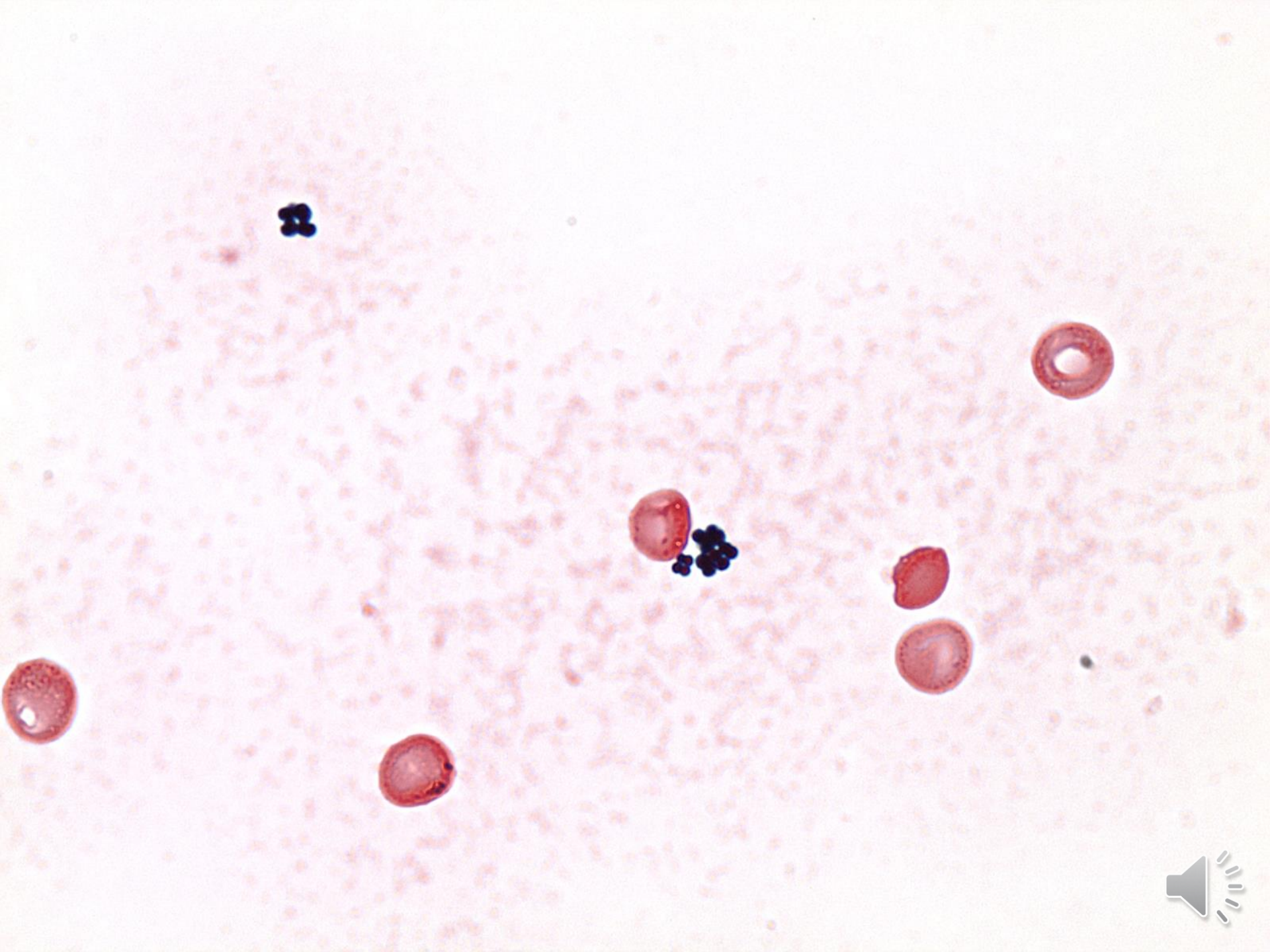
Staphylococcus & Micrococcus

Susan M . Harrington, Ph.D D(ABMM), MLS(ASCP)^{CM}

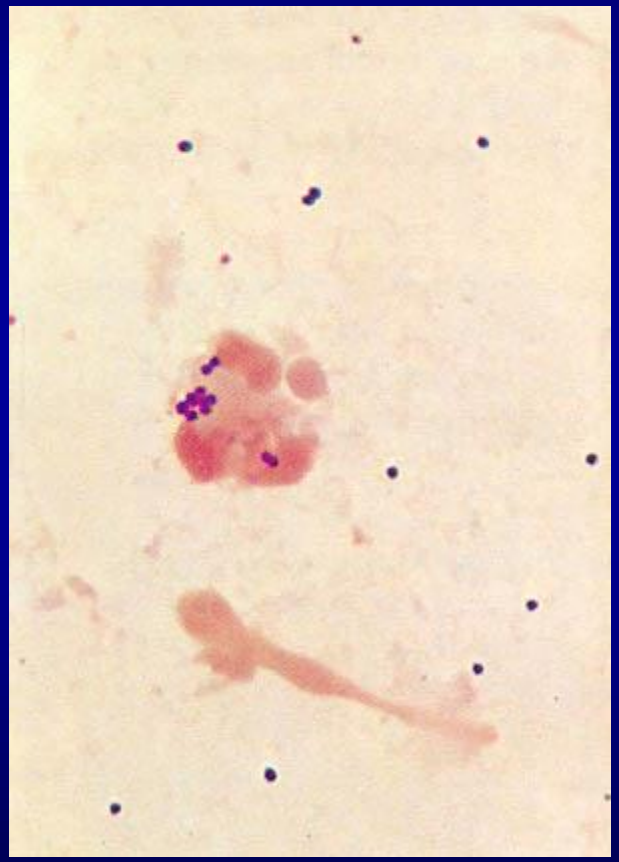
1/8/20

Staphylococcus spp.

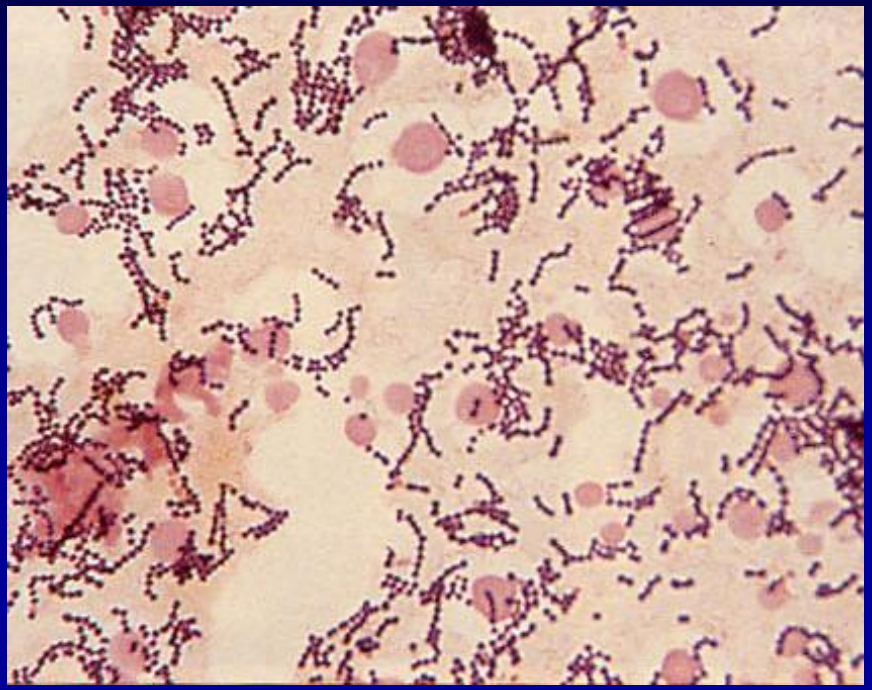
- Gram-positive cocci (GPC)
- Single cocci, pairs, tetrads, clusters
- Aerobic or facultatively anaerobic
- One exception: *S. saccharolyticus* is obligate anaerobe
- Grow in media with high concentration of salt (10% NaCl)
- Catalase positive
- Susceptible to lysostaphin

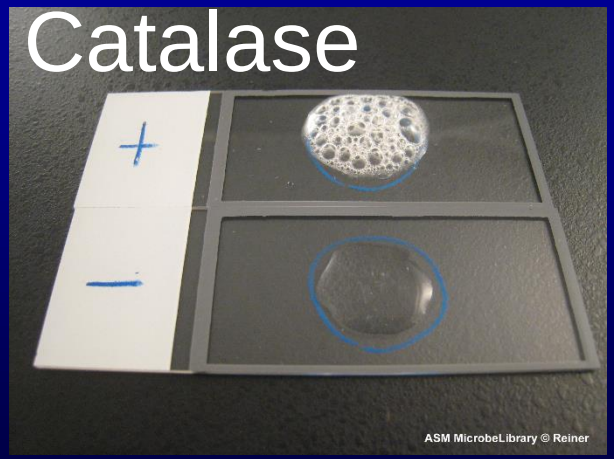


Cocci in single, pairs, clusters; intracellular



Cocci in chains





ASM Image Library

Staphylococcus spp.

- 49 species and 25 subspecies
- Ubiquitous, present on human skin and mucous membranes
- *S. aureus* colonization may be assessed with cultures:
  - anterior nares
  - umbilicus
  - axilla
  - perianal/rectal
  - inguinal folds

Opportunistic Pathogen

Disease most often associated with entry of host tissue through:

- Trauma of cutaneous barriers
- Inoculation with needles
- Direct implantation of medical devices
- Transfer from pt/HCW flora to skin & surgical sites
- Ventilator use

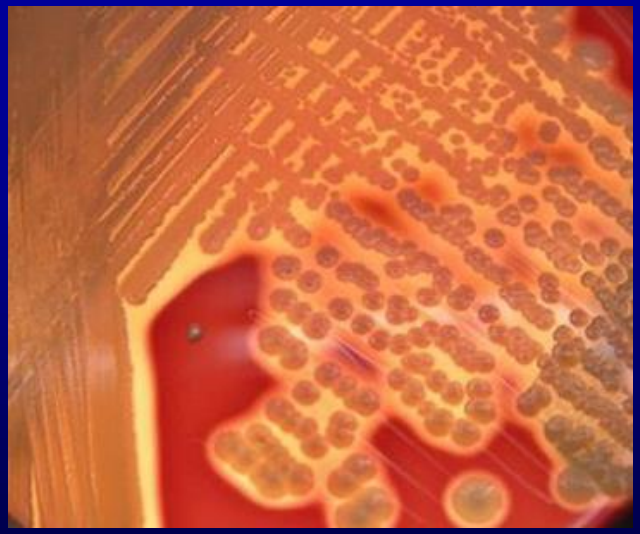
Staphylococcus spp.

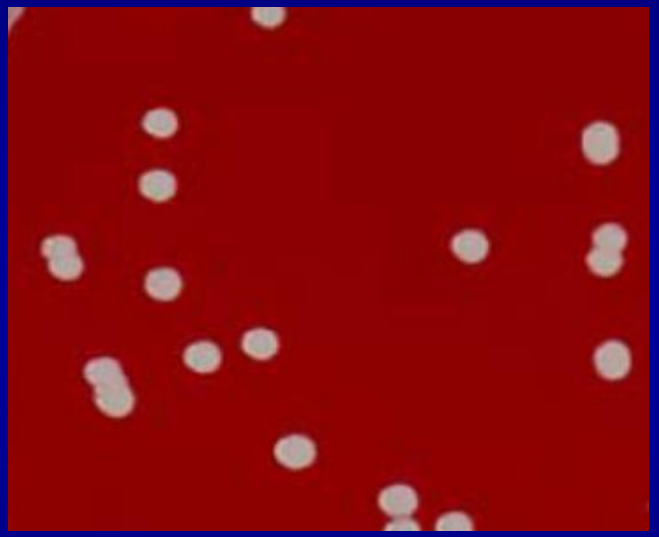
- Opportunistic Pathogen
  - Skin
  - Soft tissues
  - Bones
  - Urinary tract
  - Pneumonia
  - Device related infections

Staphylococcus spp. most common associated with human disease

- *S. aureus*
  - coagulase positive
  - most virulent
- Coagulase-negative staphylococci
  - *S. epidermidis*
  - *S. lugdunensis*
  - *S. saprophyticus*
  - *S. hominis*
  - *S. capitis*
  - *S. haemolyticus*

S. aureus vs. Coag-neg staph



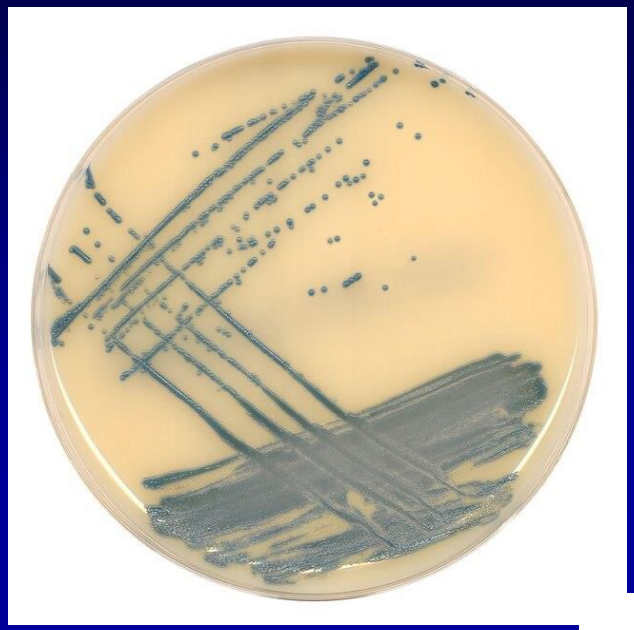


Mannitol-salt agar

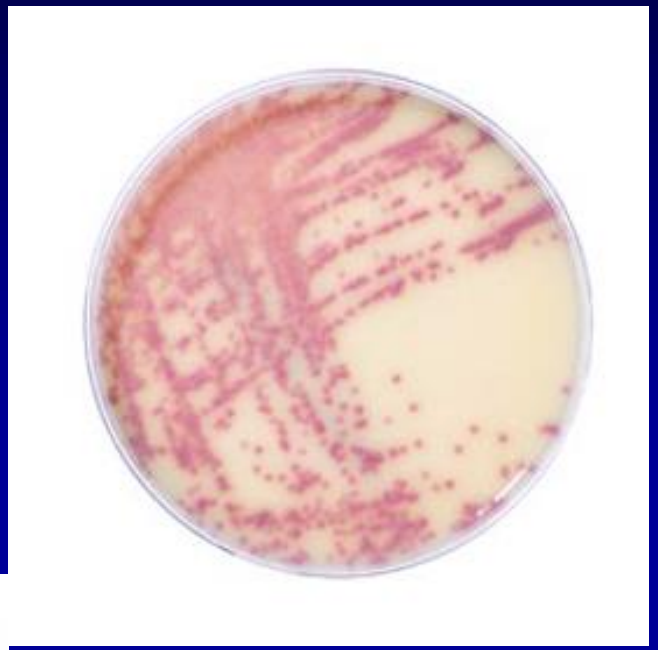
- 7.5% NaCl
- Recover *S. aureus* from contaminated specimens
- *S. aureus (*and *S. saprophyticus)* ferments mannitol into acid (pH indicator red to yellow)

http://cmgm.stanford.edu/micro

Commercial ChromAgars



Remel
Spectra
MRSA



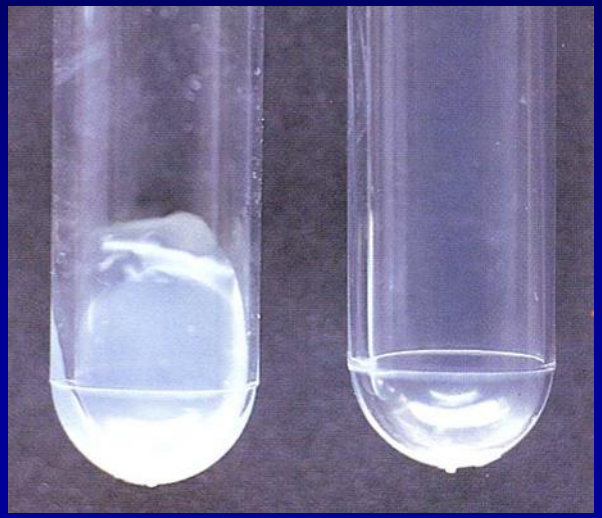
bioRad
MRSA
Select

Images from
manufacturer
websites

CHROMAgar
Staph aureus

Tube Coagulase

- Detects extracellular, free coagulase
- *S. aureus* is pos; considered definitive test
- A few other species assoc w/animals also pos e.g. *S. intermedius*
- observe after 4 h & 18 h incub at 37°C
- false neg staphylokinase



S. aureus: Slide Coagulase (clotting of plasma)

- Detects cell wall bound coagulase (i.e., clumping factor)
- Converts fibrinogen in rabbit plasma to fibrin
- *S. aureus* & some *S. lugdunensis* & *S. schleiferi* are positive
- Not definitive

Latex Agglutination

- Latex test: detects clumping factor & protein A
- Latex particle coated with fibrinogen & IgG

Staphylococci
Abbreviated Biochemical Classification

Species	Colony Pigment	β-Hemolysis	Clumping factor	Coagulase	Heat-stable nuclease	Alkaline phosphatase	PYR	Ornithine decarboxylase	Urease	β-Galactosidase	Novobiocin resistance	Polymyxin B resistance	Trehalose	Mannitol
S. aureus	+	+	+	+	+	+	-	-	d	-	-	+	+	+
S. epidermidis	-	-	-	-	-	+	-	(d)	+	-	-	+	-	-
S. haemolyticus	d	+	-	-	-	-	+	-	-	-	-	-	+	d
S. lugdunensis	d	-	(+)	-	-	-	+	+	d	-	-	d	+	-
S. schleiferi	-	-	+	-	+	+	+	-	-	(+)	-	-	d	-
S. saprophyticus	d	-	-	-	-	-	-	-	+	+	+	-	+	d

Example Automated Systems for Identification

bioMerieux
Vitek 2

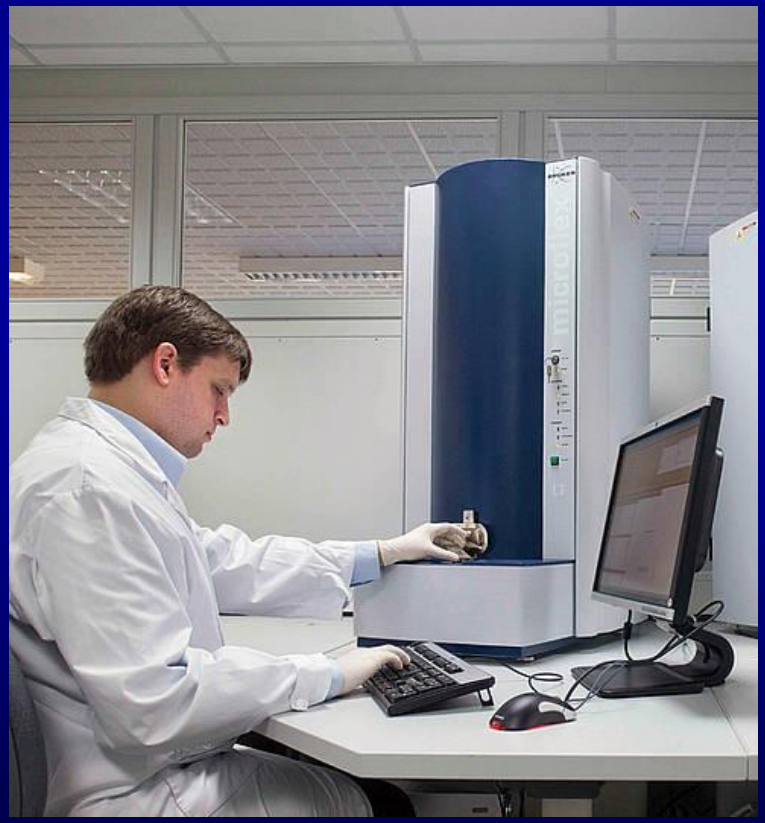
BD Phoenix

bioMerieux
API

Tools for Identification
MALDI TOF MS

Matrix Assisted Laser Desorption Ionization –
Time of Flight Mass Spectrometry

Bruker Biotyper

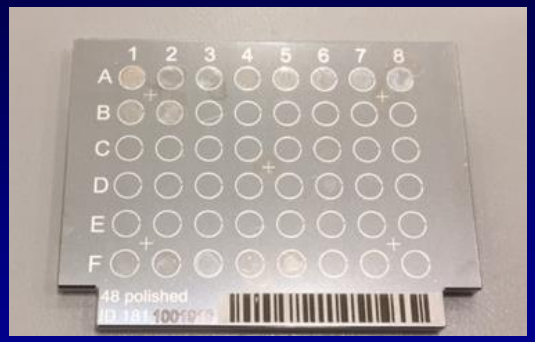


Bruker Daltonics at www.bdal.com

VITEK MS

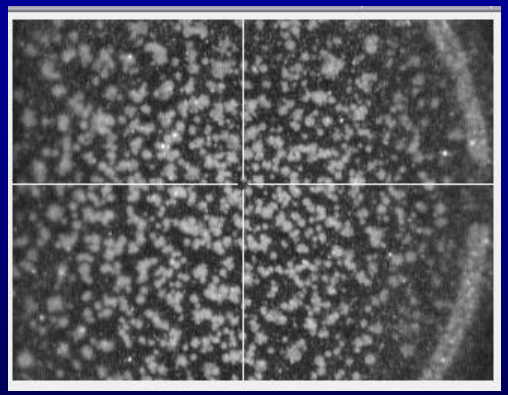
Photos courtesy of bioMérieux, Inc.

MALDI TOF MS



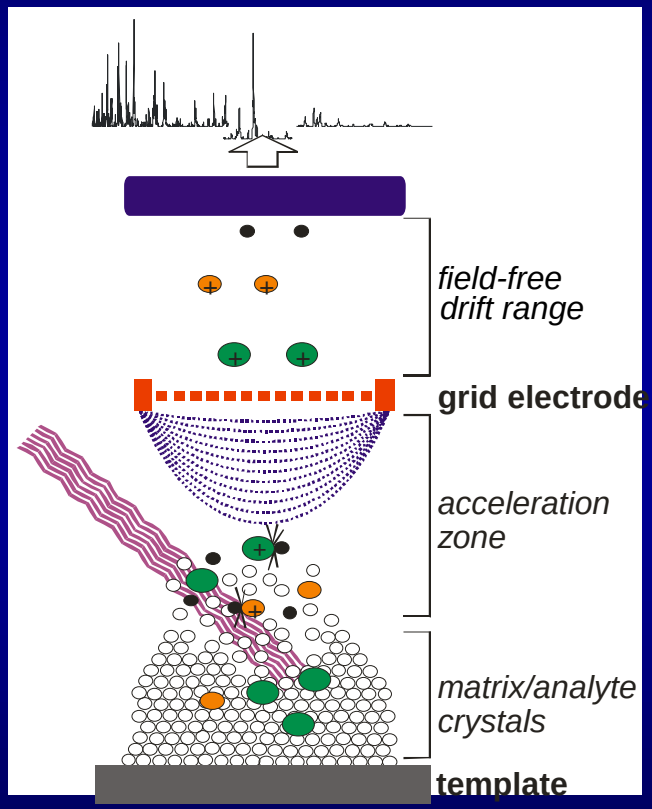
1. Organism is smeared onto target well. Matrix overlay

2. Target is placed into instrument.



3. Laser fires. Camera Image of spot as laser fires.

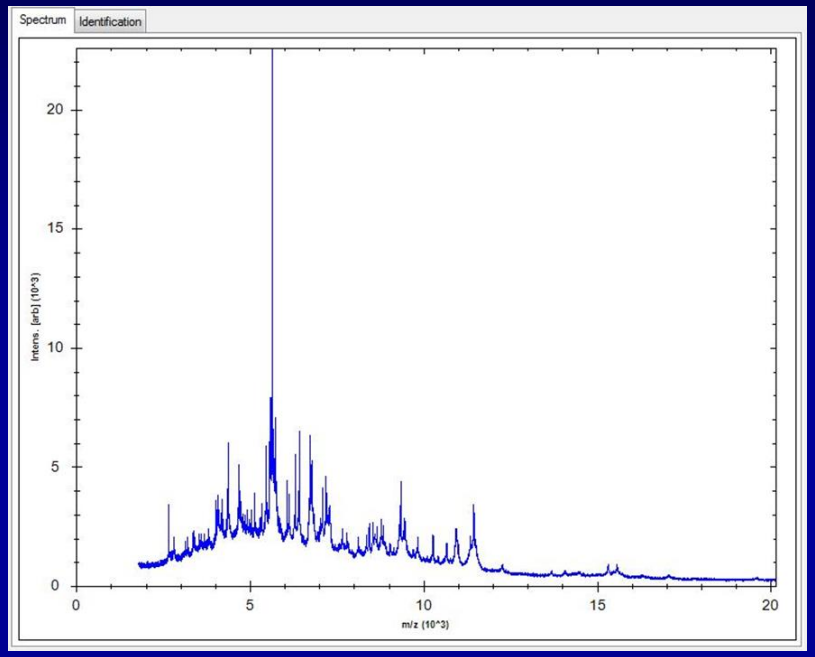
4. Ionization & separation



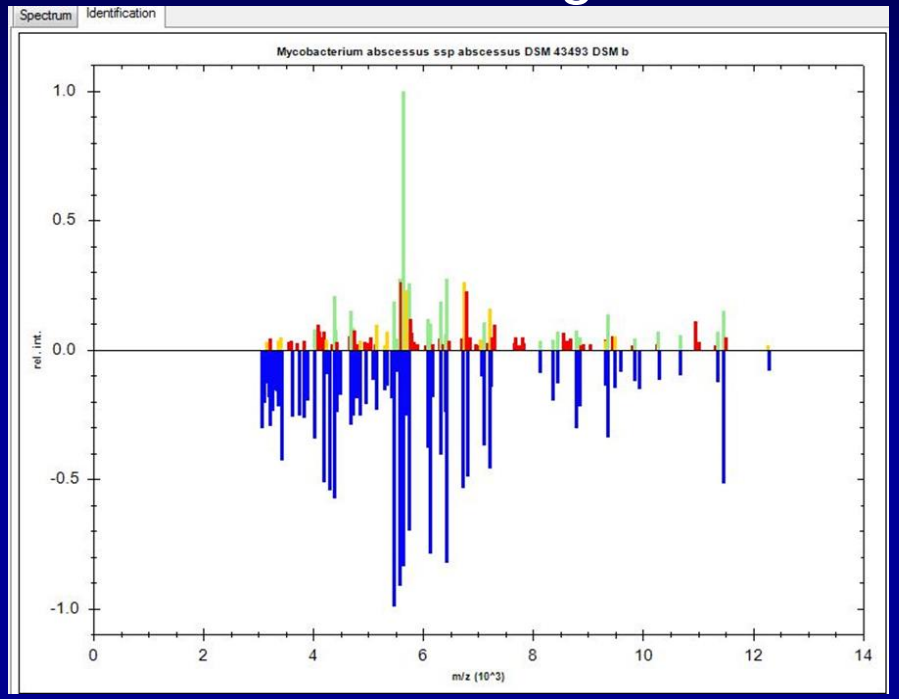
Courtesy bioMerieux

Analysis of Spectra (Bruker)

Raw spectrum



Peak matching



Best Matches in Database (example)

Mix	Detected Species	Log(Score)
	Mycobacterium abscessus ssp abscessus DSM 43493 DS...	2.180
	Mycobacterium abscessus ssp abscessus BER2 DCSS b	2.160
	Mycobacterium abscessus AFB034 MCW b	2.130
	Mycobacterium abscessus ssp bolletii Meche 51906 DCSS b	2.080
	Mycobacterium abscessus NIH S004	2.080
	Mycobacterium abscessus RV423 C I 2011 MVD b	2.030
	Mycobacterium abscessus NIH ATCC 19977T	1.990
	Mycobacterium abscessus CCUG 55245 CCUG b	1.980
	Mycobacterium abscessus ssp massiliense DSM 45103T D...	1.980
	Mycobacterium abscessus NIH S005	1.960

S. aureus: Virulence factors

- Capsule
- Protein A (binds IgG, prevents clearance)
- Cytotoxins (Panton-Valentine leukocidin)
- Exfoliative toxins (ETA, ETB) - SSSS
- Enterotoxins – food poisoning
- Toxic shock syndrome toxin-1 (TSST-1)
- Coagulase
- Hyaluronidase
- Fibrinolysin (staphylokinase)

Disease Manifestations

- Toxin Mediated
  - Food poisoning, SSSS, TSS
- Skin Infections
  - Furuncles, Cellulitis, Impetigo, SSI
- Infections of Deep sites
  - BSI, Osteomyelitis, Arthritis, Endocarditis
- Pneumonia
- Infections of the Urinary Tract
  - (descends from blood)

Staphylococcal Food Poisoning

- Symptoms: Nausea, vomiting, abdominal pain & diarrhea
- Incubation time: 2-8 hrs; resolves in 24-48 hrs
- Ingestion of heat-stabile enterotoxin (Staph enterotoxin esp. A,B,D)
- Associated foods:
  - Salads such as egg, tuna, chicken, potato, and macaroni
  - Meats, esp. w/high salt content
  - Poultry and egg products
  - Bakery products such as cream-filled pastries & pies
  - Milk and dairy products

S. aureus: Toxic-shock-syndrome

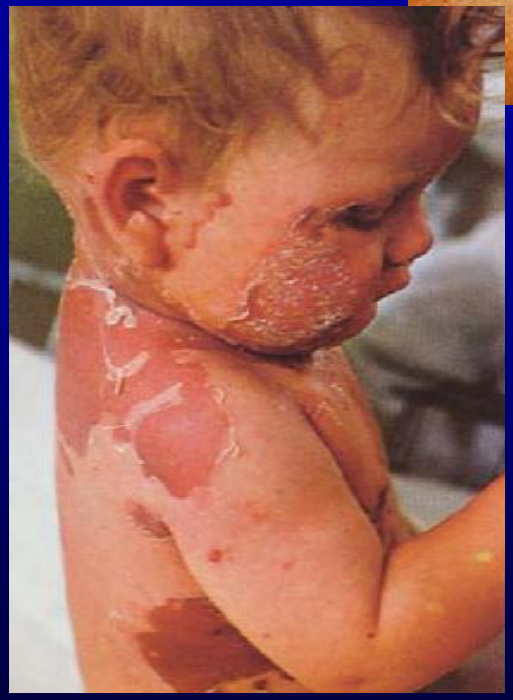
Local colonization/infection with systemic, toxin-mediated disease; associated w/high absorbancy tampons

Symptoms: high fever, vomiting, diarrhea, dehydration, edema, rash (trunk, but spreads) that desquamates. Can lead to hypotension, hypoalbuminemia, shock

SuperAg Toxin: TSST-1

Scalded Skin Syndrome

Blisters are negative for *S. aureus*



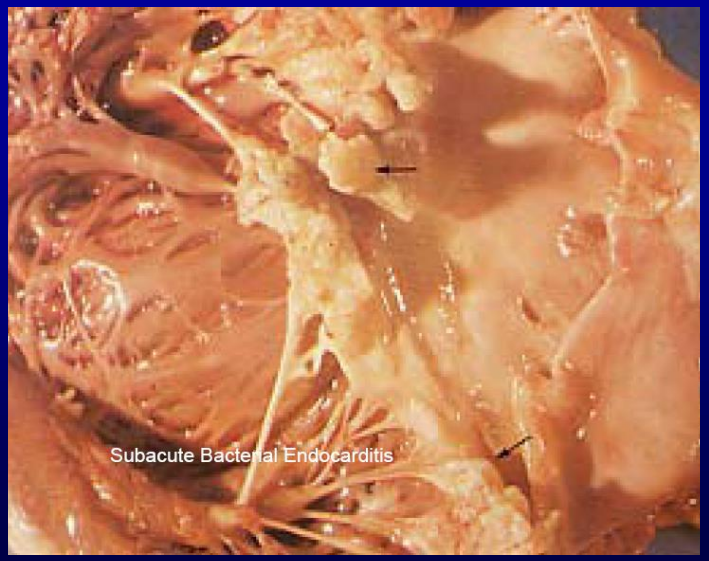
- Mostly seen in neonates and small children.
- Exfoliative toxins; ETA & ETB
- Fragile blisters which rupture and lead to skin loss.
- Associated with secondary infections.
- Usually self-limited, wanes in ~5 days.

Skin and Soft Tissue Infections

- Folliculitis
- Boil/Furuncle
- Carbuncle
- Impetigo
- Cellulitis
- Erysipelas
- Paronychia
- Wounds

Deep Infections

- BSI – primarily nosocomial; catheters
- Osteomyelitis
- Septic arthritis
- Prosthetic devices
- Endocarditis



Pneumonia

- VAP
- CAP

S. lugdunensis

- Skin/soft tissue infections
- Endocarditis
- Arthritis
- Bacteremia
- UTIs
- other

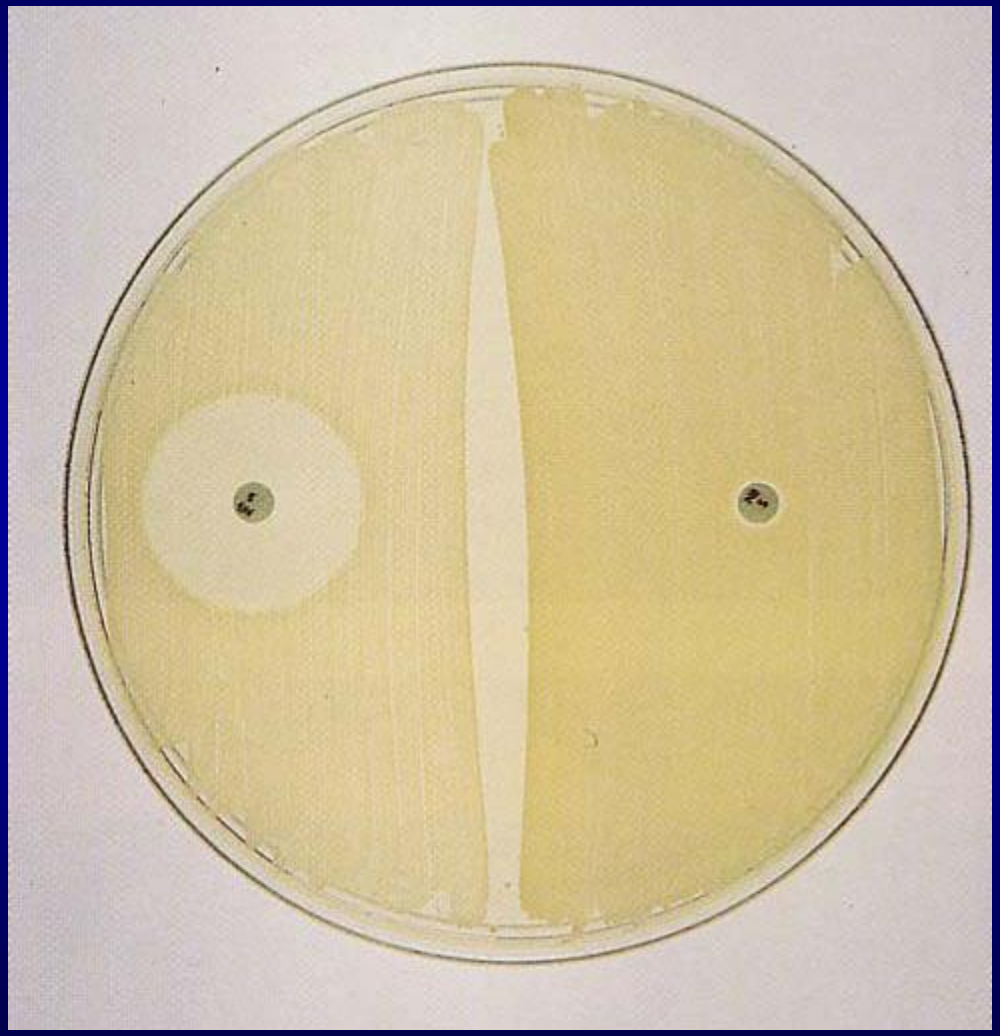
Dermatol Ther (Heidelb). 2017 Dec; 7(4): 555–562.

Identification:
Ornithine decarboxylase positive
PYR positive
May have positive clumping factor rxn

S. saprophyticus

- UTIs (2nd most common cause of uncomplicated cystitis in young women)
- Opportunistic infections
- Novobiocin resistant

S. saprophyticus: Novobiocin R (zone of inhibition ≤ 16 mm)



S. epidermidis & other CoNS

- Bacteremia
- Endocarditis
- Surgical wounds
- UTIs
- Bone and joint infections

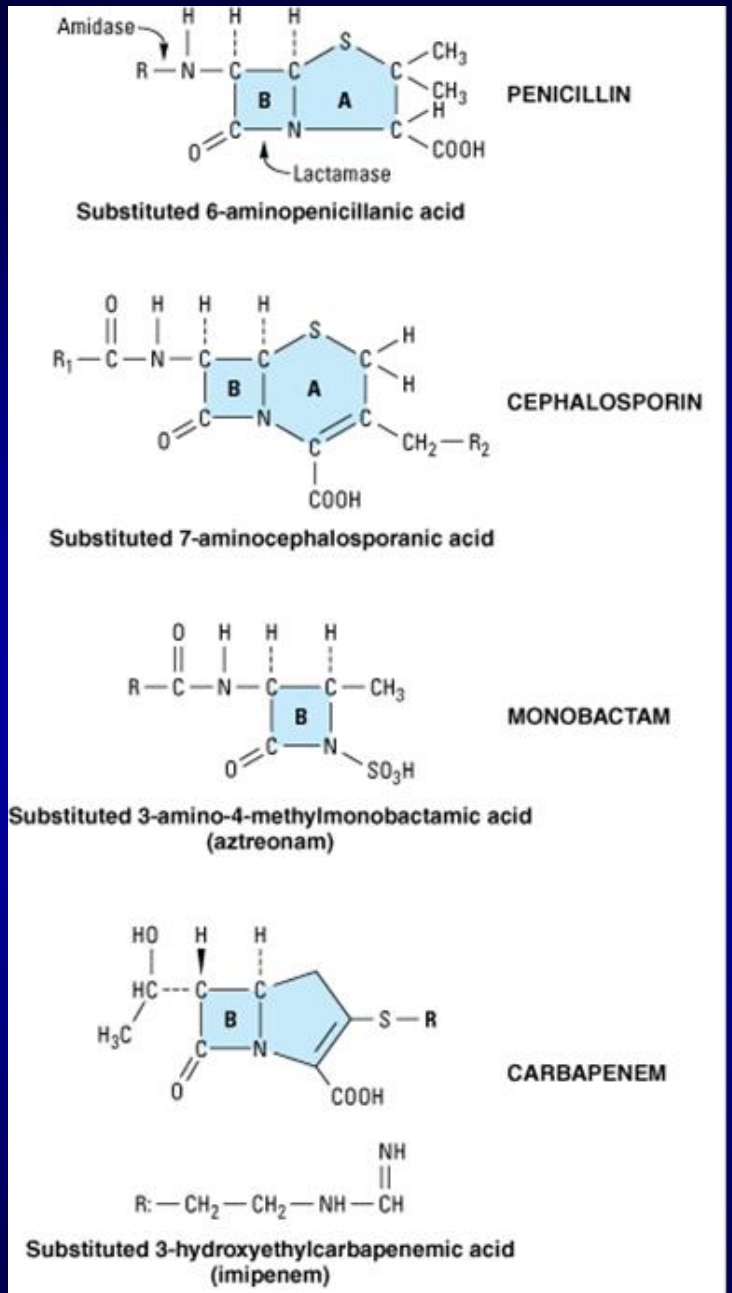
Opportunistic Infections:

- Catheters
- Shunts
- Prosthetic devices
- Peritoneal dialysates

THERAPY AND ANTIBIOTIC RESISTANCE IN STAPHYLOCOCCI

Beta-lactam antibiotics

- Bind to penicillin binding proteins in cell wall to kill bacteria by preventing cell wall synthesis.
- Most *Staph spp.* are resistant to penicillin due to presence of penicillinase.
- For >90% of Staph penicillin is not effective. Treat w/penicillinase-resistant penicillin or cephalosporin.



Source: Katzung BG: *Basic & Clinical Pharmacology*, 10th Edition: http://www.accessmedicine.com

Penicillins

- Penicillin

Penicillinase-resistant:

- oxacillin
- methicillin
- nafcillin
- dicloxacillin
- ampicillin
- amoxicillin
- amoxicillin-clavulanate
- ampicillin-sulbactam
- piperacillin-tazobactam

Cephalosporins

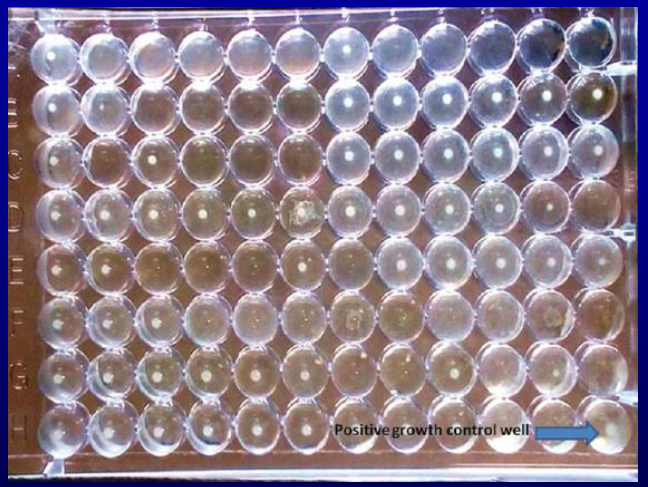
- cefazolin
- cephalexin
- cefuroxime
- cefotaxime
- cefoxitin

And many more

Some given oral; some IV. May be recommended for different types of infections.

Susceptibility Testing

- Antibiotics are usually part of a panel.
- Each Staph is tested against the panel.
- The Staph is reported as Susceptible (S), Intermediate (I) or Resistant (R) to each drug.



Antibiotics Tested for Staphylococci
(example)

- √ Cefoxitin
- Clindamycin
- Doxycycline
- Erythromycin
- Gentamicin
- Linezolid
- Nitrofurantoin
- √ Oxacillin
- Rifampin
- Trimethoprim/sulfamethoxazole
- Tetracycline
- Vancomycin

MRSA/ORSA
Methicillin/Oxacillin Resistant Staph aureus

- Cefoxitin and/or oxacillin are used to predict if Staph will be susceptible (S) to penicillins & cephalosporins
- If Staph tests S to cefox and Oxa, usually treat with a penicillinase-resistant penicillin
- If Staph tests R to cefox or oxa this is MRSA.
- Usually treat with vancomycin, linezolid, daptomycin or ceftaroline. Penicillins & cephalosporins will not be active.

MRSA = ORSA

- Mechanism of resistance is *mecA* gene.
- *mecA* causes Staph to produce an altered penicillin-binding protein (PBP2a or PBP2').
- PBP2a in cell wall will not bind the penicillin/cephalosporin so the antibiotic cannot kill the bacterium by preventing cell wall synthesis.

Optimized *In Vitro* Antibiotic Susceptibility Testing Method for Small-Colony Variant *Staphylococcus aureus*

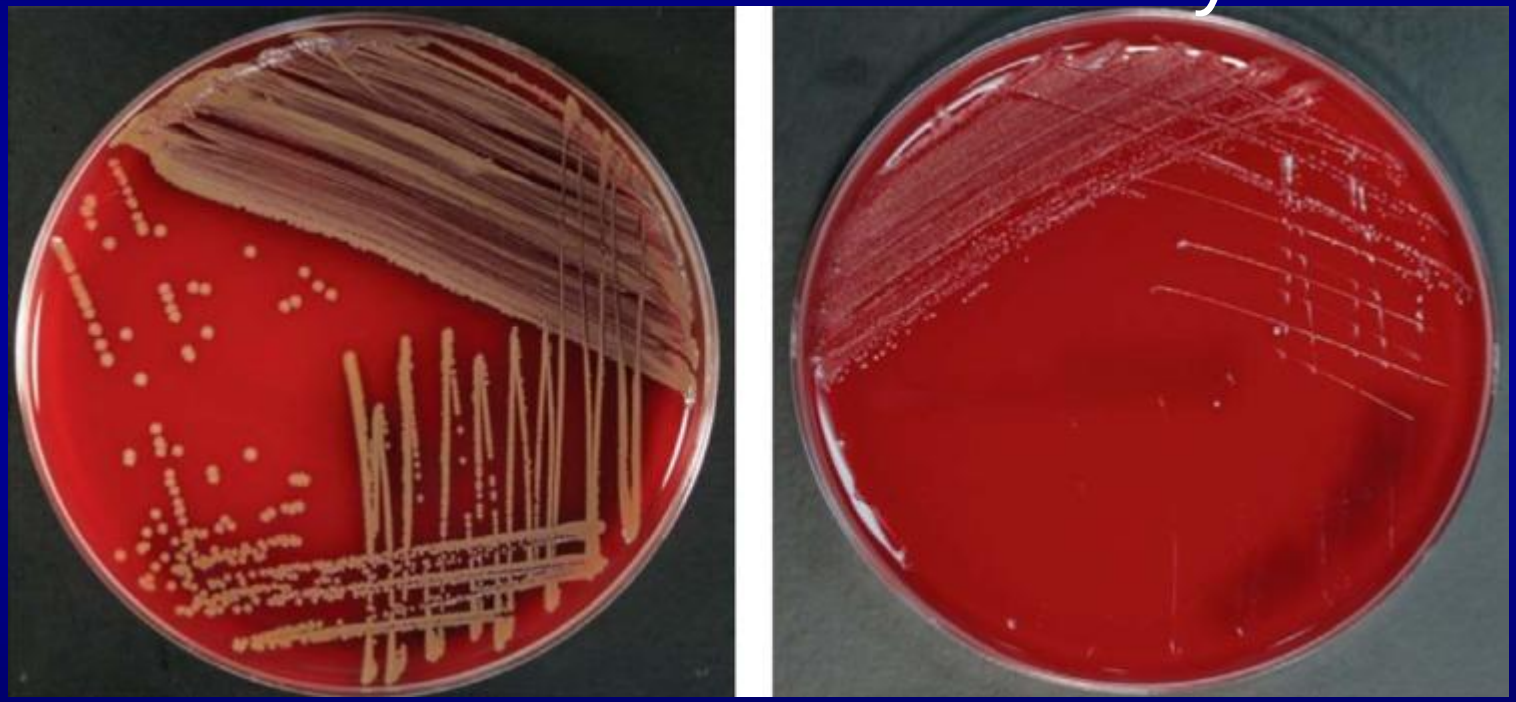
Mimi R. Precit,[a] Daniel J. Wolter,[a,b] Adam Griffith,[b] Julia Emerson,[a,b] Jane L. Burns,[a,b] Lucas R. Hoffman[a,b]

University of Washington, Seattle, Washington, USA[a]; Seattle Children's Hospital, Seattle, Washington, USA[b]

Antimicrob Agents Chemother 2016; 60:1725.

Normal

Small-colony variant (SCV)



- SCVs may require hemin, menadione, or thymidine for optimal growth
- Small colony, reduced hemolysis, decreased pigmentation

Community Acquired MRSA

- MRSA an important hospital-acquired pathogen. (HA- MRSA)
  - Usually resistant to other antibiotics, as well.
- Some acquired in the community.
  - Usually cause skin infection
  - Usually more susceptible to other antibiotics (e.g. trim/sulfa, clindamycin, tetracycline) compared to HA-MRSA.

Cleveland Clinic Antibiogram

Staphylococci	% Susceptible									
Organism (number tested):	Oxacillin[a]	Vancomycin	Linezolid	Daptomycin	Gentamicin	Erythromycin	Clindamycin	Trimethoprim/ Sulfamethoxazole	Doxycycline	Tetracycline
Staphylococcus aureus (5,092)	61	99	99	99	98	42	69	97	97	92
Oxacillin-resistant *S. aureus* (MRSA) (1,981)	0	99	99	99	97	12	59	95	95	89
Oxacillin-susceptible *S. aureus* (MSSA) (3,111)	100	100	99	99	99	64	76	98	98	95
Staphylococcus lugdunensis (381)	93	100	99	99	100	84	84	99	99	94
Staphylococcus capitis (87)	84	100	-	-	93	71	83	99	99	94
Staphylococcus epidermidis (1,234)	44	100	-	-	85	37	60	58	87	82
Staphylococcus haemolyticus (73)	48	100	-	-	74	23	69	67	89	71
Staphylococcus hominis (109)	67	100	-	-	97	33	64	77	93	70
Staphylococcus simulans (44)	84	100	-	-	100	41	43	100	100	98
Staphylococcus warneri (41)	85	100	-	-	100	68	95	98	100	90

[a] Oxacillin-susceptible staphylococci are susceptible to other penicillinase-stable penicillins (e.g., nafcillin, dicloxacillin), ß-lactam/ß-lactamase inhibitor combinations, relevant cephalosporins, and carbapenems.

Micrococcus spp.

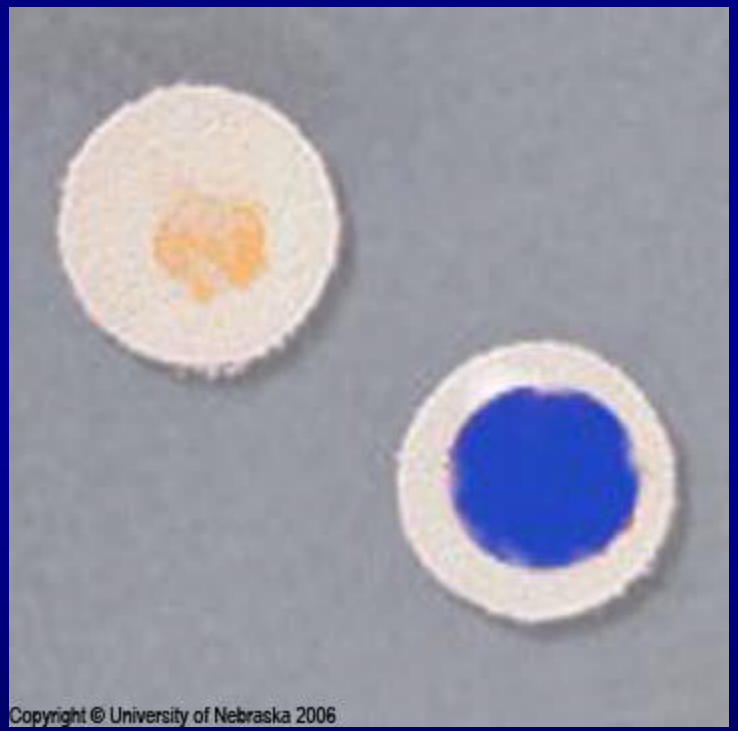
- Aerobic, catalase-positive GPC pairs, clusters, & tetrads
- Strict aerobe
- Modified oxidase positive
- Furazolidone & lysostaphin R
- Bacitracin S
- Usually clinically insignificant contaminants, but may cause opportunistic infections

Micrococcus spp.

- Skin flora (96% of humans, *M. luteus* most common)
- Frequent pigment (yellow, orange, pink-red, cream)
- *Micrococcus* genus
  - *Micrococcus luteus* (Yellow)
  - *Micrococcus lylae* (Nonpigment or cream)
  - Still considered “micrococci”: Other former species now in *Kocuria* genus, *Nesterenkonia* genus, *Dermacoccus* genus, & *Kytococcus* genus (

Microdase disk (modified oxidase test)

- Detects cytochrome C
- *Staphylococcus* spp: negative
- *Micrococcus*: positive

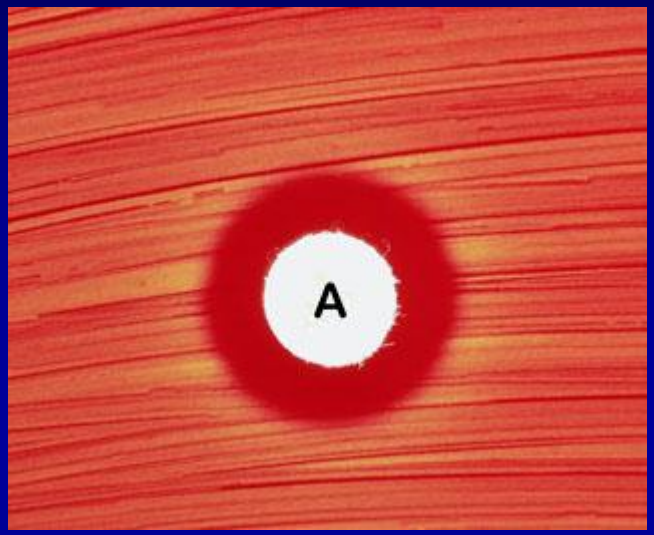


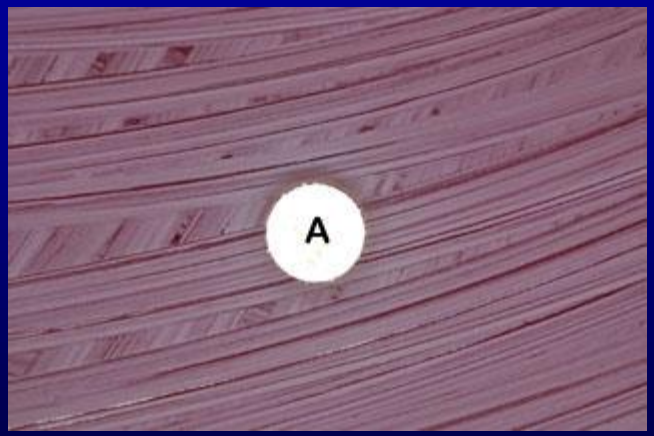
http://webmedia.unmc.edu

Bacitracin disk test

- *Micrococcus* spp. are bacitracin S and do not ferment glucose

- Staphylococci are bacitracin R and ferment glucose

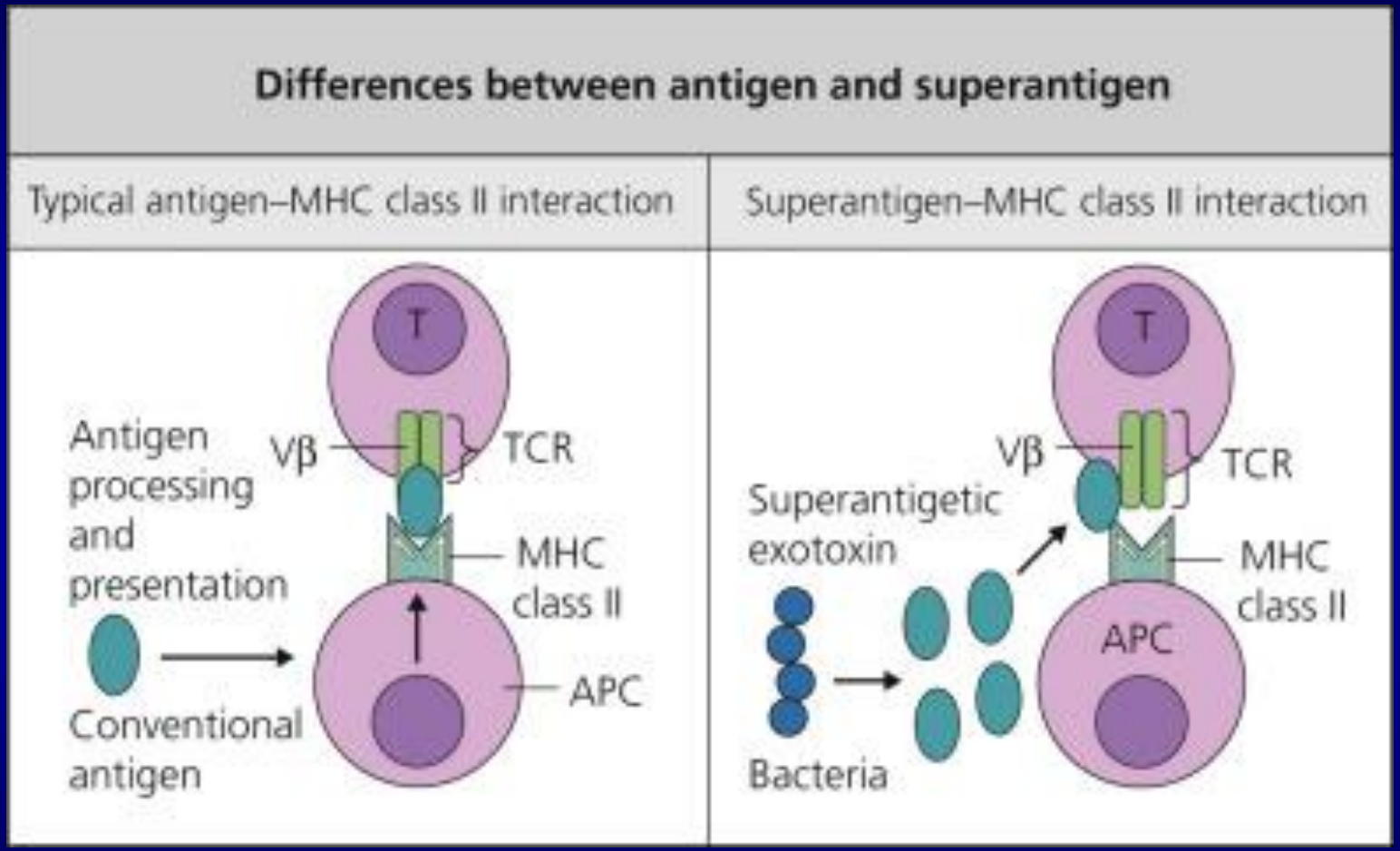




Acknowledgements

- Dr. Kathleen Stellrecht
- Dr. Sandra Richter

Superantigen



Sciencedirect.com/topics/nursing-and-health-professions/superantigen