

Overview of medically important bacteria



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Family *Micrococcaeae*

Staphylococcus (>40 species)

- 0.5-1.5 μm in diameter
- single, pair, tetrad, short chain or irregular grape-like cluster
- facultative anaerobe EXCEPT *S. aureus* subsp. *anaerobius* and *S. saccharolyticus*

Micrococcus (2 species)

- 1-1.8 μm in diameter
- pair, tetrad or irregular cluster
- strictly aerobe

Family *Micrococcaeeae*

Natural habitats

- environment
- skin
- mucous membrane
- mouth
- mammary gland
- gastrointestinal tract
- genitourinary tract
- upper respiratory tract

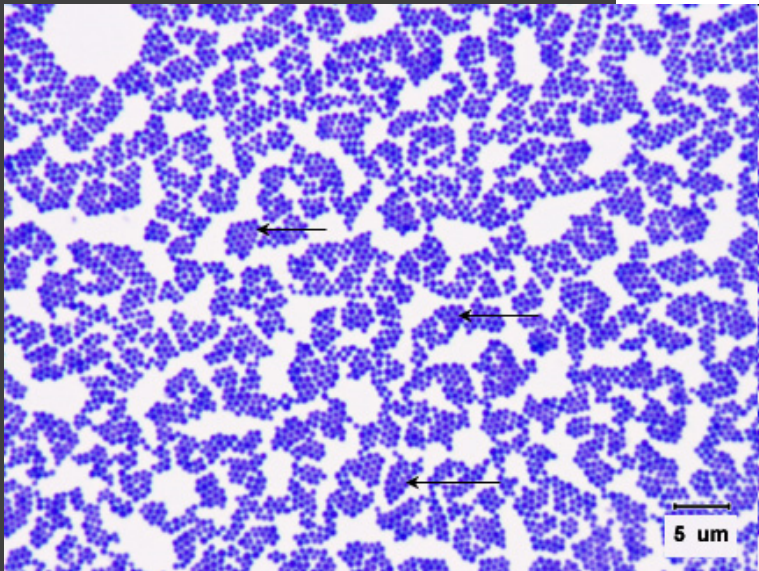
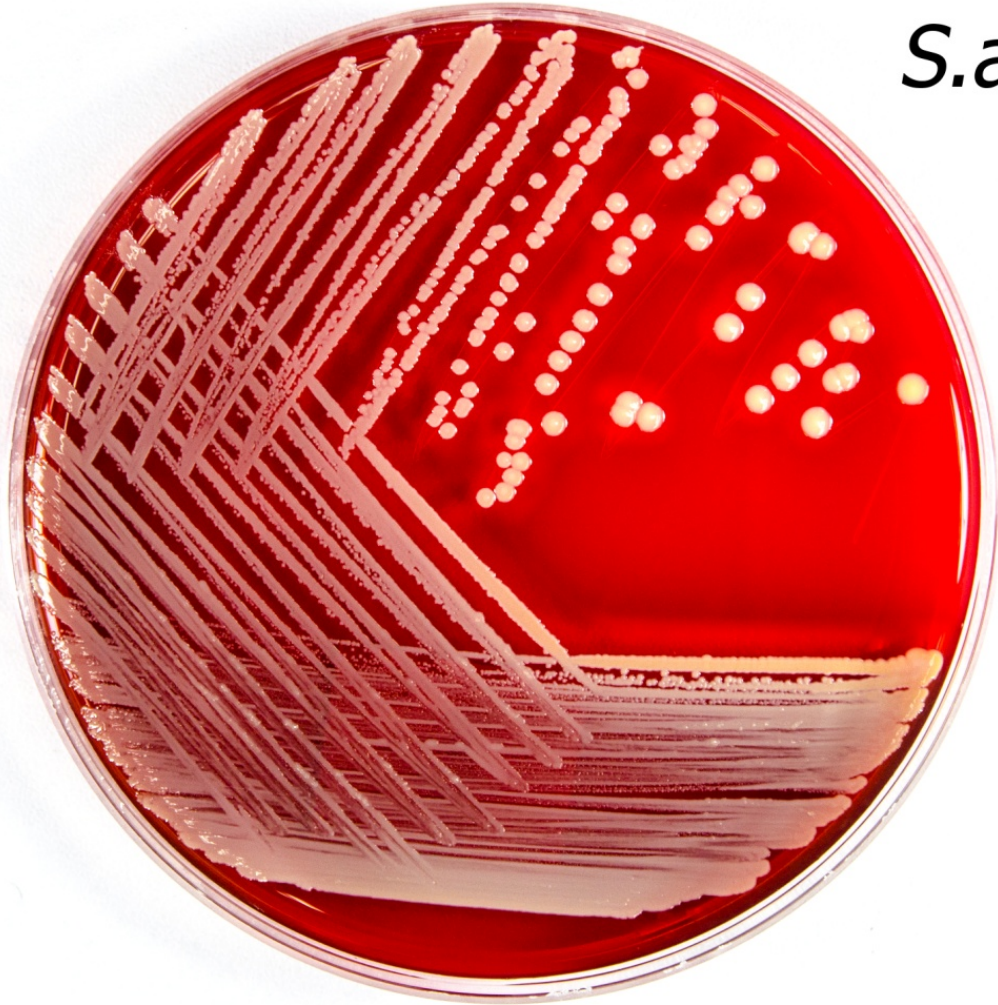
Gram positive cocci, catalase positive, large white to yellow colonies

	colony	Glucose O/F	Bacitracin	Polymyxin B
<i>Staphylococcus</i> spp.	varies	+/+	R	V
<i>Micrococcus</i> spp.	Bright yellow	+/-	S	S
<i>Rothia mucilaginosa</i> *	White, sticky	+/+	S	R

Rothia mucilaginosa : formerly *Stomatococcus mucilaginosus*

Staphylococcus aureus

S.aureus



Staphylococcus aureus

Pneumonia

Meningitis

Sepsis

Acute endocarditis

Acute & pyrogenic localized infection

Scalded skin syndrome



epidermolytic toxin

Toxic shock syndrome



TSST-1 toxin

Food poisoning

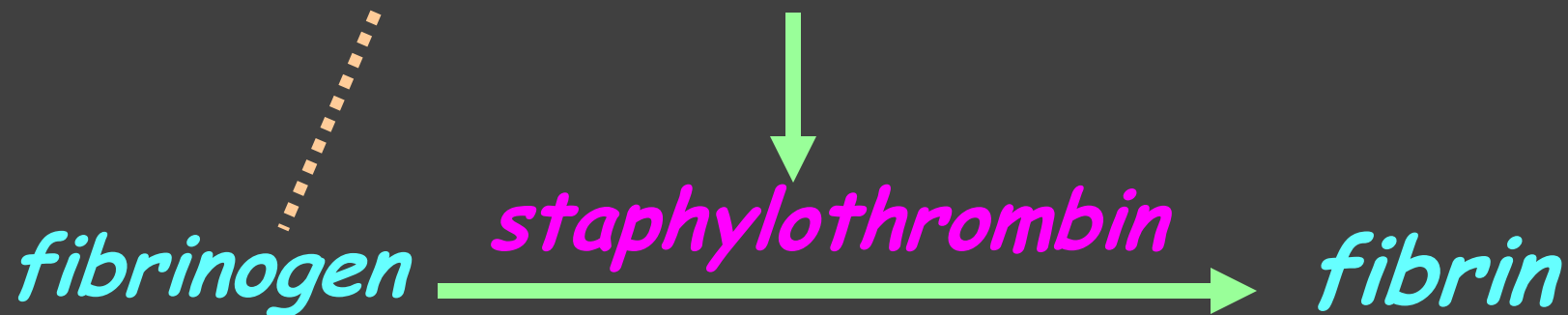


enterotoxin

S. aureus extracellular enzymes

Coagulase

coagulase + human prothrombin

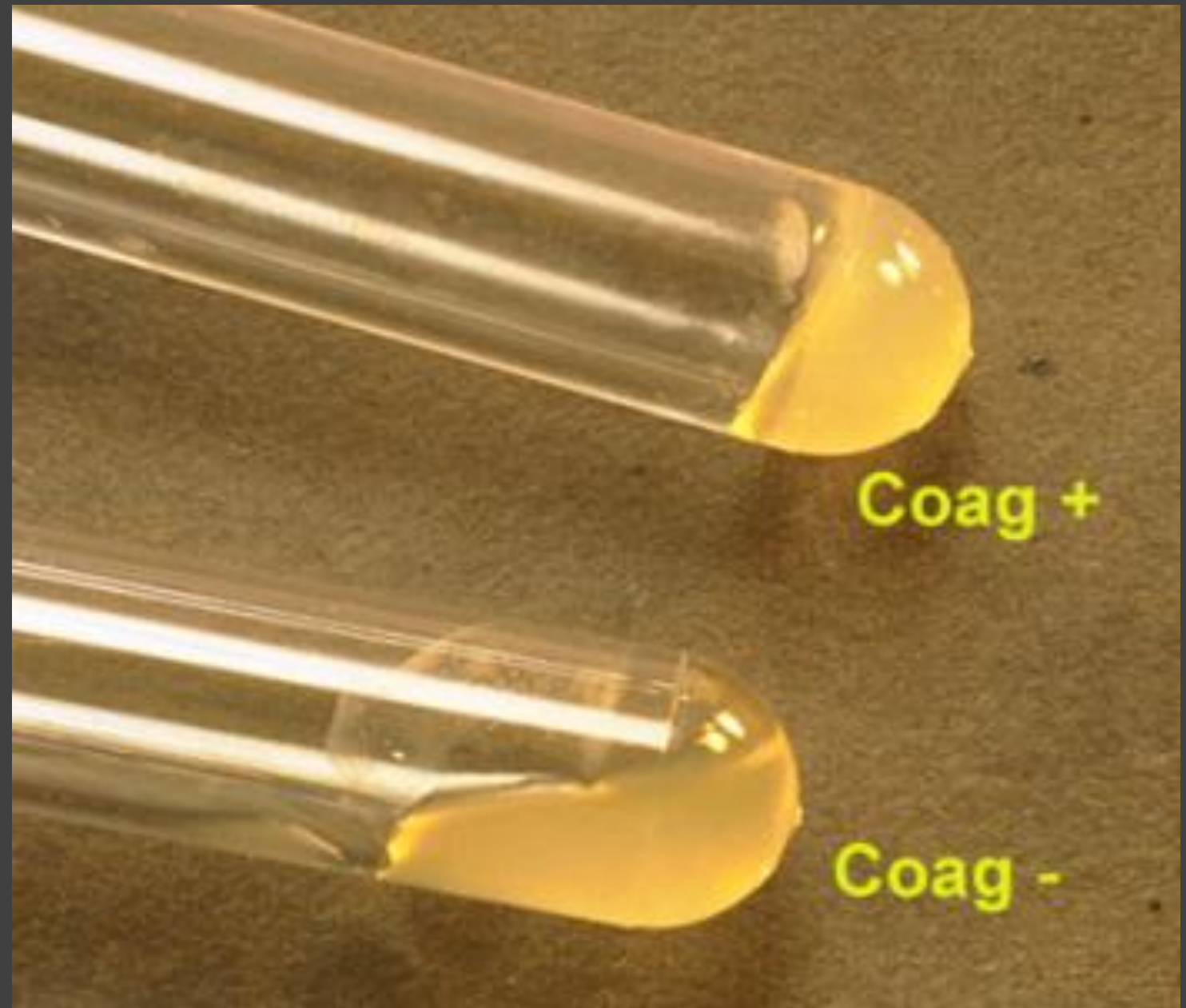


: 8 serotypes

: causing fibrin clotting around infection sites

=> protect bacteria from host defense

Coagulase test



Staphylococcus aureus

Extracellular enzymes

- : coagulase
- : staphylokinase (Sak)
- : protease
- : lipase
- : hyaluronidase
- : nuclease

Staphylococcus aureus

High risk group

: *burn*

: *diabetes*

: *cellulitis*

: *impetigo*

: *operation*

** *S. aureus* carrier

Staphylococcus aureus carrier



CHROMagar MRSA-

Results in rose to mauve MRSA colonies

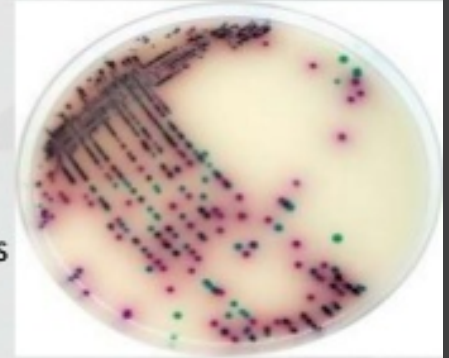


Denim Blue agar-

Produces denim blue colonies of MRSA

MRSA Ident agar

Dusky pink or ruby-colored MRSA colonies



Detection of MRSA

- : MRSA screening plate: MHA containing 6 $\mu\text{g/ml}$ of oxacillin
- : *mecA* mediated MRSA by cefoxitin
- : Chromogenic agar for MRSA screening
- : Latex agglutination for PBP2' produced by MRSA
- : Genotyping method for MRSA

Phenotypic Methods for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.

Organism	Phenotypic Methods for Detection of Methicillin (Oxacillin)-Resistant <i>Staphylococcus</i> spp.				
	Cefoxitin MIC	Cefoxitin disk diffusion	Oxacillin MIC	Oxacillin disk diffusion	Oxacillin salt agar
<i>S. aureus</i>	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	Yes (24 h)
<i>S. lugdunensis</i>	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	No
<i>S. epidermidis</i>	No	Yes (24 h)	Yes (24 h)	Yes (16-18 h)	No
<i>S. pseudintermedius</i>	No	No	Yes (24 h)	Yes (16-18 h)	No
<i>S. schleiferi</i>	No	No	Yes (24 h)	Yes (16-18 h)	No
<i>Staphylococcus</i> spp. (not listed above or not identified to the species level)	No	Yes ^a (24 h)	Yes ^a (24 h)	No	No

S. aureus* complex: *S. aureus*, *S. argenteus*, *S. schweitzeri

CLSI-M100, 31st ed., 2021

Oxacillin MIC: For all species

Oxacillin disk diffusion: Only for *S. epidermidis*, *S. schleiferi*, *S. pseudintermedius*

Oxacillin salt agar: Only for *S. aureus*

Cefoxitin MIC: Only for *S. aureus* and *S. lugdunensis*


Cefoxitin disk diffusion: For all species EXCEPT *S. pseudintermedius* and *S. schleiferi*

Pyrrolidonyl- α -naphthylamide (PYR) hydrolysis

PYR $\xrightarrow[\text{35}^\circ\text{C 2h}]{\text{Pyrrolidonyl peptidase}}$ β -naphthylamine

0.01% cinnamaldehyde reagent
(*p*-Dimethylaminocinnamaldehyde)

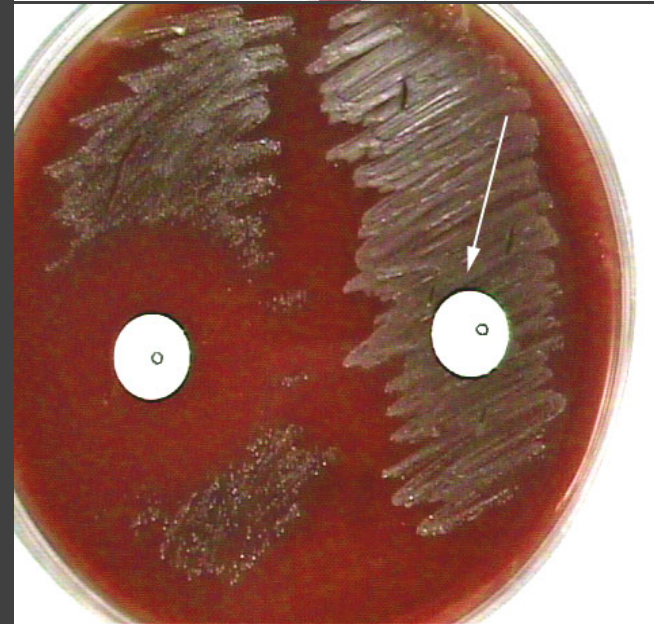
Red complex
(within 2 mins)

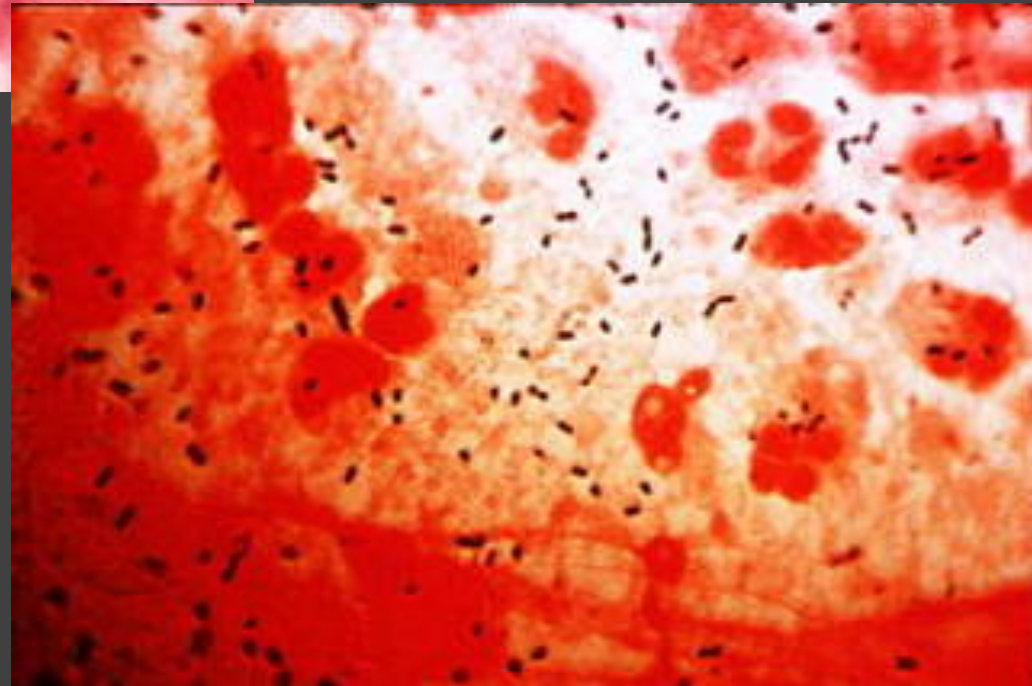
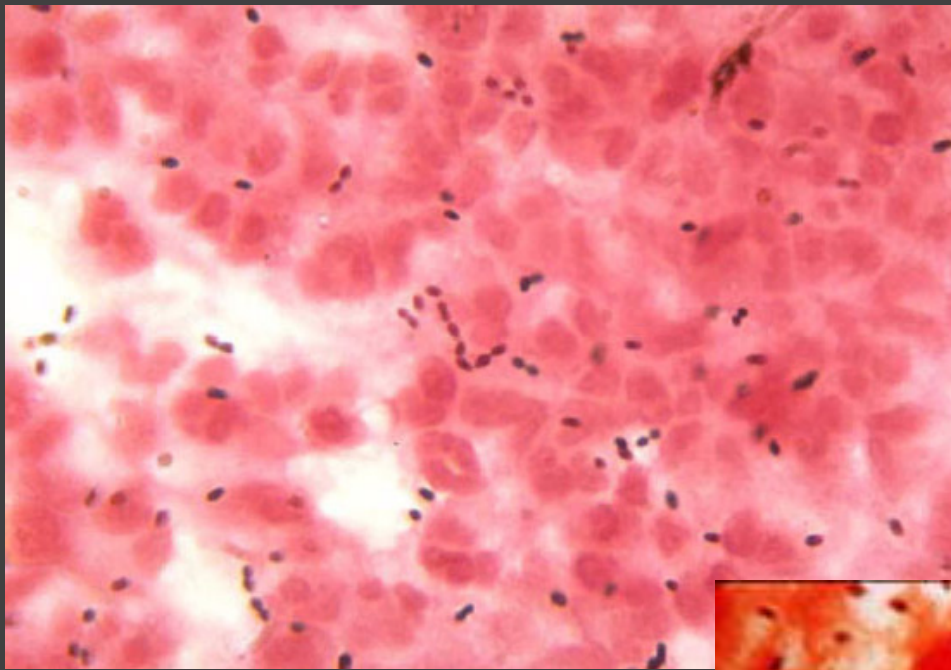


The image shows two test tubes side-by-side. The left tube has a blue minus sign below it and contains a yellow-orange liquid. The right tube has a pink plus sign below it and contains a red liquid. A yellow arrow points from the text '0.01% cinnamaldehyde reagent' to the right tube.

Streptococcus pneumoniae

- α -hemolysis
 - Optochin susceptible
 - Pair with capsule
 - Autolysis
 - community-acquired pneumonia
 - oropharyngeal carrier
- => difficult to interpret infection





Bile solubility test

0.5 ml. of bacterial suspension in saline
(1 McFarland standard)

+

0.5 ml of 10% sodium deoxycholate (bile)

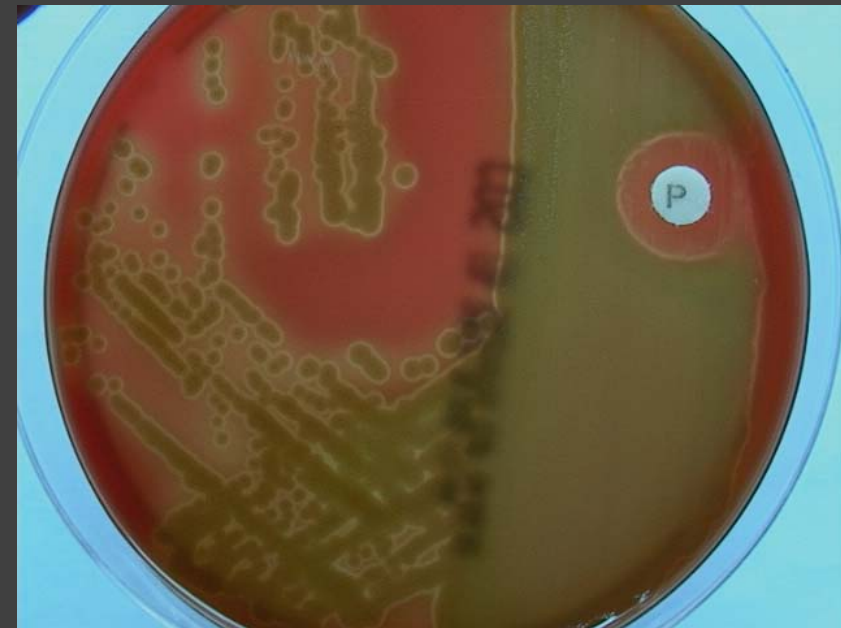
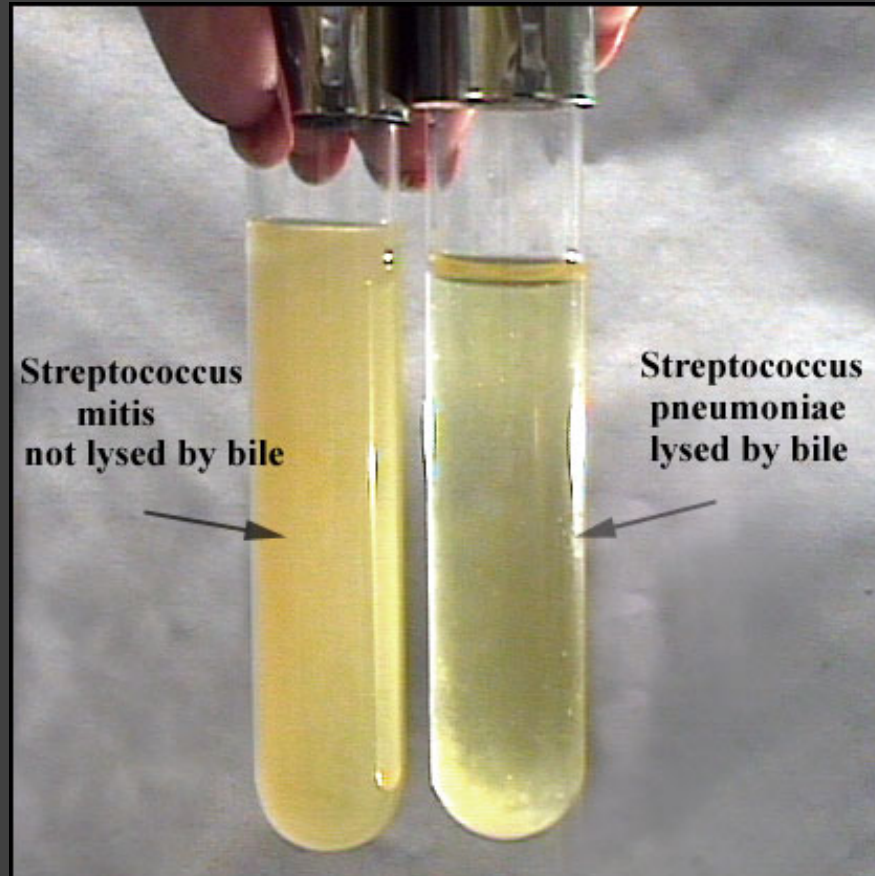


5-15 min

Positive = Clear suspension

Negative = Turbid suspension

Bile solubility test



Streptococcus pneumoniae

- pneumonia
- otitis media
- sinusitis
- Meningitis => in children
- endocarditis

Streptococcus pneumoniae

Treatment

Generally susceptible to many antibiotics

Emerging of penicillin resistance

=> multidrug resistance

Vaccine

Capsular polysaccharide

Viridans *Streptococcus*

5 groups

- *mutans* group
- *salivarius* group
- *bovis* group
- *mitis* group
- *anginosus* (previously milleri group)
(includes small colony-forming beta-hemolytic
Streptococcus group A, C, F and G)

Enterococcus spp.

- Formerly group D *Streptococcus*
- Schleifer & Kilpper-Balz (1984)
- 17 species
 - => *E. faecalis*, *E. faecium*
- Gram positive cocci
- single, pair or short chain
- facultative anaerobe

Enterococcus spp.

- α , β or γ -hemolysis
- grow in 6.5% NaCl
- bile-esculin hydrolysis
- grow at 10-45°C
- habitat
 - : soil, water, food, plants, animals
 - GI tract, female genital tract

Enterococcus spp.

Clinical significance

UTI : ~10% of all cases
~16% of nosocomial UTI

Intra-abdominal
Pelvic wound } polymicrobial

Septicemia => elderly patients with
serious underlying dis.

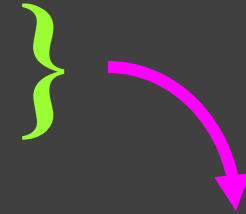
Endocarditis

Enterococcus spp.

Isolation using selective media

Colistin-nalidixic acid agar (CNA)

Phenylethyl alcohol agar (PEA)



Inhibit Gram negative

Enterococcosel-vancomycin broth



Brain heart infusion-vancomycin agar



VRE (Vancomycin resistance enterococci)

Vancomycin Resistant Enterococci

First described in Europe in 1988

In the USA: increase >20 folds in 5 years
(from 0.3 % in 1989 to 7.9% in 1993)

Six resistant genes have been described
(VanA, VanB, VanC, VanD, VanE, VanG)

VRE (Vancomycin-resistant enterococci)

Enterococcus spp.

Van A & Van B

E. faecium and *E. faecalis*

Transferable

Van C

E. gallinarum, *E. casseliflavus*,
E. flavescens

Non-transferable

Level and type of resistance to vancomycin in enterococci.

Strain characteristic	Acquired resistance level, type					Intrinsic resistance, low level, type VanC1/C2/C3
	High, VanA	Variable, VanB	Moderate, VanD	Low		
				VanG	VanE	
MIC, mg/L						
Vancomycin	64–100	4–1000	64–128	16	8–32	2–32
Teicoplanin	16–512	0.5–1	4–64	0.5	0.5	0.5–1
Conjugation	Positive	Positive	Negative	Positive	Negative	Negative
Mobile element	Tn1546	Tn1547 or Tn1549
Expression	Inducible	Inducible	Constitutive	Inducible	Inducible	Constitutive Inducible
Location	Plasmid chromosome	Plasmid chromosome	Chromosome	Chromosome	Chromosome	Chromosome
Modified target	D-Ala-D-Lac	D-Ala-D-Lac	D-Ala-D-Lac	D-Ala-D-Ser	D-Ala-D-Ser	D-Ala-D-Ser

NOTE. D-Ala-D-Lac, D-alanine-D-lactate; D-Ala-D-Ser, D-alanine-D-serine.

Enterococcus species

	Mannitol	Sorbose	AD
Group I	+	+	-
Group II	+	-	+
Group III	-	-	+
Group IV	-	-	-
Group V	+	-	-

AD = Arginine dihydrolase

Enterococcus gr. I (Mannitol +, Sorbose +, AD -)

	Ara	Sor	Raff	Su	Pyru	Tel	Mot	Pig	MGP
<i>E. avium</i>	+	+	-	+	+	-	-	-	V
<i>E. raffinosus</i>	+	+	+	+	+	-	-	-	V
<i>E. gilvus</i>	-	+	+	+	+	-	-	+	-
<i>E. pallens</i>	-	+	+	+	-	-	-	+	+
<i>E. saccharolyticus</i>	-	+	+	+	-	-	-	-	+
<i>E. malodoratus</i>	-	+	+	+	+	-	-	-	V
<i>E. pseudoavium</i>	-	+	-	+	+	-	-	-	+
<i>E. hawaiiensis</i>	-	+	-	-	+	-	-	-	-

Ara = Arabinose, Sor = Sorbitol, Raff = Raffinose, Su = Sucrose, Pyru = Pyruvate,
 Tel = 0.04% tellurite, Moti= Motile, Pig = Pigment, MGP = methyl- α -D-glucopyranoside

Enterococcus gr. II (Mannitol +, Sorbose -, AD +)

	Ara	Sor	Raff	Su	Pyru	Tel	Mot	Pig	MGP
<i>E. faecium</i>	+	V	V	+	-	-	-	-	-
<i>E. casseliflavus</i>	+	V	+	+	V	-	+	+	+
<i>E. gallinarum</i>	+	-	+	+	-	-	+	-	+
<i>E. mundtii</i>	+	V	+	+	-	-	-	+	-
<i>E. faecalis</i>	-	+	-	+	+	+	-	-	-
<i>E. haemoperoxidus</i>	-	-	-	+	-	-	-	-	+
<i>E. sanguinicola</i>	-	-	-	+	-	+	-	-	-
<i>Lactococcus spp.</i>	-	-	-	V	-	-	-	-	-

Ara = Arabinose, Sor = Sorbitol, Raff = Raffinose, Su = Sucrose, Pyru = Pyruvate,
 Tel = 0.04% tellurite, Moti= Motile, Pig = Pigment, MGP = methyl- α -D-glucopyranoside

Enterococcus gr. III (Mannitol -, Sorbose -, AD +)

	Ara	Sor	Raff	Su	Pyru	Tel	Mot	Pig	MGP
<i>E. dispar</i>	-	-	+	+	+	-	-	-	+
<i>E. hirae</i>	-	-	+	+	-	-	-	-	-
<i>E. durans</i>	-	-	-	-	-	-	-	-	-
<i>E. ratti</i>	-	-	-	-	-	-	-	-	-
<i>E. villorum</i>	-	-	-	-	-	-	-	-	-

Ara = Arabinose, Sor = Sorbitol, Raff = Raffinose, Su = Sucrose, Pyru = Pyruvate,
Tel = 0.04% tellulite, Moti= Motile, Pig = Pigment, MGP = methyl- α -D-glucopyranoside

Enterococcus gr. IV (Mannitol -, Sorbose -, AD -)

	Ara	Sor	Raff	Su	Pyru	Tel	Mot	Pig	MGP
<i>E. cecorum</i>	-	-	+	+	+	-	-	-	-
<i>E. pheoniculicola</i>	+	-	+	+	-	-	-	-	+
<i>E. sulfureus</i>	-	-	+	+	-	-	-	+	+
<i>E. asini</i>	-	-	-	+	-	-	-	-	-
<i>E. caccae</i>	-	-	-	+	+	-	-	-	+

Ara = Arabinose, Sor = Sorbitol, Raff = Raffinose, Su = Sucrose, Pyru = Pyruvate,
 Tel = 0.04% tellulite, Moti= Motile, Pig = Pigment, MGP = methyl- α -D-glucopyranoside

Enterococcus gr. V (Mannitol +, Sorbose -, AD -)

	Ara	Sor	Raff	Su	Pyru	Tel	Mot	Pig	MGP
<i>E. canis</i>	+	-	-	+	+	-	-	-	+
<i>E. columbae</i>	+	+	+	+	+	-	-	-	-
<i>E. moraviensis</i>	+	-	-	+	+	-	-	-	+
<i>E. hermannienseis</i>	-	-	-	-	-	-	-	-	-
<i>E. italicus</i>	-	V	-	+	+	-	-	-	+
<i>Vagococcus fluvialis</i>	-	+	-	+	-	-	+	-	+

Ara = Arabinose, Sor = Sorbitol, Raff = Raffinose, Su = Sucrose, Pyru = Pyruvate,
 Tel = 0.04% tellulite, Moti= Motile, Pig = Pigment, MGP = methyl- α -D-glucopyranoside

Gram positive cocci (cluster, catalase -)

	PYR	BE	Esc	Van	LAP	NaCl
<i>Aerococcus</i>	V	V	V	S	V	+
<i>Dolosigranulum</i>	+	-	+	S	+	+
<i>Facklamia</i>	+	-	-	S	+	+
<i>Helcococcus</i>	+	-	-	S	-	V
<i>Pediococcus</i>	-	+	+	R	+	V
<i>Tetragenococcus</i>	-	+	+	S	+	+

PYR = pyrrolidonyl arylamidase, BE = Bile-esculin, Esc = Esculin
Van = Vancomycin, LAP = Leucine aminopeptidase,
AD = Arginine dihydrolase, β -Gur = β -glucuronidase

Reference: Murray, P.R., Baron, E.J., Tenover, M.C., Tenover, F.C. 2007.
Manual of Clinical Microbiology, 9th ed. ASM Press, Washington DC.

Gram positive cocci (chain, catalase -)

	Sat	Mot	Van	PYR	LAP	NaCl	BE	
<i>Abiotrophia</i>	+			+	+	-	-	
<i>Granulicatella</i>	+			+	+	-	-	
<i>Vagococcus</i>		+		+	+	V+	+	
<i>Leuconostoc</i>			R	-	-	+	-	AD -
<i>Dolosicoccus</i>				+	-	-	NA	
<i>Gemella</i> (48 hr)				+	+	-	-	
<i>Globicatella</i>				+	-	+	V	
<i>Ignavigranum</i>				+	+	+	-	
<i>Lactococcus</i>				V+	+	V	V+	
<i>Weisella</i>			R	-	-	+	+	AD +

Sat = Satellite phenomenon, Mot = Motility, Van = Vancomycin, PYR = pyrrolidonyl arylamidase, LAP = Leucine aminopeptidase, NaCl = 6.5% NaCl, BE = Bile-esculin

Other Gram positive cocci

- normal flora : oral cavity, GI tract, respiratory tract, skin
- associated with immunocompromised
- either "staph-like" or "strep-like"
- none are β -hemolysis
- facultative anaerobe

Leuconostoc & Pediococcus

Glycopeptide resistance

=> vancomycin & teicoplanin

Leuconostoc spp.

- : associate with host defense impairment
- : neonatal sepsis

Pediococcus spp. (*P. acidilactici*)

- : sepsis
- : hepatic abscess

Gram positive cocci (cluster, catalase -)

	PYR	BE	Esc	Van	LAP	AD	β -Gur
<i>Aerococcus</i>	V	V	V	S	V	-	+
<i>Dolosigranulum</i>	+	ND	+	S	+		
<i>Facklamia</i>	+	-	-	S	+		
<i>Helcococcus</i>	+	-	-	S	-		
<i>Pediococcus</i>	-	+	+	R	+		
<i>Tetragenococcus</i>	-	+	+	S	+		

PYR = pyrrolidonyl arylamidase, BE = Bile-esculin, Esc = Esculin
Van = Vancomycin, LAP = Leucine aminopeptidase,
AD = Arginine dihydrolase, β -Gur = β -glucuronidase

Gram positive cocci (chain, catalase -)

	Sat	Mot	Van	PYR	LAP	NaCl
<i>Abiotrophia</i>	+			+	+	-
<i>Granulicatella</i>	+			+	+	-
<i>Vagococcus</i>		+		+	+	V
<i>Leuconostoc</i>			R	-	-	+
<i>Dolosicoccus</i>				+	-	-
<i>Gamella</i>				+	+	-
<i>Globicatella</i>				+	-	+
<i>Ignavigranum</i>				+	+	+
<i>Lactococcus</i>				V	+	V

Sat = Satellite phenomenon, Mot = Motility, Van = Vancomycin,
PYR = pyrrolidonyl arylamidase, LAP = Leucine aminopeptidase,
NaCl = 6.5% NaCl

Enterobacteriales

Description

- Gram negative rod
- grow well on plain media and selective differential (i.e. MacConkey agar)
- facultative anaerobic bacteria
- produce peritrichous flagella
(in motile organisms)

Enterobacteriaceae

Description

- Glucose fermenter (with/without gas)
- catalase positive
- oxidase negative
- reduce NO_3 to NO_2
- G+C content of DNA 39-59%

Enterobacteriaceae

Isolation

: blood agar

: MacConkey or EMB agar

=> lactose fermenters

: *E.coli, Klebsiella, Enterobacter, etc.*

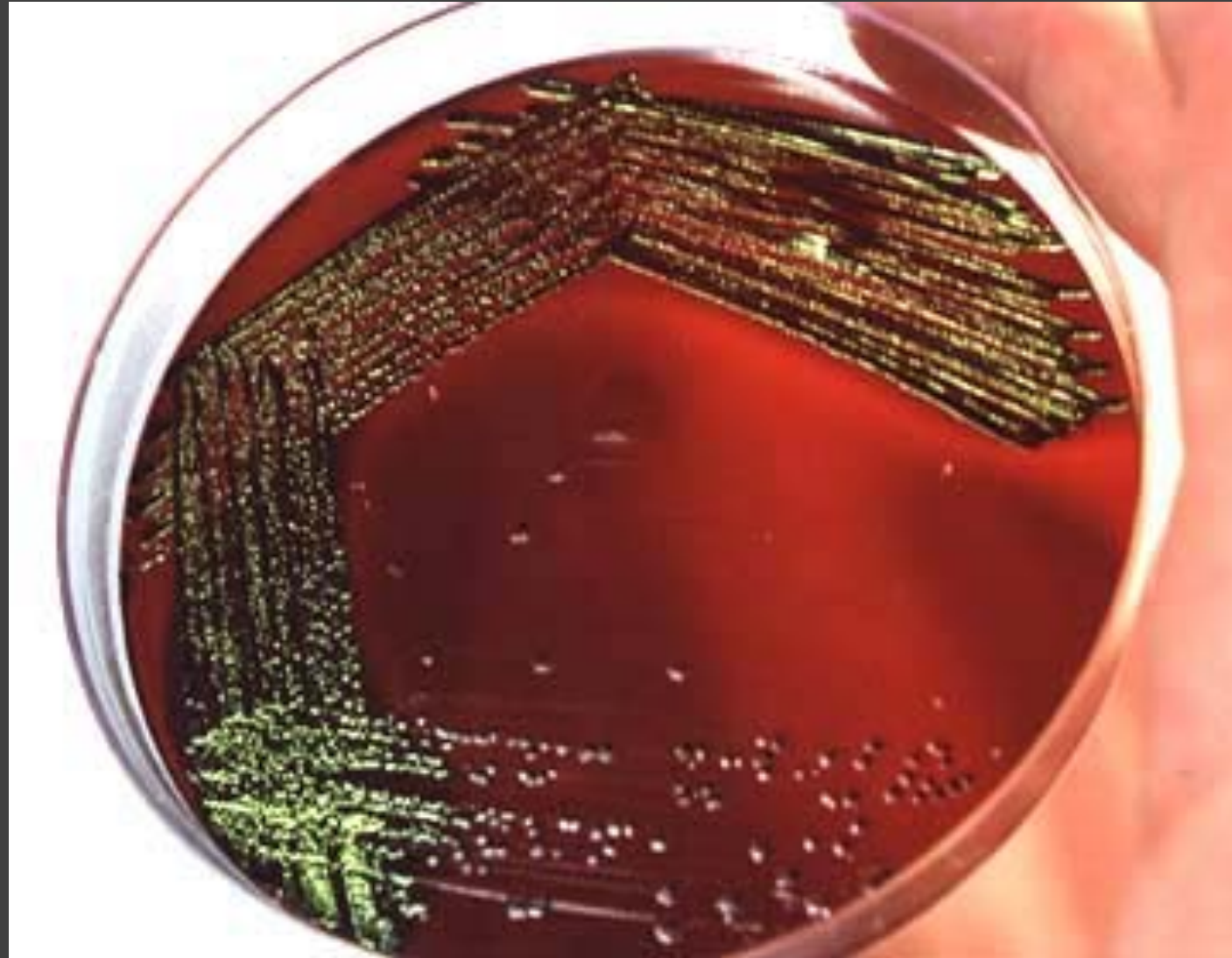
=> non-lactose fermenters

: *Salmonella, Shigella, Proteus, etc.*

Escherichia coli on MacConkey agar



Escherichia coli on EMB agar



Escherichia coli

8 groups of *E.coli*

- Enteropathogenic *E.coli* (EPEC)
- Enterotoxigenic *E.coli* (ETEC)
- Enteroinvasive *E.coli* (EIEC)
- Enterohaemorrhagic *E.coli* (EHEC)
=> Shiga Toxin-producing *E.coli* (STEC)
- Enteroaggregative *E.coli* (EAaggEC)
- Diffusely adherent *E.coli* (DAEC)
- Cytotoxic necrotizing factor-producing *E.coli* (CNFEC)
- Cytolethal distending toxin-producing *E.coli* (CDTEC)

Enterohaemorrhagic *E. coli* (EHEC)

- produce cytotoxin for Hela & Vero cells
=> called as "Verotoxin or Shiga-like toxin"
- often cause bloody diarrhea
- serotype O157:H7 is the most important
 - : haemorrhagic colitis
 - : extraintestinal diseases

***E. coli* O157:H7**

- 1982 - First recognized as pathogen
- 1985 - Associated with hemolytic uremic syndrome
- 1990 - Outbreak from drinking water
- 1991 - Outbreak from apple cider
- 1993 - Multi-state outbreak from fast food hamburgers
- 1995 - Outbreak from fresh produce
- 1996 - Outbreak in Japan
 - Multi-state outbreak from unpasteurized apple juice

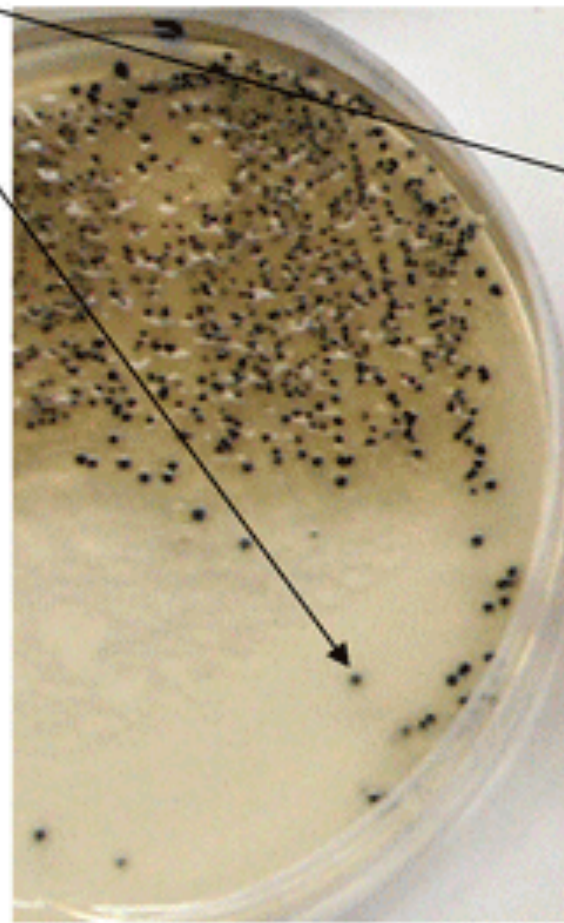
E. coli O157:H7

- 1982: two outbreaks of haemorrhagic colitis
- cause severe abdominal pain & cramps, watery diarrhea followed by bloody diarrhea
- cause colonic inflammation (colitis) but little or no fever
- selective media : McConkey sorbitol agar
=> produce colorless colony
- serology test with anti-serum

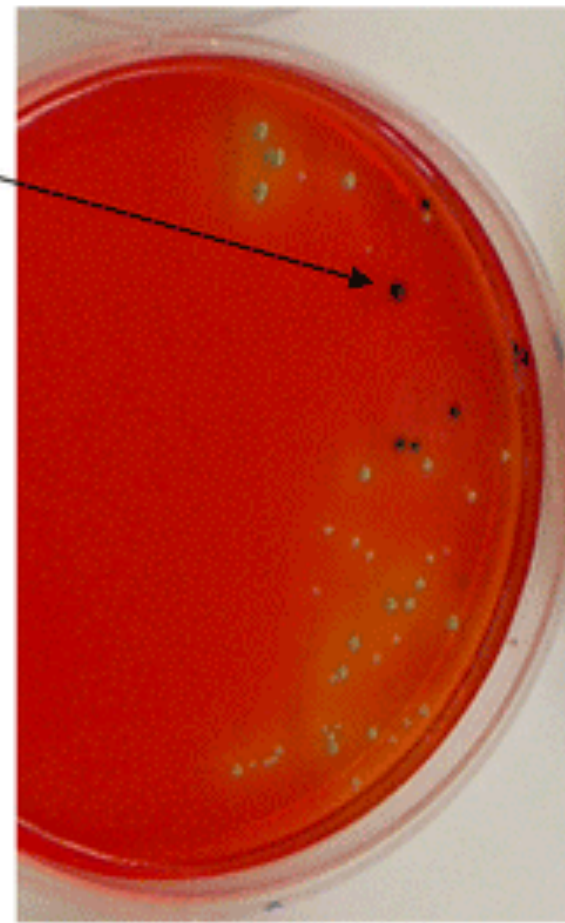
Typical *E. coli* O157:H7 colonies on selective agars



TC-SMAC



Rainbow® Agar
O157



R&F® *E. coli* O157:H7

Escherichia spp.

	<i>E.coli</i>	<i>E.fergusonii</i>	<i>E.hermannii</i>	<i>E.vulneris</i>	<i>E.blattae</i>
OD	65/20	100	100	0	100
LD	90/40	95	6	85	100
Lactose	95/25	0	45	15	0
Sorbitol	94/75	0	0	1	0
Cellobiose	2/2	96	97	100	0
KCN	3/1	0	94	15	0
Pigment	0/0	0	95	50	0
Indole	98/80	98	99	0	0
Malonate	0/0	35	0	85	100
Mannitol	98/93	98	100	100	0
H ₂ S	1/1	0	0	0	0

Reference: Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, J.C., Tenover, R.H. 2007.
Manual of Clinical Microbiology, 9th ed. ASM Press, Washington DC.

SHIGELLA SPECIES

- cause "bacillary dysentery"
- low infective dose (10-100 CFU)
- severe cramping, abdominal pain, diarrhea with blood and mucus
- invade mucosal cells & causing cell death
- rarely invade beyond mucosa
- person to person transmission
- NO animal reservoir

SHIGELLA SPECIES

4 species

- *S. dysenteriae* (serogroup A)
- *S. flexneri* (serogroup B)
 - => higher in **developing** countries
- *S. boydii* (serogroup C)
- *S. sonnei* (serogroup D)
 - => higher in **developed** countries

very close related to *E. coli* => same species??

SHIGELLA SPECIES

Culture media

- MacConkey agar
- XLD (xylose-lysine-deoxycholate) agar
- SS (Salmonella-Shigella) agar



Colorless colony

Klebsiella spp.

- Non motile
- Produce mucoid colony
- Lactose fermenter
- Intrinsic resistance to Ampicillin
- May cause nosocomial infection
- Extended Spectrum Beta-lactamase (ESBL) production in some strains

Klebsiella spp. and formerly *Klebsiella*

- *K. pneumoniae*
- *K. oxytoca*
- *K. ozanae*
- *K. rhinoscleromatis*
- *Raoultella ornithinolytica*
- *Raoultella planticola*
- *Raoultella terrigena*

Klebsiella spp.

	<i>K.pneu</i>	<i>K.oxy</i>	<i>R.orni</i>	<i>K.plan</i>	<i>K.ozan</i>	<i>K.rhi</i>	<i>R.terri</i>
Urease	95	90	100	98	10	0	0
Citrate	98	95	100	100	30	0	40
LD	98	99	100	100	40	0	100
OD	0	0	100	0	3	0	20
Indole	0	99	100	20	0	0	0
Lactose	98	100	100	100	30	0	100
MR	10	20	96	100	98	100	60
VP	98	95	70	98	0	0	100
Malonate	93	98	100	100	3	95	100
Gas	97	97	100	100	50	0	80
Sucrose	99	100	100	100	20	75	100

K.pneu = *K. pneumoniae*, *K.oxy* = *K. oxytoca*, *R.orni* = *Raoutella ornithinolytica* (*K. ornithinolytica*),
K.plan = *K. planticola*, *K.czan* = *K. ozanae*, *K.rhi* = *K. rhinoscleromatis*,
R.terri = *Raoutella terrigena* (*K. terrigena*)

Reference: Murray, P.R., Baron, E.J., Tenover, M.C., Tenover, J.C., Tenover, J.C., Tenover, J.C., Tenover, J.C. 2007.
 Manual of Clinical Microbiology, 9th ed. ASM Press, Washington DC.

SALMONELLA SPECIES

Nomenclature

contains one species => *S. enterica*
with >2000 serotype

Some serotypes are frequent associated with severe diseases & bacteremia

- *S. enterica* Choleraesuis
- *S. enterica* Paratyphi A
- *S. enterica* Typhi
- *S. enterica* Enteritidis

SALMONELLA SPECIES

Epidemiology

- found in human and animal **EXCEPT**
S. typhi and *S. paratyphi A*
- transmitted to human via contaminated food
- human to human transmission also occur
=> especially in a family

LABORATORY DIAGNOSIS FOR *SALMONELLA* SPECIES

Isolation

- low-selectivity media
: MacConkey or EMB
- intermediate-selectivity media
: XLD or SS
- highly-selective media
: Brilliant green, MSRV

With or without enrichment broth
: Selenite F broth

LABORATORY DIAGNOSIS FOR *SALMONELLA* SPECIES

Identification

- Biochemical reaction
 - : H₂S production
 - : LD (+)
 - : Phenylalanine deaminase (PD) (-)
- Serodiagnosis
 - : Widal test => typhoid and paratyphoid
 - : anti-serum for agglutination of colonies

Recommended biochemical tests for NFB

Urease

Citrate

TSI

LIA

MIO

10% glucose slant

10% lactose slant

Litmus milk

Nitrate reductase

Starch hydrolysis

DNase

Esculin hydrolysis

Carbohydrate O/F (glu, xyl, mal, lac, suc, man)

Malonate

Common NFB organisms (based on 10% glucose and 10% lactose results)

10% Glucose	10% Lactose	Organism	Remarks
A	A	<i>Burkholderia</i> spp., <i>Ralstonia</i> spp.	colistin resistant, oxidase positive
		<i>Acinetobacter baumannii</i> complex	non-motile, oxidase negative
		<i>Sphingomonas</i> spp., <i>Sphingobacterium</i> spp.	yellow pigment, esculin hydrolysis positive
A	Alk	<i>Pseudomonas</i> spp., <i>Achromobacter xylosoxidans</i> , <i>Ochrobactrum</i> spp.	
Alk	Alk	<i>Stenotrophomonas maltophilia</i>	motile, oxidase negative, carbapenem resistant
		<i>Acinetobacter lwoffii</i> and other asaacharolytic <i>Acinetobacter</i> spp.	non-motile, oxidase negative
		<i>Moraxella</i> spp.	Non-motile, oxidase positive, PenG susceptible
		<i>Shewanella</i> spp.	H ₂ S producing, oxidase positive
		<i>Elizabethkingia meningoseptica</i>	indole positive, oxidase positive, non-motile, vancomycin susceptible
		<i>Brevundimonas</i> spp., <i>Delftia</i> spp., <i>Comamonas</i> spp.	yellow-brown pigment

Gram negative non-ferment bacilli (NFB)

rRNA homology of *Pseudomonas* : 5 groups

Group I : *Pseudomonas*

Group II : *Burkholderia, Ralstonia*

Group III : *Delftia, Comamonas, Acidovorax*

Group IV : *Brevundimonas*

Group V : *Stenotrophomonas*

General description

Aerobic Gram negative bacilli

Straight or slightly curve

Motile with polar flagella

=> except *B. mallei* is non-motile

Catalase positive

Oxidase positive => except *Stenotrophomonas*
B. gladioli

Grow on MacConkey

=> except *B. versicularis* (43% grow)

Glucose oxidizer => except *B. diminuta*
D. acidovorans
Comamonas spp.

Pseudomonas spp.

Fluorescent group

6 species: *P. aeruginosa*, *P. fluorescens*, *P. putida*,
P. veronii, *P. monteilii*, *P. mosselii*

Produce **pyoverdin**

(yellow-green or yellow-brown, water soluble pigment)

Only *P. aeruginosa* can produce **pyocyanin**
(blue, water soluble pigment)

P. aeruginosa may produce **pyorubin**
(red, water soluble pigment)

P. aeruginosa may produce **pyomelanin**
(brown-black, water soluble pigment)

Pseudomonas aeruginosa

Distinctive colony color: usually **green**

Produce **grape-like / corn taco-like** odor

Usually **flat colonies with serrated edge**

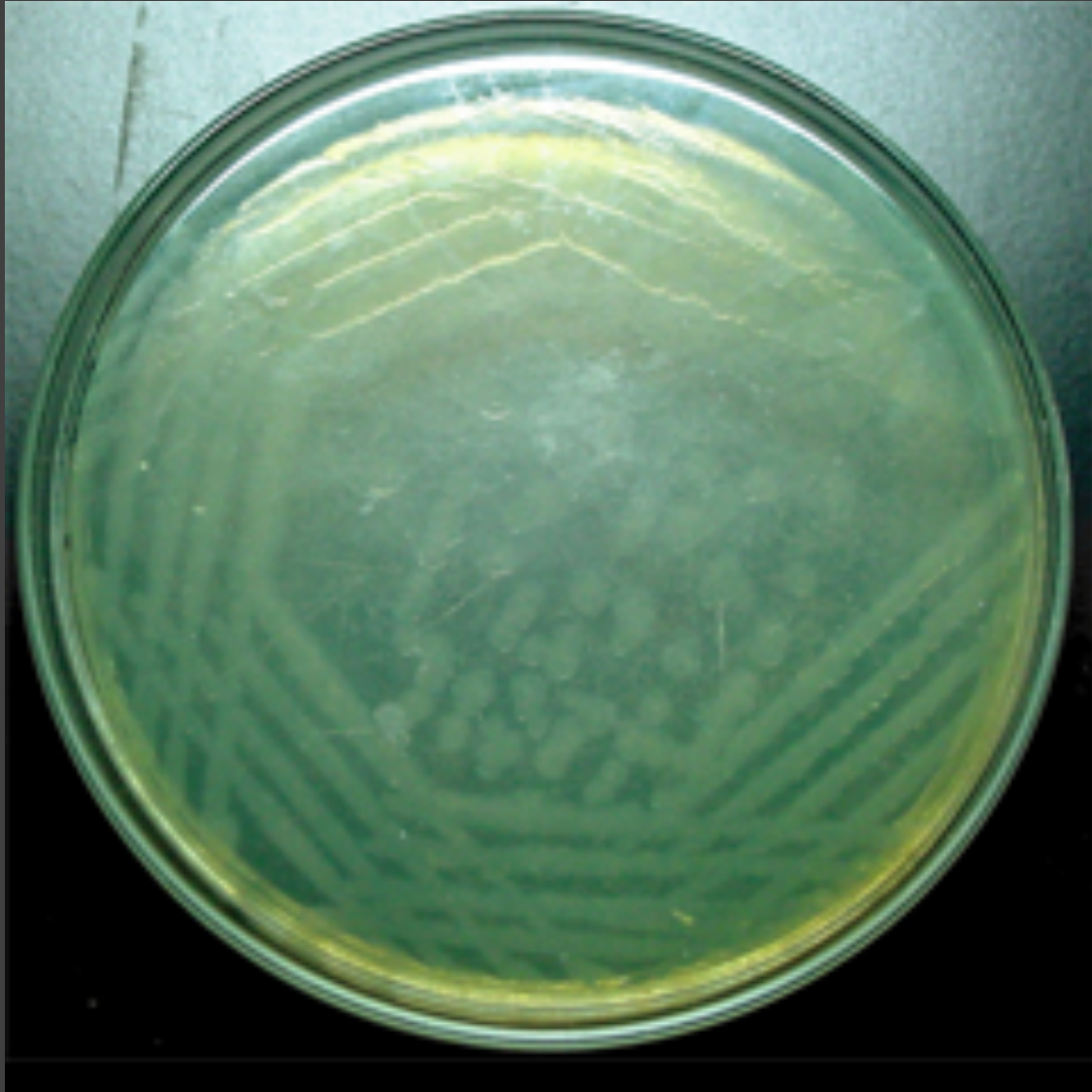
Mucoid colonies usually seen in cystic fibrosis patient

Grow at 42°C

Usually **nitrate and nitrite reductase**
(reduced nitrate to nitrogen gas)



Pseudomonas aeruginosa



Pseudomonas spp.



1 = urea, 2 = citrate, 3 = 10% glucose, 4 = 10% lactose,
5 = TSI, 6 = LIA, 7 = MIO

Pseudomonas aeruginosa



1 = urease (+), 2 = citrate (+), 3 = 10% glucose (Acid), 4 = 10% lactose (Alkaline), 5 = TSI (K/K, K/N),
6 = LIA (-/NA), 7 = MIO (+/-/NA), 8 = mannitol oxidation (+), 9 = maltose oxidation (-),
10 = glucose fermentation (-), 11 = glucose oxidation (+), 12 = lactose oxidation (-), 13 = xylose oxidation (+),
14 = sucrose oxidation (-), 15 = nitrate reductase (+ with gas), 16 = litmus milk (peptonized),
17 = esculin hydrolysis (-)

Gram negative non-ferment bacilli (NFB)

- I. Oxidase negative group
- II. Oxidase positive, indole negative, asaccharolytic, coccoid shape
- III. Oxidase positive, indole negative, asaccharolytic, rod shape
- IV. Oxidase positive, indole negative, saccharolytic, motile, rod shape
- V. Oxidase positive, indole negative, saccharolytic, non-motile, rod or coccoid shape
- VI. Oxidase positive, indole positive, yellow pigment
- VII. Halophilic nonfermenters
- VIII. Pink-pigmented group

Gram negative non-ferment bacilli (NFB)

I. Oxidase negative group

Acinetobacter spp.

=> non-motile, coccobacilli, NO₃ negative

CDC group EO-5 (Eugonic oxidizer)

=> Similar to *A.baumannii*

except no growth on MacConkey agar and
some strains produce yellow soluble pigment

CDC group NO-1 (Nonoxidizer)

=> Similar to *A.lwoffii*, except NO₃ positive

Bordetella spp.

=> *B.holmesii*, *B.parapertussis*,
B.trematum(CDC group NO-2)

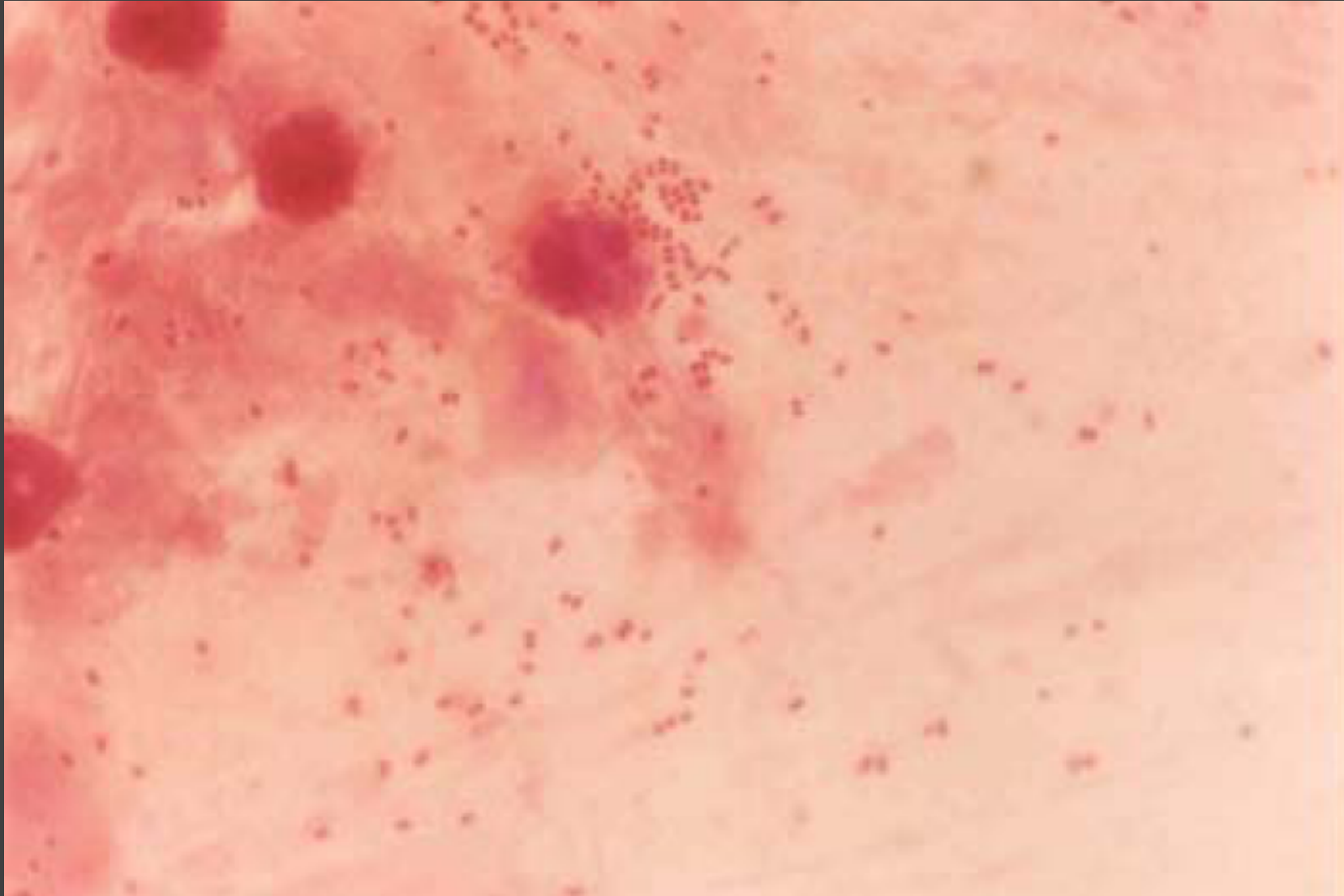
I. Oxidase negative group (*Acinetobacter* spp.)

	<i>A. cal</i> (8)	<i>A. bau</i> (121)	<i>A. haeno</i> (23)	<i>A. jun</i> (17)	<i>A. john</i> (23)	<i>A. lwof</i> (34)	<i>A. noso</i> (15)
37°C	100	100	100	100	0	100	100
41°C	0	100	0	90	0	0	100
44°C	0	100	0	0	0	0	0
Gelatin	0	0	96	0	0	0	0
Glucose	100	95	52	0	0	6	100
Hemolysis	0	0	100	0	0	0	0
Citrate	100	100	91	82	100	0	100
Malonate	100	98	0	0	13	0	87

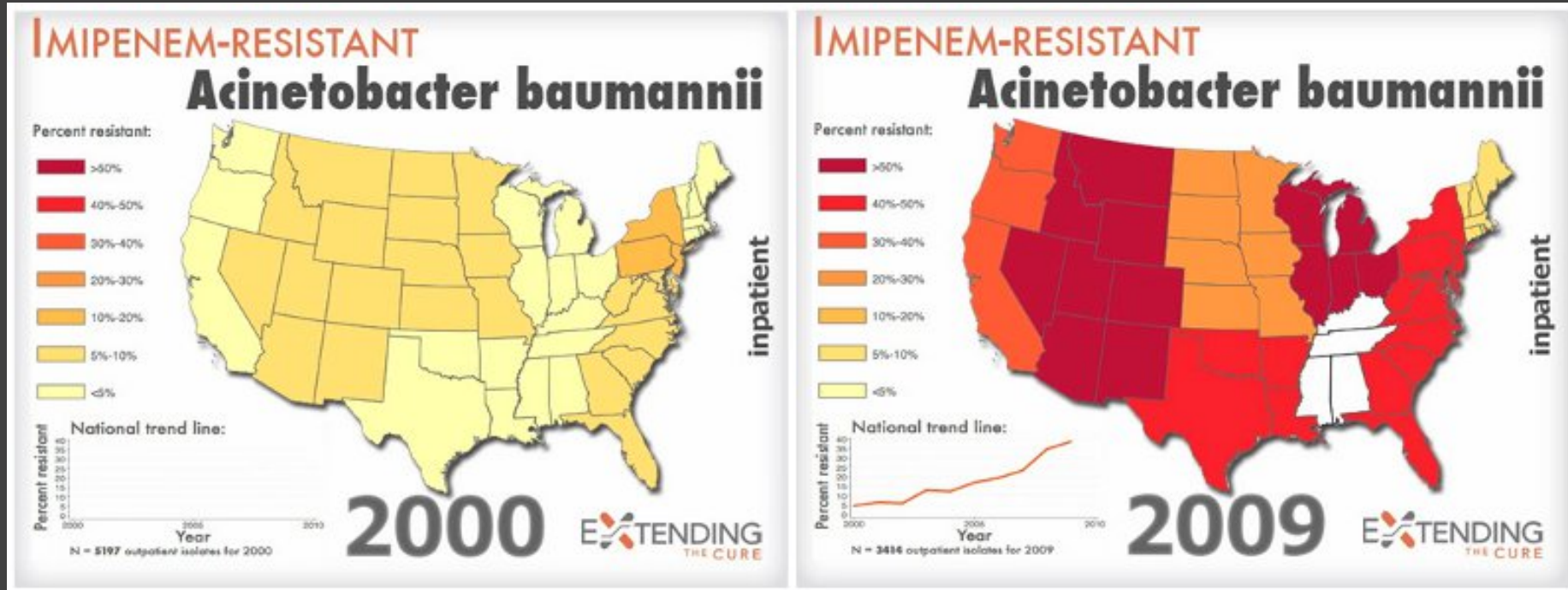
A. cal = *A. calcoaceticus*, *A. bau* = *A. baumannii*, *A. haemo* = *A. haemolyticus*, *A. jun* = *A. junii*,
A. john = *A. johnsonii*, *A. lwof* = *A. lwoffii*, *A. noso* = *A. nosocomialis* (formerly genomospecies 3)

A. baumannii complex: *A. baumannii*, *A. calcoaceticus*, *A. lactucaae*, *A. nosocomialis*, *A. pittii*, *A. seifertii*

Acinetobacter baumannii



Acinetobacter baumannii



Acinetobacter baumannii

TABLE 1

Proportion of *Acinetobacter baumannii* isolates exhibiting resistance to various antimicrobial agents; data from European countries

Country	Collection period	No of isolates tested	Ceftazidime	Cefepime	Ampicillin/Sulbactam	Imipenem	Meropenem	Ciprofloxacin	Piperacillin/Tazobactam	Tobramycin	Amikacin	Polymyxin B	Reference
11 European countries ^a	1997-2002	490	58	NA ^b	NA	16	18	60	66	40	NA	NA	19
30 European centres	2001-2004	851	60.3	56.1	51.6	26.3	29.6	61.3	NA	NA	45	2.7	18
12 European countries ^c	2006	433	68.8	NA	NA	42.5	43.4	67.9	65.1	48.4	28.6	NA	20
Sweden	2001-2004	128	79	NA	NA	4	NA	11	60	9 ^d	NA	NA	100
Spain	2000-2003	92	41.3	28.3	28.3	47.8	44.6	87	70.7	56.5	37	NA	101
Germany	2004-2008	86	17.4	16.3	NA	2.3	NA	20 ^e	14	NA	7	NA	36
Italy	2004-2008	98	58.2	61.2	NA	26.3	NA	50 ^e	41.8	NA	37.8	NA	36
United Kingdom	2004-2008	42	50	47.6	NA	16.7	NA	45.2 ^e	45.2	NA	14.3	NA	36
France	2004-2008	113	29.2	31.9	NA	1.8	NA	38.1 ^e	23	NA	2.4	NA	36
Turkey	2000-2003	779	84	76	NA	48	42	79	82	57	NA	NA	102
Greece ^f	February 2006	*	96.9	96.6	67.4	85	NA	97.8	95	86.6	87.3	NA	GSSAR ^g

^a Belgium, Bulgaria, Czech Republic, Germany, Italy, Poland, Russia, Sweden, Switzerland, Turkey, United Kingdom.

^b NA = not applicable

^c Belgium, Croatia, Czech Republic, Finland, Germany, Greece, Poland, Russia, Spain, Sweden, Turkey, United Kingdom.

^d Netilmicin was tested.

^e Levofloxacin was tested.

^f Data refers to blood isolates from intensive care unit (ICU).

^g Greek System for Surveillance of Antimicrobial Resistance, available at: <http://www.mednet.gr/whonet/>

* The number of isolates submitted to susceptibility testing varied from 46 to 224 depending on the antimicrobial agent.

Automated Identification and antimicrobial susceptibility testing

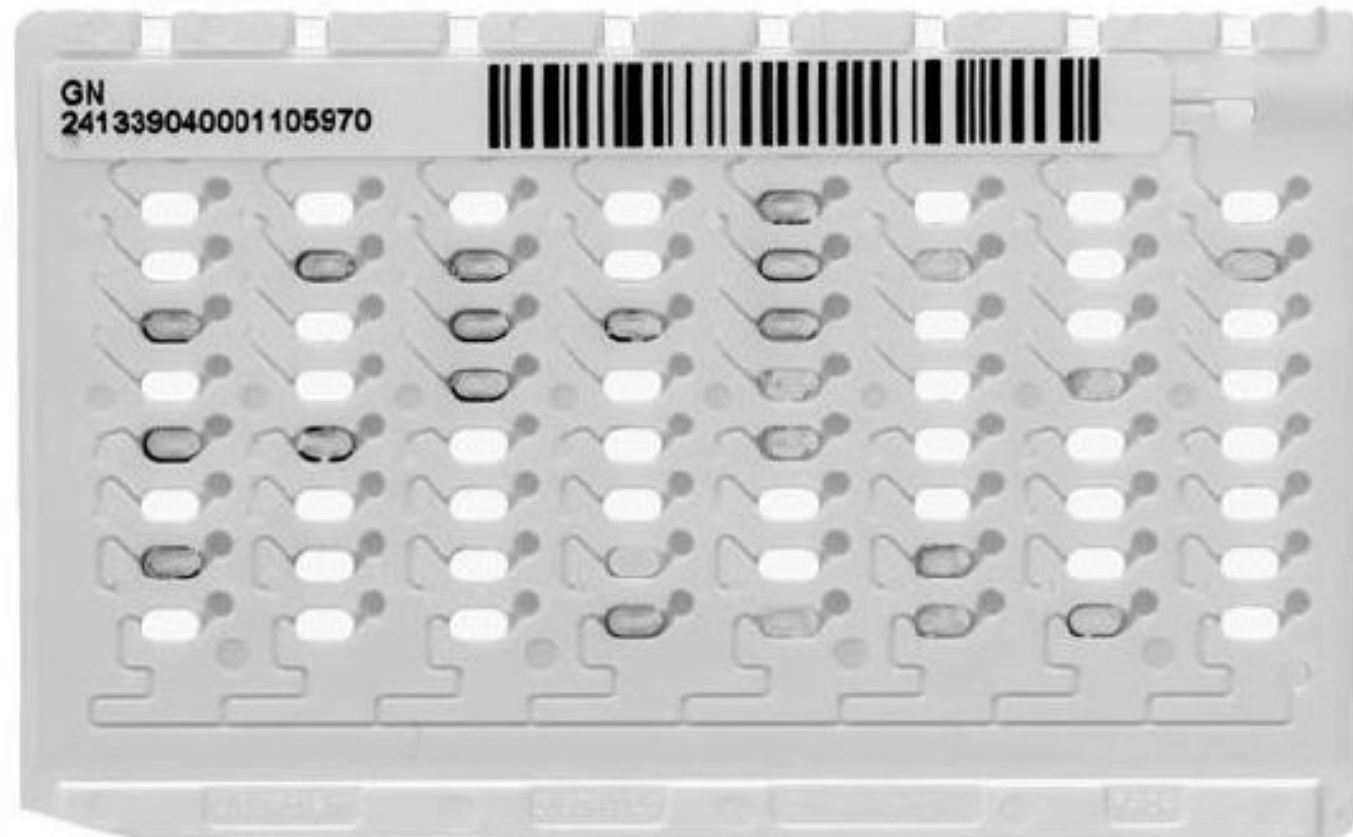


Vitek



Vitek

Figure 2. VITEK 2 GN Colorimetric Identification Card.



Vitek

Figure 3. VITEK 2 Compact Cassette Loaded with 10 Cards and Suspension Tubes and Bar Code Scanner for Data Entry.

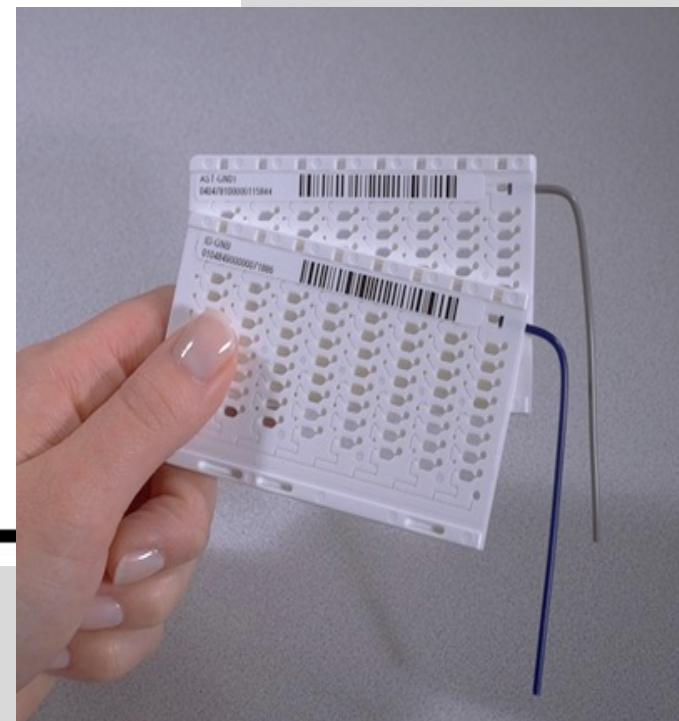


Figure 4. VITEK 2 Cassette Loaded with Cards and Suspension Tubes Being Loaded Into the Automatic Transport System.



Vitek

Table 2. Identification Levels.

ID Message Confidence Level	Choices	% Probability	Comments
Excellent	1	96 to 99	N/A
Very Good	1	93 to 95	N/A
Good	1	89 to 92	N/A
Acceptable	1	85 to 88	N/A
Low Discrimination	2 to 3	Sum of choices = 100; after resolution to one choice, percent probability reflects the number associated with the selected choice.	2 to 3 taxa exhibit the same biopattern. Separate by supplemental testing.
Unidentified Organism	> 3 or 0	N/A	Either > 3 taxa exhibit the same biopattern or Very atypical biopattern. Does not correspond to any taxon in the database. Check Gram stain and purity.

Automated Identification and antimicrobial susceptibility testing



Phoenix



Phoenix

- AST based on broth susceptibility with specific cards
- ID currently biochemical
- Growth/reactions “continuously” monitored



Automated Identification and antimicrobial susceptibility testing

Phoenix



Phoenix

The Phoenix panel is comprised of a 51 well ID side and an 85 well AST side. The ID side contains 45 wells with dried biochemical substrates and 2 fluorescent control wells. The AST side contains 84 wells with dried antimicrobial agents and 1 growth control well. Panels are available as ID only (Phoenix™ NID Panels, Phoenix™ PID Panels), AST only (Phoenix™ NMIC Panels, Phoenix™ PMIC Panels), or ID/AST combination (Phoenix™ NMIC/ID Panels, Phoenix™ PMIC/ID Panels). Unused wells are reserved for future use.

MicroScan Walkaway -40/96 system



MicroScan Walkaway -40/96 system



- 96 well plates
- AST based on conventional microbroth dilution MIC
- ID currently biochemical test

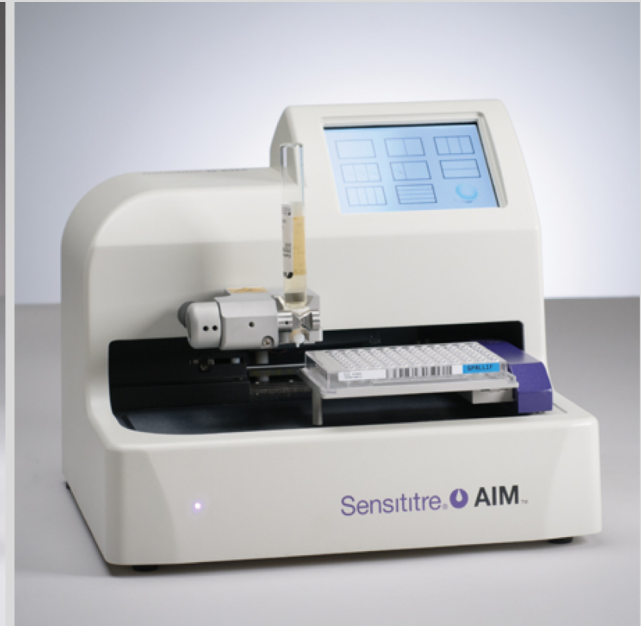
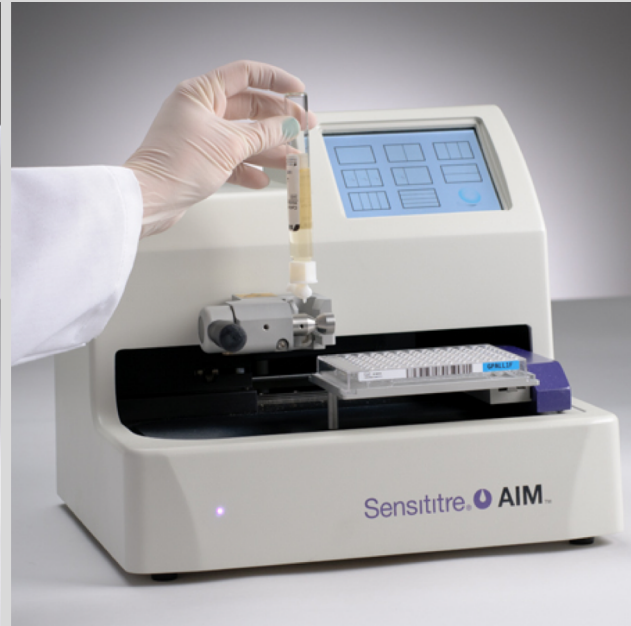
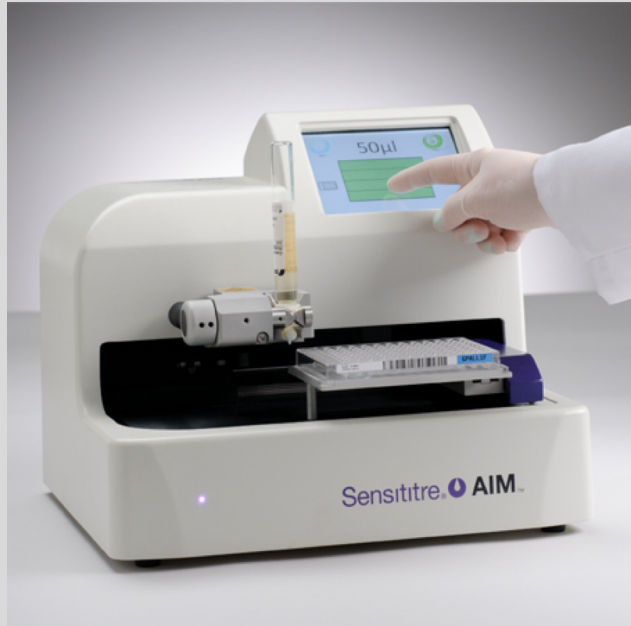
MicroScan Walkaway -40/96 system

- **Conventional panels**
 - Enterobacteriaceae, gram-negative non-fermenters, staphylococci, enterococci
 - Combo, MIC-only, ID-only
- **Rapid panels**
 - 2 - 2.5 hour ID (pre-formed enzymes)
 - AST for Gram negative organisms (MIC \geq 4 hrs)
- **Synergies plus panels**
 - 2 - 2.5 hour ID (pre-formed enzymes)
 - Broth microdilution "Read-when-ready" MIC
 - Resistance detection flagged in as few as 4.5 hours
 - All results finalized in 16/18 hours
- **Additional**
 - Yeast ID, Anaerobe ID, HNID
 - MICroSTREP *plus*® - dry format
 - ES \square L *plus*® -ES \square L Confirmatory Panel

Automated Identification and antimicrobial susceptibility testing

Sensititre system





Sensititre system



Sensititre MIC panels

- Identification panels (3 organisms / plate)
- Susceptibility testing panels
 - Gram positive (THAP1F / THAP2F)
 - Gram negative (THAN1F / THAN2F / THAMDR)
 - Anaerobic bacteria
 - Actinomyces & Rapid grower mycobacteria
 - Slow grower mycobacteria (NTM)
 - MTB complex
 - Fungi (Yeast10)
- Combination panels

What is MALDI-TOF MS?

Matrix-Assisted Laser Desorption/Ionization Time Of Flight Mass Spectrometry

MALDI

Peptides are converted into ions by either addition or loss of one proton based on “soft ionization” method

....

TOF

The m/z ratio of an ion is measured by determining the time required for it to travel the length of the flight tube

....

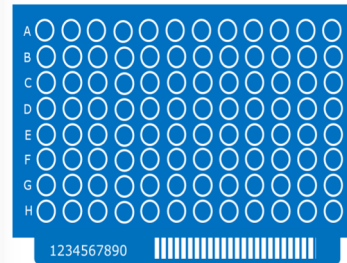
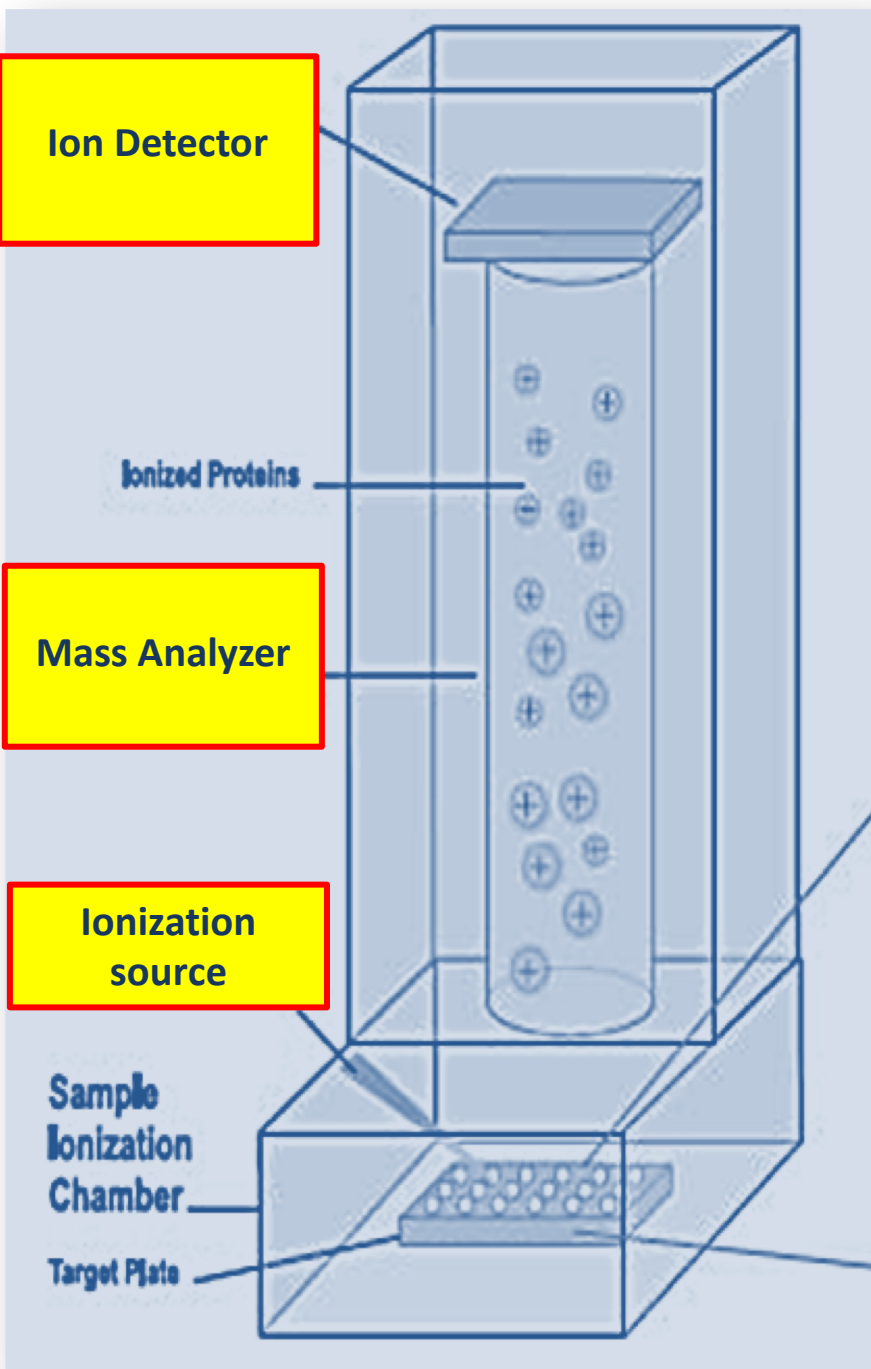
Invented by **Tanaka** (Nobel prize awarded 2002), **Hillenkamp**, and **Karas** in the mid 1980s

Principles of MALDI-TOF MS

1) Ionisation: Matrix Assisted Laser Desorption Ionization

1. Sample (S) is mixed with excess matrix (MH⁺) and dried on a MALDI target plate
2. Laser flash ionizes matrix molecules
3. Sample molecules are ionized by proton transfer from matrix: $MH^+ + S \rightarrow M + SH^+$

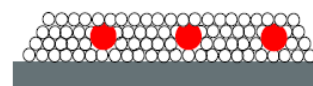
Matrix = solution of an energy-absorbent, organic compound



MALDI target plate

Sample embedded in light-absorbing matrix

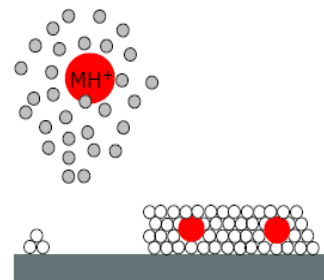
- Sample molecule
- Matrix molecule



Excitation of matrix molecules by laser light

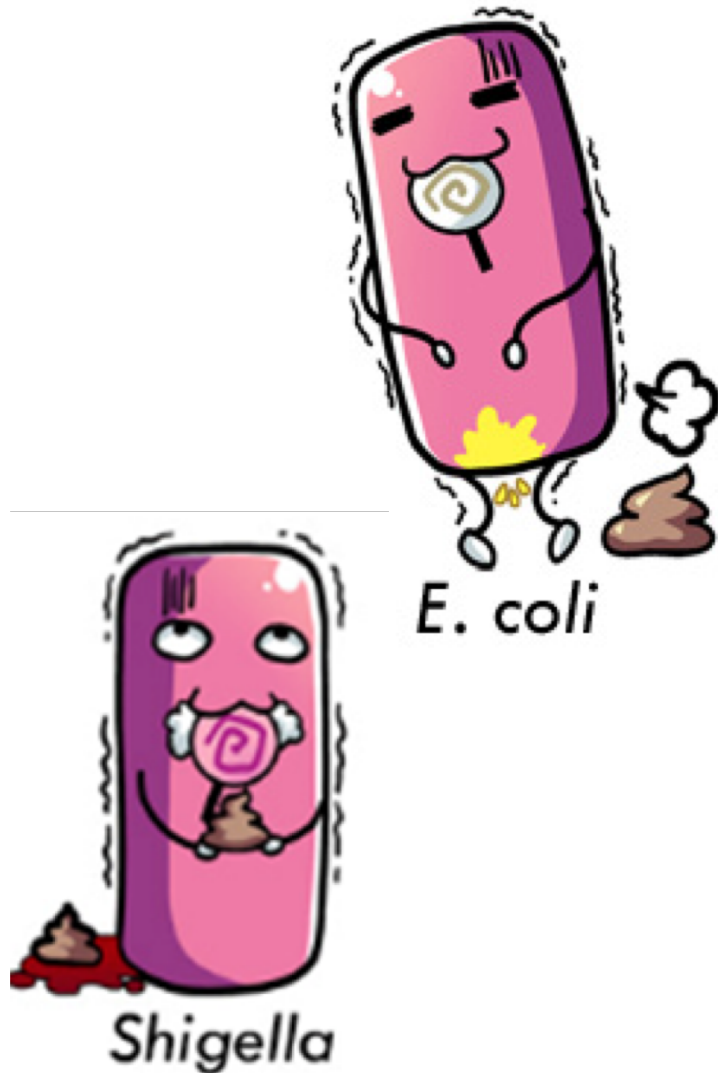


Desorption/protonation of sample molecules



Formation of alternative adducts depends on the presence of respective cations (either being ubiquitous present or actively added – depending on type of sample):
[M+Na]⁺; [M+K]⁺; [M+Cu]⁺; [M+Li]⁺; [M+Ag]⁺

Identification Pitfalls



E. coli and *Shigella* spp.

Limitation/Pitfall

MALDI-TOF MS cannot resolve these organism.

Shigella spp. are identified as *E. coli*

Possible Approaches to Resolution

Lactose fermenter : *E. coli*

Non-lactose fermenter : *E. coli* / *Shigella* spp.

Biochemical testing : indole, motile

Stool : *E. coli* / *Shigella* spp.

Blood, Wounds, Urine : *E. coli*

Identification Pitfalls



S. pneumoniae

Streptococcus mitis group

S. mitis, *S. sanguinis*, *S. parasanguinis*,
S. gordonii, *S. crinitatus*, *S. oralis*,
S. infantis, *S. peroris*, *S. australis*,
S. sinensis, *S. orisratti*, *S. oligofermentans*,
S. massiliensis, *S. pseudopneumoniae*,
S. pneumoniae

Streptococcus pneumoniae

VS

Streptococcus mitis group

Limitation/Pitfall

S. pneumoniae and *S. mitis* group are highly similar at the genetic level. Some MALDI-TOF MS misidentify nonpneumococcal *S. mitis* group isolates as *S. pneumoniae* or vice versa.

Possible Approaches to Resolution

Optochin disk testing
Bile solubility testing

Identification Pitfalls



Score ▼	Detected Species
2.271	Salmonella sp (enterica st Anatum) 11 LAL
2.208	Salmonella sp (enterica st Hadar) Sa05_506 VAB
2.196	Salmonella sp (typhimurium) 12 LAL
2.171	Salmonella sp (enterica st Dublin) Sa05_188 VAB
2.163	Salmonella sp (enterica st Houtenae) DSM 9221T HAM
2.143	Salmonella sp (enteritidis) 25089078 (PX) MLD
2.083	Salmonella sp (enterica st Enterica) DSM 17058T HAM
2.027	Salmonella sp (choleraesuis) 08 LAL
2.007	Salmonella sp (enterica st Stanley) 15 LAL

Salmonella spp.

Limitation/Pitfall

- *Salmonella* can only be identified on genus level.
- MALDI-TOF MS is not reliable for serotyping (as required for public health and epidemiology purposes).

Possible Approaches to Resolution

- Consider reporting as *Salmonella* spp.
- Send isolates for confirmatory testing and/or serotyping as directed by government regulations.

Identification pitfalls

Score	Detected Species	
6483	D8 0 Burkholderia metallica	2.187 is a member of Burkholderia cepacia complex
2.187	Burkholderia metallica DSM 23519T DSM	is a member of Burkholderia cepacia complex
2.150	Burkholderia vietnamiensis LMG 10929T HAM	is a member of Burkholderia cepacia complex
2.143	Burkholderia seminalis DSM 23518T DSM	is a member of Burkholderia cepacia complex
2.142	Burkholderia cepacia LMG 2161 HAM	is a member of Burkholderia cepacia complex
2.140	Burkholderia cenocepacia LMG 12614 HAM	is a member of Burkholderia cepacia complex
2.097	Burkholderia cepacia DSM 50181 DSM	is a member of Burkholderia cepacia complex
2.095	Burkholderia cepacia DSM 7288T DSM	is a member of Burkholderia cepacia complex
2.044	Burkholderia pyrrocinia LMG 14191T HAM	is a member of Burkholderia cepacia complex
2.032	Bur	<p><i>Burkholderia cepacia</i> complex</p> <p>Distinguishing among species within the complex is not reliable using some MALDI-TOF MS-MIS or databases. However, accurate species-level identification may be important for stratifying prognosis in patients with CF.</p> <ul style="list-style-type: none"> As needed, send isolates to an established referral laboratory for genomic testing and/or as directed for designated CF centers. Consider reporting to group or complex level when isolated from other hosts.
2.028	Bur	

Identification pitfalls

Score	Detected Species	
883	H2 0 Citrobacter freundii	2.167 Species of this genus have very similar patten
2.167	Citrobacter freundii 22054_1 CHB	Species of this genus have very similar patterns:
2.126	Citrobacter braakii 20663_2 CHB	Species of this genus have very similar patterns:
2.126	Citrobacter freundii DSM 15979 DSM	Species of this genus have very similar patterns:
2.119	Citrobacter youngae DSM 17578T HAM	Species of this genus have very similar patterns:
2.104	Citrobacter freundii 13158_2 CHB	Species of this genus have very similar patterns:
2.062	Citrobacter freundii DSM 30039T HAM	Species of this genus have very similar patterns:
2.036	Citrobacter braakii 9314_2 CHB	Species of this genus have very similar patterns:
1.978	Citrobacter murlinae DSM 13695T HAM	Species of this genus have very similar patterns:
1.948	Citrobacter gillenii DSM 13694T HAM	Species of this genus have very similar patterns:
1.810	Citrobacter koseri 9553_1 CHB	Species of this genus have very similar patterns:

Citrobacter freundii complex

Large complex of closely related organisms; specific clinical significance and/or accuracy of MALDI-TOF MS identification within the complex is not well defined.

- Consider reporting as *C. freundii* complex.

Identification pitfalls

Score	Detected Species	
1318	E7 0 Enterobacter hormaechei	2.108 is a member of Enterobacter cloacae complex
2.108	Enterobacter hormaechei ssp hormaechei DSM 12409T DSM	is a member of Enterobacter cloacae complex
2.106	Enterobacter cloacae 13159_1 CHB	is a member of Enterobacter cloacae complex
2.105	Enterobacter cloacae MB11506_1 CHB	is a member of Enterobacter cloacae complex
2.064	Enterobacter cloacae DSM 46348 DSM	is a member of Enterobacter cloacae complex
1.954	Enterobacter cloacae MB_8779_05 THL	is a member of Enterobacter cloacae complex
1.941	Enterobacter kobei DSM 13645T DSM	is a member of Enterobacter cloacae complex
1.927	Enterobacter cloacae MB_5277_05 THL	is a member of Enterobacter cloacae complex
1.890	Enterobacter cloacae DSM 30062 DSM	is a member of Enterobacter cloacae complex
1.885	Enterobacter cloacae 20105_2 CHB	is a member of Enterobacter cloacae complex
1.869	Enterobacter asburiae DSM 17506T DSM	is a member of Enterobacter cloacae complex



Enterobacter

Enterobacter cloacae complex

Large complex of closely related organisms; specific clinical significance and/or accuracy of MALDI-TOF MS identification within the complex is not well defined.

- Consider reporting as *E. cloacae* complex.

Identification pitfalls

***Staphylococcus aureus* complex:**
Staphylococcus aureus
Staphylococcus schweitzeri
Staphylococcus argenteus

For routine purposes, no additional efforts are necessary to distinguish within the *S. aureus* complex.

These novel species are reported as 'members of the *S. aureus* complex'.

If methicillin-resistant, they should be reported and handled as MRSA



S. aureus

Acinetobacter baumannii complex

1. *Acinetobacter baumannii*
2. *Acinetobacter calcoaceticus*
3. *Acinetobacter nosocomialis*
4. *Acinetobacter pittii*
5. *Acinetobacter seifertii*
6. *Acinetobacter dijkschoorniae*



A. baumannii



K. pneumoniae


**How important to get a correct
identification?**

Pseudo-outbreak of Acinetobacter Iwoffii Infection in a Tertiary Care Center in Thailand •

June 2007 · Infection Control and Hospital Epidemiology 28(5):637-9

DOI: 10.1086/513620

Source · [PubMed](#)

Anucha Apisarnthanarak ·  Pattarachai Kiratisin · Kanokporn Thongphubeth ·

[Show all 5 authors](#) ·  Linda M Mundy

Pseudo-outbreak of *Acinetobacter lwoffii*

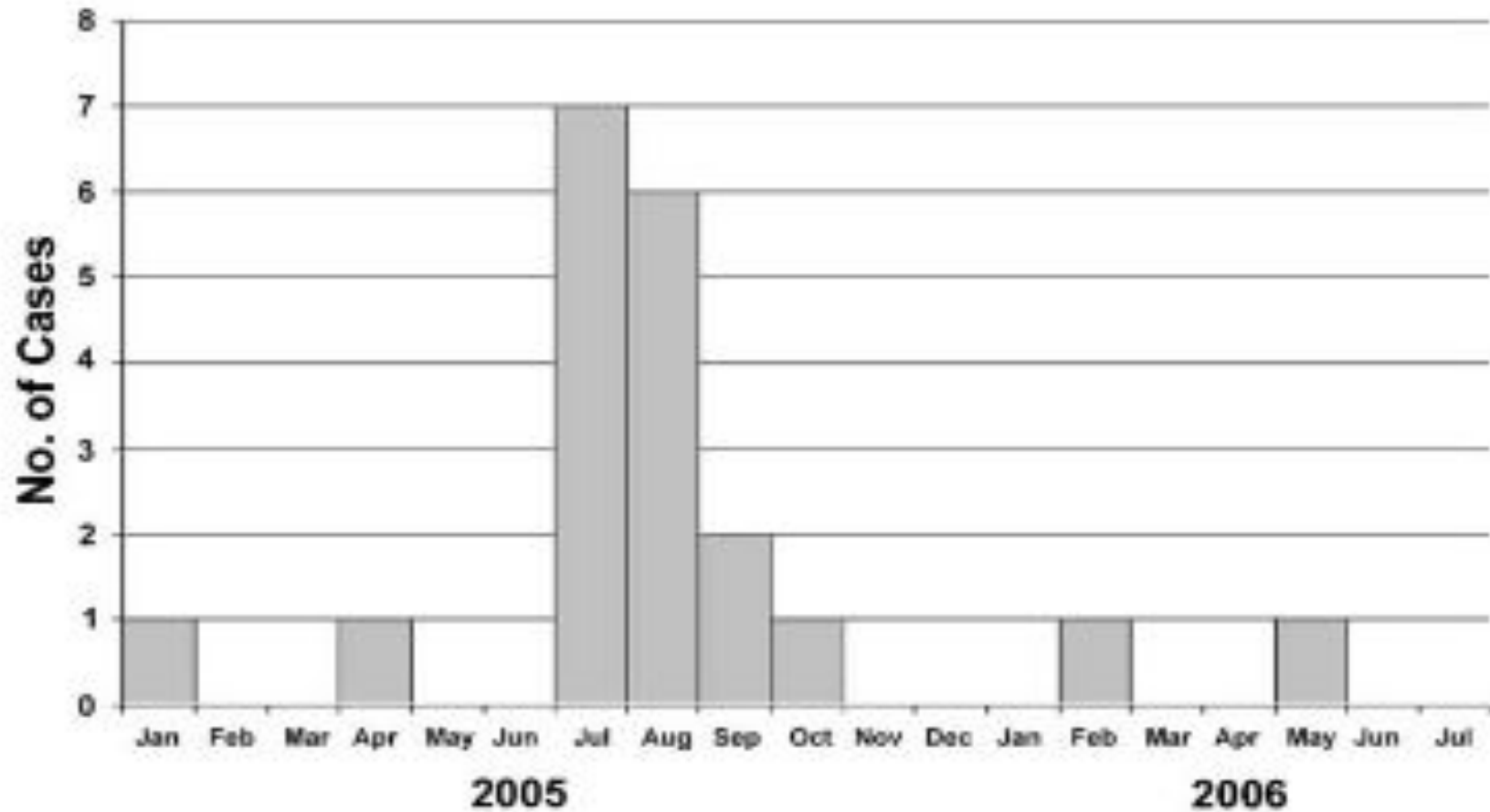


FIGURE. Epidemic curve of the pseudo-outbreak of *Acinetobacter lwoffii* infection.

Pseudo-outbreak of *Acinetobacter lwoffii*

TABLE. Characteristics of a Pseudo-outbreak of *Acinetobacter lwoffii* Infection and Colonization Over a 3.5-Month Period in 2005 at a Tertiary Care Center in Thailand

Patient	Age, years; sex	Unit	Culture finding		Type of acquisition ^a	Drugs the isolate was susceptible to ^b	Patient outcome	
			Date	Specimen				Isolate
1	23; M	Surgery	Jul 3	Wound	<i>A. xylosoxidans</i>	Colonization	Pip-Taz, Cbp	Survived
2	32; F	OBG	Jul 5	Blood	<i>P. putida</i>	Colonization	Pip-Taz, Cbp, Cip, Amgl	Survived
3	36; F	Orthopedics	Jul 10	Wound	<i>C. acidovorans</i>	Colonization	Ctz, Cpz, Cbp, Cip	Survived
4	78; M	Medicine	Jul 14	Sputum	<i>O. anthropi</i>	Infection	Cbp, Amgl, Cip, TMP-SMX	Died
5	45; M	MICU	Jul 16	Sputum	<i>P. stutzeri</i>	Colonization	Cbp, Amgl	Survived
6	43; F	Orthopedics	Jul 24	Wound	<i>A. junii</i>	Colonization	Pip-Taz, Cpz, Ctz, Cbp	Survived
7	41; M	SICU	Jul 26	Urine	<i>O. anthropi</i>	Infection	Cbp, Amgl, Cip, TMP-SMX	Survived
8	29; F	OBG	Aug 2	Blood	<i>A. junii</i>	Colonization	Pip-Taz, Cbp, Cip	Survived
9	68; F	Medicine	Aug 6	Blood	<i>S. maltophilia</i>	Infection	TMP-SMX	Died
10	70; M	SICU	Aug 12	Sputum	<i>S. maltophilia</i>	Colonization	TMP-SMX	Survived
11	44; F	Surgery	Aug 19	Wound	<i>P. putida</i>	Colonization	Pip-Taz, Cbp, Cip	Survived
12	45; F	SICU	Aug 24	Urine	<i>A. xylosoxidans</i>	Colonization	Pip-Taz, Cbp, Cip	Survived
13	66; F	CCU	Aug 26	Blood	<i>P. stutzeri</i>	Colonization	Cbp, Amgl	Survived
14	75; F	MICU	Sep 6	Blood	<i>A. xylosoxidans</i>	Infection	Pip-Taz, Cbp	Died
15	33; M	Medicine	Sep 12	Sputum	<i>C. acidovorans</i>	Colonization	Ctz, Cpz, Cbp, TMP-SMX	Survived
16	64; M	CCU	Oct 13	Sputum	<i>A. junii</i>	Colonization	Pip-Taz, Cbp, Cip	Survived

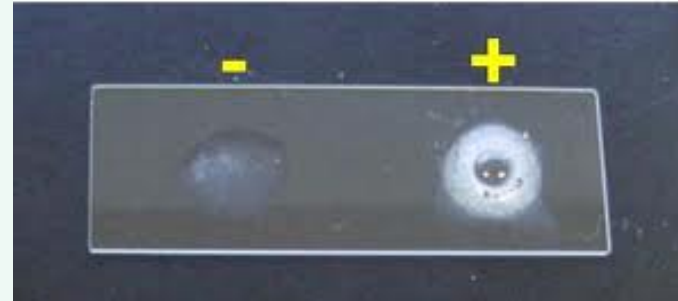
NOTE. *A. xylosoxidans*, *Achromobacter xylosoxidans*; *A. junii*, *Acinetobacter junii*; Amgl, aminoglycoside; *C. acidovorans*, *Comamonas acidovorans*; Cbp, carbapenem; CCU, coronary care unit; Cip, ciprofloxacin; Cpz, cefoperazone; Ctz, ceftazidime; MICU, medical intensive care unit; OBG, obstetrics and gynecology; *O. anthropi*, *Ochrobactrum anthropi*; *P. putida*, *Pseudomonas putida*; *P. stutzeri*, *Pseudomonas stutzeri*; *S. maltophilia*, *Stenotrophomonas maltophilia*; Pip-Taz, piperacillin-tazobactam; SICU, surgical intensive care unit; TMP-SMX, trimethoprim-sulfamethoxazole.

^a Centers for Disease Control and Prevention definitions for nosocomial infection were used to distinguish between nosocomial infection and colonization.⁹

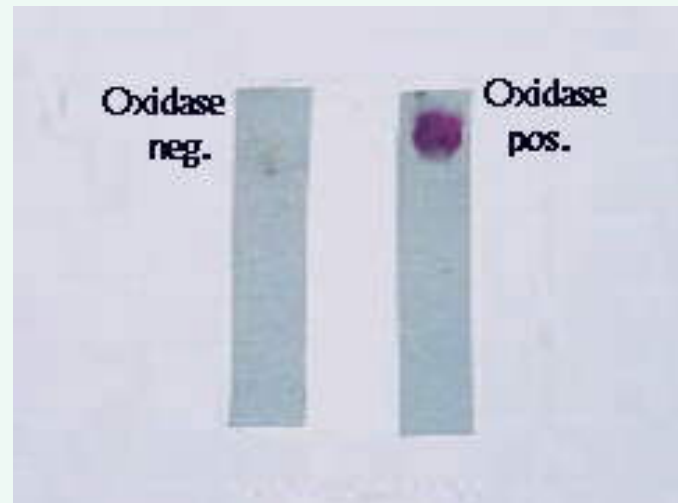
^b Identification of the susceptibility result was performed according to the guideline for *P. aeruginosa* from the Clinical Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards).

Simple rapid tests

: catalase



: oxidase



List of organisms which addressed in M100-32nd ed. (2022)

- *Enterobacterales*
- *Pseudomonas aeruginosa*
- *Acinetobacter* spp.
- *Burkholderia cepacia* complex
- *Stenotrophomonas maltophilia*
- Non-*Enterobacterales*
- *Haemophilus influenzae* & *H. parainfluenzae*
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Staphylococcus* spp.
- *Enterococcus* spp.
- *Streptococcus pneumoniae*
- *Streptococcus* spp. (β -hemolytic)
- viridans *Streptococcus*
- Anaerobes

Thank you and Happy Loi Krathong Festival

