Antimicrobial Susceptibility Tests

Test Methods for Antimicrobial Susceptibility

- Disc Diffusion Method (Kirby-Bauer Antibiogram Test)
  - Mostly used method in lab!!!
- Broth Dilution Method (Minimal Inhibition Concentration Test, The MIC Test)
  - Most precise method!!!
- E-test
- Genetic testing with molecular methods
- Automated Testing (Maldi-Tof)

Antimicrobial Susceptibility Testing

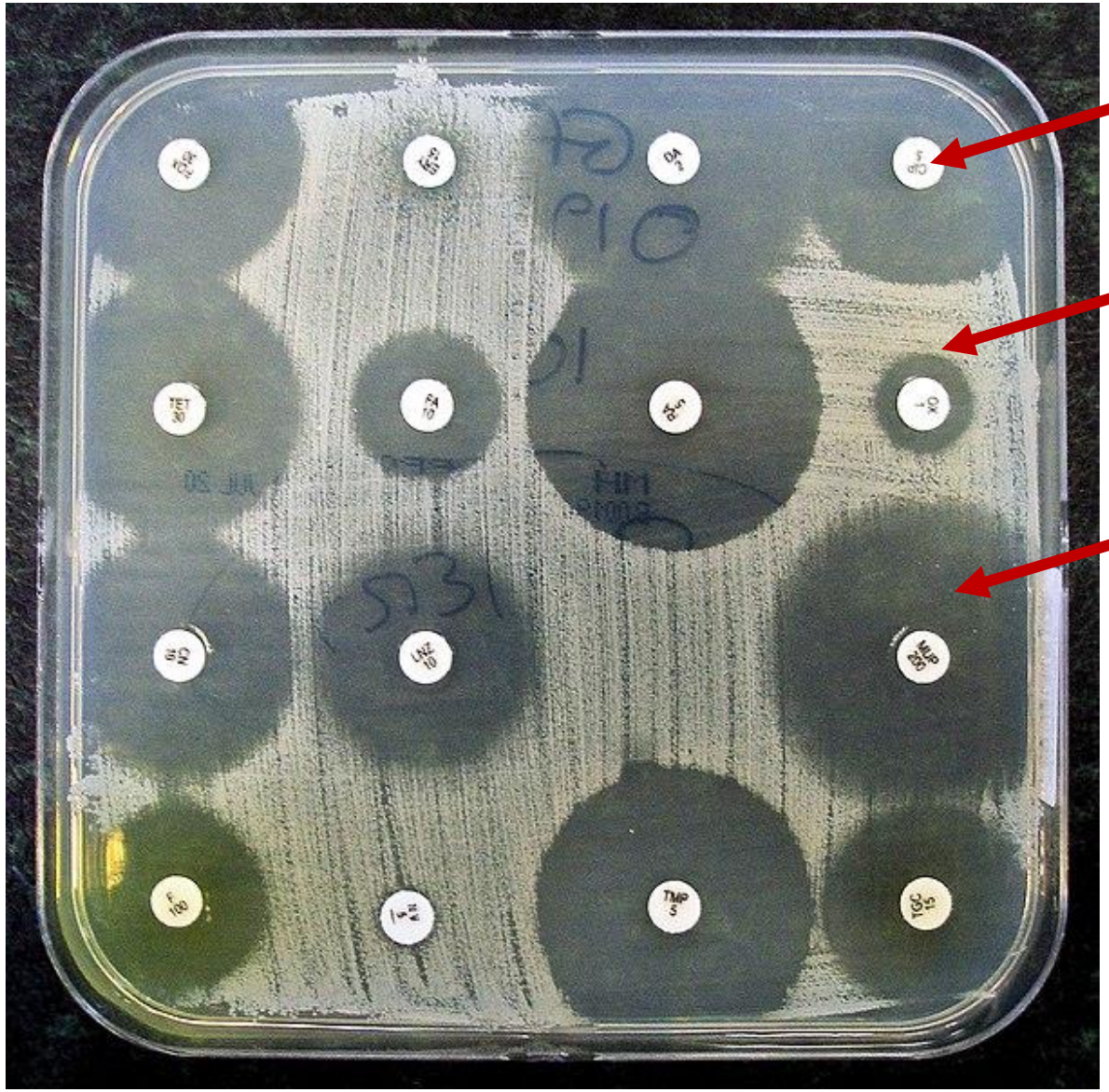
- **Antibiotic sensitivity testing** or **antibiotic susceptibility testing** is the measurement of the susceptibility of bacteria to antibiotics.
- It is used because bacteria may have resistance to some antibiotics.
- Sensitivity testing results can allow a clinician to change the choice of antibiotics from **empiric therapy**, which is when an antibiotic is selected based on clinical suspicion about the site of an infection and common causative bacteria, to **directed therapy**, in which the choice of antibiotic is based on knowledge of the organism and its sensitivities

Antimicrobial Susceptibility Testing

- Sensitivity testing usually occurs in a microbiology laboratory, and uses **culture methods** that expose bacteria to antibiotics, or **genetic methods** that test to see if bacteria have genes that confer resistance.
- Culture methods often involve measuring the diameter of areas without bacterial growth, called **zones of inhibition**, around **paper discs containing antibiotics** on agar culture dishes that have been evenly inoculated with bacteria.
- The minimum inhibitory concentration (MIC), which is **the lowest concentration of the antibiotic that stops the growth of bacteria**, can be estimated from the size of the zone of inhibition.

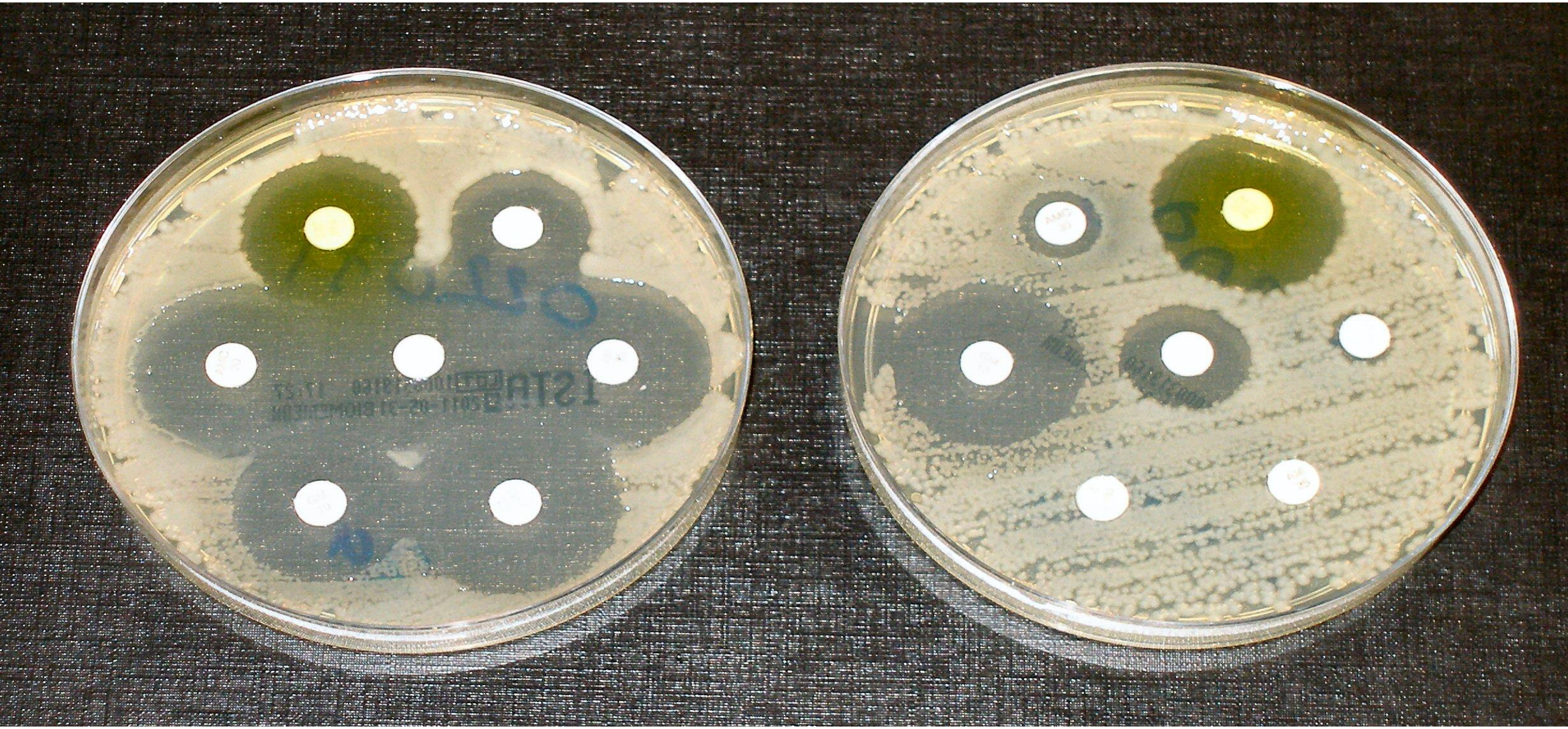
Antimicrobial Susceptibility Testing

- Antibiotic susceptibility testing has been needed since the discovery of the beta-lactam antibiotic penicillin.
- Initial methods were **phenotypic**, and involved **culture or dilution**.
- The **E-test**, an antibiotic impregnated strip, has been available since the 1980s, and genetic methods such as **polymerase chain reaction (PCR)** testing have been available since the early 2000s.
- Research is ongoing into improving current methods by making them faster or more accurate, as well as developing new methods for testing, such as **microfluidics**.

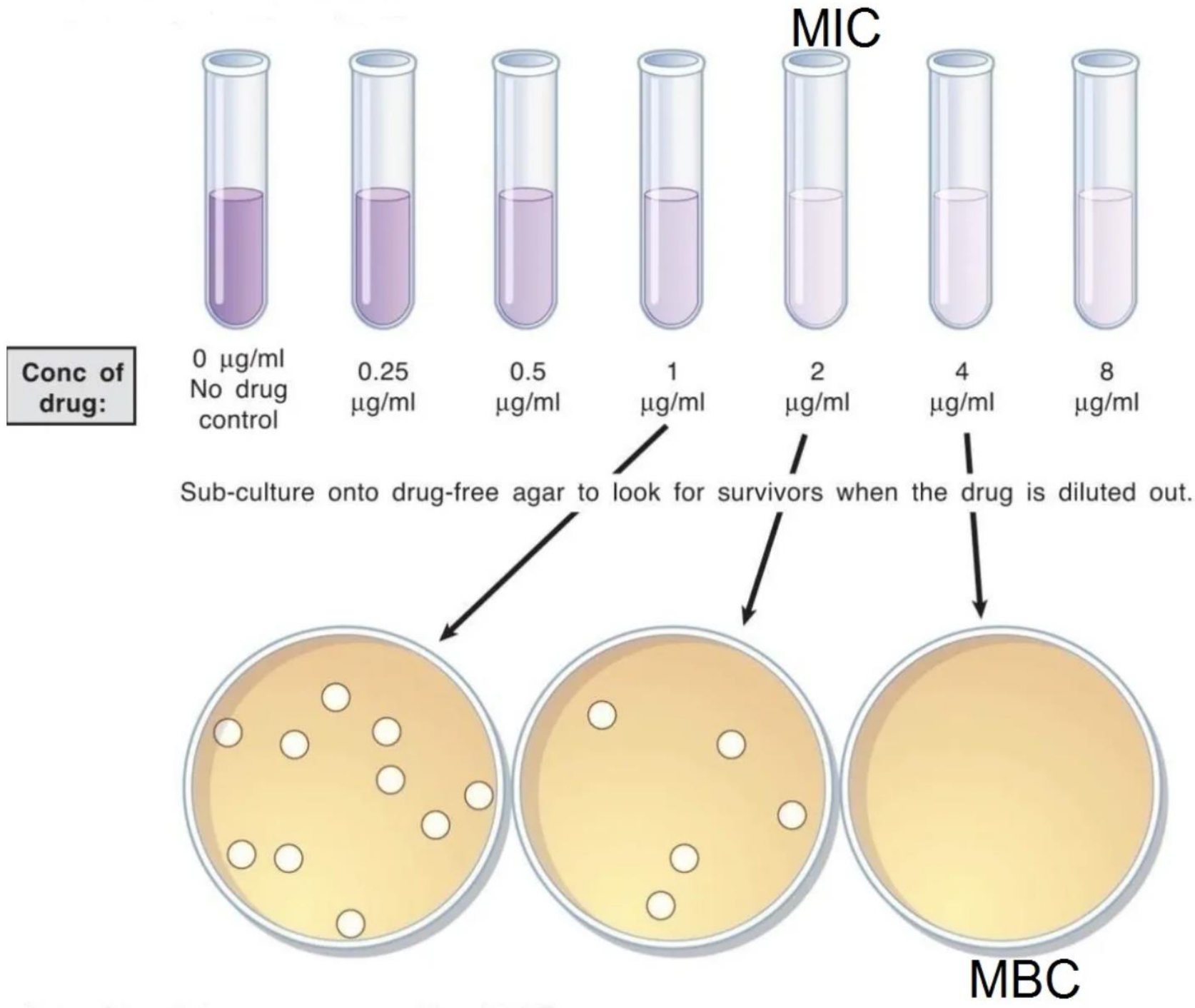


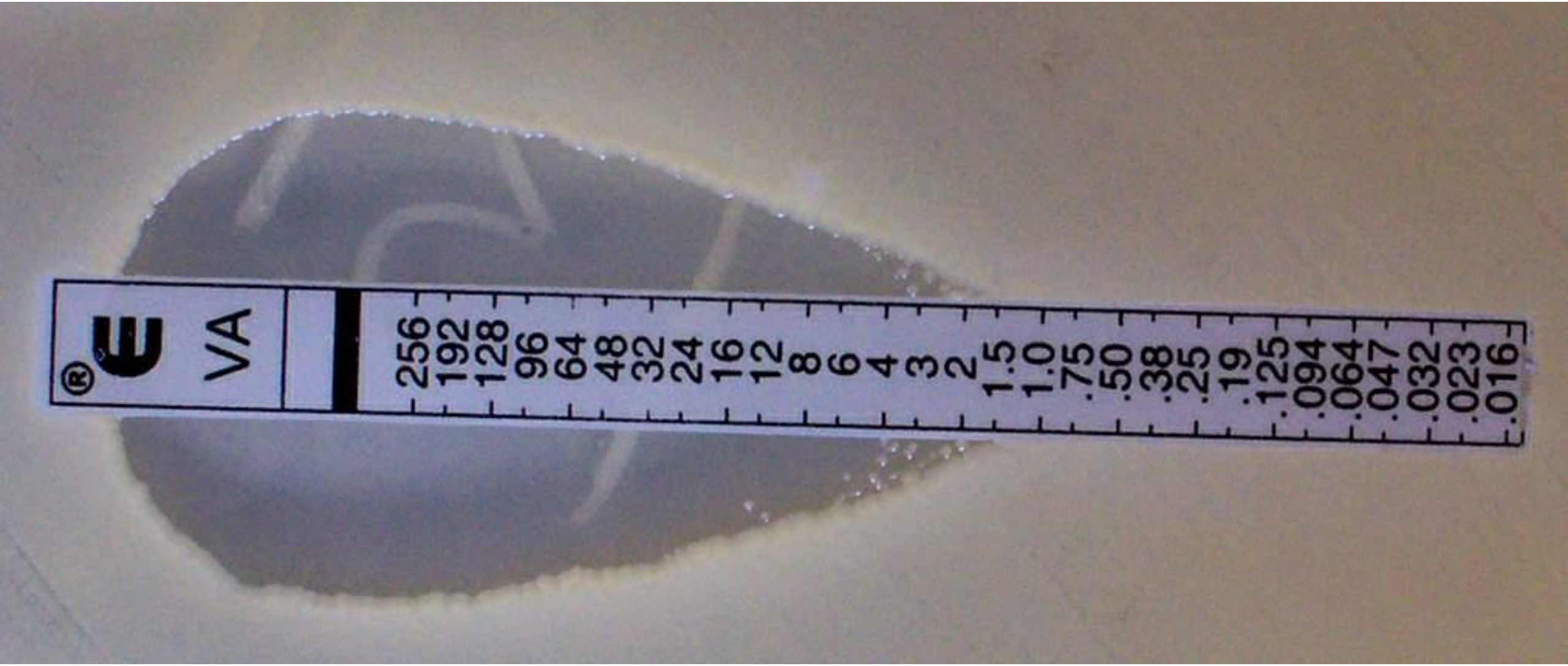
Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Staphylococcus aureus growing on a nutrient agar. The discs contain an antibiotic. The bacterium is completely resistant to three of the antibiotics. The zones around the discs are where the bacteria have not grown because they are sensitive to the antibiotic in each of the discs of filter paper.



Antibiotic resistance tests: Bacteria are streaked on dishes with white disks, each impregnated with a different antibiotic. Clear rings, such as those on the left, show that bacteria have not grown—indicating that these bacteria are not resistant. The bacteria on the right are fully resistant to all but two of the seven antibiotics tested.


MIC
Conc of drug:
0 µg/ml No drug control
0.25 µg/ml
0.5 µg/ml
1 µg/ml
2 µg/ml
4 µg/ml
8 µg/ml
Sub-culture onto drug-free agar to look for survivors when the drug is diluted out.
MBC



Etest. After the required incubation period, when an even lawn of growth is distinctly visible, the MIC value is read where the pointed end of the inhibition ellipse intersects the side of the strip.

Investigation of aminoglycoside modifying enzyme genes in methicillin-resistant staphylococci

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KEYWORDS
MRSA;
MRCNS;
Aminoglycoside resistance;
mecA;
Aminoglycoside modifying enzyme

Summary

Methicillin-resistant staphylococci may also be resistant to some other antibiotics as well as β-lactams. In this study, co-existence of resistance to methicillin and aminoglycosides was genetically investigated in staphylococci. A total of 50 staphylococci from in-patients, 17 *Staphylococcus aureus* and 33 coagulase negative staphylococci (CNS) that contained *mecA* (gene encoding PBP 2a, an altered penicillin-binding protein) determined by polymerase chain reaction (PCR) were included in the study. Aminoglycoside modifying enzyme (AME) genes were investigated using multiplex-PCR. Aminocyclitol-6′-acetyltransferase-aminocyclitol-2″-phosphotransferase [*aac(6′)/aph(2″)*] gene (encoding bifunctional acetyltransferases/phosphotransferases) was determined in 66% of the isolates, aminocyclitol-4′-adenylytransferase (*ant(4′)-Ia*) gene (encoding phosphotransferases) in 24%, and aminocyclitol-3′-phosphotransferase (*aph(3′)-IIIa*) gene (encoding nucleotidyltransferases) in 8%. Two isolates contained all these three genes. Thirty-six (72%) isolates had at least one of these genes. Three CNS and one *S. aureus* isolates sensitive to oxacillin had the *mecA* gene. In conclusion, a high rate of aminoglycoside resistance was determined in methicillin-resistant staphylococci. The *aac(6′)/aph(2″)* was the most frequently detected.

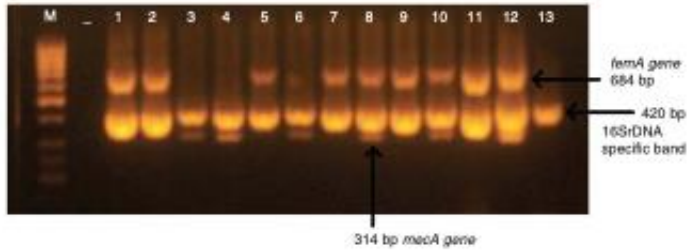

Figure 1. Demonstration of the *mecA*, *femA* and 16SrDNA genes in agarose gel M, Molecular size marker; -, *mecA* negative standard strain, 1–13 test isolates.

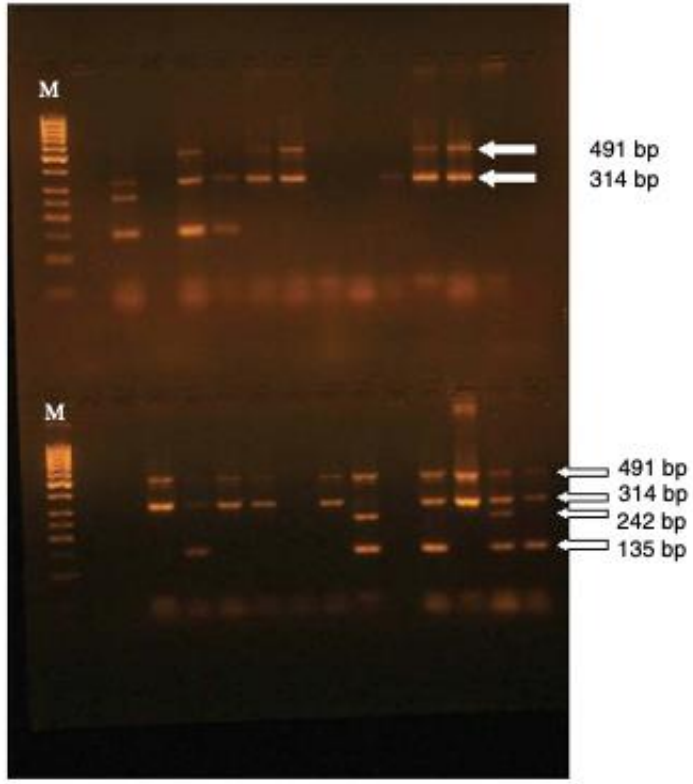

Figure 2. Demonstration of AME genes determined with multiplex-PCR in agarose gel. M, Molecular size marker, 491 bp band, *aac(6′)/aph(2″)* gene; 314 bp band, *mecA* gene; 242 bp band, *aph(3′)-IIIa* gene; 135 bp band, *ant(4′)-Ia* gene.

Table 1. Oligonucleotide sequences belonging to the primers used in the determination of methicillin resistance

Target genes		Oligonucleotide sequences[a]	Size of target region (bp)
mecA	Forward	CCT AGT AAA GCT CCG GAA	314
	Reverse	CTA GTC CAT TCG GTC CA	
16S rDNA	Forward	CAG CTC GTG TCG TGA GAT GT	420
	Reverse	AAT CAT TTG TCC CAC CTT CG	
femA	Forward	CTTACTTACTGCTGTACCTG	684
	Reverse	ATCTCGCTTGTTATGTGC	

[a]Oligonucleotide sequences were obtained from the IONTEK company.

Table 2. Oligonucleotide sequences belonging to the primers used in the determination of AME resistance

Target genes		Oligonucleotide sequences[a]	Size of target region (bp)
aac(6′)/aph(2″)	Forward	GAA GTA CGC AGA AGA GA	491
	Reverse	ACA TGG CAA GCT CTA GGA	
aph(3′)-IIIa	Forward	AAA TAC CGC TGC GTA	242
	Reverse	CAT ACT CTT CCG AGC AA	
ant(4′)-Ia	Forward	AAT CGG TAG AAG CCC AA	135
	Reverse	GCA CCT GCC ATT GCT A	

[a]Oligonucleotide sequences were obtained from the IONTEK company.

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Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci

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Abstract

In this study, erythromycin [*erm*(A) and *erm*(C)] and tetracycline [*tet*(K) and *tet*(M)] resistance genes were investigated by multiplex polymerase chain reaction (PCR) in a total of 56 methicillin-resistant (*mecA*+) staphylococcal hospital isolates, 28 of which were determined to be *Staphylococcus aureus* (MRSA) and the other 28 were coagulase-negative staphylococci (MRCNS). Internal control primers amplifying a specific fragment of 16S rDNA of staphylococci were included in the multiplex PCR protocol to ensure the efficacy of amplification and to determine any PCR inhibition. No resistance genes were detected in 5 of 56 (8.9%) isolates in the study. In the study, *tet*(K) genes were detected widely (42.9%) in MRCNS, whilst *tet*(M) genes were detected in MRSA (50.0%). Regarding the erythromycin resistance genes, whilst *erm*(A) genes were detected in most (71.4%) MRSA isolates, detection rates of *erm*(C) genes were the same (64.3%) both in MRCNS and MRSA. The resistance rates for tetracycline and erythromycin were 57.1% and 78.6%, respectively, in MRSA isolates. In conclusion, in this study, the multiplex PCR technique including an internal control is shown to be a fast, sensitive, reliable, practical, reproducible and economic technique for the detection of erythromycin and tetracycline resistance in staphylococcal isolates.

Keywords: MRSA; MRCNS; Erythromycin; Tetracycline; Resistance genes

Table 1
Oligonucleotide sequences of the primers used in the detection of methicillin resistance

Target genes		Oligonucleotide sequences[a]	Size of target region (bp)	Reference
mecA	Forward	CCT AGT AAA GCT CCG GAA	314	Choi et al. [4]
	Reverse	CTA GTC CAT TCG GTC CA		
16S rDNA	Forward	CAG CTC GTG TCG TGA GAT GT	420	Strommenger et al. [5]
	Reverse	AAT CAT TTG TCC CAC CTT CG		
femA	Forward	CTT ACT TAC TGC TGT ACC TG	684	
	Reverse	ATC TCG CTT GTT ATG TGC		
erm(C)	Forward	AAT CGT CAA TTC CTG CAT GT	299	
	Reverse	TAA TCG TGG AAT ACG GGT TTG		
erm(A)	Forward	AAG CGG TAA ACC CCT CTG A	190	
	Reverse	TTC GCA AAT CCC TTC TCA AC		
tet(K)	Forward	GTA GCG ACA ATA GGT AAT AGT	360	
	Reverse	GTA GTG ACA ATA AAC CTC CTA		
tet(M)	Forward	AGT GGA GCG ATT ACA GAA	158	
	Reverse	CAT ATG TCC TGG CGT GTC TA		

[a] Oligonucleotide sequences were obtained from the Iontek Company.

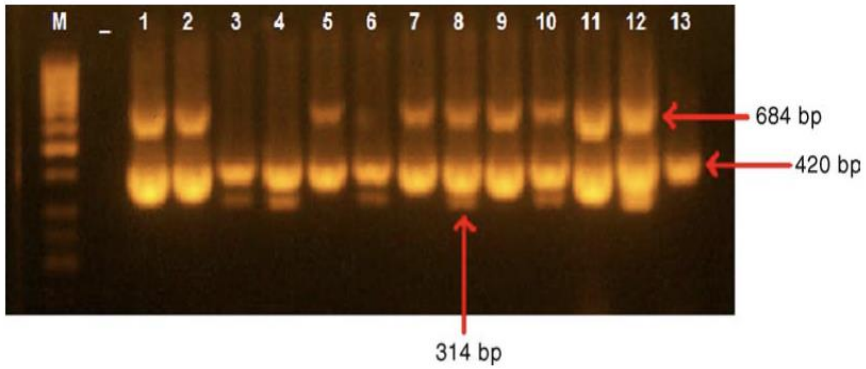

Fig. 1. Image of the *mecA*, *femA* and 16S rDNA genes in agarose gel as a result of the PCR test. M, molecular marker; –, *mecA*-negative standard strain; 1–13, test isolates.

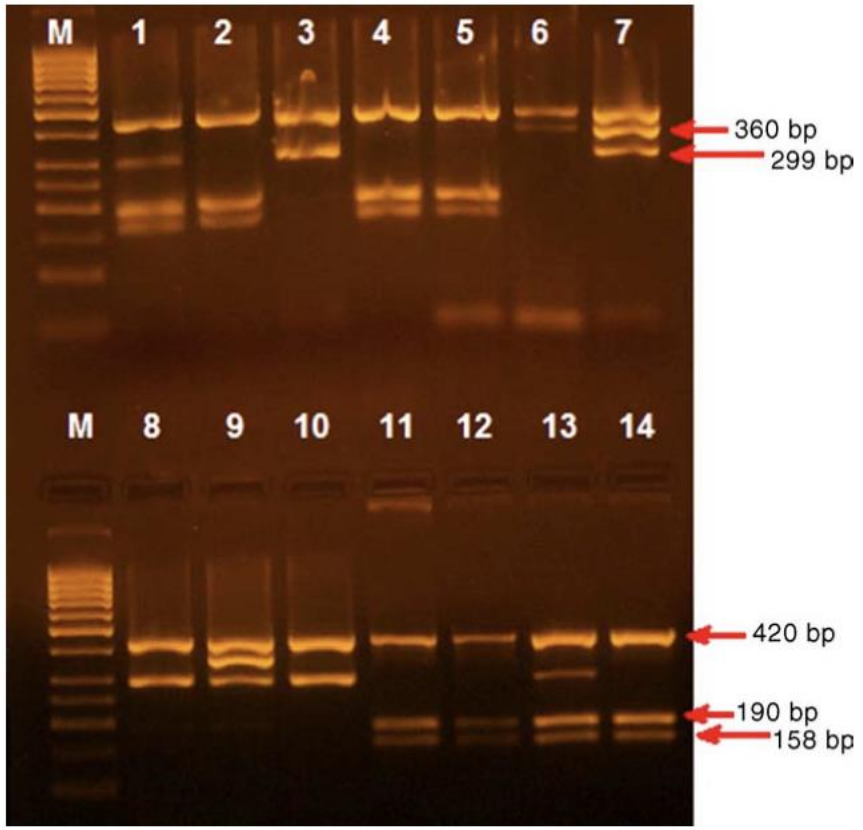

Fig. 2. Image of erythromycin [*erm*(C) and *erm*(A)] and tetracycline [*tet*(K) and *tet*(M)] genes in agarose gel. M, molecular marker; 1–14, test isolates.

Folia Microbiol. **53** (4), 363–367 (2008) http://www.biomed.cas.cz/mbu/folia/

Detection of Methicillin and Mupirocin Resistance in Staphylococcal Hospital Isolates with a Touchdown Multiplex Polymerase Chain Reaction

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ABSTRACT. Staphylococcal hospital isolates (n = 166) were tested in a touchdown multiplex-polymerase chain reaction assay for the identification of methicillin and mupirocin resistance and discrimination of *S. aureus* (*femA* gene) from coagulase negative staphylococci and other bacteria. All isolates harbored the 16SrDNA (*Staphylococcus* genus specific internal control) gene, and 130 (78 %) the *mecA* (methicillin resistance) gene. Fifty-seven (44 %) of these were determined as methicillin-resistant *S. aureus*, while the remaining 73 (56 %) were methicillin-resistant coagulase-negative staphylococci. Seventy-five (45 %) isolates harbored the *ileS-2* (high-level mupirocin resistance) gene and were determined as mupirocin-resistant. This assay represents a simple, rapid, reliable approach for the detection and discrimination of methicillin- and mupirocin-resistant staphylococci.

Table I. Oligonucleotide primers used

Gene	Primer	Oligonucleotide sequence (5′–3′)	Product size, bp	Reference
femA	F1	CTT ACT TAC TGC TGT ACC TG	684	Vannufel *et al.* 1995
	F2	ATC TCG CTT GTT ATG TGC		
mecA	Met1	CCT AGT AAA GCT CCG GAA	314	Choi *et al.* 2003
	Met2	CTA GTC CAT TCG GTC CA		
ileS-2	M1	GTT TAT CTT CTG ATG CTG AG	237	Nunes *et al.* 1999
	M2	CCC CAG TTA CAC CGA TAT AA		
16S rDNA	16s1	CAG CTC GTG TCG TGA GAT GT	420	Strommenger *et al.* 2003
	16s2	AAT CAT TTG TCC CAC CTT CG		

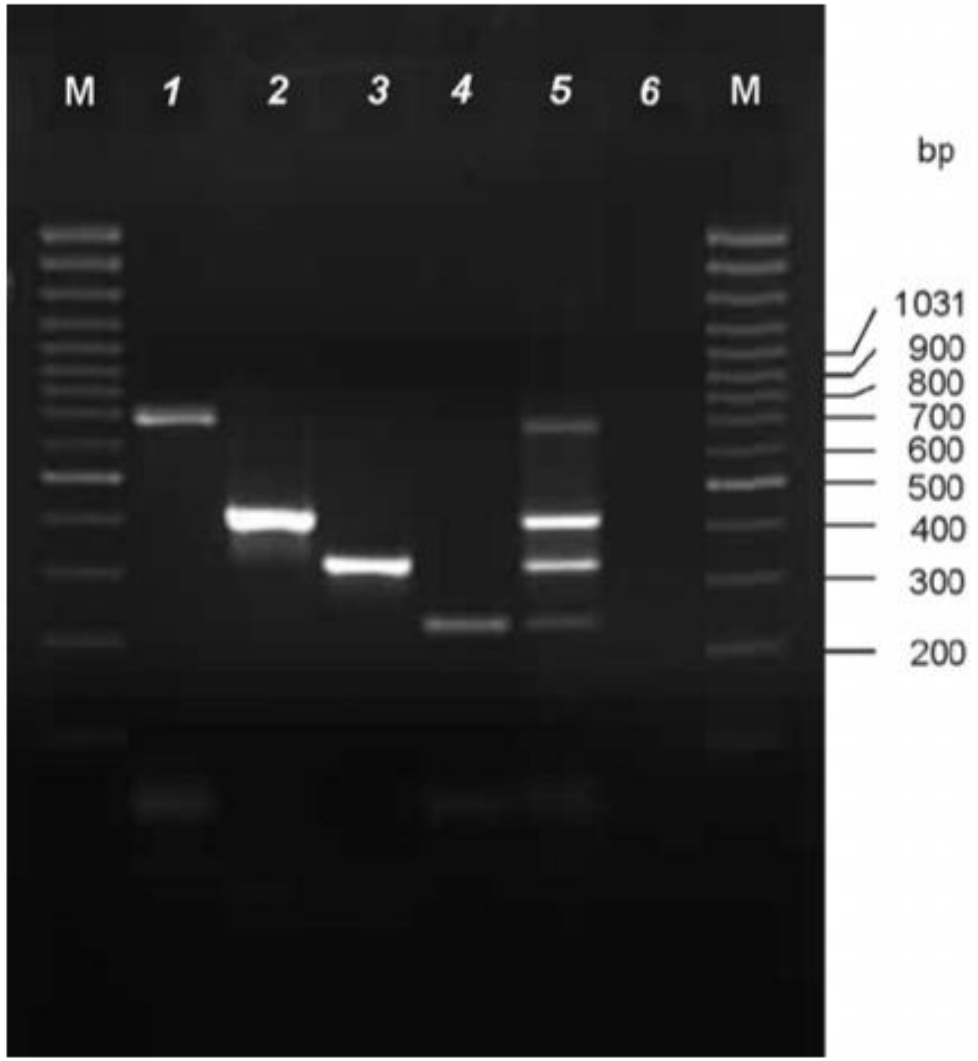

Fig. 1. Agarose gel electrophoresis patterns showing single PCR and mPCR amplification products; *1–4*: PCR amplicons from *femA* (684 bp), *16SrDNA* (420), *mecA* (314), *ileS-2* (237); *5*: quadriplex PCR amplicon, *i.e.*, *femA*, *16S rDNA*, *mecA* and *ileS-2* simultaneously amplified; M: DNA molecular size markers (100-bp ladder); *6*: negative control.

MENU

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Review Article | Published: 01 March 2010

Resistance to and synthesis of the antibiotic mupirocin

Christopher M. Thomas ✉, Joanne Hothersall, Christine L. Willis & Thomas J. Simpson

Nature Reviews Microbiology **8**, 281–289(2010) | Cite this article

Key Points

- The spread of methicillin-resistant *Staphylococcus aureus* (MRSA) necessitates the development of new antibiotics.
- The control of mupirocin production in soil bacteria is in proportion to bacterial cell density.
- Mupirocin inhibits isoleucyl-tRNA synthetase, and spontaneous mupirocin-resistant mutants are generally less fit than wild-type bacteria.

25 Sareyyupoglu, B., Ozyurt, M., Hanzedaroglu, T. & Ardic, N. Detection of methicillin and mupirocin resistance in staphylococcal hospital isolates witha touchdown multiplex polymerase chain reaction. *Folia Microbiol. (Praha)* **53**, 363–367 (2008). **An important demonstration of the value of rapid PCR techniques for identifying resistance genes and highlighting the prevalence of *mupA* , a gene conferring high-level mupirocin resistance.**

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Importance of Antibiotics in Veterinary Medicine

- Treatment of animal infections
- Prevention of zoonotic transmission
- Animal welfare and food safety
- Contribution to global antimicrobial use

Thank you for your patience&interest.