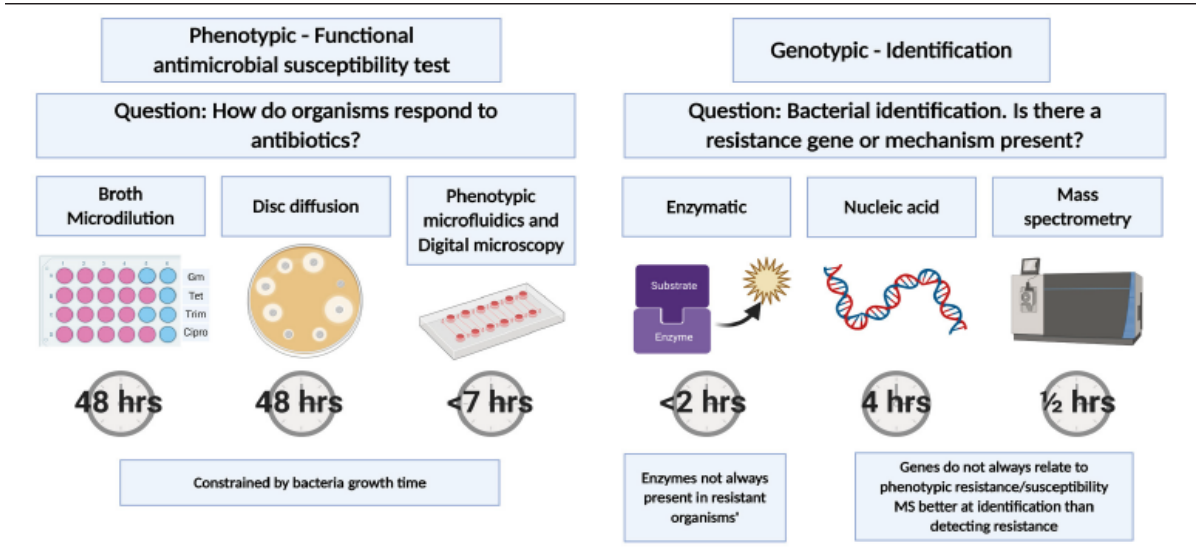


FIGURE 1 | Antimicrobial susceptibility tests. Tests can be split into phenotypic or genotypic tests with traditional AST relying on bacterial growth. Phenotypic microfluidics test times based on commercially available Accelerate PhenoTest™ BC Kit. Genotypic tests are typically faster than phenotypic tests and are more suited to bacterial identification and the identification of genes or enzymes that confer antibiotic susceptibility. Created with BioRender.com.

INTRODUCTION

- RCA is a linear amplification technique for the replication of DNA circles
- Padlock probes are oligonucleotides with target-specific ends, which upon perfect target recognition can be enzymatically joined .
- Reacted probes can be amplified by rolling circle amplification (RCA).

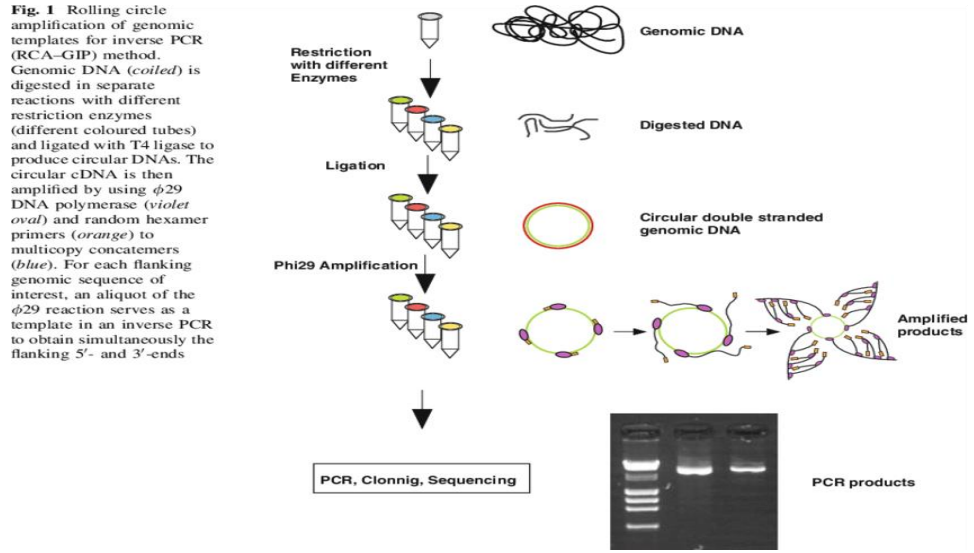


Fig. 1 Rolling circle amplification of genomic templates for inverse PCR (RCA–GIP) method. Genomic DNA (*coiled*) is digested in separate reactions with different restriction enzymes (different coloured tubes) and ligated with T4 ligase to produce circular DNAs. The circular cDNA is then amplified by using ϕ29 DNA polymerase (*violet oval*) and random hexamer primers (*orange*) to multicopy concatemers (*blue*). For each flanking genomic sequence of interest, an aliquot of the ϕ29 reaction serves as a template in an inverse PCR to obtain simultaneously the flanking 5′- and 3′-ends

Material and methods

(A) growth of a sample in antibiotic-free LB (−AB) and in LB supplemented with different antibiotics.

(B) NaOH was added

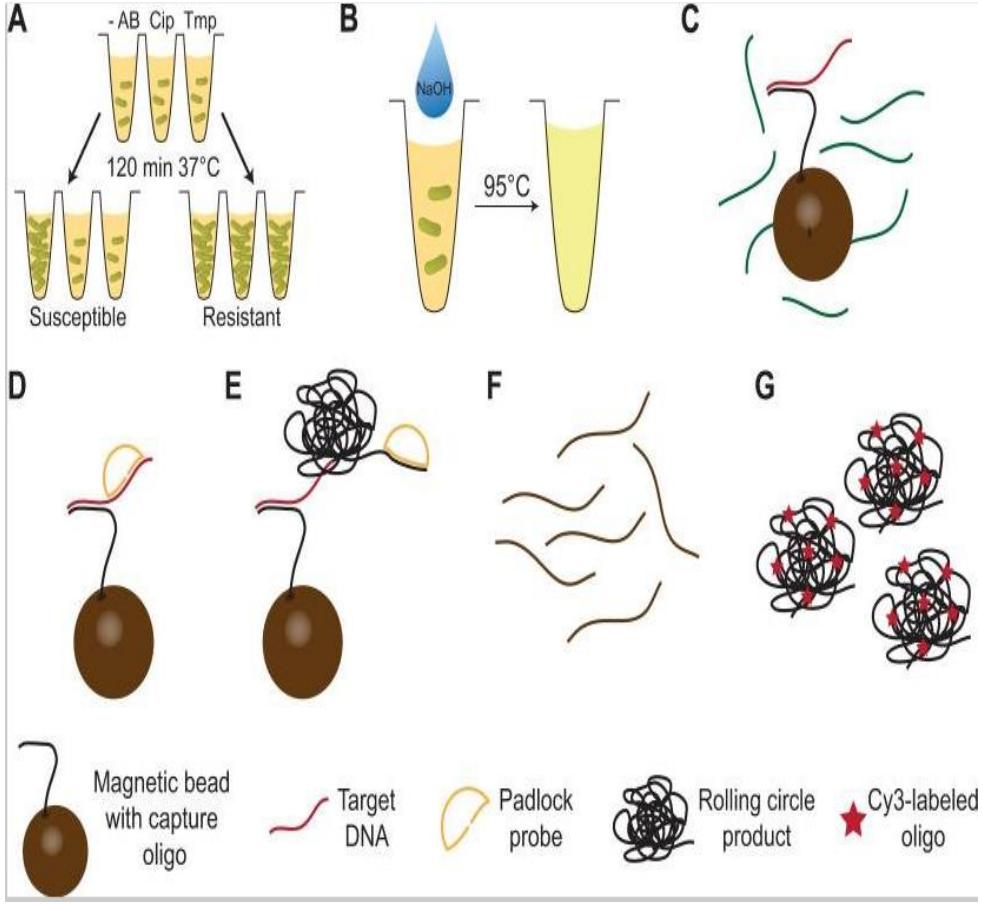
(C) Magnetic bead particles

(D) To target the bacterial DNA, padlock probes were added.

(E) Correctly hybridized padlock probes were circularized by a thermostable DNA ligase.

(F) second cycle of RCA and simultaneous fluorescence labeling by hybridizing Cy3-tagged oligonucleotides to the generated RCPs.

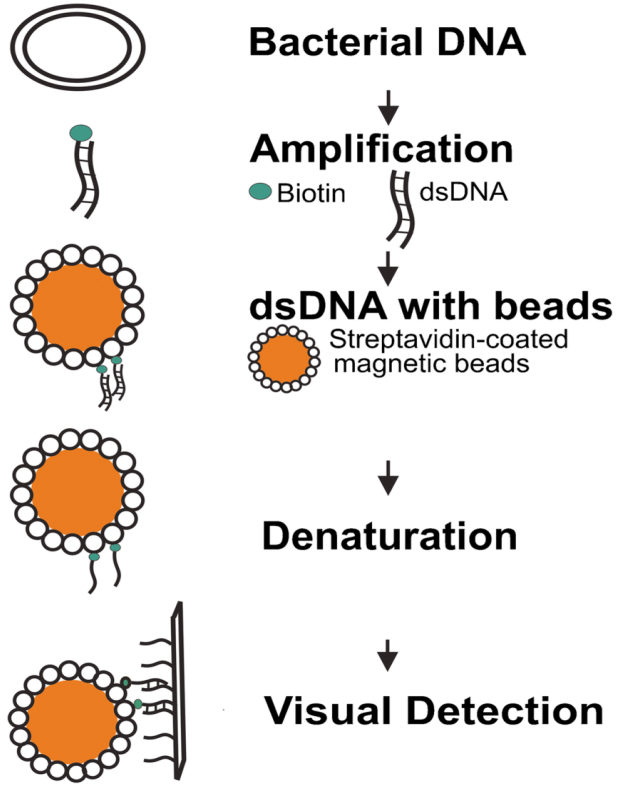
(G) The fluorescence-labeled RCPs were analyzed using a high-throughput reader that digitally enumerates



Material and methods

- This step distinguishes a susceptible strain from a resistant strain, as only bacteria with a resistant phenotype can grow in the presence of the antibiotic and thereby generate more genomes (rRNA genes).

- To enrich for the target sequences, the cell lysates were mixed with complementary biotinylated capture oligonucleotides and streptavidin-coated magnetic beads

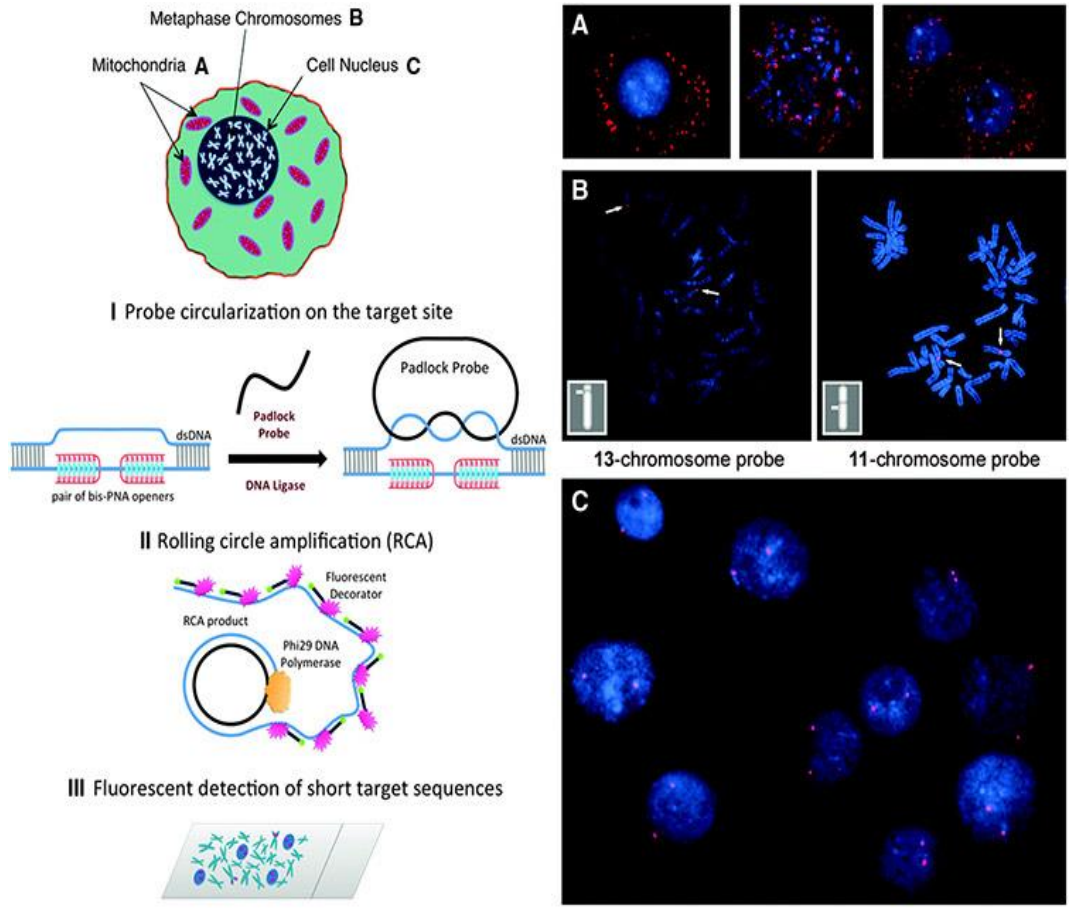


Material and methods

➢ Padlock probe

- technique called Padlock probe, invented by Mats Nilsson and Ulf Landegren.

- The padlock probe technique using a 70 to 90 nucleotides (nt) long oligonucleotide with target-complementary ends as primers.

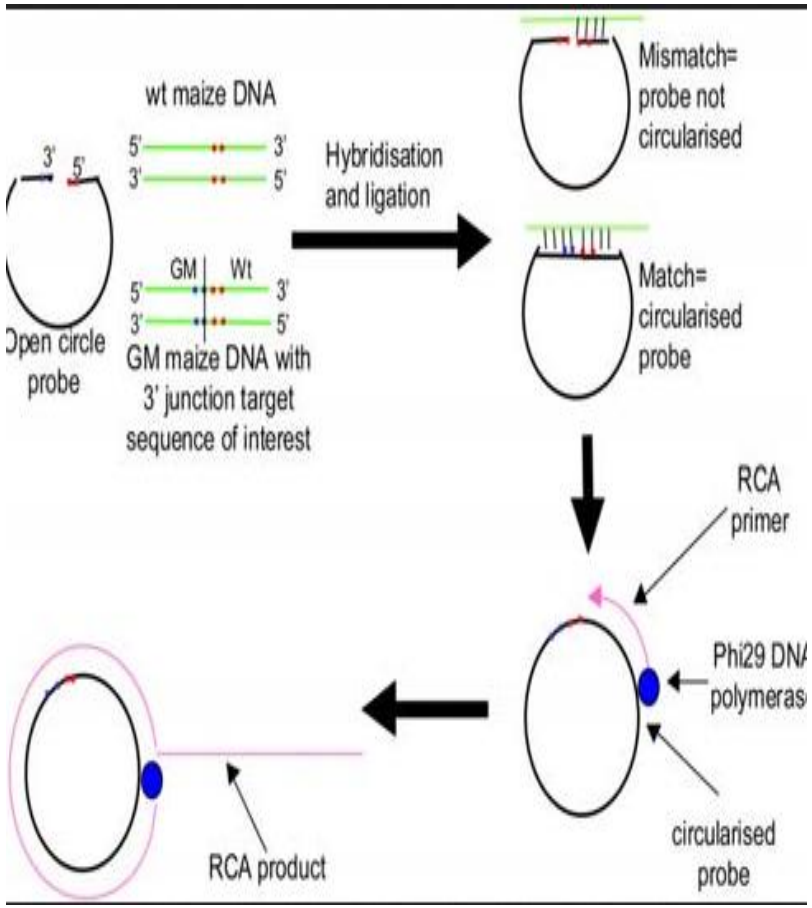


Material and methods

➢ **Padlock probe phosphorylation**

- padlock probes was phosphorylated in 1× polynucleotide kinase (PNK) buffer.
- **Phosphorylation of the probe prior to hybridization is therefore necessary in order for ligation to occur.**

- **Hybridization and ligation of padlock probe**
- **The padlock probe hybridizes in an end to tail conformation on the target molecule, with 15 to 20 nt on each end segment.**
- ligation of padlock probe by T4 DNA Ligase.



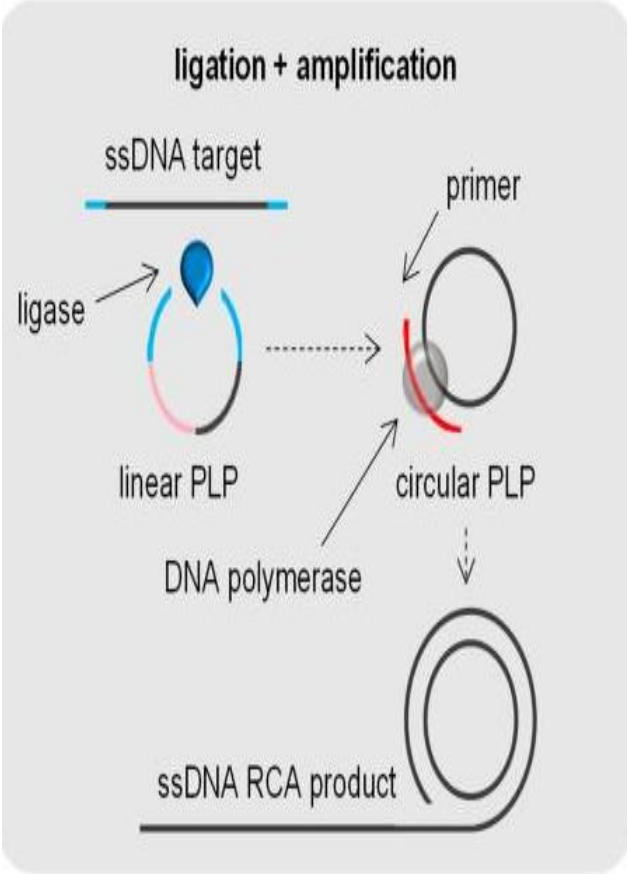
Material and methods

➢ Rolling Circle Amplification (RCA) RCA

- **RCA was reported for the first time in 1995.**

- **Rolling circle amplification (RCA) is an efficient enzymatic isothermal reaction that using circular probe as a template to generate long tandem single-stranded DNA or RNA products.**

- **This circularisation process demands a ligase for the specific circularisation of the PLP (with 3′-hydroxy and 5′-phosphate) using the target sequence of the genomic DNA as a template.**



Material and methods

- **Detection of RCA products**.

- visualization using fluorescence based techniques including fluorescence spectroscopy, microscopy, and flowcytometry Similar to fluorescent probe hybridization, nanoparticles including gold nanoparticles (AuNPs), magnetic.

- Magnetic beads functionalized with complementary DNA sequences can be hybridized to the RCA product to produce a diffractometric signal for the detection of the RCA product .

advantages

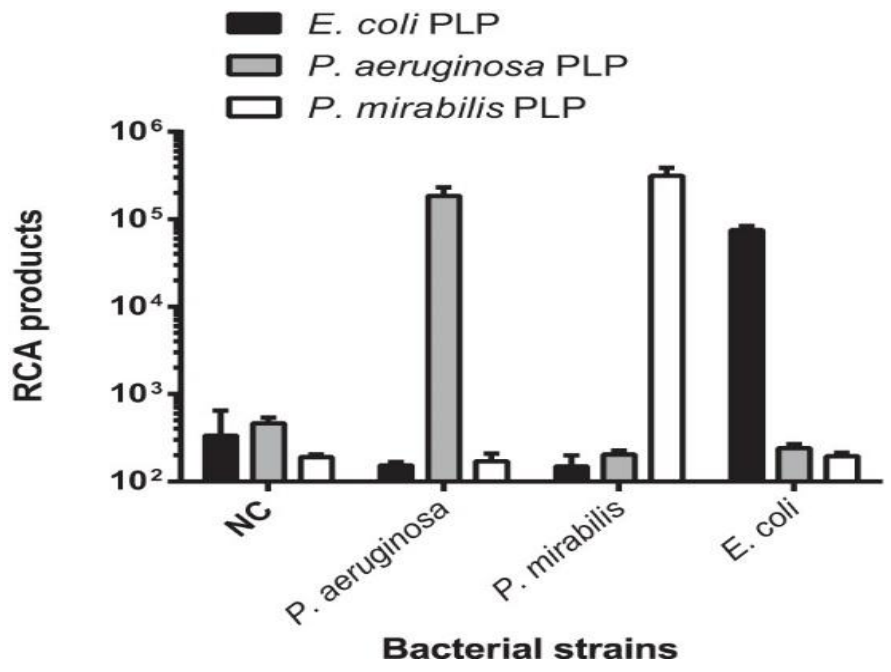
RCA has many advantages

1) because of the strict complementarity required in ligation of a padlock probe, it endows the RCA reaction with high specificity and can even be utilized to distinguish single base mismatches.

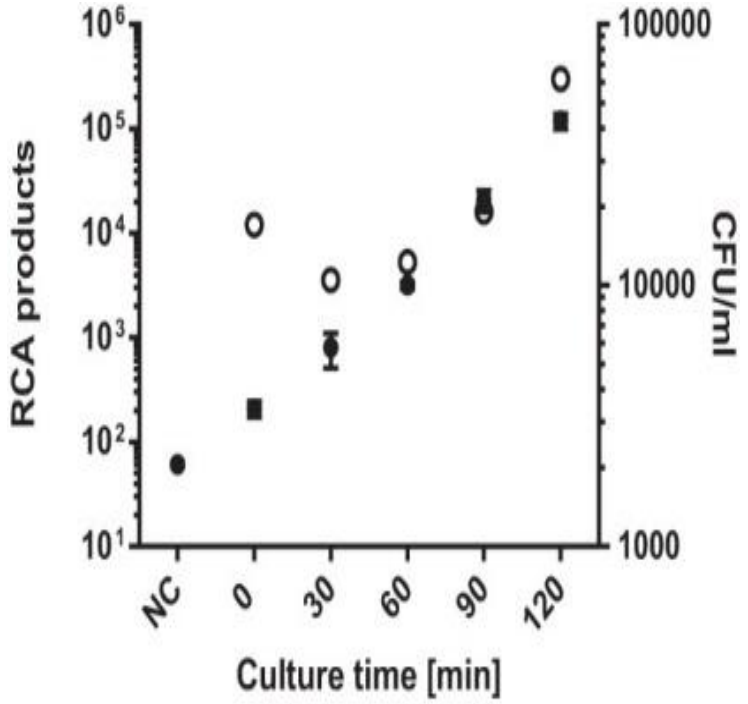
2) through the introduction of multiple primers, exponential amplification can be achieved easily and leads to a good sensitivity.

3) RCA products can be customized by manipulating circular templates to generate functional nucleic acids such as aptamer, DNAzymes and restriction enzyme sites.

RESULTS

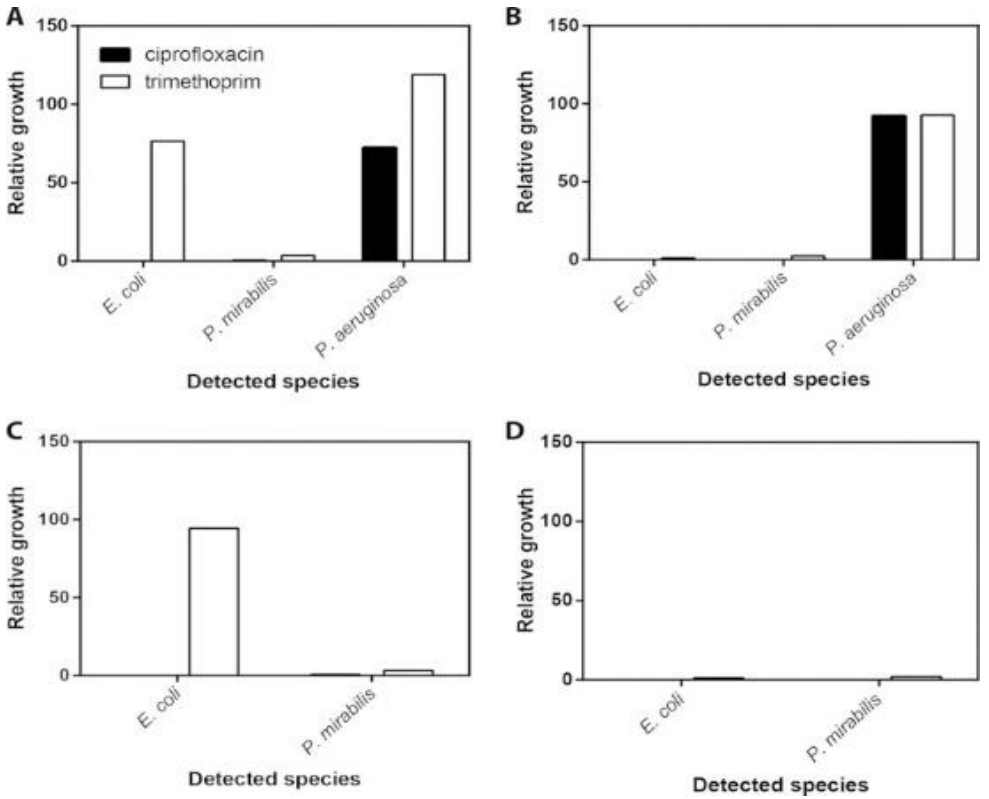


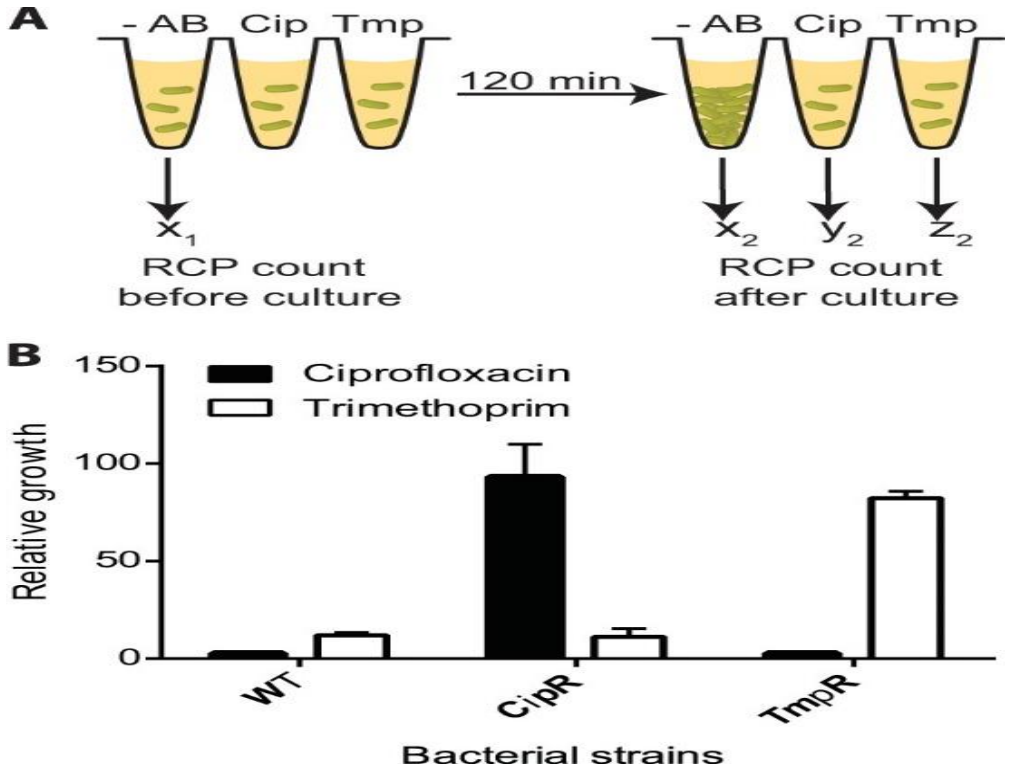
Specificity



Detection of growth inhibition

Antibiotic susceptibility profile





DISCUSSION

diagnosing and screening bacteria for ASP based on the combined information from culture in the absence and presence of antibiotics and highly sensitive species-specific detection of 16S rRNA gene sequences.

padlock probes identify only bacterial species and not the resistance genes, the antibiotic susceptibility profiling is independent of genotype and resistance mechanism.

Another major advantage of our assay is the ability to use patient samples directly without the need for any purification.

REFERENCES

1) Mezger A, Gullberg E, Göransson J, Zorzet A, Herthnek D, Tano E, Nilsson M, Andersson DI. A general method for rapid determination of antibiotic susceptibility and species in bacterial infections. Journal of clinical microbiology. 2015 Feb;53(2):425-32.

2) Leonardo S, Toldrà A, Campàs M. Biosensors based on isothermal DNA amplification for bacterial detection in food safety and environmental monitoring. Sensors. 2021 Jan;21(2):602.

3) Kühnemund M, Witters D, Nilsson M, Lammertyn J. Circle-to-circle amplification on a digital microfluidic chip for amplified single molecule detection. Lab on a Chip. 2014;14(16):2983-92.

4) Jarvius J, Melin J. Go ransson J, Stenberg J, Fredriksson S, Gonzalez-Rey C, Bertilsson S, Nilsson M. 2006. Digital quantification using amplified single-molecule detection. Nature Methods.;3:725-7.

5) Mach KE, Mohan R, Baron EJ, Shih MC, Gau V, Wong PK, Liao JC. A biosensor platform for rapid antimicrobial susceptibility testing directly from clinical samples. The Journal of urology. 2011 Jan;185(1):148-53.

6) Nilsson M, Malmgren H, Samiotaki M, Kwiatkowski M, Chowdhary BP, Landegren U. Padlock probes: circularizing oligonucleotides for localized DNA detection. Science. 1994 Sep 30;265(5181):2085-8

7) Mohsen MG, Kool ET. The discovery of rolling circle amplification and rolling circle transcription. Accounts of chemical research. 2016 Nov 15;49(11):2540-50.

8) Toldrà A, O'Sullivan CK, Campàs M. Detecting harmful algal blooms with isothermal molecular strategies. Trends in biotechnology. 2019 Dec 1;37(12):1278-81

9) Xiang Y, Zhu X, Huang Q, Zheng J, Fu W. Real-time monitoring of mycobacterium genomic DNA with target-primed rolling circle amplification by a Au nanoparticle-embedded SPR biosensor. Biosensors and Bioelectronics. 2015 Apr 15;66:512-9.

10) Wang T, Zhang Z, Li Y, Xie G. Amplified electrochemical detection of mecA gene in methicillin-resistant Staphylococcus aureus based on target recycling amplification and isothermal strand-displacement polymerization reaction. Sensors and Actuators B: Chemical. 2015 Dec 31;221:148-54

11) Gu L, Yan W, Liu L, Wang S, Zhang X, Lyu M. Research progress on rolling circle amplification (RCA)-based biomedical sensing. Pharmaceuticals. 2018 Jun;11(2):35.

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