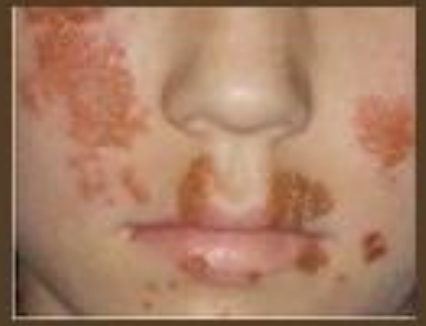
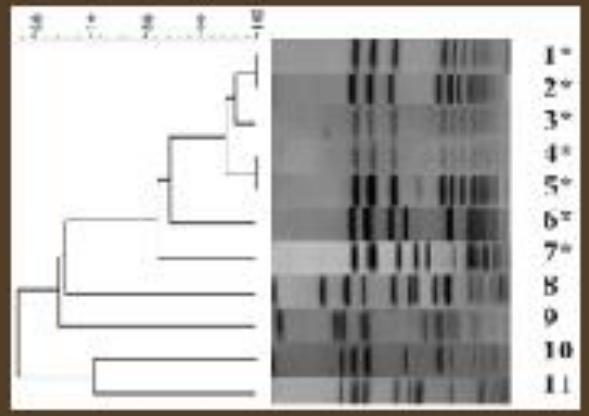
Toipc Number three

Methicillin-resistant *Staphylococcus aureus*





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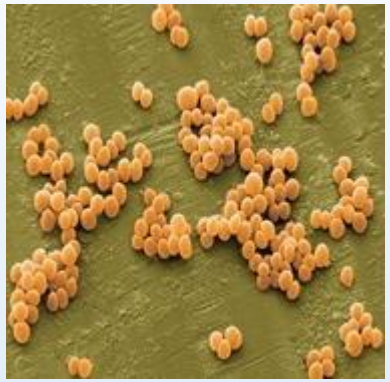
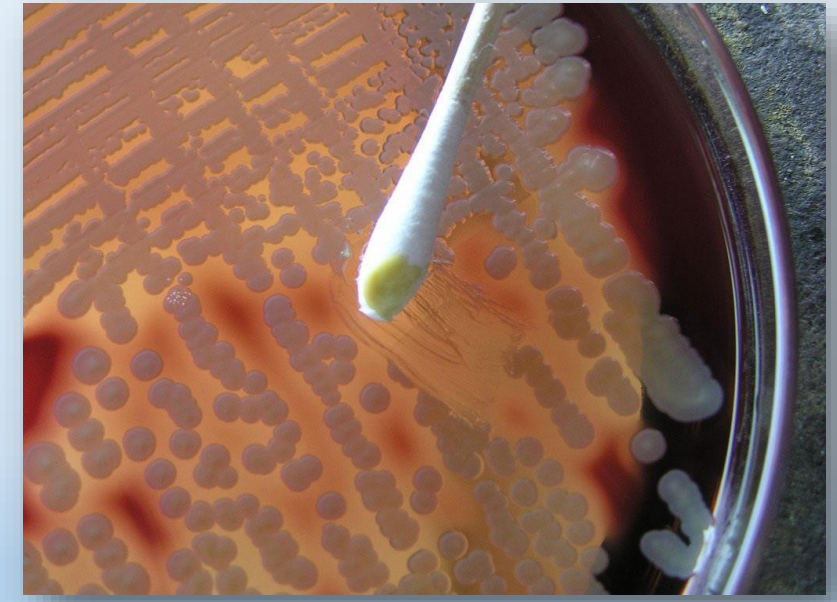
Staphylococcus aureus

- The name Staphylococcus comes from the Greek staphyle, meaning a bunch of grapes, and kokkos, meaning berry.
- Under the microscope, it looks like a bunch of grapes or little round berries.
- The organisms *S. aureus* are gram-positive, facultative anaerobic, usually unencapsulated cocci.
- Strains of *Staphylococcus aureus* produce a golden yellow pigment

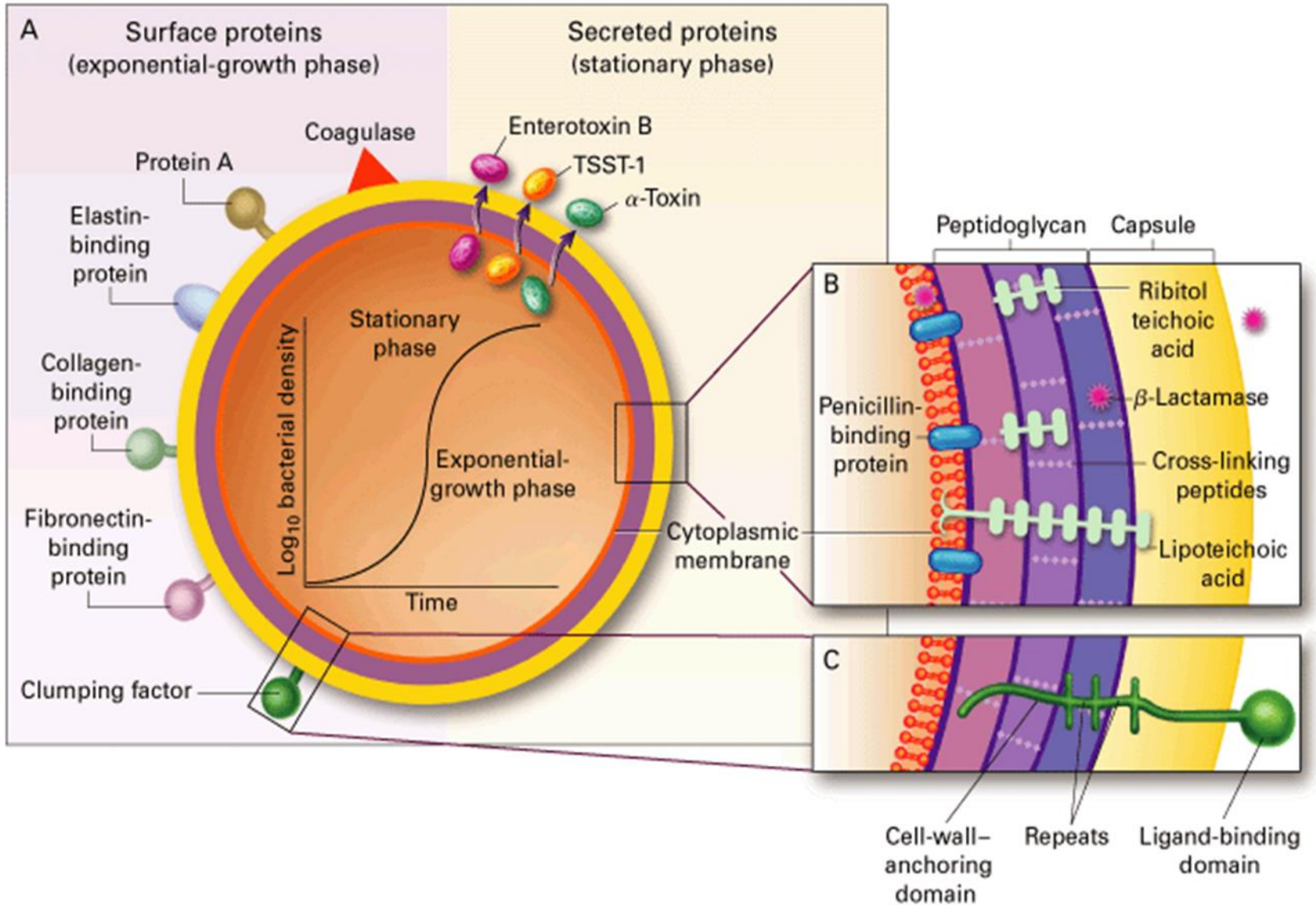




Epidemiology

- The primary reservoir of staphylococci is thought to be the anterior nares.
- Nasal carriage rates of 25 % - 40 % have been reported, and are known to be influenced by ethnicity, age, exposure to antibiotics and the hospital environment.
- MRSA carriage have a greater risk for the development of *S. aureus* infection than does MSSA carriage.
- This could be a result of the resistance itself and increased intrinsic virulence of MRSA compared with MSSA
- Elimination of nasal carriage would theoretically reduce the infection rates in populations.

Virulence Factors



Cell Wall Component and Microcapsules

Cell Wall Component

- ❑ Endotoxin-like activity →Sepsis
- ❑ Macrophage cytokine release
- ❑ Complement activation
- ❑ Platelet aggregation

Microcapsule

- ❑ 11 types
- ❑ 75% of human infection caused by types 5 and 8
- ❑ Most MRSA isolates are type 5
- ❑ Consist of antiphagocytic polysaccharides

Surface Proteins

Protein A

- Found in cell wall
- Binds to Fc part of IgG
- Blocks phagocytosis

Coagulase

- Clumping factor

Specific Binding Proteins

- Collagen-facilitates bone and joint invasion
- Fibronectin-facilitates adherence to catheters
- Collagen adhesin protein-development of infected thrombi/adherence to lung epithellium

Extracellular Enzymes

- Beta-lactamase, cuts the beta lactam wall of certain antibiotics

Secreted proteins: Toxins and Enzymes

1. Superantigens: enterotoxins and toxic shock syndrome toxin1. Enterotoxin

- causes food poisoning characterized by prominent vomiting and watery, nonbloody diarrhea.
- Enterotoxin is heat-resistant and is therefore usually not inactivated by brief cooking.
- There are six immunologic types of enterotoxin, types A–F.

Toxic shock syndrome toxin (TSST)

- Causes toxic shock.
- TSST is a superantigen and causes toxic shock by stimulating the release of large amounts of IL-1, IL-2, and tumor necrosis factor (TNF).

2. Epidermolytic (exfoliative) toxin (ET)

- It is "epidermolytic" and acts as a protease leading to the separation of the epidermis at the granular cell layer.

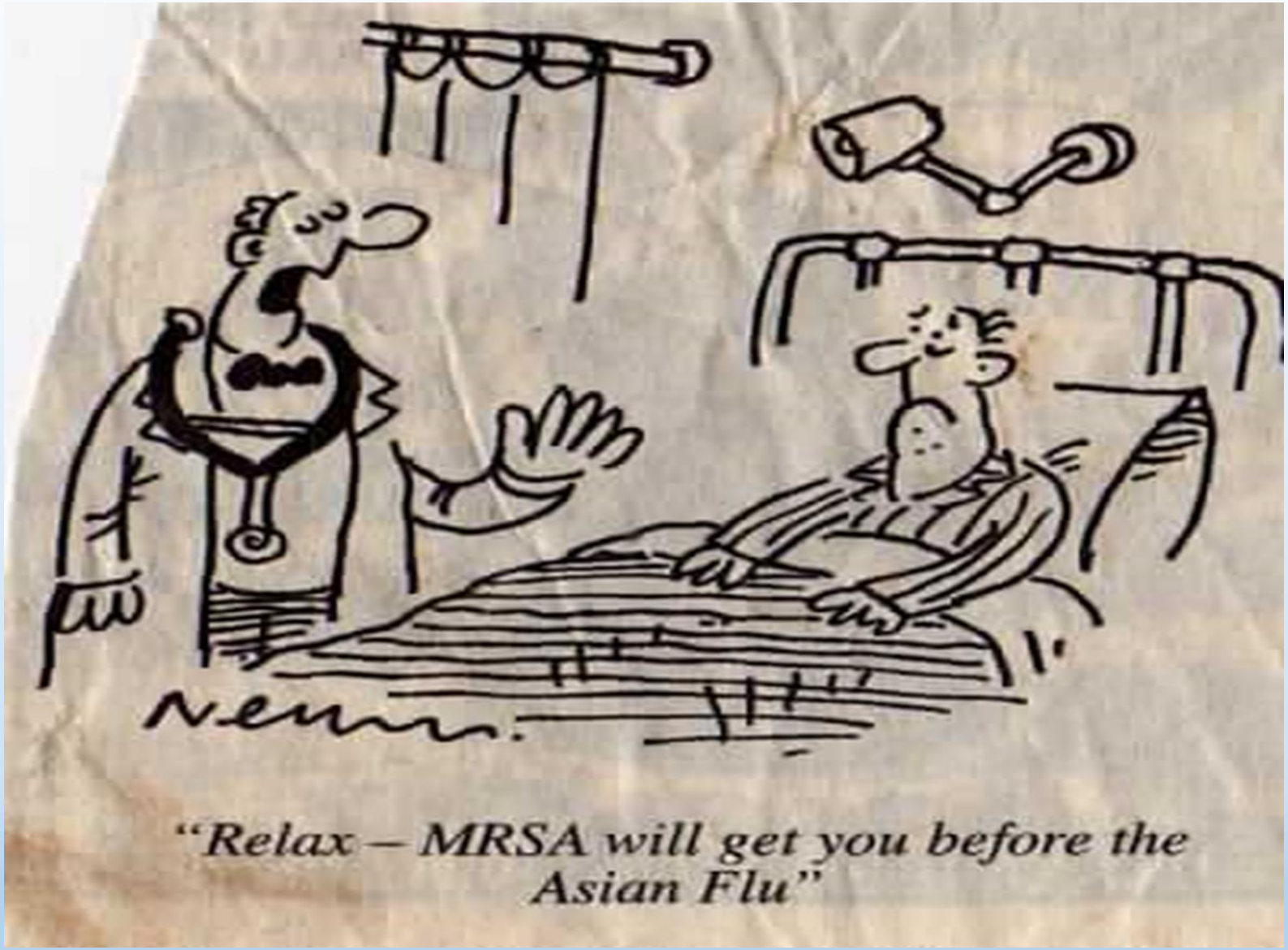
(con’t)

3. Membrane Damaging Toxins

- Alpha toxin, which causes marked necrosis of the skin and hemolysis.
- P-V leukocidin, is a pore-forming toxin that kills cells, especially white blood cells, by damaging cell membranes.

4. Extracellullar Enzymes include

- Hyaluronidase, hydrolyzes hyaluronic acid in connective tissue allowing spread of infection
- Staphylokinase, Fibrinolysin which allows spread of infection
- Lipase, allows colonization by acting on lipids present on the surface of the skin.
- Coagulase, by clotting plasma, serves to wall off the infected site, thereby retarding the migration of neutrophils into the site.
- Staphylokinase is a fibrinolysin that can lyse thrombi



"Relax – MRSA will get you before the Asian Flu"

Staphylococcal infections

Pyogenic infections

1. The furuncle or boil

- Superficial skin infection that typically develops in a hair follicle, sebaceous gland, or sweat gland.
- Multiple boils become a carbuncle

2. Impetigo

- Highly infectious skin disease which causes sores and blisters
- Bullous impetigo is a localized form of staphylococcal scalded skin syndrome

3. Deep infections

- Acute osteomyelitis, Pneumonia , Bacteremic spread and endocarditis are most common in drug abusers

(con't)

Toxin mediated infections

1. Scaled Skin Syndrome

- In this syndrome the organisms release exfolitative toxin, which is resposible for the extensive intraepidermal splitting and necrosis of the tissue.

2. Toxic Shock Syndrome

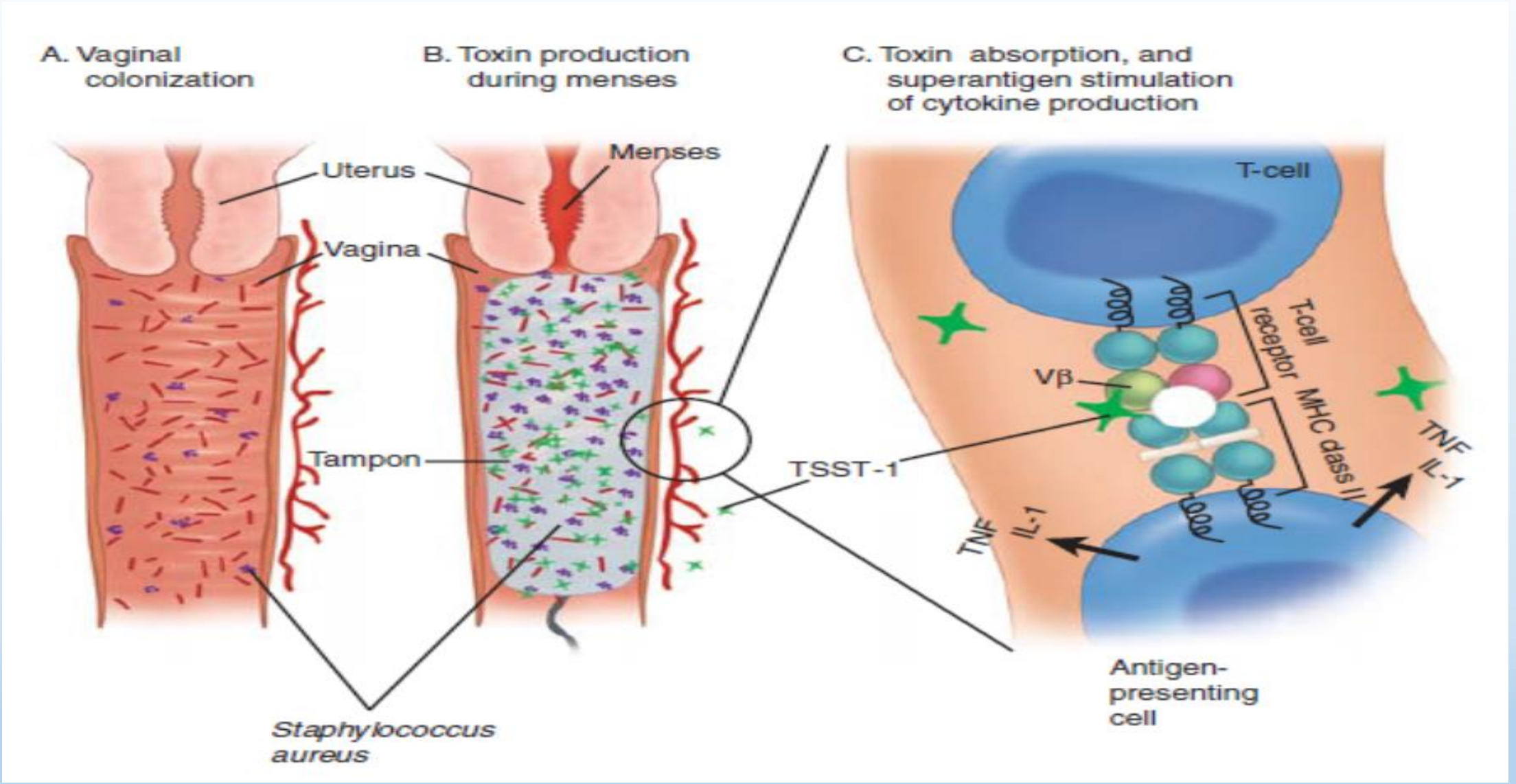
- The disease is initiated with the localized growth of toxin-producing strains of *S.aureus* in the vagina or a wound, followed by release of the toxin into the blood stream.
- The disease is characterized by high fever, hypotension, and a diffuse macular erythematous rash

3. Staphylococcal Food Poisoning

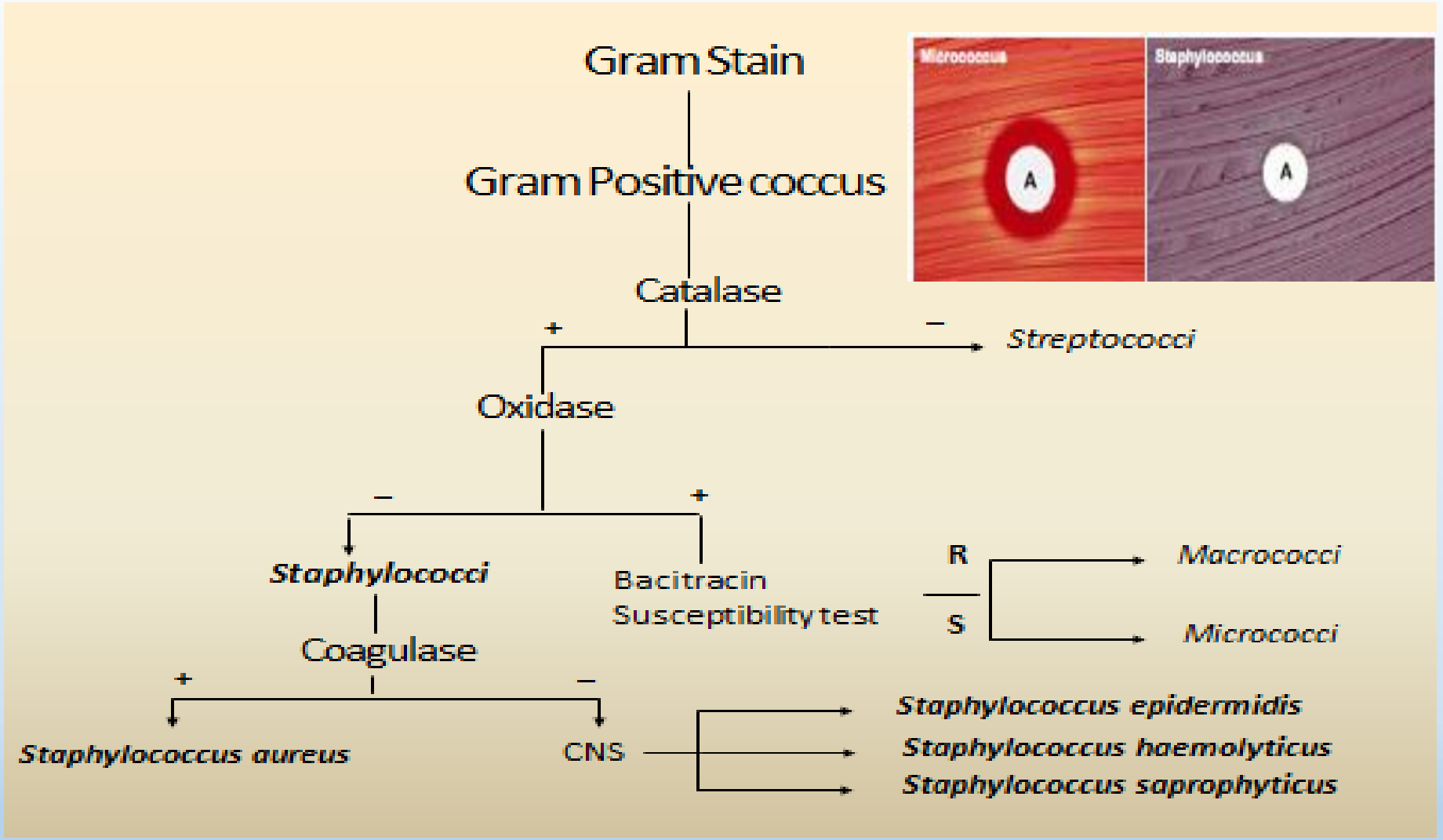
- Ingestion of staphylococcal enterotoxin–contaminated food results in acute vomiting without fever and diarrhea within 1 to 5 hours.

Supperantigen mechanism of action

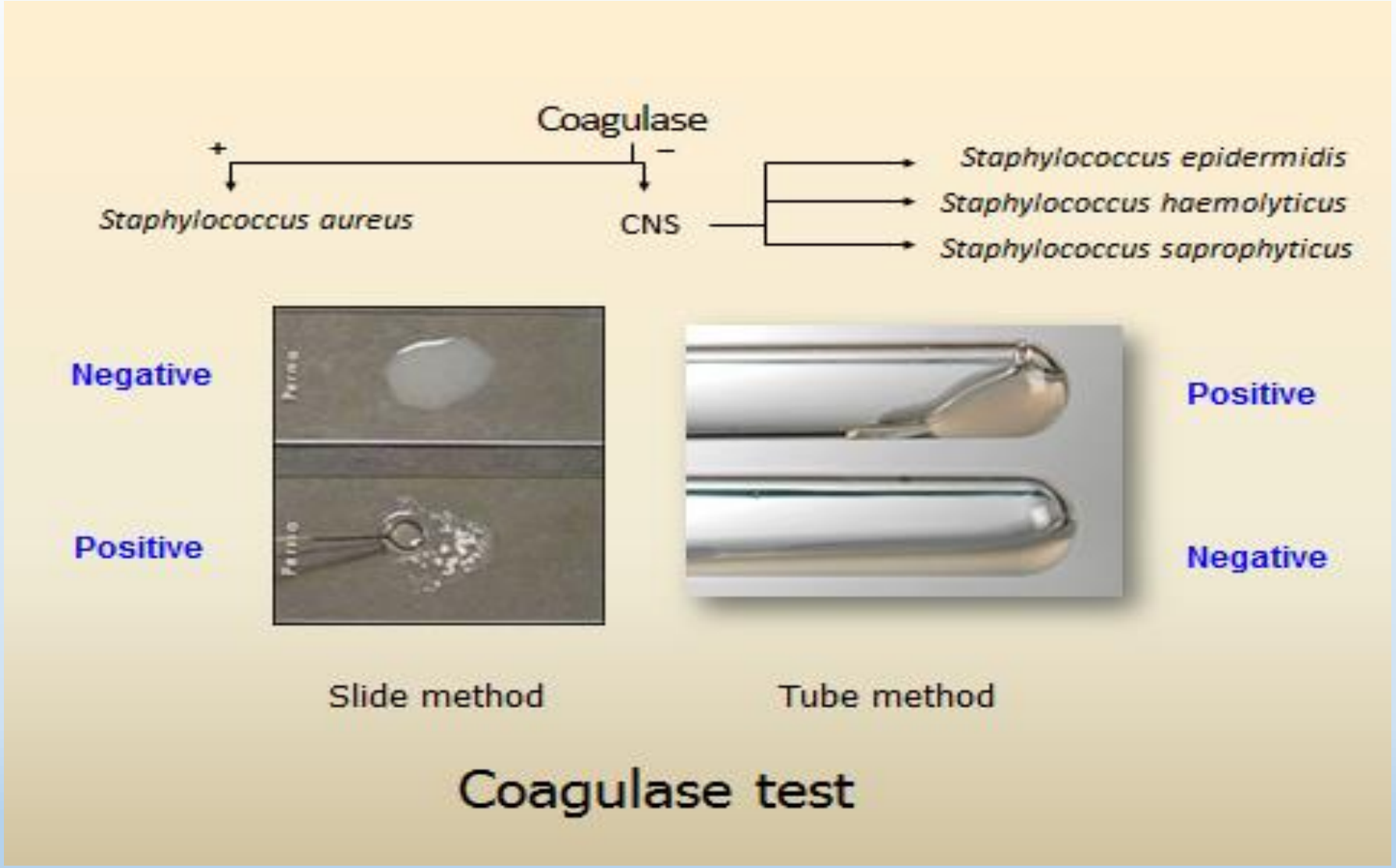
- Superantigens bind to MHC class II molecules of antigen presenting cells (i.e., macrophages) and Vβ region of T-cell receptor in a non-antigen-specific manner
- This leads to massive release of cytokines and chemokines, as well as the clonal expansion of certain clonal types of T cells.
- Based on the site of infection, TSS can be divided into two categories, menstrual and non-menstrual TSS.
- Menstrual is associated with tampon usage in women colonized vaginally by superantigen-producing *S. aureus*.
- Non-menstrual TSS occurs as a complication of *S. aureus* infections after surgical procedures, burns or post-influenza pneumonia.


A. Vaginal colonization
B. Toxin production during menses
C. Toxin absorption, and superantigen stimulation of cytokine production
Uterus
Vagina
Tampon
Menses
Staphylococcus aureus
T-cell
T-cell receptor
MHC class II
Vβ
TSST-1
TNF
IL-1
TNF
IL-1
Antigen-presenting cell

Identification of Staphylococci



Coagulase test



Novobiocin resistance test

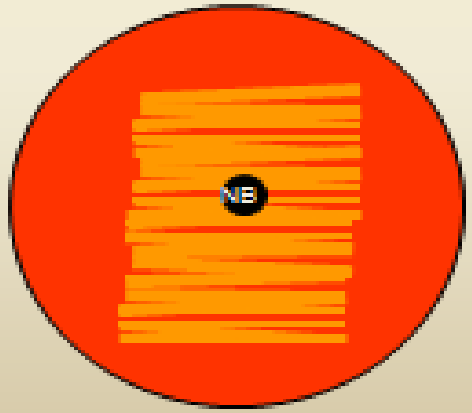
Coagulase Negative Staph

- *Staph saprophyticus*
- *Staph heamolyticus*
- *Staph epidermidis*

Novobiocin resistance Test:

Procedure:

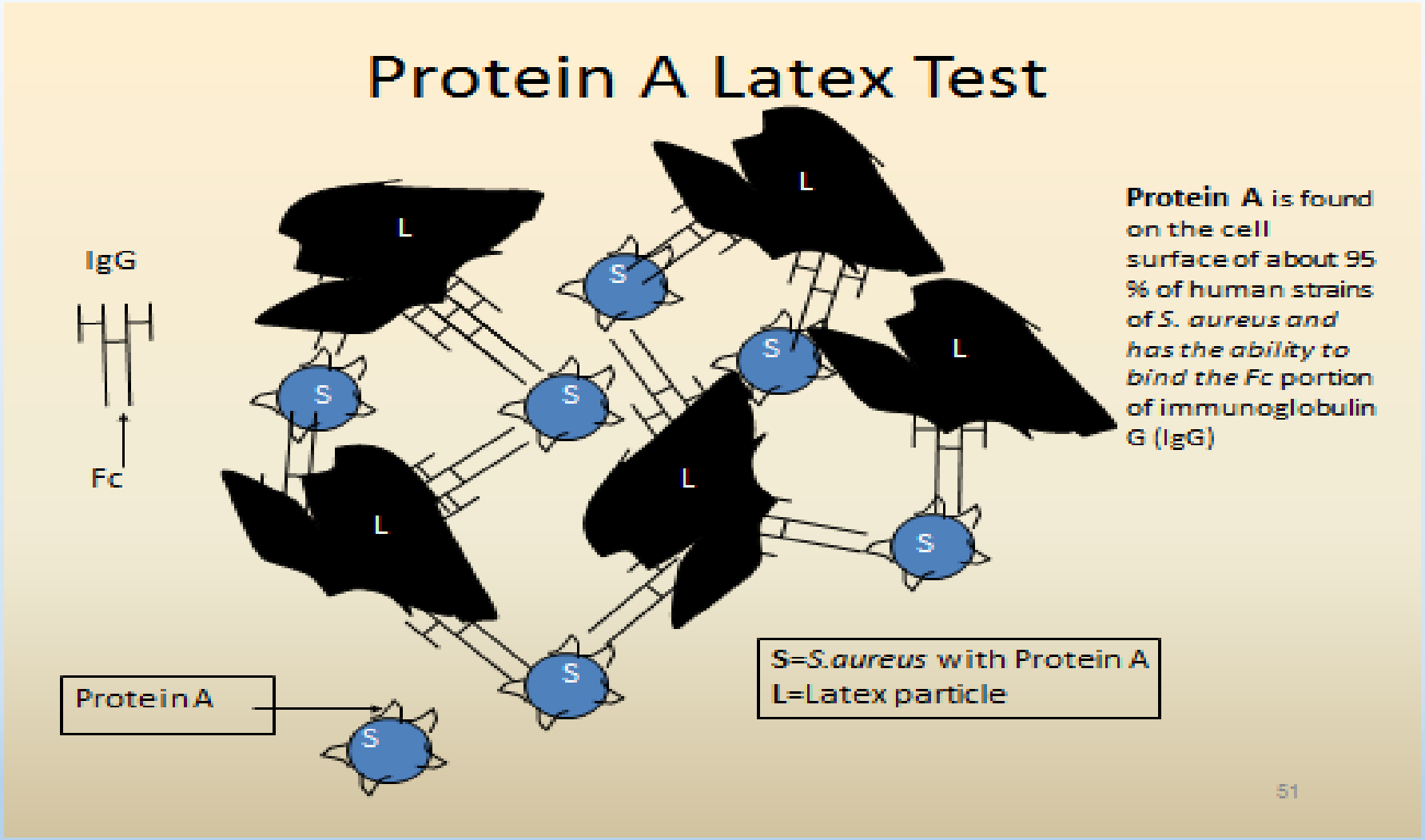
1. Inoculate blood agar plate with the test organism.
2. Aseptically apply Novobiocin disc onto the center of the streaked area.
3. Incubate the plate at 37°C for 24 hrs.



MANNITOL SALT AGAR (MSA)

- Mannitol Salt Agar (MSA) is a selective and differential medium.
- The high concentration of salt (7.5%) selects for members of the genus Staphylococcus, since they can tolerate high saline levels.
- MSA also contains the sugar mannitol and the pH indicator phenol red.
- If an organism can ferment mannitol, an acidic byproduct is formed that will cause the phenol red in the agar to turn yellow.
- *Staphylococcus aureus* ferments mannitol. Most non-pathogenic staphylococci don't ferment mannitol.

Protein A Latex Test



Staphyloslide Latex Test

Staphyloslide™ Latex Test for Staphylococcus aureus

Latex Test consists of latex particles coated with human fibrinogen and IgG. On mixing the latex reagent with colonies of staphylococci which have clumping factor or Protein A present, cross-linking will occur giving visible agglutination of the latex particles.

Such agglutination will occur notably with *S. aureus*.

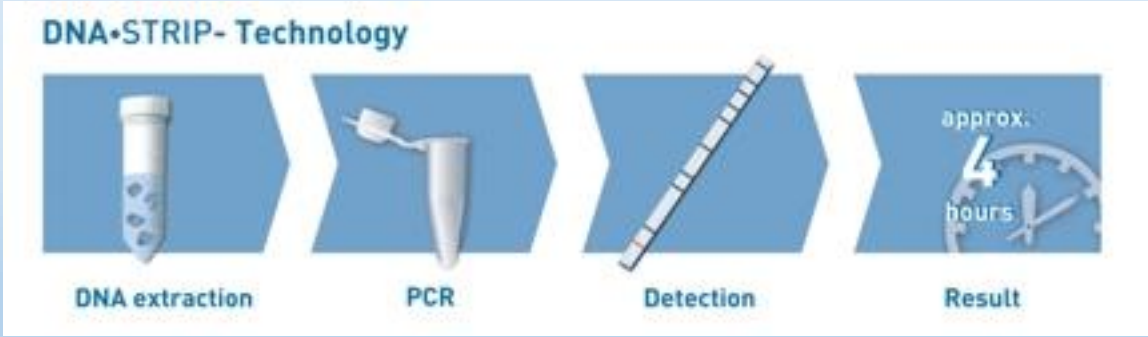
If neither clumping factor nor Protein A are present, no agglutination will occur and the result will be regarded as negative.

GenoType® MRSA – The basic test for culture confirmation

- Identification of *S. aureus* and *S. epidermidis*
- Detection of the *mecA* gene as well as a PVL-specific fragment. Thus, it can be used to distinguishes CA-MRSA from nosocomial MRSA therefore makes sense from a therapeutic and epidemiological viewpoint.
- Rapid result: Performing the test from the overnight culture provides you with a result in only 4 hours and thus guarantees rapid diagnosis.

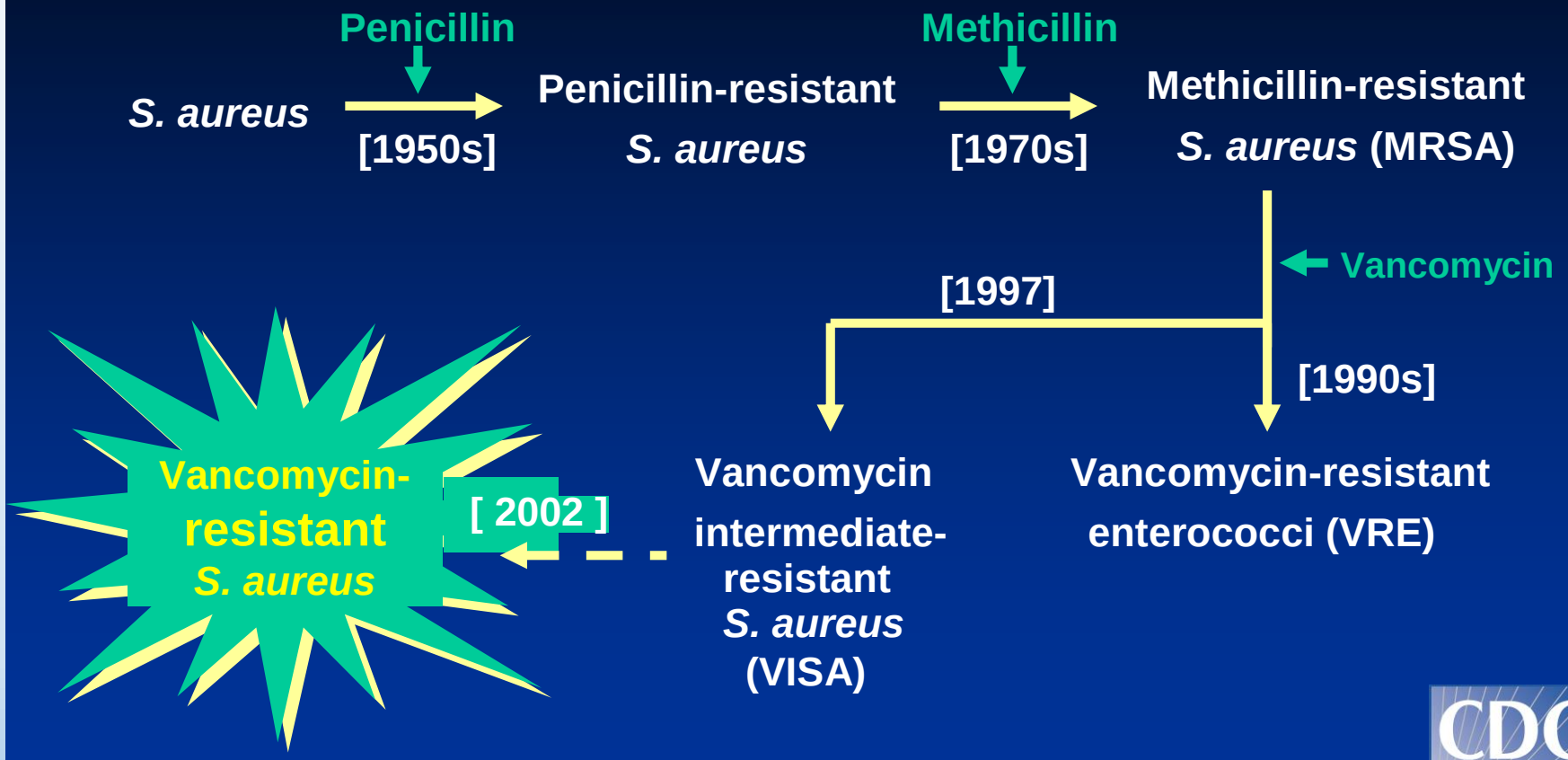
GenoType® MRSA method

- Following DNA isolation, nucleic acids are amplified, then the amplicons are chemically denatured
- The DNA-STRIP is coated with highly specific probes which are complementary to selectively amplified nucleic acids.
- The single-stranded amplicon binds specifically to the analog probes during hybridization
- During the conjugate reaction, the specifically bound amplicon is marked with the enzyme alkaline phosphatase and is then made visible in a colorimetric detection reaction



Break Time!!!

Evolution of Drug Resistance in *S. aureus*



CDC
Centers for Disease Control and Prevention

MRSA-terminology

- Methicillin, a semi-synthetic Beta-lactam is no longer in use.
- Strictly speaking ORSA as oxicillin has superceded methicillin in practice.
- *mecA* gene encodes penicillin binding protein which is intrinsically insensitive to methiciliin and beta-lactams such as cephalosporins, cefamycins and carbapenems

Risk factors for MRSA infection

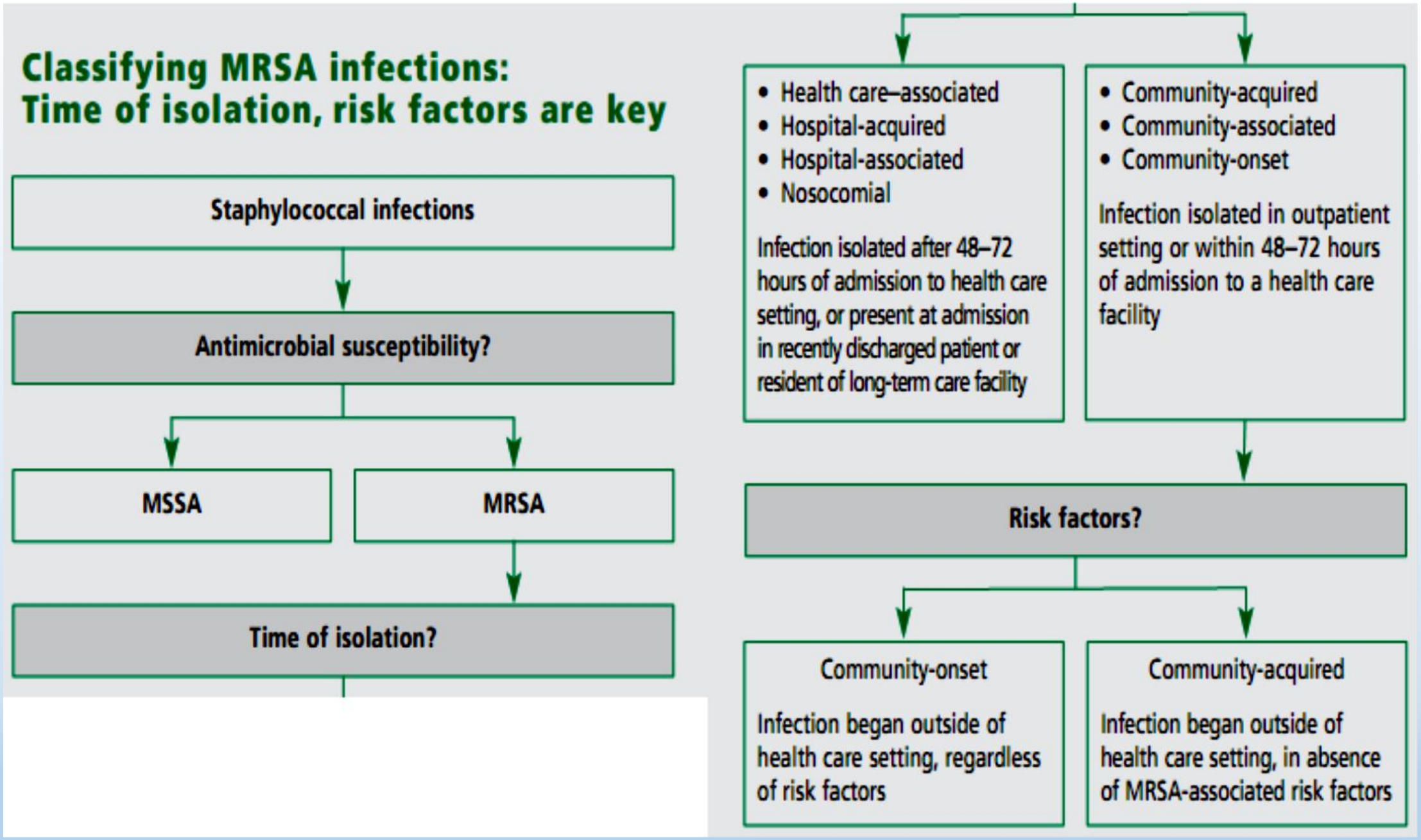
- ❑ Hospitalization during prior year
- ❑ Surgical procedure
- ❑ Dialysis patients
- ❑ Chronic Indwelling vascular catheter
- ❑ Exposure to broad spectrum antibiotics
- ❑ Close contact with any individual with the previous risk factors

Classifying MRSA infections

Two primary factors currently used in the categorization of MRSA infections are time of infection isolation and the presence or absence of MRSA-related risk factors

- Nosocomial, Hospital acquired, Hospital associated or Health care–associated MRSA (HA-MRSA) MRSA: MRSA infection 48 to 72 hours post admission to the hospital or those present at the time of admission in recently discharged patients
- Community-onset: Infections that begin outside of the health care setting (regardless of the presence of risk factors for MRSA)
- Community-acquired, community-associated “true” CA-MRSA: Infections occurring in a community setting in the absence of risk factors for MRSA are considered by some to represent cases of “true” CA-MRSA.

Classifying MRSA infections: Time of isolation, risk factors are key



Updated definitions

1. CA-MRSA without health-care associated risk factors
   - ❑ Tend to be more susceptible to non-Beta-lactam antibiotics
   - ❑ Account for most outpatient infections in the USA
2. CA-MRSA with health care associated risk factors
   - ❑ Many of these may be typical nosocomial MRSA strains
3. Nosocomial MRSA
   - ❑ Typically the most antibiotic resistant strains

Characteristics of community-acquired MRSA

Patients with CA-MRSA infection tend to have all of the following characteristics:

- ❑ Diagnosis of MRSA made in the outpatient setting or on the basis of a positive culture for MRSA within 48 hours after hospital admission
- ❑ No medical history of MRSA infection or colonization
- ❑ No history in the preceding year of hospitalization, dialysis, surgery,or hospital
- ❑ No permanent indwelling catheters or medical devices that pass through the skin into the body.

HOW COMMUNITY-ACQUIRED MRSA DIFFERS FROM HEALTH CARE–ASSOCIATED STRAINS

- At a genetic level, CA-MRSA is more similar to methicillin-susceptible *S aureus* (MSSA) than to traditional MRSA, and its emergence appears to be due to the acquisition, by an MSSA strain, of the staphylococcal cassette chromosome (SCC) carrying *mecA* he gene encoding the methicillin-resistant penicillin binding protein.
- CA-MRSA are more frequently susceptible to a variety of non–beta-lactam antibiotics.
- CA-MRSA carry SCC*mec* type V,IV, which are smaller in size than HA-MRSA (types I, II, and III).
- Small size of SCC allow for more efficient transfer of resistance, a factor that may be relevant in the rapid emergence of CA-MRSA.

(con't)

- The potential of CA-MRSA strains to cause serious illness is further underscored by their production of a relatively greater number of recognized staphylococcal virulence factors compared with HA-MRSA.
- Most notably, CA-MRSA strains frequently carry the Panton-Valentine leukocidin genes that produce cytotoxins associated with tissue necrosis and leukocyte destruction
- Based on pulsed-field gel electrophoresis, almost all CA-MRSA strains are from a single clone (USA 300).

TABLE 1
Microbial profiles of health care–associated and community-acquired strains of MRSA

Strain	SCC*mec* gene	Antibiotic resistance	PFGE type	Toxins	PVL genes	Infection spectrum
HA-MRSA	Types I, II, and III	Multidrug-resistant	USA 100	Fewer	Rare	Bloodstream, respiratory tract, urinary tract infections
CA-MRSA	Types IV and V	Resistance typically limited to beta-lactams and erythromycin, although multidrug resistance can occur	USA 300	More	Common	*Commonly:* skin and soft-tissue infections *Occasionally:* necrotizing fasciitis, necrotizing pneumonia

HA-MRSA = health care–associated methicillin-resistant *Staphylococcus aureus*
CA-MRSA = community-acquired methicillin-resistant *Staphylococcus aureus*
SCC*mec* = staphylococcal cassette chromosome *mec*
PFGE = pulsed-field gel electrophoresis
PVL = Panton-Valentine leukocidin

Differing spectrums of disease

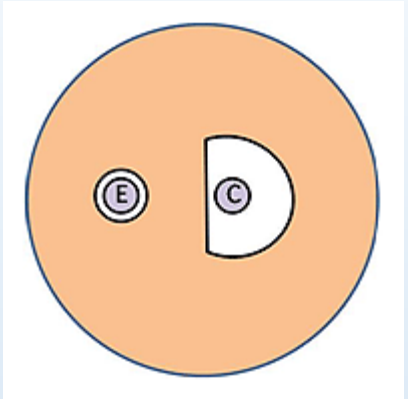
HA-MRSA	CA-MRSA
Skin and soft tissue, 36%	Skin and soft tissue, 74%
Respiratory tract, 22%	Otitis media, 7%
Urinary tract, 20%	Respiratory tract, 6%
Bloodstream, 9%	Bloodstream, 4%
Others, 13%	Others, 9%

Differing resistance patterns

- HA-MRSA is usually resistant to multiple classes of antimicrobials, whereas the usual pattern for CA-MRSA is resistance to the beta-lactams and erythromycin but susceptibility to other drugs tested.
- CA-MRSA strains are often susceptible to clindamycin, but the emergence of resistance during therapy has been reported, especially among erythromycin-resistant strains.
- Thus, an erythromycin-induction test (D-test) should be performed on such isolates to determine the presence of in vitro inducible resistance.
- Although these CA-MRSA infections are generally mild in nature, more serious infections leading to hospitalization or death have occasionally been described, including bacteremia, necrotizing fasciitis, and necrotizing pneumonia.

Staphylococcus aureus and Inducible Resistance to Clindamycin

- CA-MRSA isolates may be resistant to erythromycin, but not clindamycin,via an *mrsA* gene-mediated efflux pump
- It is also recommended that all staphylococcal isolates with results of erythromycin-resistant and clindamycin-susceptible should be further determined for inducible clindamycin resistance due to *erm* gene-mediated ribosomal methylation by a D-zone test The D-zone is based on the disk diffusion method. Briefly, erythromycin and clindamycin disks are placed with 15-26 mm apart. After 16-18 hours of incubation at 35 degree Celsius, flattening of the clindamycin inhibition zone only at the side that is adjacent to the erythromycin disk indicates inducible clinidamycin resistance.
- If this is the case, the isolate should be reported as clindamycin-resistant even though it was originally tested as susceptible



D-zone test. A D-shape appearance of clindamycin inhibitory zone with flattening zone adjacent to erythromycin disk indicates inducible clindamycin resistance. (E, erythromycin; C, clindamycin

Vancomycin resistance

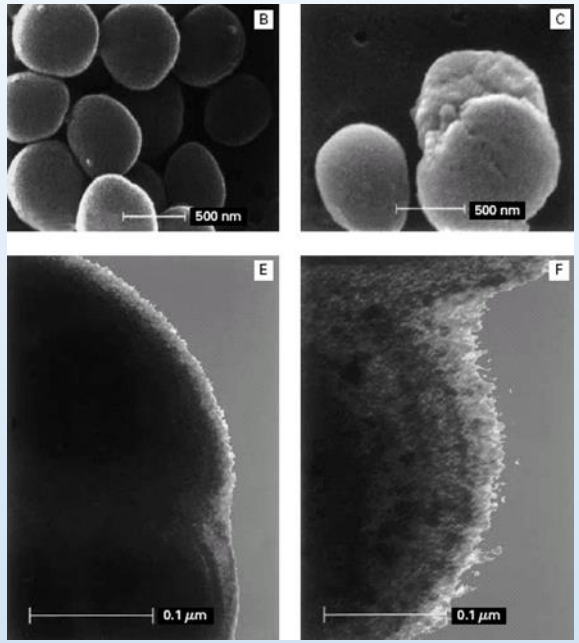
Vancomycin binds to cell wall precursors ending in D-Ala-D-Ala and prevents their incorporation into cell wall synthesis

Vancomycin-intermediate resistant *S. aureus* (VISA)

- ❑ First documented in Japan 1996, US in 1997
- ❑ Increased cell wall thickness limiting glycopeptide access to site of cell wall synthesis

Vancomycin-resistant *S. aureus* (VRSA)

- ❑ Isolated in June 2002
- ❑ Contained *vanA* resistance gene identical to *vanA* gene in patient's vancomycin-resistant Enterococcus faecalis
- ❑ van genes encode for precursors with alternative termini that have low affinity for vancomycin (eg. *vanA* encodes D-Ala-D-Lac



Mechanism of methicillin resistance

- Resistance of methicillin-resistant *S. aureus* to all β-lactam antibiotics is related to the "acquired" penicillin-binding protein PBP-2a.
- PBP2a is encoded by the *mecA* gene, which is carried by a large mobile genetic element that is designated staphylococcal cassette chromosome *mec* (SCC*mec*)
- PBPs are involved in the assembly of the bacterial cell-wall peptidoglycan.
- The low-affinity PBP2a is assumed to take over the cell wall biosynthetic functions of normal PBPs in the presence of β-lactam
- PBP2a was produced in a larger amount at 32°C than at 37°C. From these results, it suggested that the mechanism of methicillin resistance depends on the induction of PBP2a

The staphylococcal cassette chromosome mec (SCCmec)

The SCCmec element is present in five (I to V) different allotypes and contains a characteristic combination of two essential genetic components, the *mec* gene complex and the *ccr* gene complex

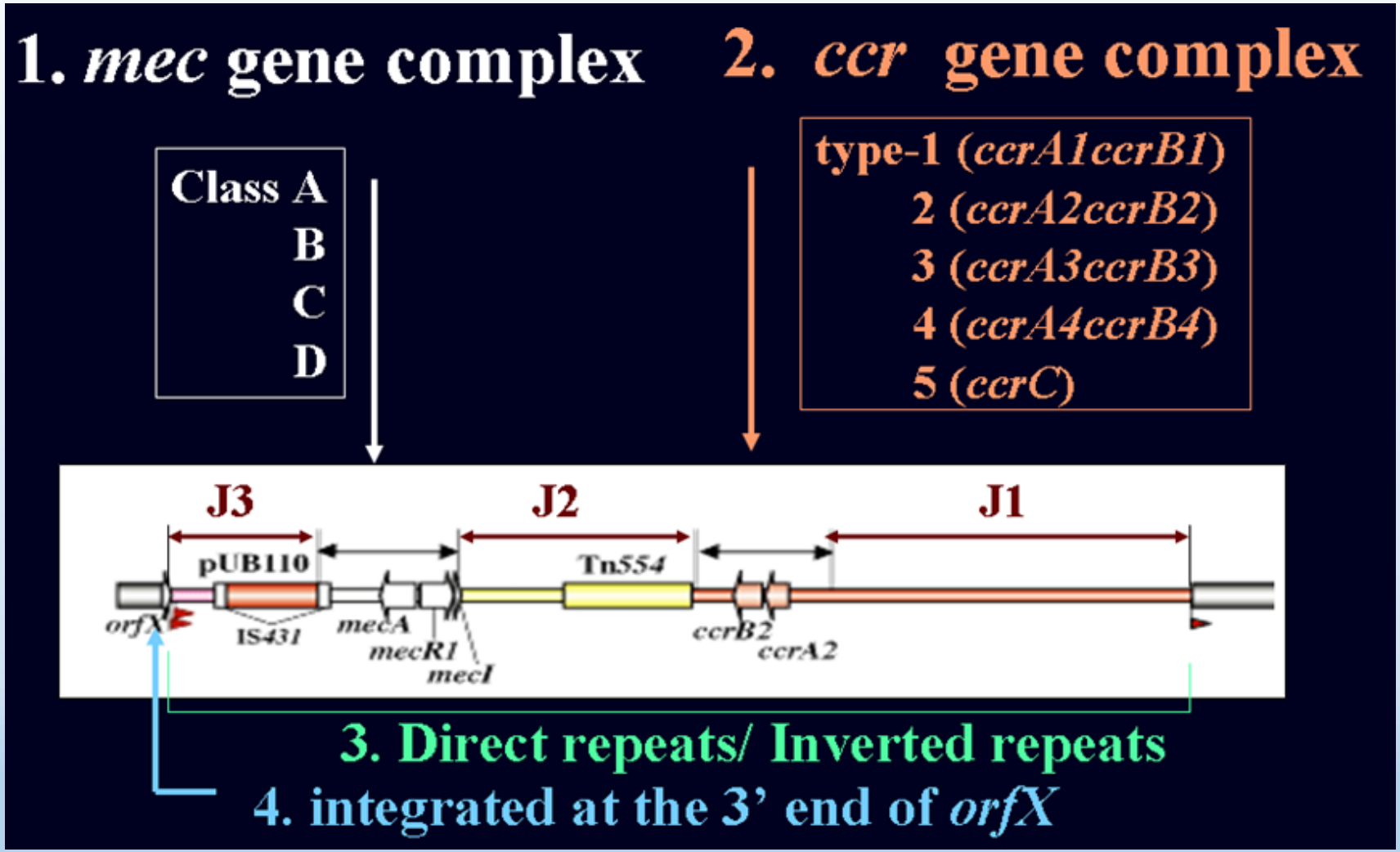
The ccr gene complex contains two site-specific recombinase genes, ccrA and ccrB, which are responsible for the mobility of SCCmec.

SCCmec may be classified into four major types according to the class of mecA gene complex and the type of ccr gene complex present.

Type I, class B and ccrAB1; type II, class A and ccrAB2; type III, class A and ccrAB3; and type IV, class B and ccrAB2.

The region other than the *mec* and *ccr* gene complexes is designated the J (junkyard) region

The essential structure of SCC*mec* elements



Panton-Valentine Leucocidin (PVL)

- The toxic effect of PVL, results from the synergistic action of two separate exoproteins, namely, LukS-PV and LukF-PV.
- These proteins are encoded by two contiguous and cotranscribed genes (LukS-PV and LukF-PV), which are carried on temperate bacteriophages.
- PVL is a pore-forming cytotoxin that targets human and rabbit mononuclear and polymorphonuclear cells.
- Most notably, CA-MRSA strains frequently carry the Panton-Valentine leukocidin genes that produce cytotoxins associated with tissue necrosis and leukocyte destruction

Molecular Techniques for MRSA Typing

- Enough information must be generated to permit the implementation of appropriate measures for control of infection, so that outbreaks can be contained.
- The most commonly used molecular typing techniques include:
    1. Pulsed field gel electrophoresis (PFGE)
    2. Multilocus sequence typing (MLST)
    3. *Spa* typing

Pulsed-field gel electrophoresis

- The “gold standard” for S. aureus strain typing mainly because of its excellent discriminatory power
- PFGE is based on the digestion of chromosomal DNA with the rare cutting restriction enzyme, followed by agarose gel electrophoresis, Why?
- The PFGE patterns are analyzed with a software package with Dice comparison and unweight pair group matching analysis (UPGMA)
- Interpretation of the results is troubled by a lack of both interlaboratory reproducibility and a common nomenclature

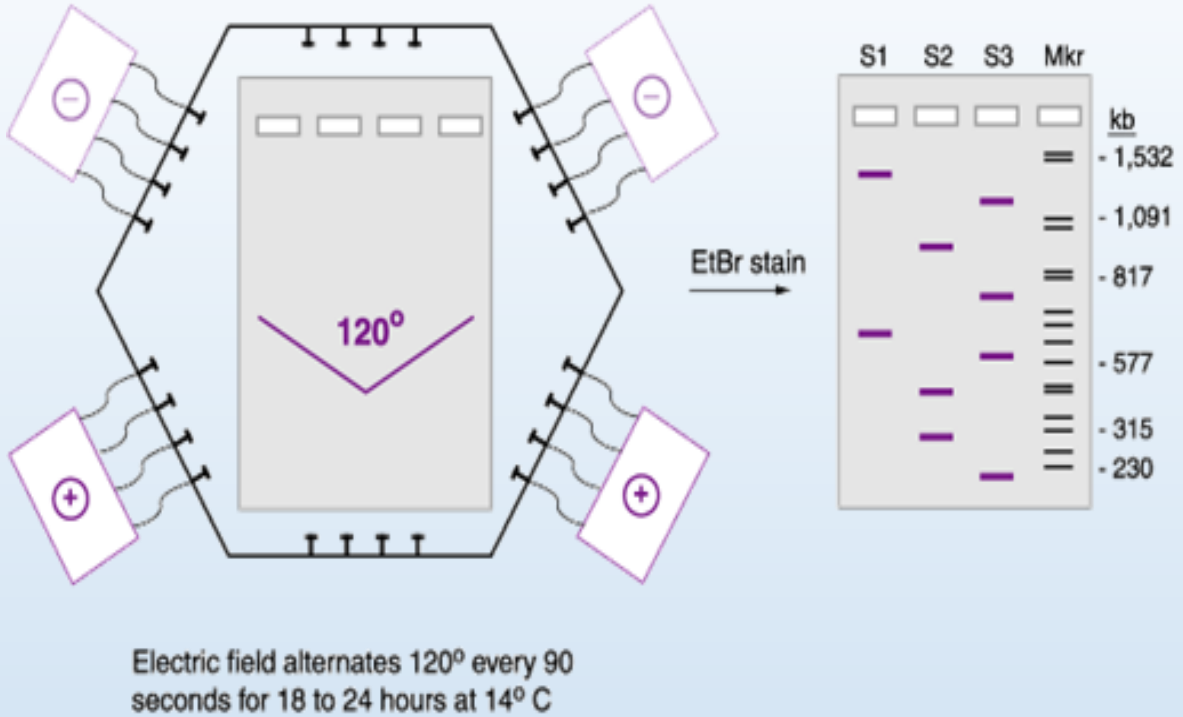
(con't)

- During continuous field electrophoresis, DNA above 30-50 kb migrates with the same mobility regardless of size. This is seen in a gel as a single large diffuse band.
- In PFGE, the DNA is forced to change direction during electrophoresis, different sized fragments can be separated.
- large DNA fragments require more time to reorient direction in an electric field than do small DNA fragments, so small DNA fragments move smoothly than large DNA fragments
- PFGE is a valuable method for total genomic analysis

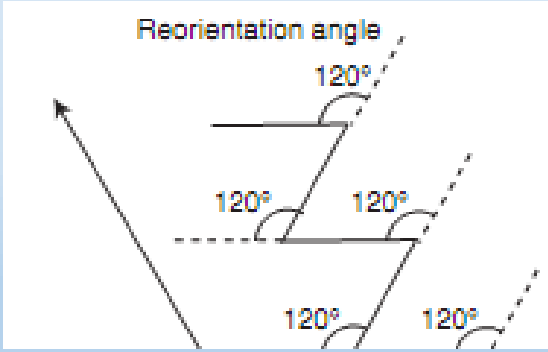
Contoured clamped homogeneous electric field (CHEF) system

This system uses a hexagonal gel box that alters the angle of the fields relative to the agarose gel 120◦ every 90 s.

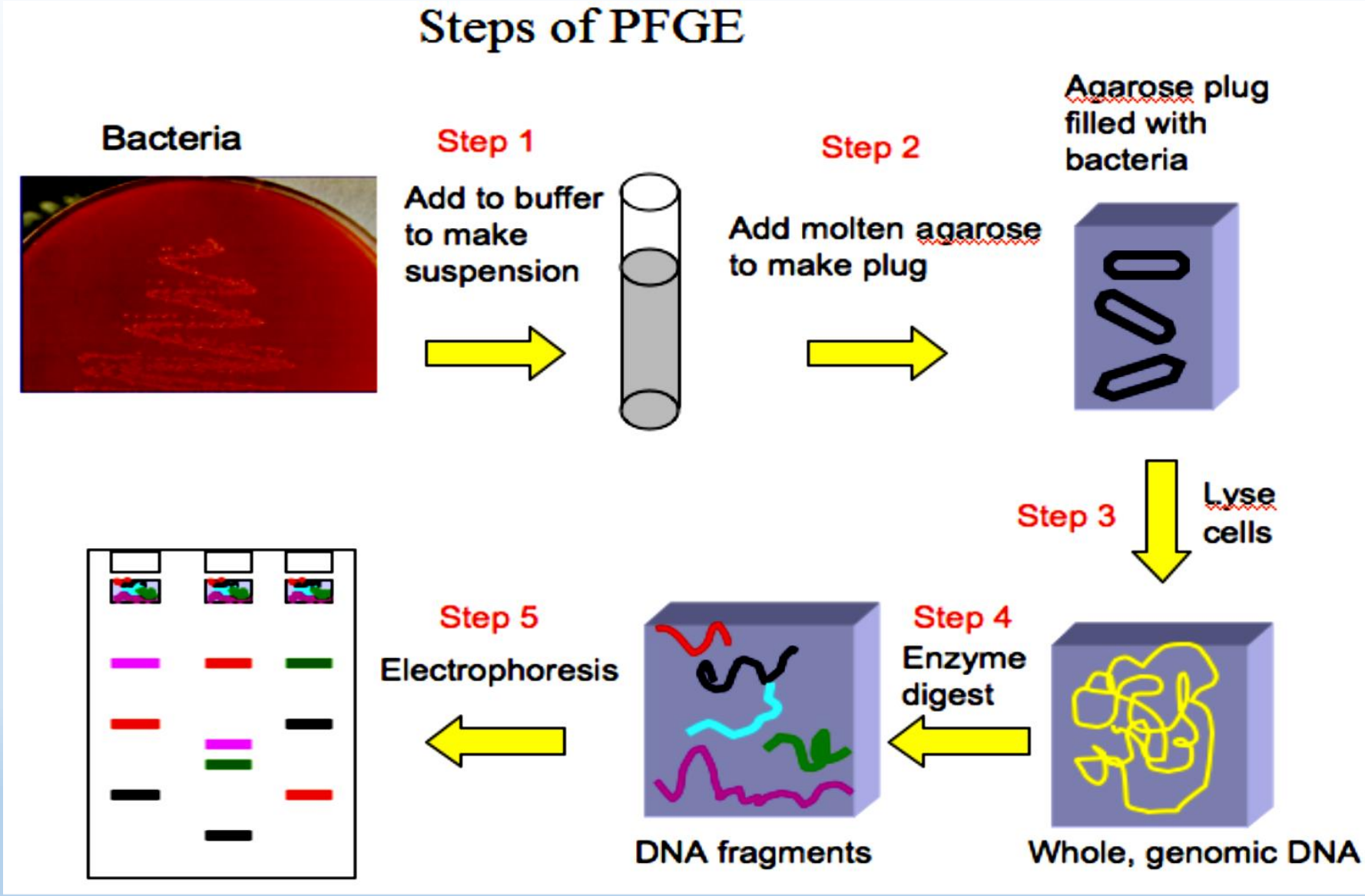
PFGE gels are loaded with DNA samples that are imbedded within agar blocks to minimize random breakage of large molecules.



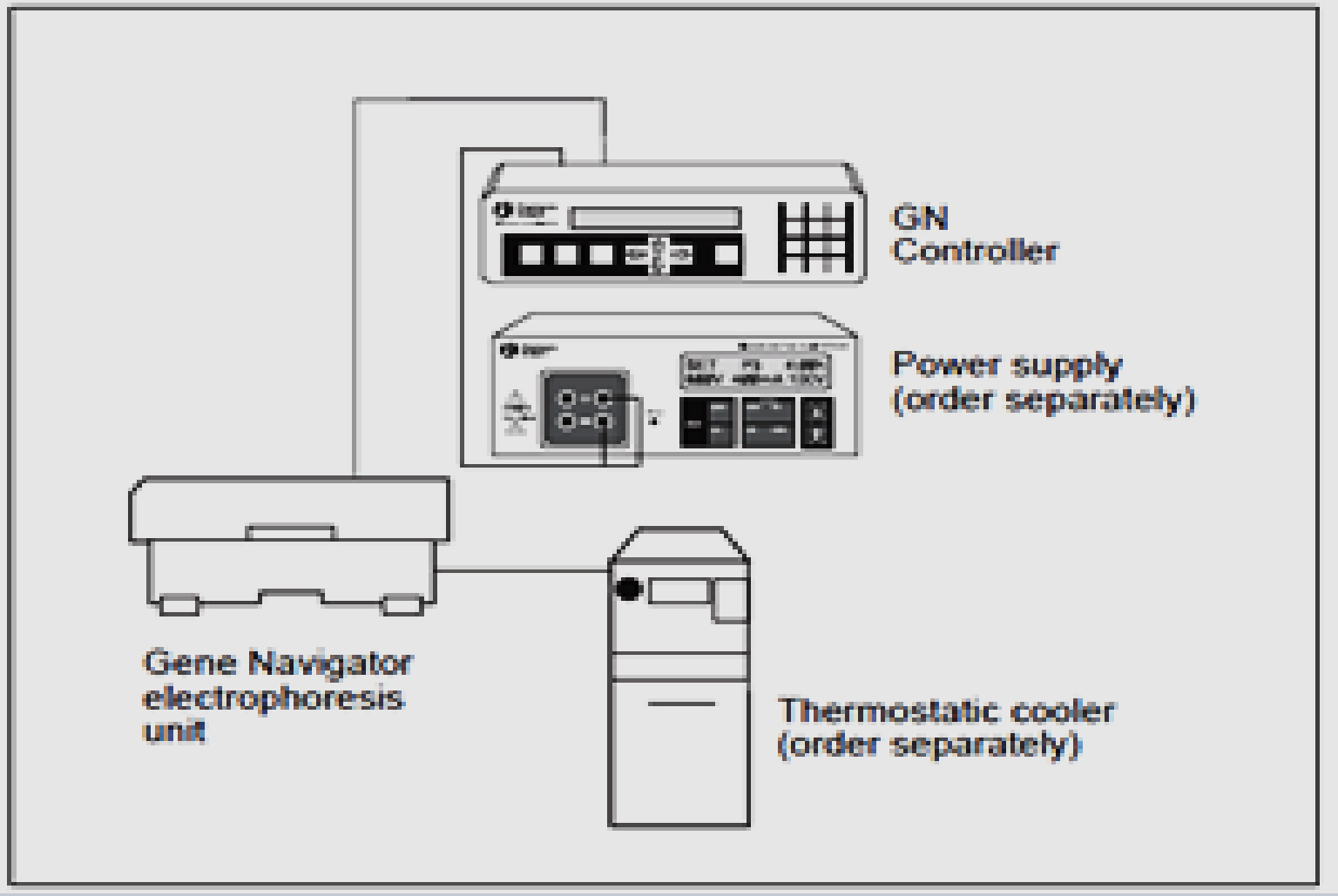
Electric field alternates 120° every 90 seconds for 18 to 24 hours at 14° C



Steps of PFGE

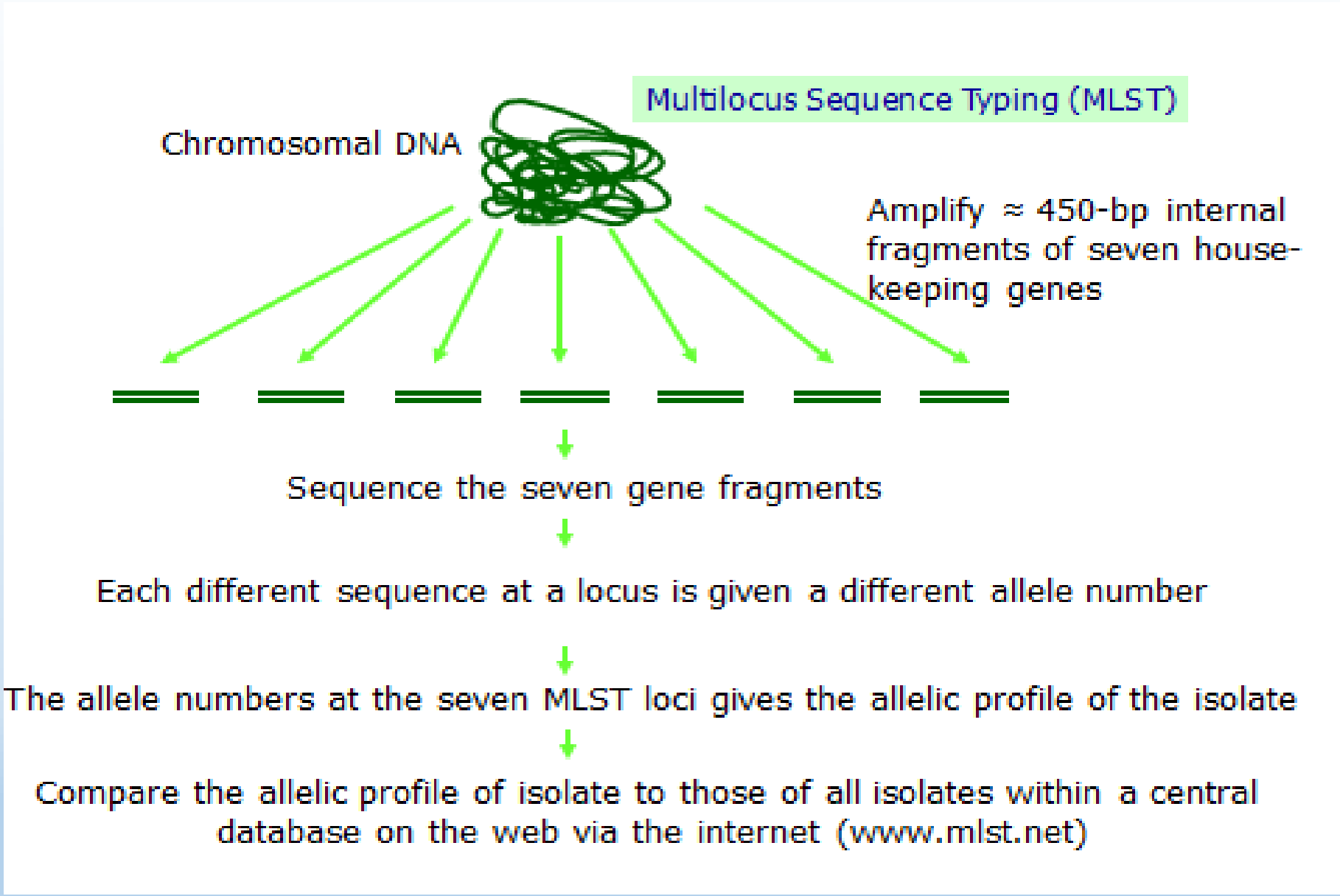


Major Components

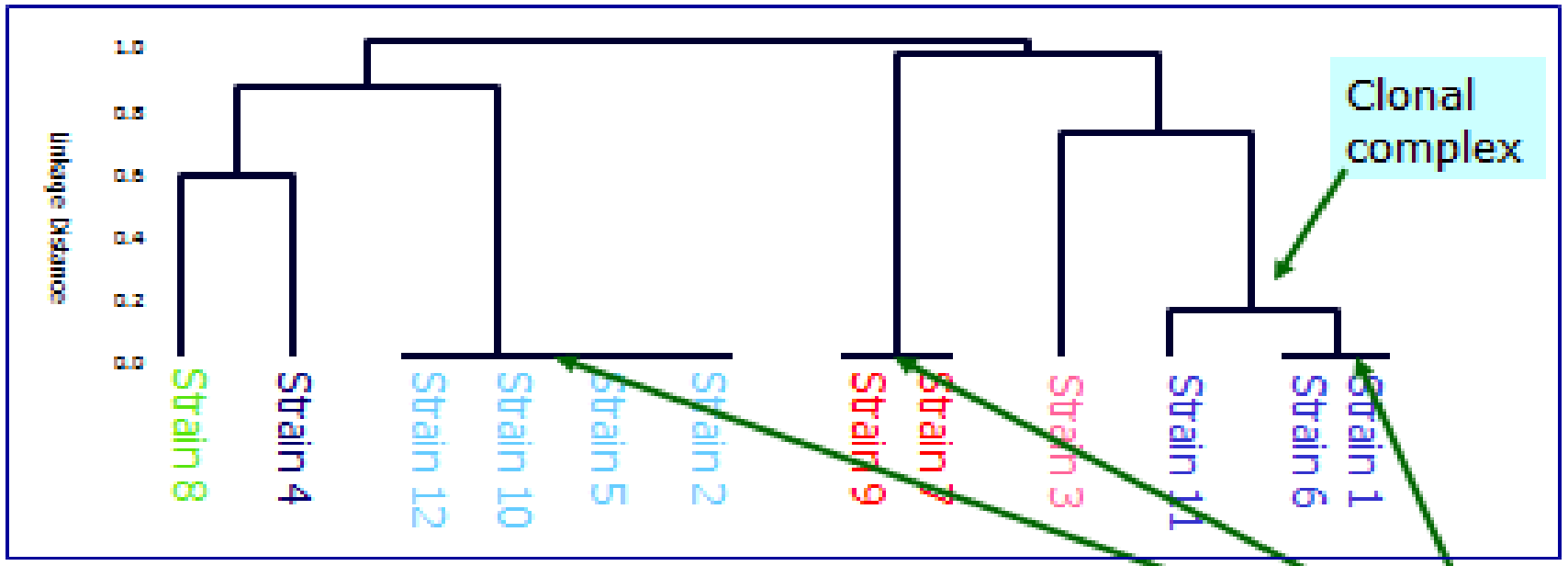


MLST

- Bsed on the sequencing of seven “housekeeping” genes, allows more precise identification of a particular strain and ready comparison of results between different laboratories.
- In molecular biology, Housekeeping genes are typically constitutive genes that are required for the maintenance of basic cellular function
- seven housekeeping genes are used in MLST typing of MRSA. These genes include carbamate kinase (arcC), shikimate dehydrogenase (aroE), glycerol kinase (glpF), guanylate kinase (gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi) and acetyl coenzyme A acetyltransferase (yqiL)
- MLST has provided valuable in the national and international epidemiology of MRSA but lacks the discriminatory power for investigating local epidemic strains such as EMRSA-15 or -16


Multilocus Sequence Typing (MLST)
Chromosomal DNA
Amplify ≈ 450-bp internal fragments of seven house-keeping genes
Sequence the seven gene fragments
Each different sequence at a locus is given a different allele number
The allele numbers at the seven MLST loci gives the allelic profile of the isolate
Compare the allelic profile of isolate to those of all isolates within a central database on the web via the internet (www.mlst.net)

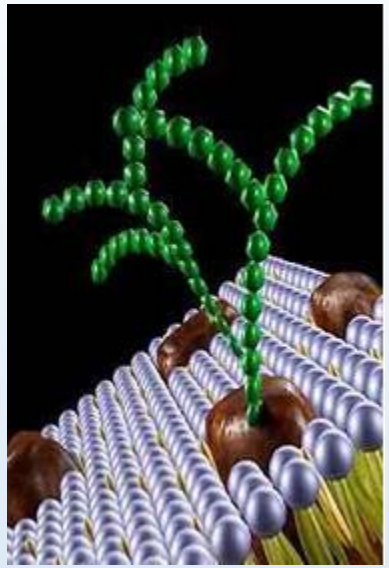
Dendrogram based on allelic mismatches between the allelic profiles

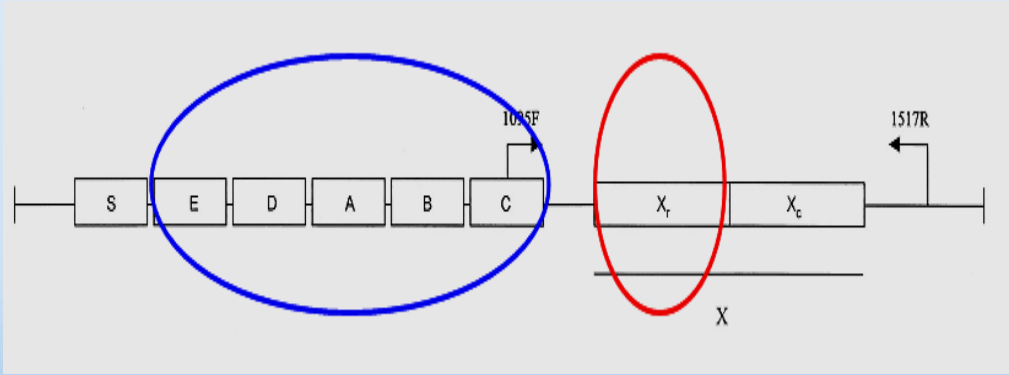


Strain 1	1	5	3	7	2	8	4
Strain 2	11	15	30	12	22	11	20
Strain 3	6	5	9	1	2	13	17
Strain 4	9	10	1	19	12	18	14
Strain 5	11	15	30	12	22	11	20
Strain 6	1	5	3	7	2	8	4
Strain 7	20	11	29	9	21	13	11
Strain 8	9	15	13	27	22	18	14
Strain 9	20	11	29	9	21	13	11
Strain 10	11	15	30	12	22	11	20
Strain 11	1	5	3	5	2	8	4
Strain 12	11	15	30	12	22	11	20

Staphylococcal Protein A (spa) typing

- Protein A or Jensen's antigen A, is a surface protein consists of approximately 2,150 base pairs
- Staphylococcal protein A is well known for its ability to interact directly with the Fc region of immunoglobulins of most mammalian species
- The C- terminal part, also called region X or short sequence repeat (SSR) region contains a polymorphic repetitious (21-24 bp in length) The sequence variation in the X region can arise from both duplications and deletions of repetitive units or by point mutations within the repeat sequence





spa repeat code	DNA sequence										Amino Acid sequence
r01	GGT	GAA	AAC	AAA	GCT	GAA	GAC	AAA	GGC	AAC	GENKAEDKGN
r02	AAA	GAA	AAC	AAA	GCT	GAA	GAT	AAA	GGC	AGC	KENKAEDKGS
r03	AAA	GAA	GAC	AAA	GCT	GAA	GAT	AAA	GGC	AGC	KEDKAEDKGS
r04	ATA	GAA	GAT	AAA	GCT	AAA	GAC	AAA	GAC	AAC	IEDKAKDKDN
r05	AAA	GAA	GGC	AAA	GCT	GCA	GAC	AAA	GGT	ATG	KEGKAADKGM
r06	AAA	GAA	GAT	AAA	GCT	AAA	GAC	AAA	GAC	AAC	KEDKAKDKDN
r07	AAA	GAA	GGC	AAA	GCT	GCA	CAC	AAA	GGT	ATG	KEGKAAHKGM
r08	AAA	GAA	GGC	AAA	GCT	GCA	AAC	AAA	GGT	ATG	KEGKAANKGM
r09	AAA	GGC	AAC	AAA	GCT	GAA	GAT	AAA	GGC	AGC	KGNKAEDKGS
r10	AAA	GAA	GAC	AAA	GCT	GAA	GAT	AAA	GGC	AAC	KEDKAEDKGN
r11	AAA	GAA	GAC	CAA	GCT	GAA	GAT	AAA	GGC	AGC	KEDQAEDKGS
r12	AAA	GAA	AAC	AAA	GCT	GAA	GAC	AAA	GGC	AAC	KENKAEDKGN
r13	AAA	GAA	GAT	AAA	GCT	GAA	GAT	AAA	GGC	AGC	KEDKAEDKGS
General Consensus	AAA	GAA	GAC	AAA	GCT	GAA	GAC	AAA	GGC	AXC	KEXKAEDKGX
Motif 1	AAA	GAA	XXX	AAA	GCT	XAA	XXX	AAA	GXX	XXX	
Motif 2	XXX	GAA	XXX	AAA	GCT	XAA	GAC	AAA	GXX	XXX	

Spa typing procedure

- DNA Amplification
- PCR Purification
- DNA sequencing
- Evaluation of spa typing results

  The spa type is identified with the letter “t” and a unique number, whereas the repeat is identified with a letter “r” and a unique number. If a spa type or repeat is unknown, it is assigned a new local spa type or repeat in the database beginning with the letters “tx” or “rx”.
- Staph-Type software are Kreiswirth-Ridom or Ridom

spaTyper

Enter sequence or sequences in FASTA format:

```
GAGAAACAACAAGCCTGGAAAGAAGACAACAACAAGCCTGGTAAAGAA
GACGGCAACAAACCTGGTAAAGAAGACAACAAAAAACCTGGCAAAGAA
GATGGCAACAAACCTGGTAAAGAAGACGGCAACAAGCCTGGTAAAGAA
GATGGCAACAAGCCTGGTAAAGAAGACGGCAACGGATACATGTCGTTA
AACCTGGTGATACAGTAAATGACATTGCAAAAGCAAACGGCACTACTG
CTG
```

or file in FASTA format:

Browse...

Submit Reset

Results

Inspect checked | Plain text

sequence	start pos[1]	repeat units[2]	len in bp[3]	repeat seq[4]	ridom type[5]
seq1	68	6	144	G1:K1:A1:O1:M1:Q1 12:16:02:25:17:24	t932

The End