



KLİNİK MİKROBİYOLOJİK TANI

Bakterilerin tanımlanması

Dr Gökhan AYGÜN

CTF

Tıbbi Mikrobiyoloji AD

Klinik Mikrobiyolojik tanı

Uygun Örnek ne olabilir, hangi testleri istemelisiniz

- Etkeni göstermek (boyama)
- Üretmek (Kültür)

Üreme etken/kolonizasyon...YORUM
Sonucun kullanımının takibi
Kontrol önlemleri

Mikrobiyolojik tanı

- Etkeni göstermek (boyama)
- Üretmek (Kültür)
- Etkene ait antijenler/yapılar
- Etkene karşı gelişen antikor (seroloji)
- Etkene özgü DNA/RNA yapıları



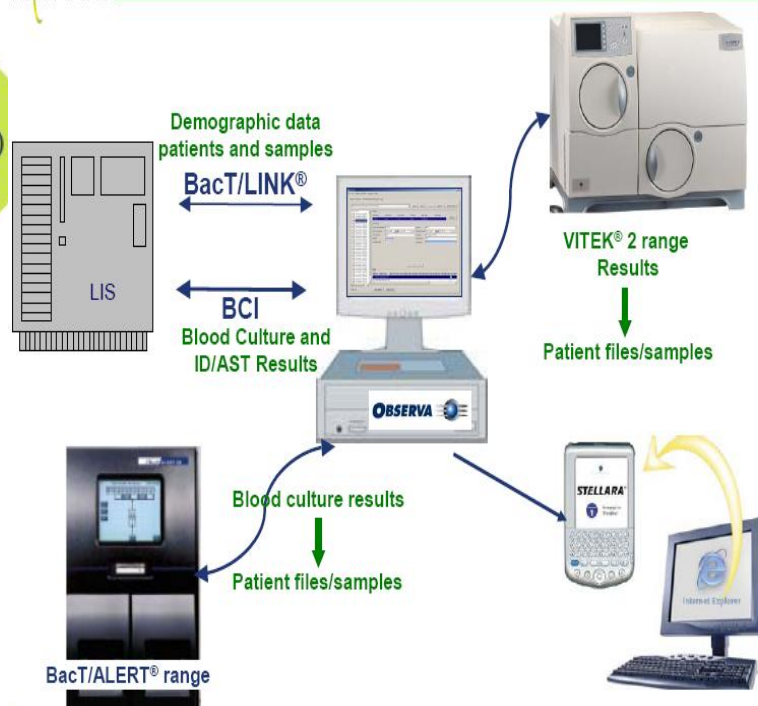
Blood and CSF sampling

Blood culture on BacT/ALERT®

Culture

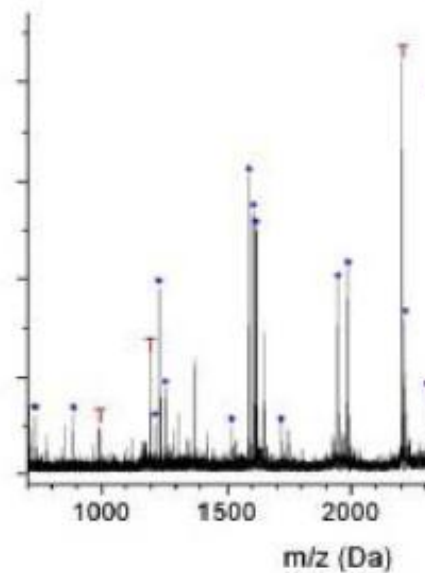
ID and AST on ATB™ Expression

Process completed in 48 hrs (CSF) and 72 hrs (blood)

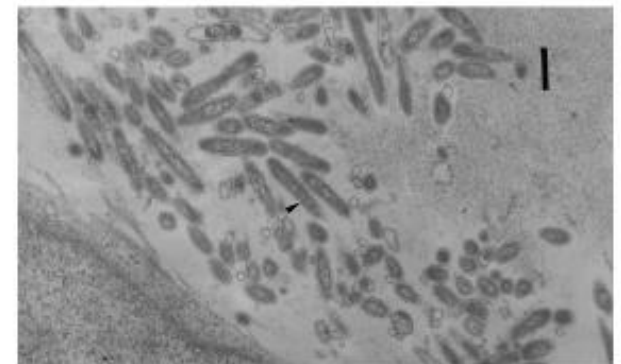
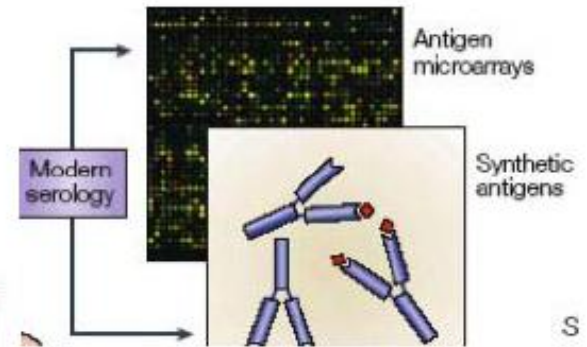


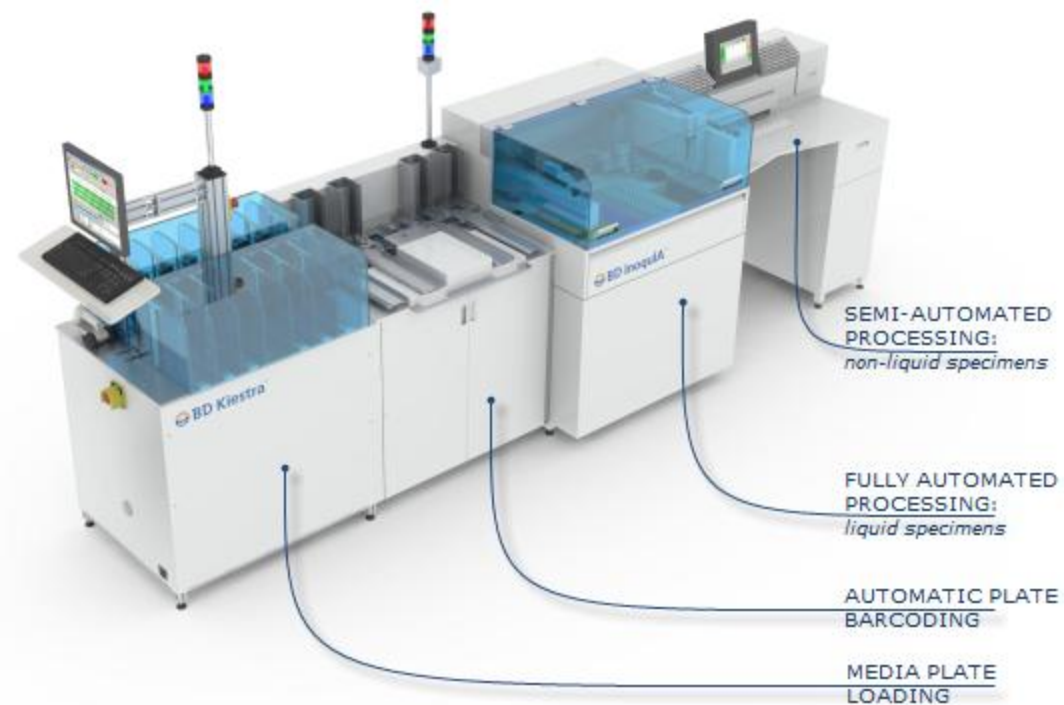
► Antigen microarrays for serology

► Mass spectrometry



► Transmission electronic microscopy





Gelecekte Klinik Mikrobiyoloji

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2002, p. 3889-3893
0095-1137/02/\$04.00+0 DOI: 10.1128/JCM.40.11.3889-3893.2002
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Vol. 40, No. 11

GUEST COMMENTARY

Clinical Microbiology in the Year 2025

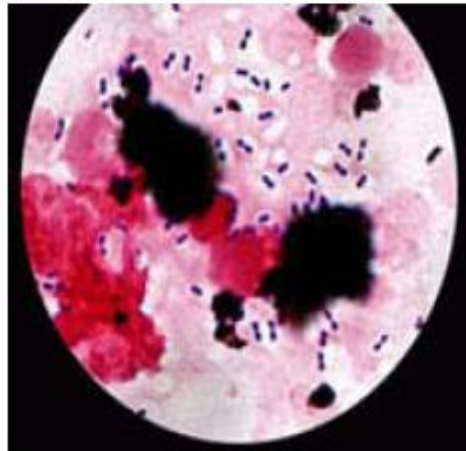
W. Michael Dunne, Jr.,^{1*} J. Keith Pinckard,¹ and Lora V. Hooper²

*Departments of Pathology and Immunology¹ and Molecular Biology and Pharmacology,²
Washington University School of Medicine, St. Louis, Missouri 63110*

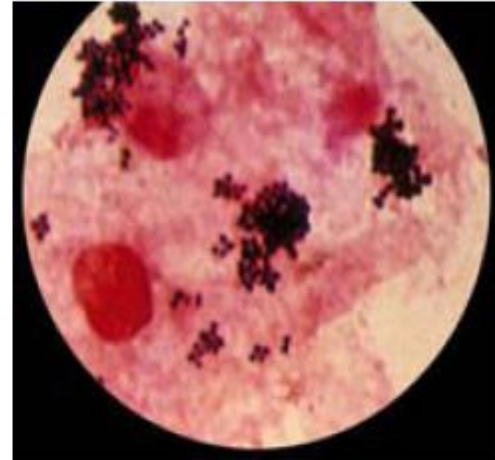




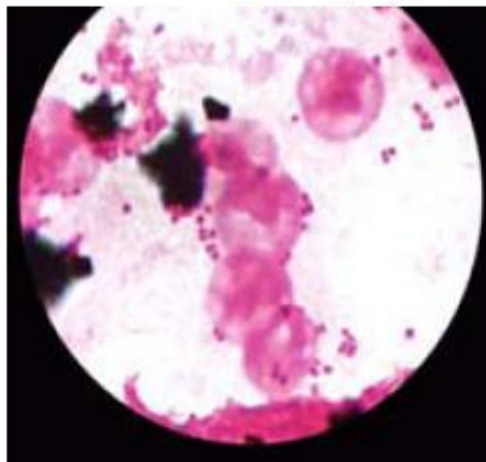
Pneumonia (A)



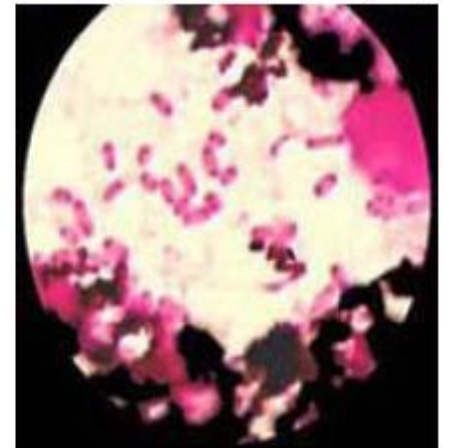
Skin abscess (B)



Meningitis (C)



Pyelonephritis (D)



Örneği ekтім...

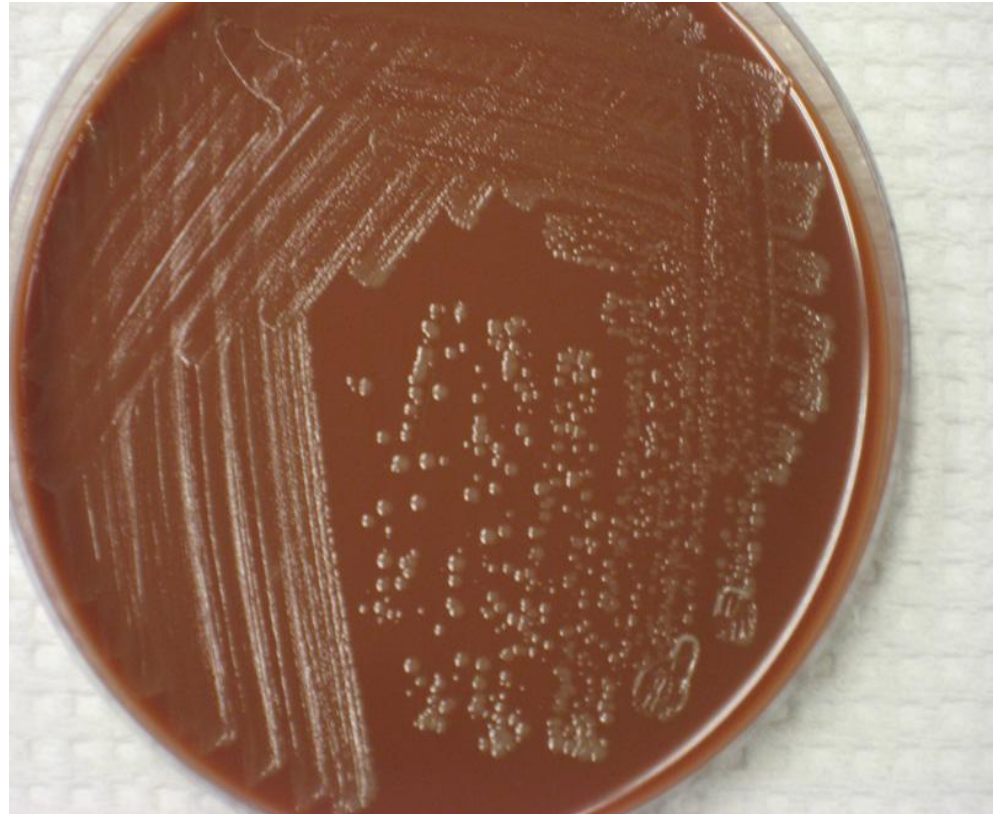
- Sadece Çukolata Agar'da üreme var
- Kanlı Agar /Mac Conkey'de üreme yok !
- Gram (-) çomak =
H.influenzae

Geç mi üredi ?

Francisella tularensis

Gram (-) diplokok:

Gonokok?



Haemophilus influenzae

X ve V faktörü

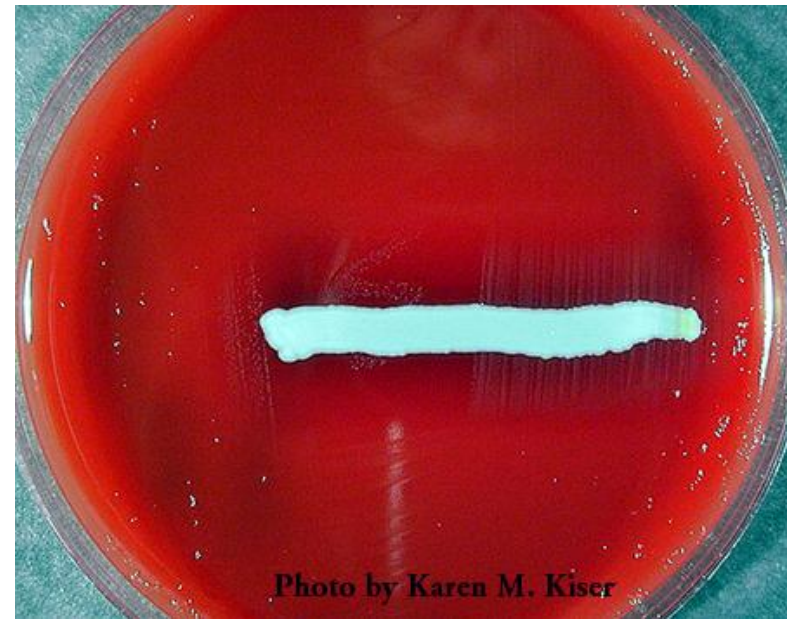
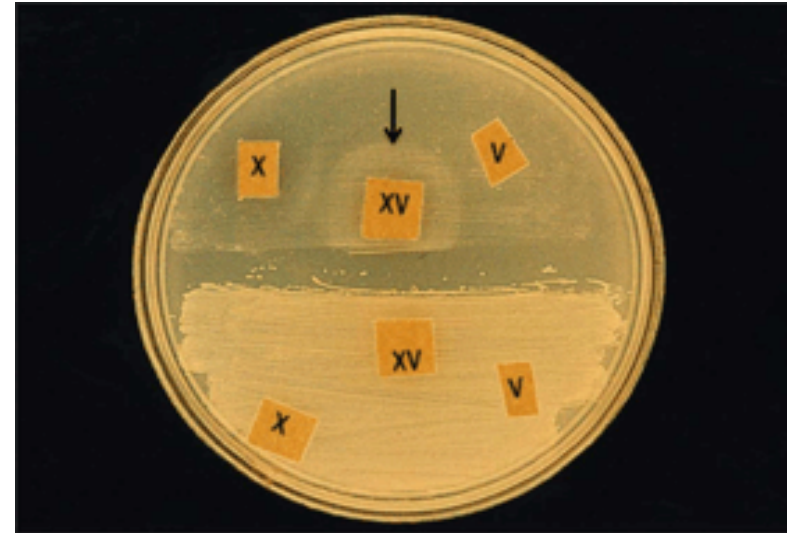
Süt annelik

Serotipleme

Diğer hemofiller

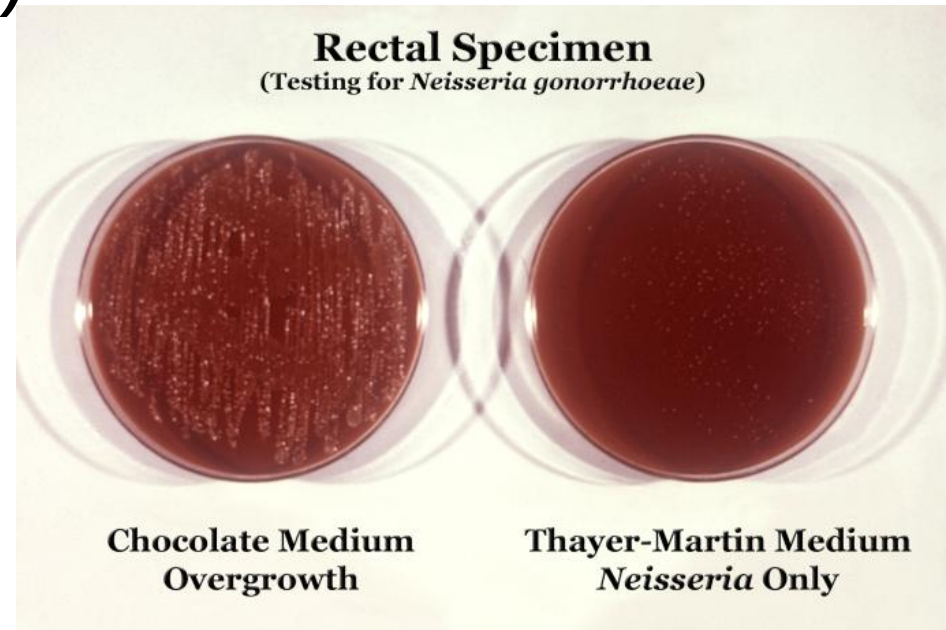
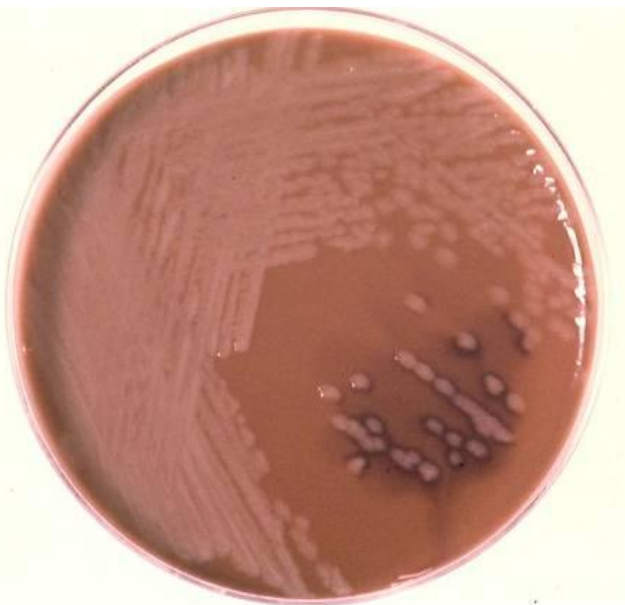
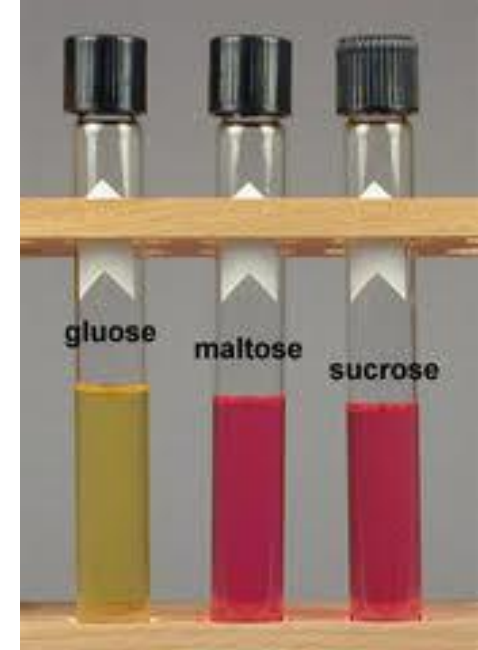
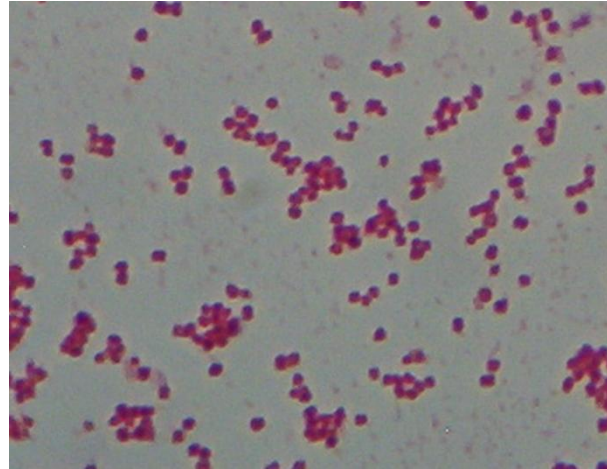
(sadece X, sadece V)

Sıkıntılı alan!



Neisseriae

- Tipik Gram
- Oksidaz (+)
- KH kullanımı
(gonokok, meningokok)



Örneği eklim...

- Sadece Çukolata, Kanlı Agarda üreme var
- Mac Conkey Agarda üreme yok

Gram (+)

Gram(-) kok (Neisseriae)

Gram (-) çomak !!!



Sadece kanlı ve çukulatada Gram-negatif çomak

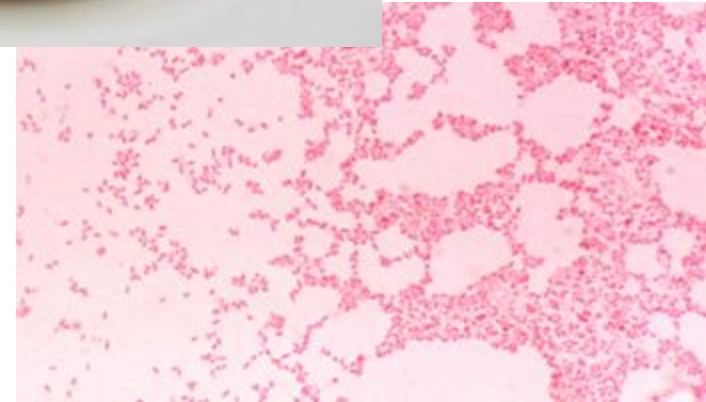
- **Brucella ?**

Oksidaz

Katalaz

Üreaz

Aglütinasyon !! (A ve M)



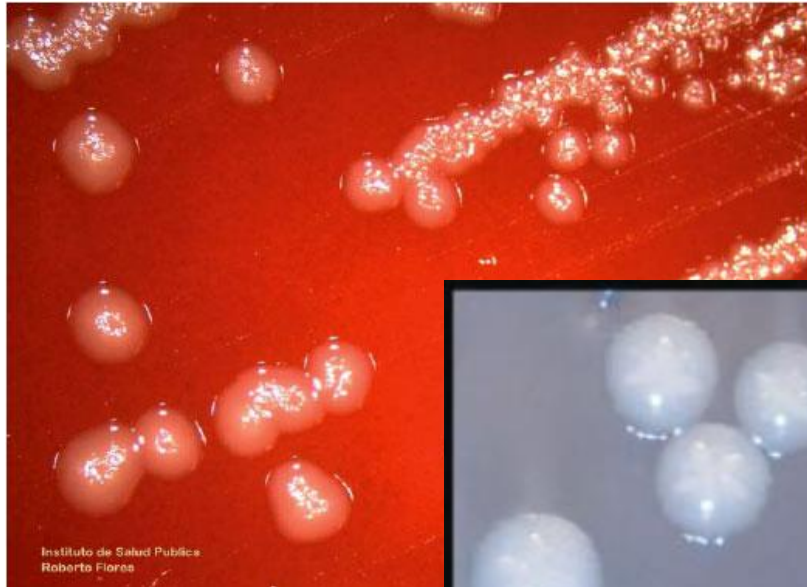
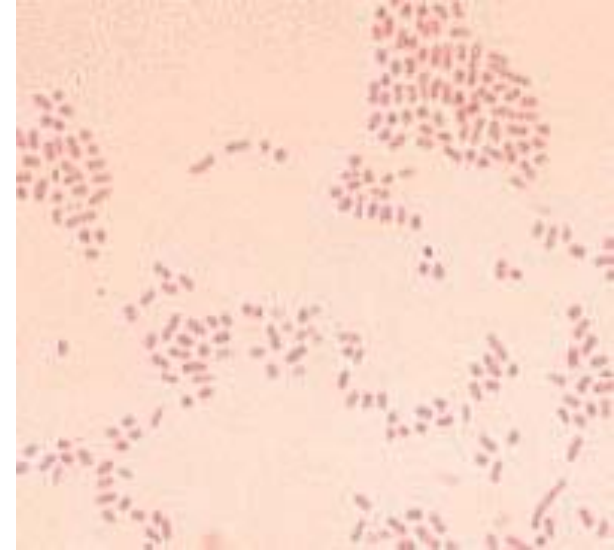
Psychrobacter/Moraxella phenylpruvicus ?

Sadece kanlı ve çukulatada Gram-negatif çomak

- HACEK
- AACEK

Aggregatibacter aphrophilus

Aggregatibacter actinomycetemcomitans



H - *Haemophilus parainfluenzae* / *H. aphrophilus*

A - *Actinobacillus actinomycetemcomitans*

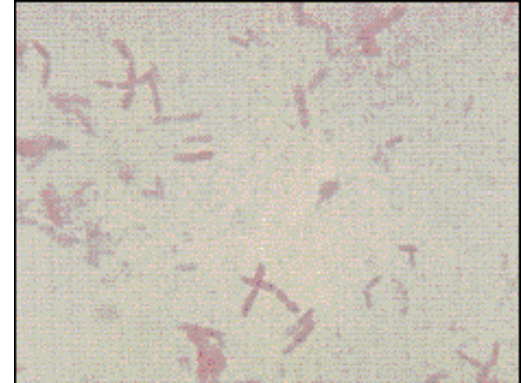
C - *Cardiobacterium hominis*

E - *Eikenella corrodens*

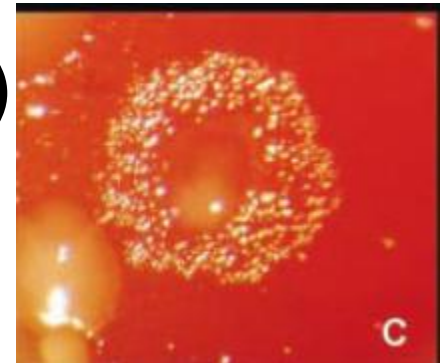
K - *Kingella kingae*

HACEK

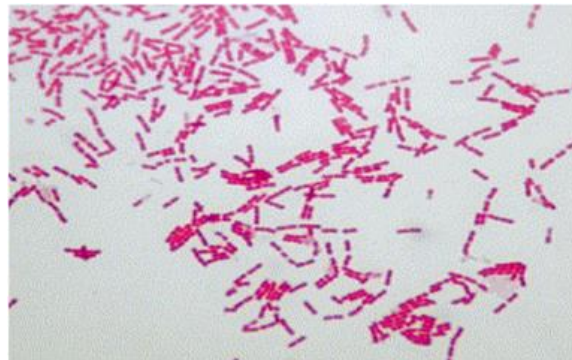
- *Cardiobacterium*



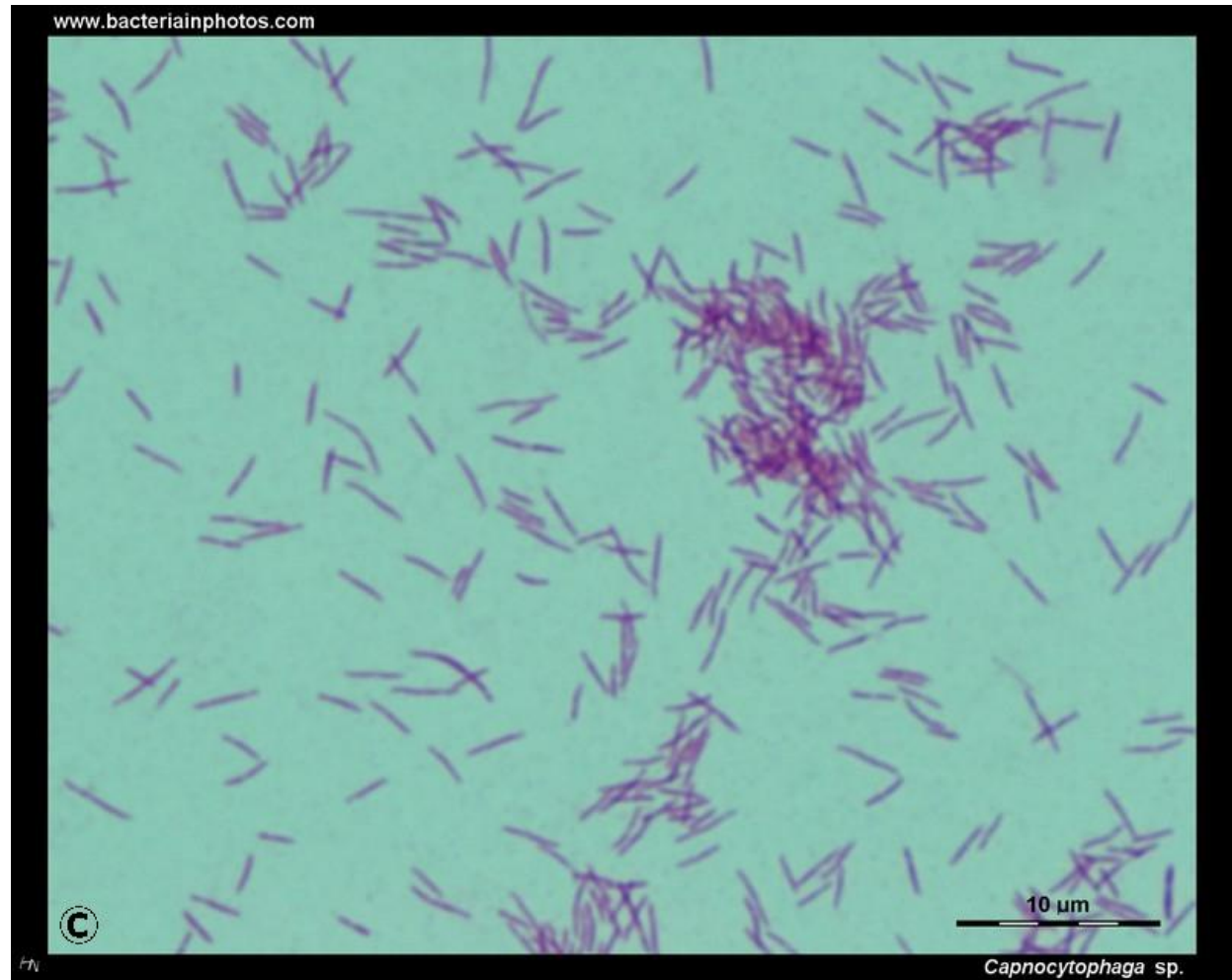
- *Eikenella* (çamaşır suyu kokusu)



- *Kingella*

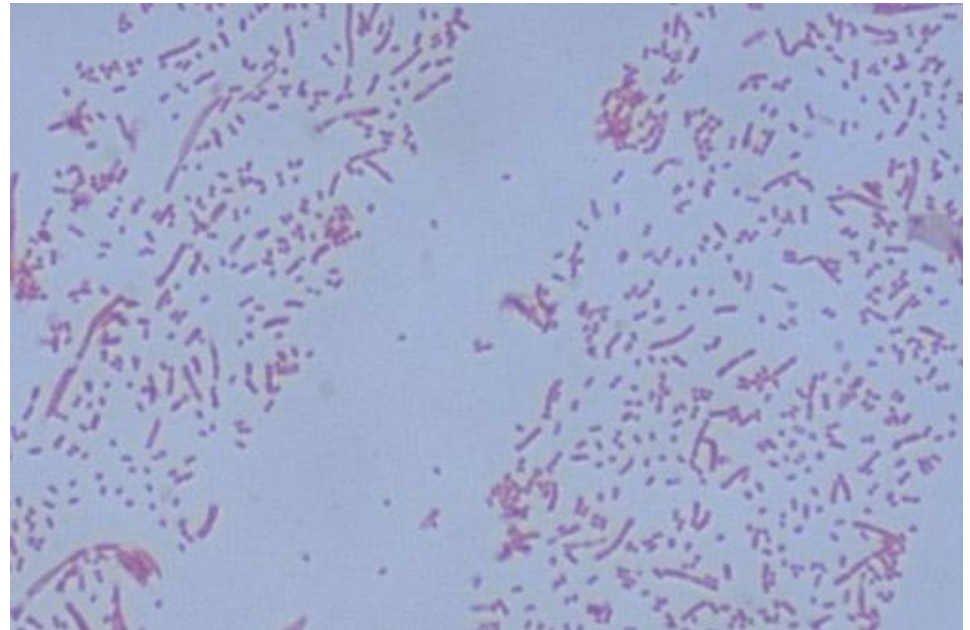
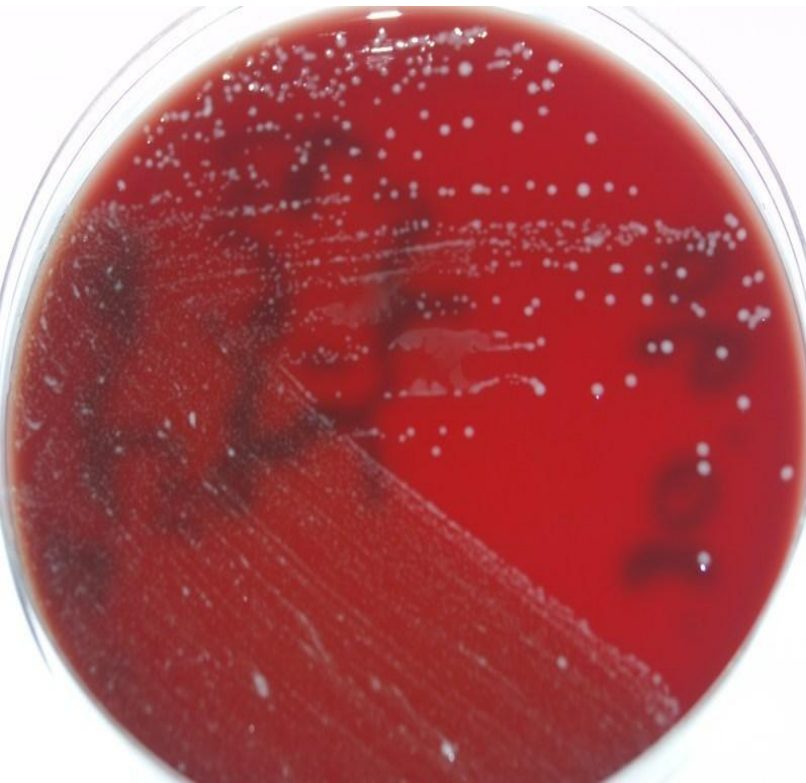


Capnocytophaga



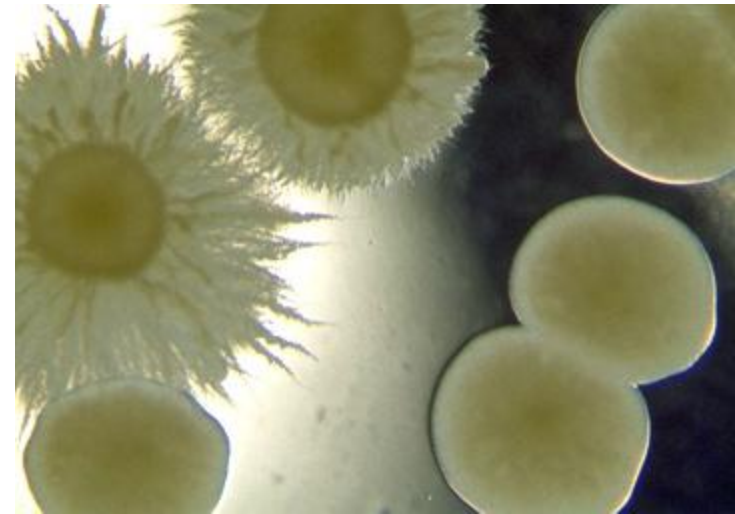
Pasteurella

- Fermentatif
- TSİ zayıf glikoz fermentasyonu
- Penisilin duyarlılığı ?



Örneği eklim...

- Çukolata, Kanlı, Mac Conkey Agarda üreme var ...
- Gram (-) çomak (fastidious:müşkülpesent olmayanlar)
- Maya ?



Gram (-) çomak ...

- Tipik koloni ile tanırım ...
EMB/Endo ..metalik röfle
E.coli



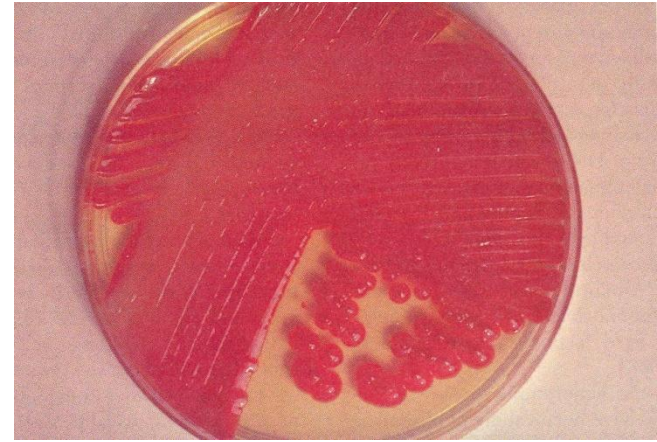
Besiyerinde yayılma
(Swarming)
Proteus mirabilis



Gram (-) çomak ...

- Tipik koloni ile tanırım (pigment) ...

Kırmızı.. *Serratia marcescens*



Yeşil..... *P. aeruginosa*



Gram (-) çomak ...

- Koklarım anlarım ...

P.aeruginosa

Proteus spp.

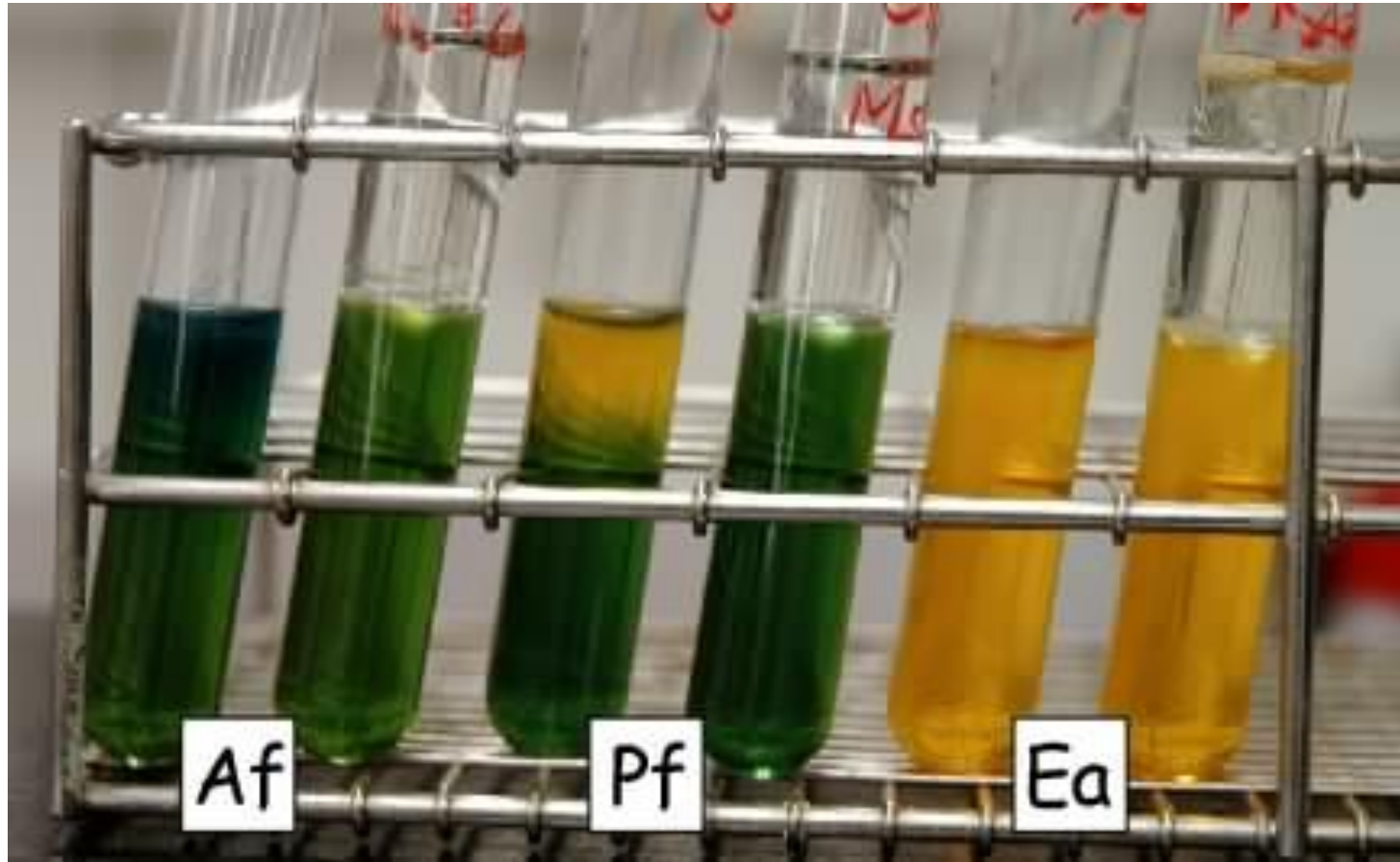
H.influenzae

Eikenella

Nocardia

.....

Gram(-) çomak



Gram negatif çomak

- Fermentatif
Oksidaz (-)
Enterobacteriaceae

- Nonfermentatif
Oksidaz (+)
Pseudomonas

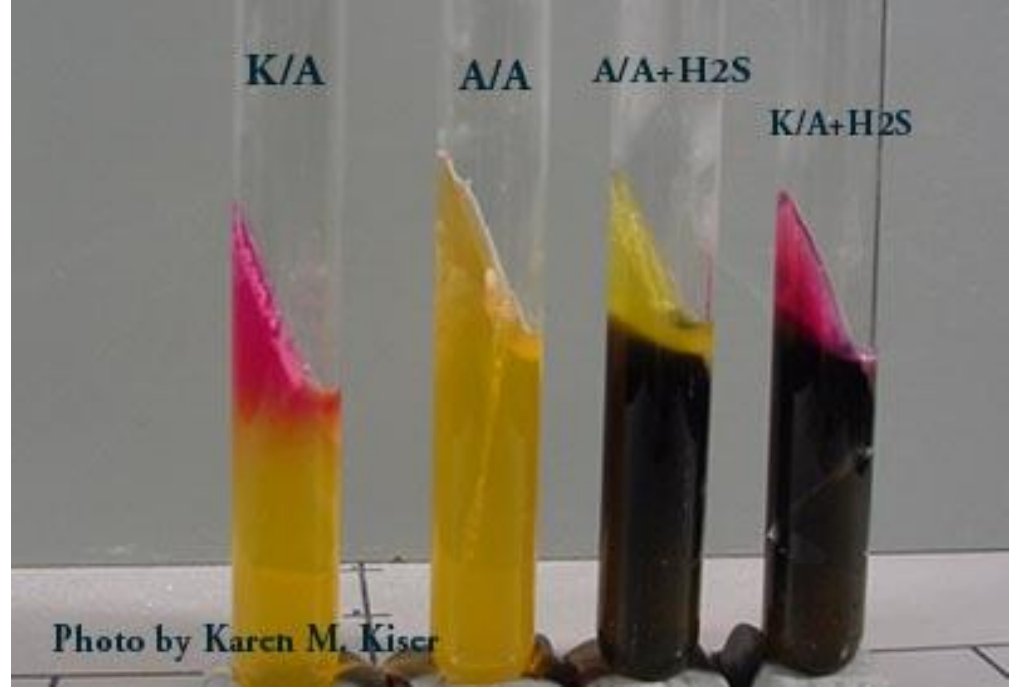
- Fermentatif
Oksidaz (+)
Vibrio ailesi

- Nonfermentatif
Oksidaz (-)
Acinetobacter
Stenotrophomonas





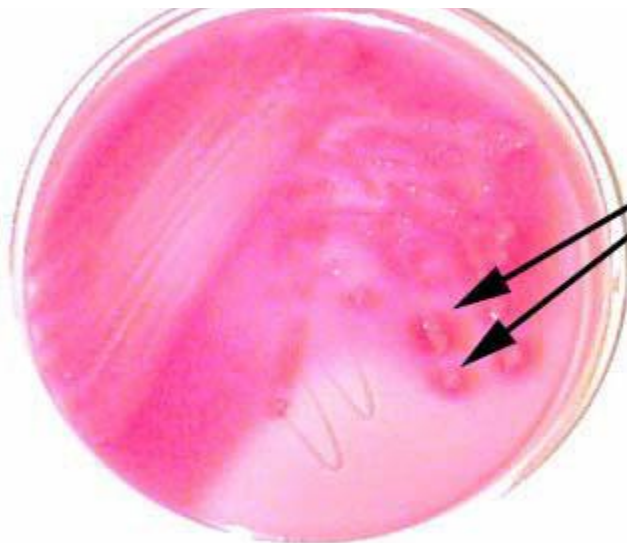
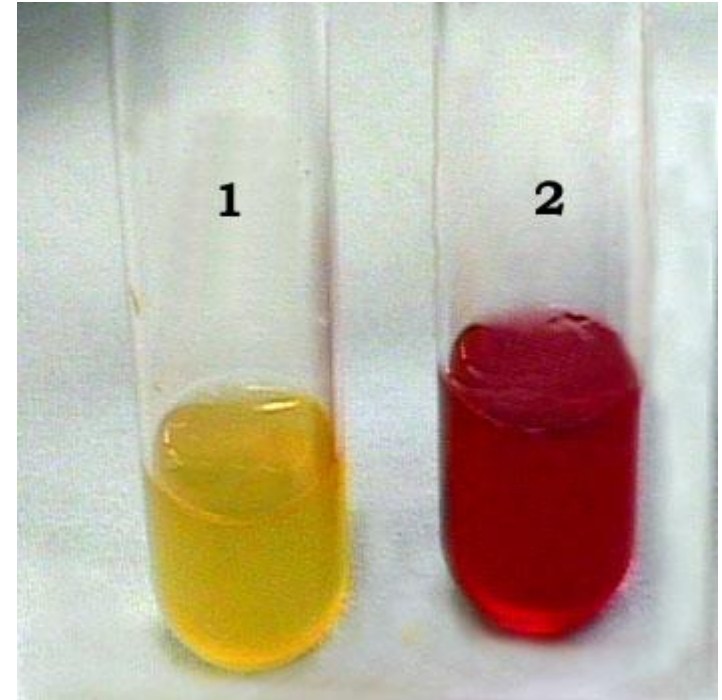
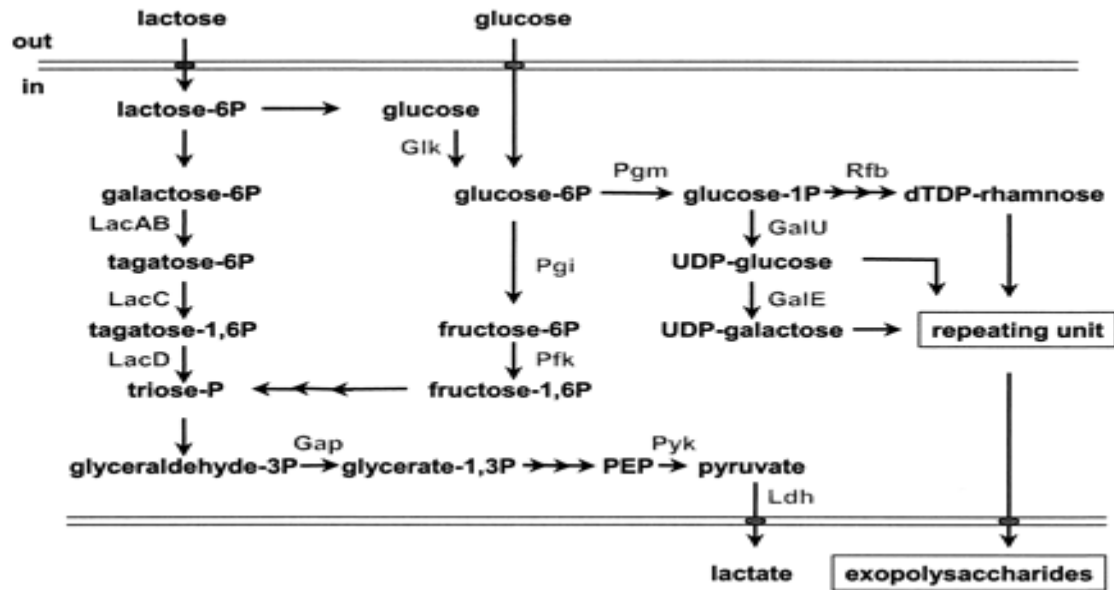
Glikoz (+)
Laktoz (+/-)



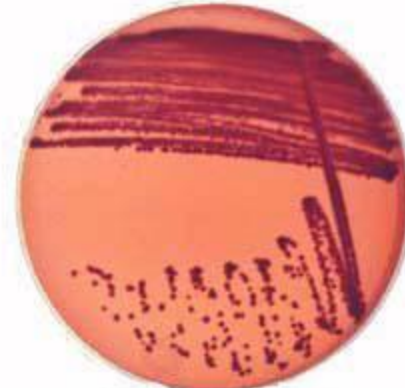
- Glikoza etki yok (-)



Laktoz fermentasyonu



Lactose-fermenting colonies





- Proteus (PAD / yayılma)
- Citrobacter

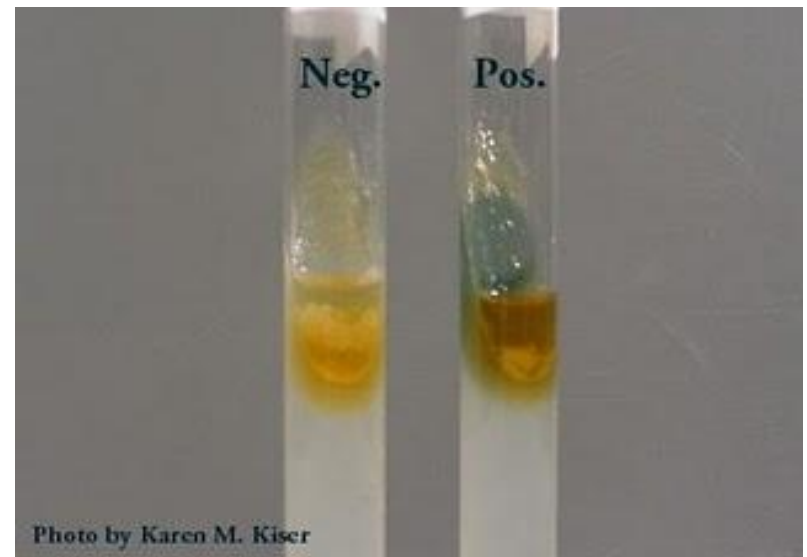
Tanıtıcı deneyler/PAD

- Proteus ailesi

Proteus

Providencia

Morganella





- Sitrat (-), Indol (+), Voges (-)....E.coli
- Sitrat (+), Voges (+)....KES
 - Hareket (-) Klebsiella (indol+ K.oxytoca)
 - Hareket (+) Enterobacter, Serratia
 - DNAze (+)...Serratia

Tanıtıcı deneyler /VP

- Klebsiella kabilesi

Klebsiella

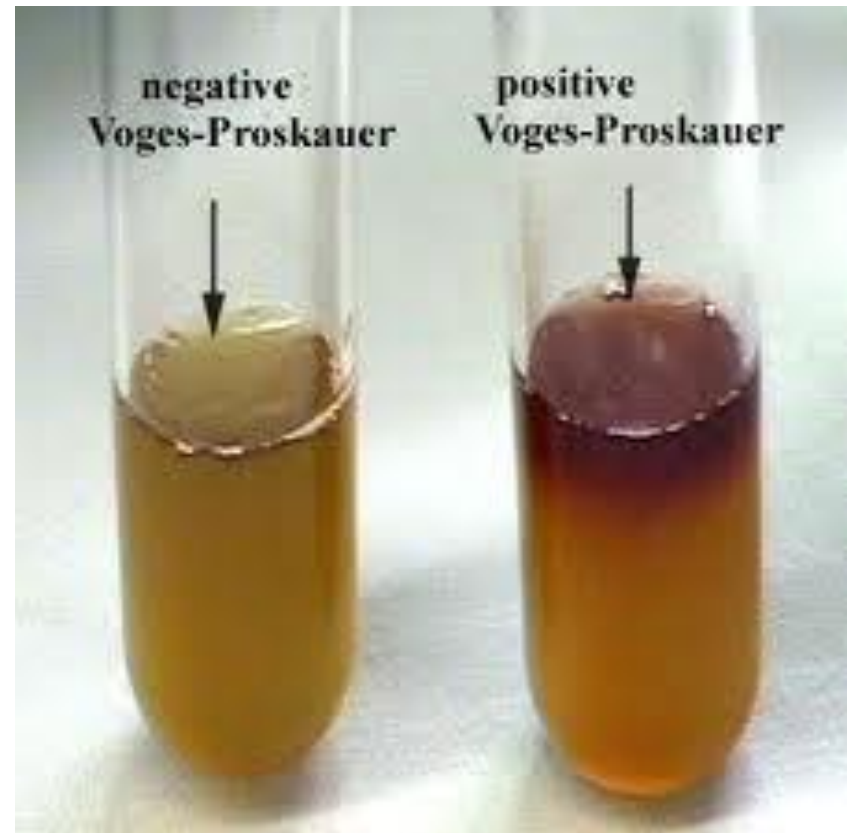
Enterobacter

Serratia

Pantoea

Hafnia

Cronobacter





- Yayılma (+) . Proteus
- Fenil alanin deaminaz(+)... Proteus sp
- PAD (-), yayılma (-), **PYR (-)** ..Salmonella?
Lam aglütinasyonu...



- Hareket (-) ... Shigella, E.coli (Aglütinasyon)
- Hareket(+)...

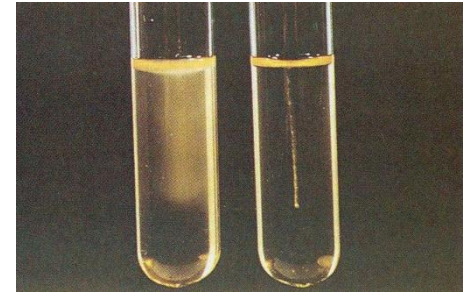
Fenil alanin deaminaz(+)... Morganella, Providencia,
(-)Enterobacter, Serratia, Yersinia,



- Oksidaz (+) ...Aeromonas , Vibrio



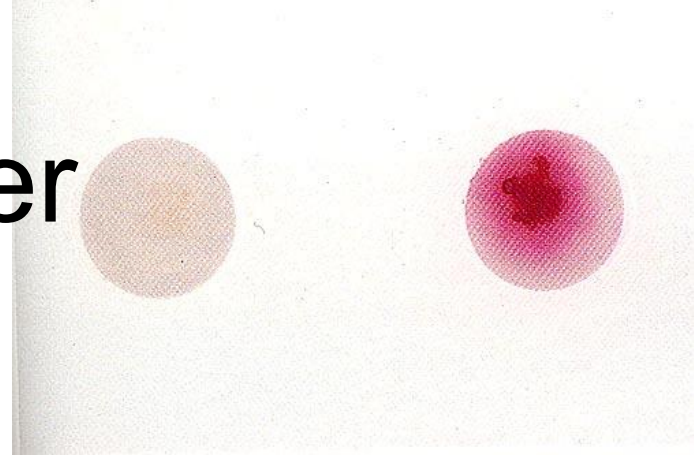
Ayırıcı testler



- Hareket.....Klebsiella / Enterobacter, Serratia
Shigella / E.coli, ...
Yersinia (37 C/22 C)
- DNaze Serratia (+) /Klebsiella kabilesi
- Sitrat (-)..... E.coli, Shigella, Morganella
Yersinia



Ayırıcı testler



- PYR (Pyrrolidonyl-beta naphthylamide)
- İndol, laktoz (+) enterik bakteri
PYR (+)E.coli
- H₂S, sitrat (+) salmonella şüpheli bakteri
PYR (+).....Citrobacter

Laktoz (-) şüpheli koloni

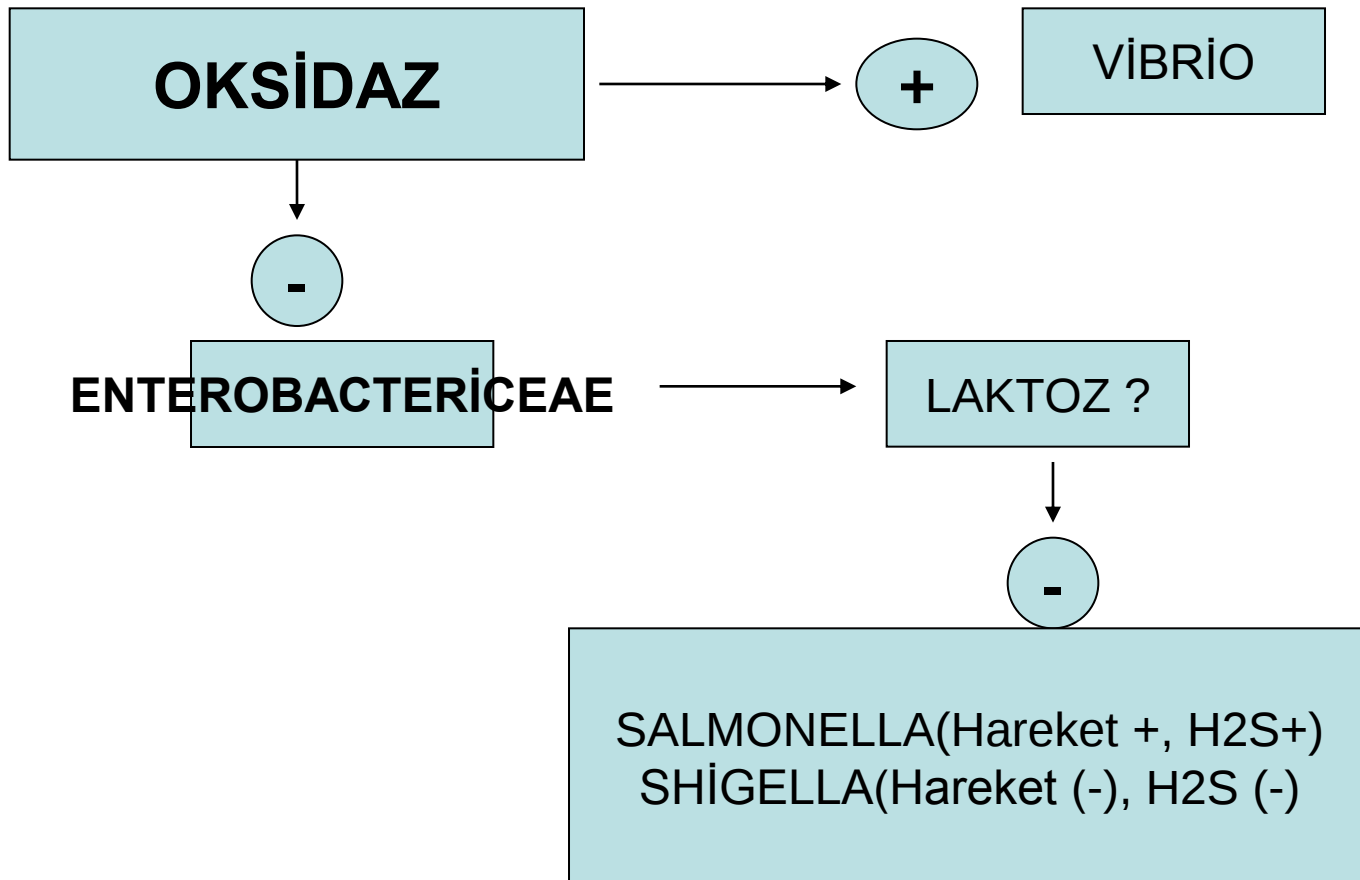
- Salmonella , Shigella
- Lam aglütinasyonu
- Serogruplama



Shigella antiserum will agglutinate strains of the same subgroup or serotype (right); in contrast, the *Shigella* suspension on the left did not agglutinate when mixed with saline.

İshal olgusu?

Kültürde Gram (-) Çomak üremesi (+)



Chrom agar



P.aeruginosa

- 42 C üreme
- Pyocyanin (+)
- Acetamide



ASM MicrobeLibrary.org © Buxton



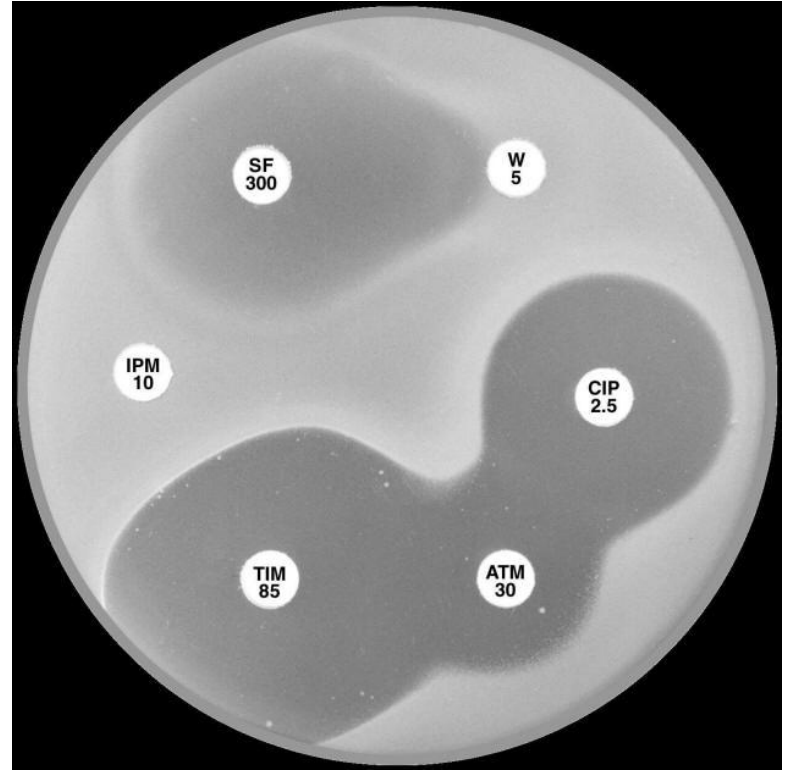
NF/ oksidaz (-)

Acinetobacter spp

- Hareketsiz
- Kok/kokobasil görünümünde (bazen Gram +)

Stenotrophomonas maltophilia

- Hareketli
- DNAZe (+)
- Lizin dekarboksilaz (+)
- Karbapenem dirençli , Kotrimoksazol duyarlı



NF(Sarı pigment)

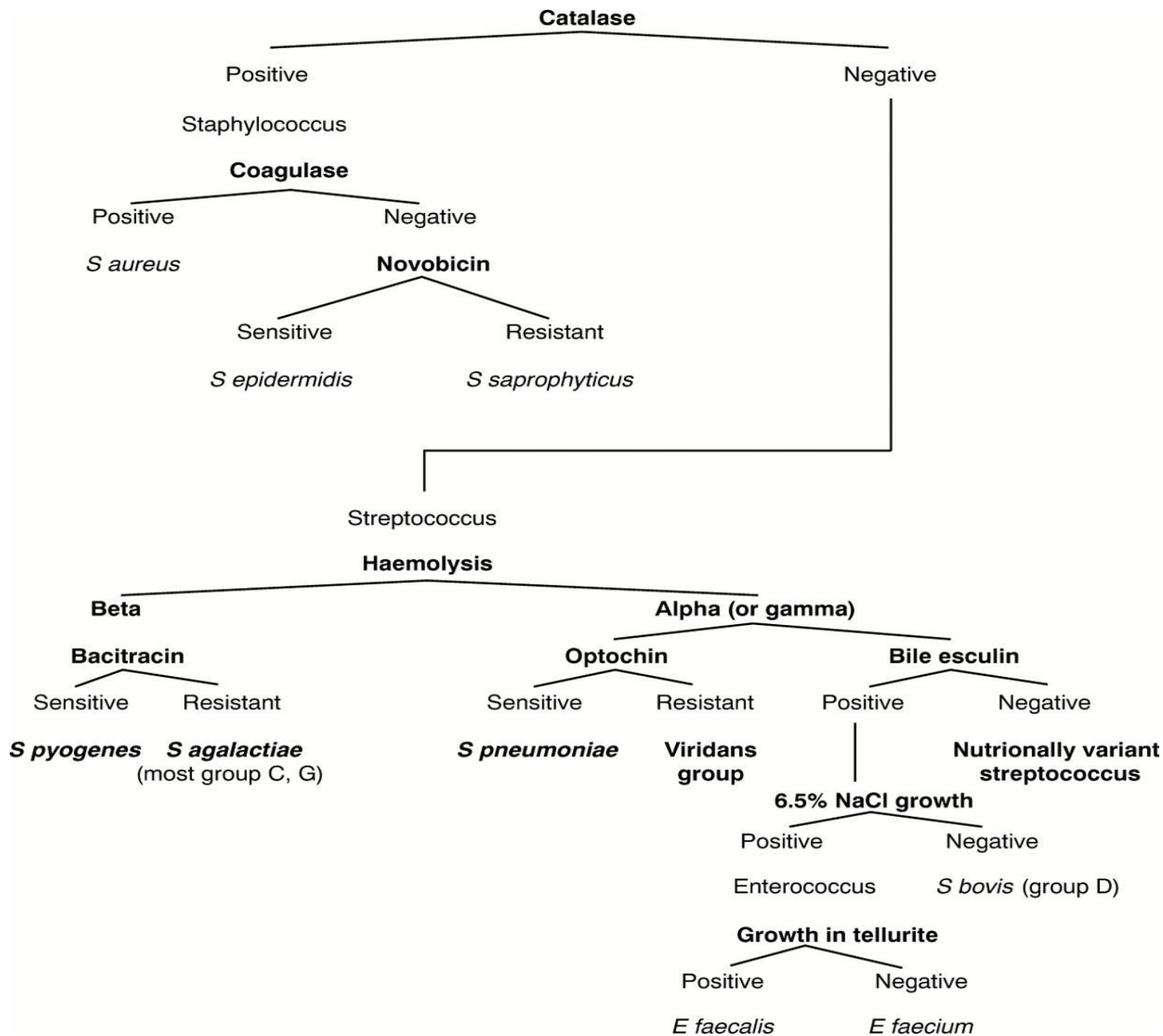
- Chyseobacterium
- Elizabethkingia
- Pseudomonas luteola
- Pseudomonas oryzihabitans
- Sphingobacterium
- P.stutzeri , B.cepacia, Ralstonia,...



NONFERMENTATİF GRAM (-) ÇOMAKLARIN PRATİK SINIFLANMASI

Polar flagella ile hareketli grup (Pseudomonaslar)	Peritrikoz flagella ile hareketli grup	Hareketsiz Oksidaz (+) bakteriler	Hareketsiz Oksidaz (-) bakteriler
Pseudomonas ailesi	Alcaligenes ailesi Alcaligenes, Achromobacter, Bordetella, Kerstersia, Oligella cinsleri	Flavobacteria ailesi Chryseobacterium, Weeksella, Myroides cinsleri ve CDC grupları	Acinetobacter spp
Burkholderia ailesi Burkholderia, Ralstonia, Cupriavidus, Pandoreae, Inquilinus, Lautropia cinsleri	Rhizobacteria ailesi Rhizobium-Agrobacterium cinsi	Sphingobacteria ailesi Sphingobacterium, Pedobacter cinsleri	CDC Grup NO-1
Comamonas ailesi Acidovorans, Facilis-Delafieldi grupları	Brucella ailesi Ochrobactrum cinsi	Moraxella ailesi Moraxella, Psychrobacter, CDC EO grupları	Bordetella holmesii
Caulobacter ailesi Brevundimonas-Diminuta grup		Neisseria cinsi Gilardi grup 1	CDC Grup EO-5
Xanthomonas ailesi Stenotrophomonas cinsi			
Sphingomonas ailesi Sphingomonas cinsi			
Oceanospirilla ailesi Balneatrix cinsi			
Oxalobacteria ailesi Massilia, Herbaspirillum cinsi			
Alteromonas ailesi Shewanella, Alischwanella grup			
Halomonas ailesi Halomonas cinsi			
Methylobacteria ailesi Methylobacterium, Roseomonas			





Katalaz (+) Gram (+) kok



- STAFİLOKOK

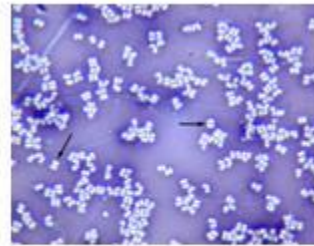
- Micrococcus

Mod oksidaz +

Basitrasin 0.04 U S

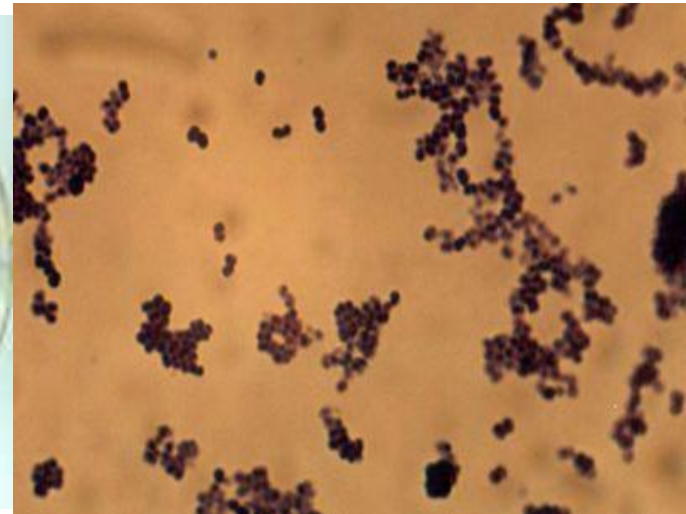
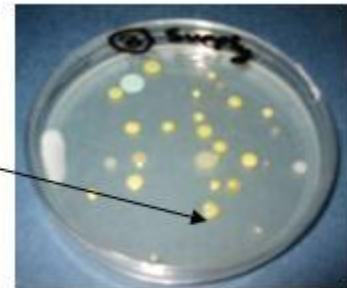
Lysostafine R

- Rothia
- Aerococcus
- Alloiococcus ?



Micrococcus sp

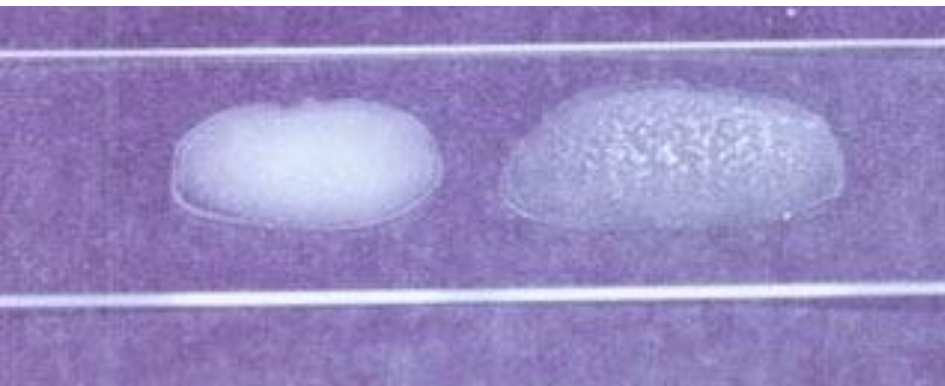
Clustered arrangement of *Micrococcus sp*



S.aureus



www.shutterstock.com · 48041722



Coagulase tubes

Staphylococcus epidermidis

Staphylococcus aureus

S.aureus

- Kümeleyici faktör : +
S.aureus, S. Lugdunensis, S.schleiferi
- Koagülaz : +
S.aureus, S.hyicus, S.intermedius

S.lugdunensis :

Kümeleyici faktör (+), **PYR (+)**

S.aureus

DNAse



Chapman besiyeri



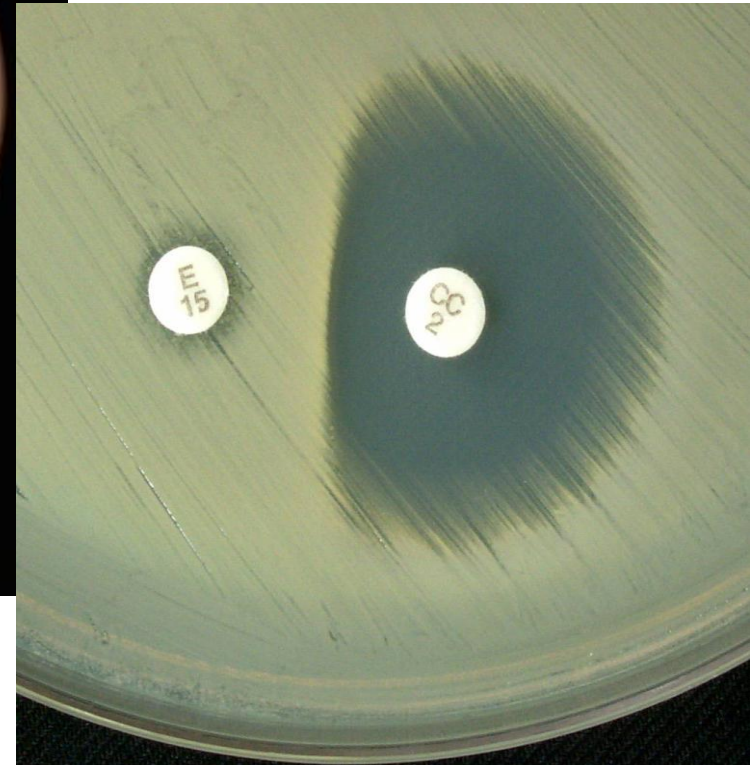
S.aureus antibiogram



strain A
Staphylococcus aureus



strain B
Staphylococcus aureus
methicillin-resistant
MRSA



Stafilokok antibiyotik direnci

- Penisilin Dirençli

Oksasilin/met Duyarlı :

PENİSİLİNAZ.... SAM; NAFS;

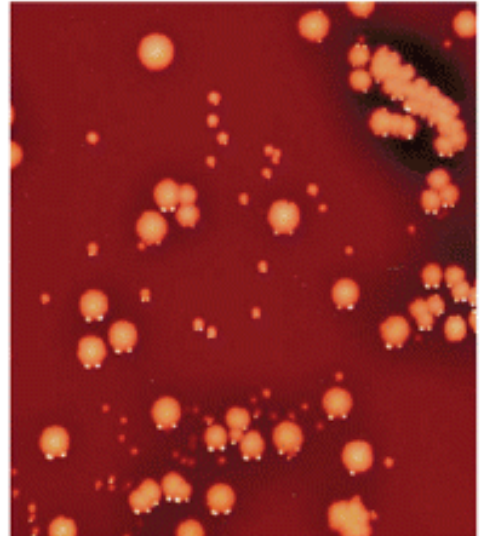
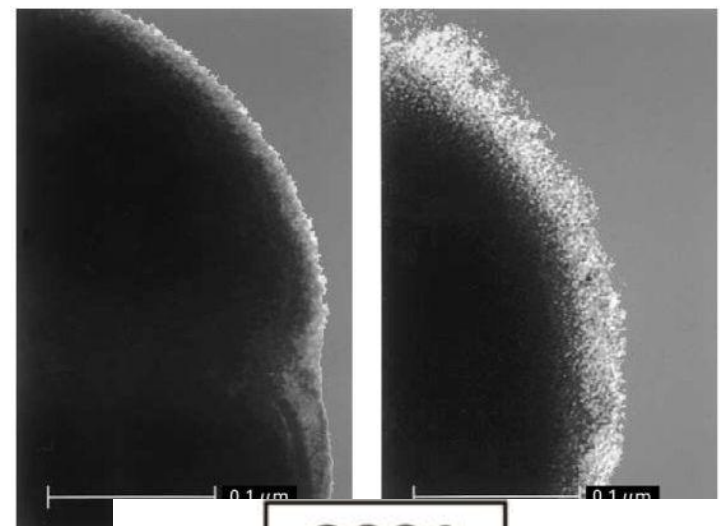
Oksasilin/metisilin Dirençli

PBP değişimi (PBP 2a) (MecA)...

VANKOMİSİN

- Nazal taşıyıcılık : MUPİROSİN
- Toksik şok : Klindamisin ?

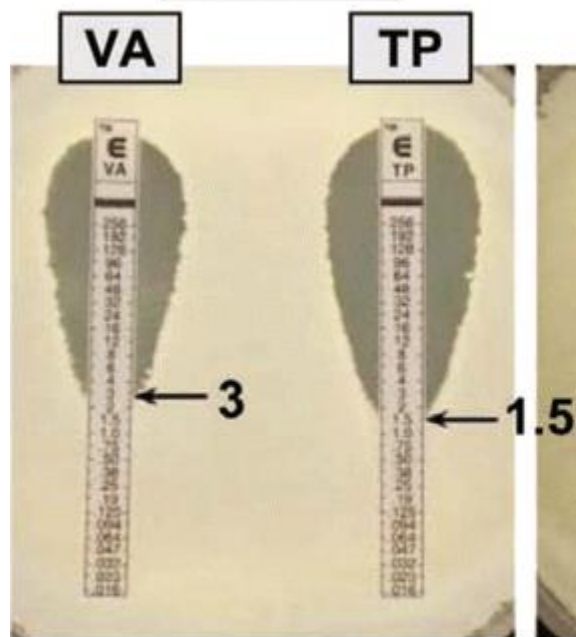
VISA



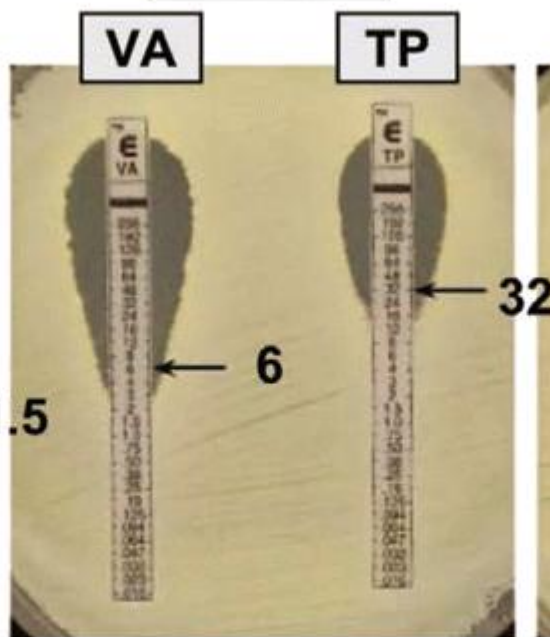
GSSA

hGISA

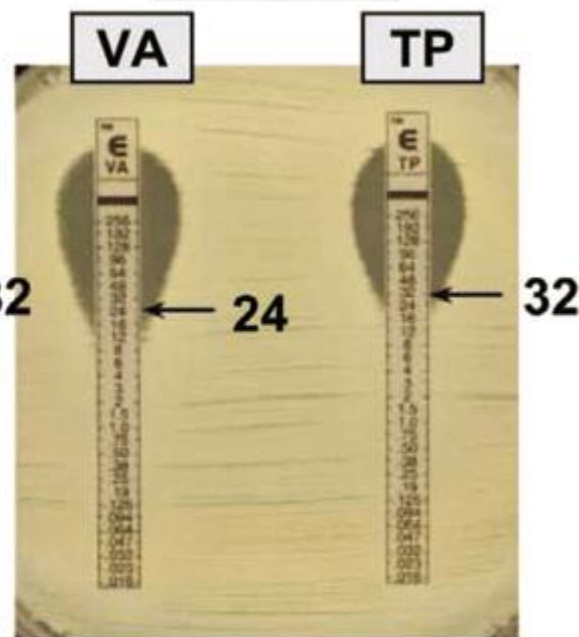
GISA



S. aureus ATCC 43300
(MRSA)

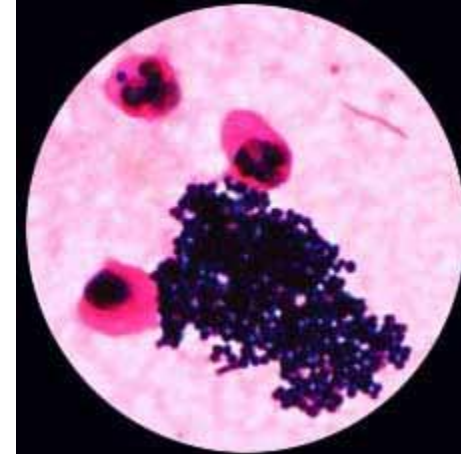


S. aureus ATCC 700698
(Mu 3 - hGISA)



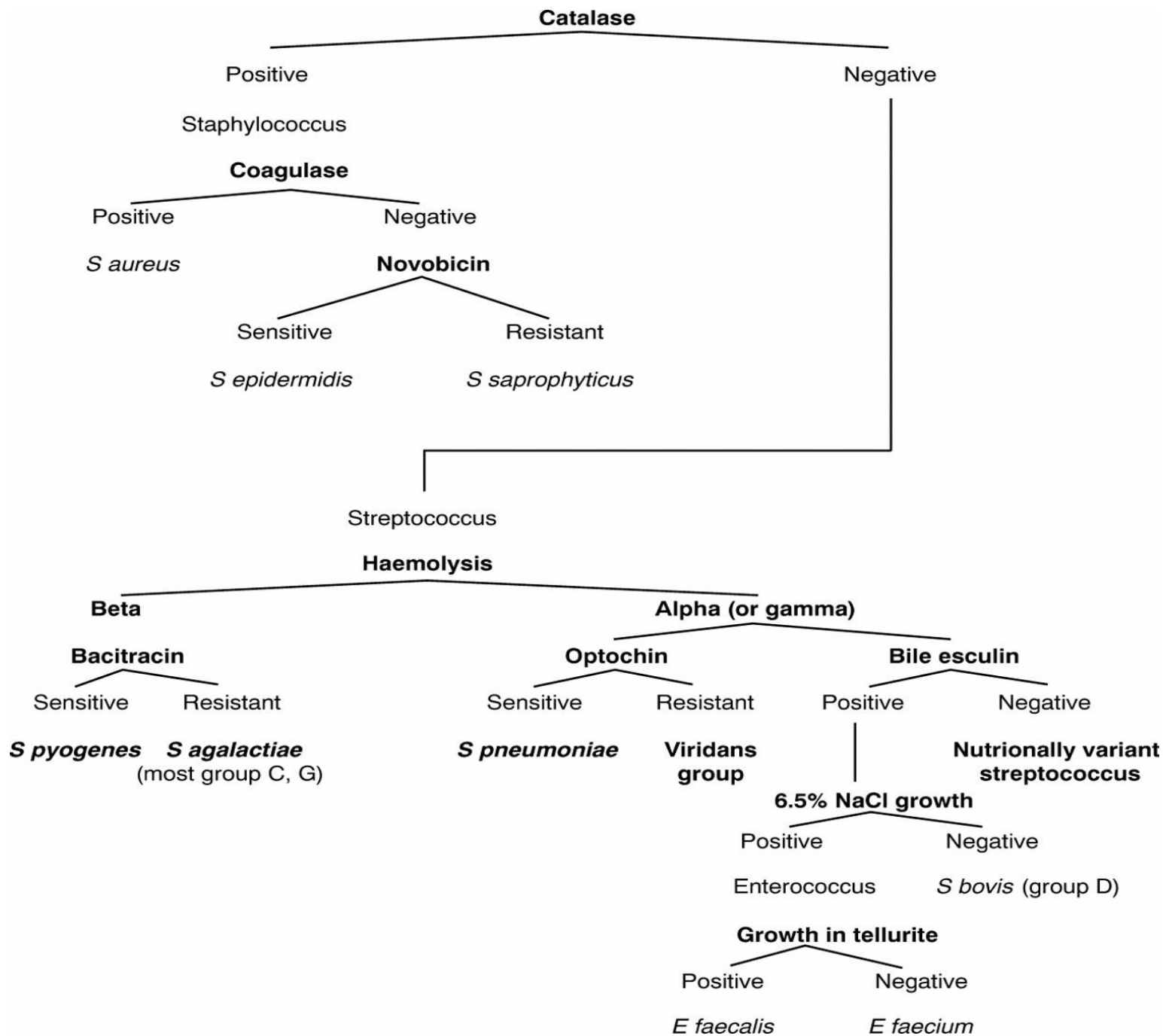
S. aureus ATCC 700699
(Mu 50 - GISA)

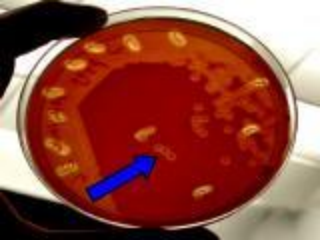
Genç kadınlarda üriner sistem enfeksiyonu



- Antibiyogram önerilmez
NF, SXT, kinolon yeterli

NB 5 ug





Beta hemolitik streptokok

- GAS (*S.pyogenes*)
- Serogruplama



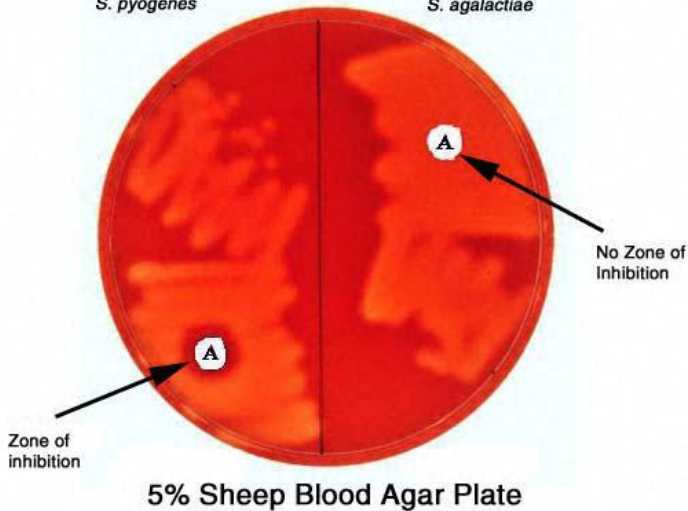
Beta hemolitik streptokok

- GBS (*S. agalactiae*)



S. pyogenes

S. agalactiae



Beta hemolitik streptokok

Basitrasine Duyarlı.....GAS

Dirençli

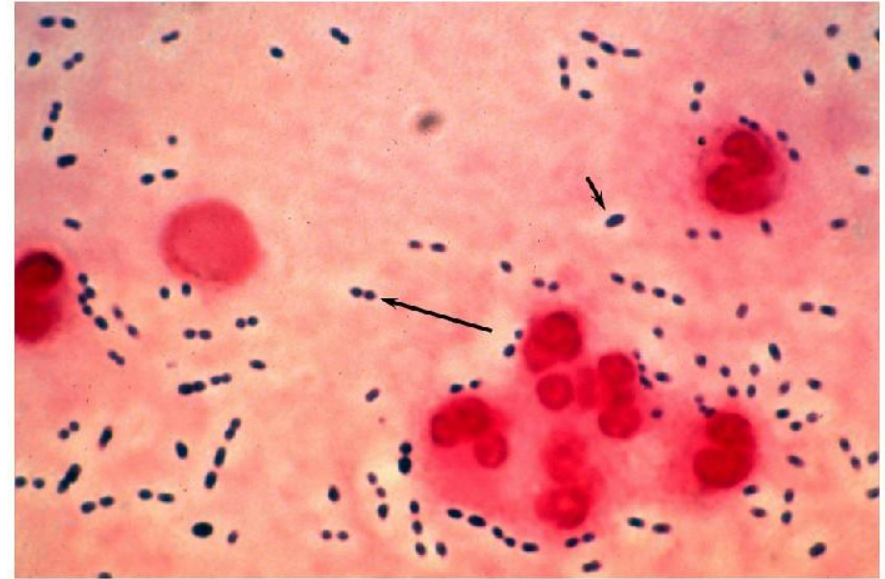
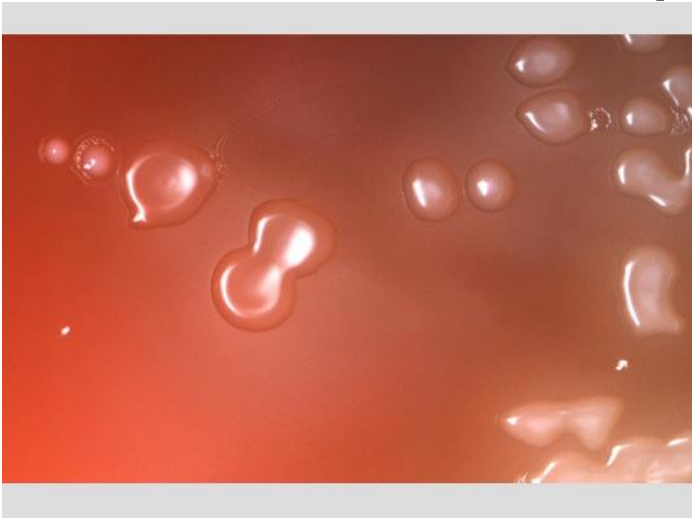
CAMP (+).....GBS

(-) ya da minik koloniler

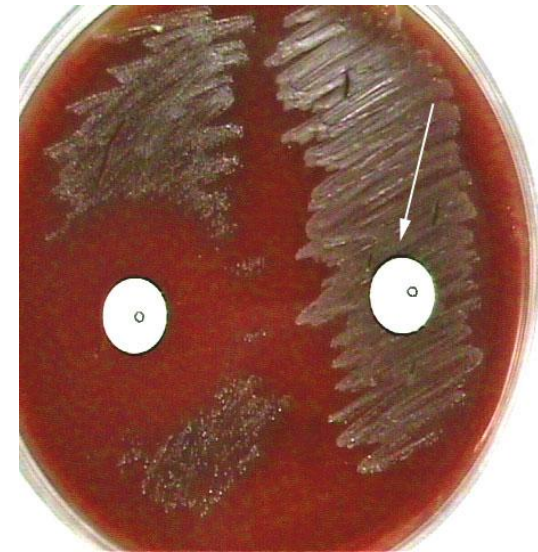
C,D,G,...diğer beta-hemolitik str grupları

Alfa hemolitik streptokok

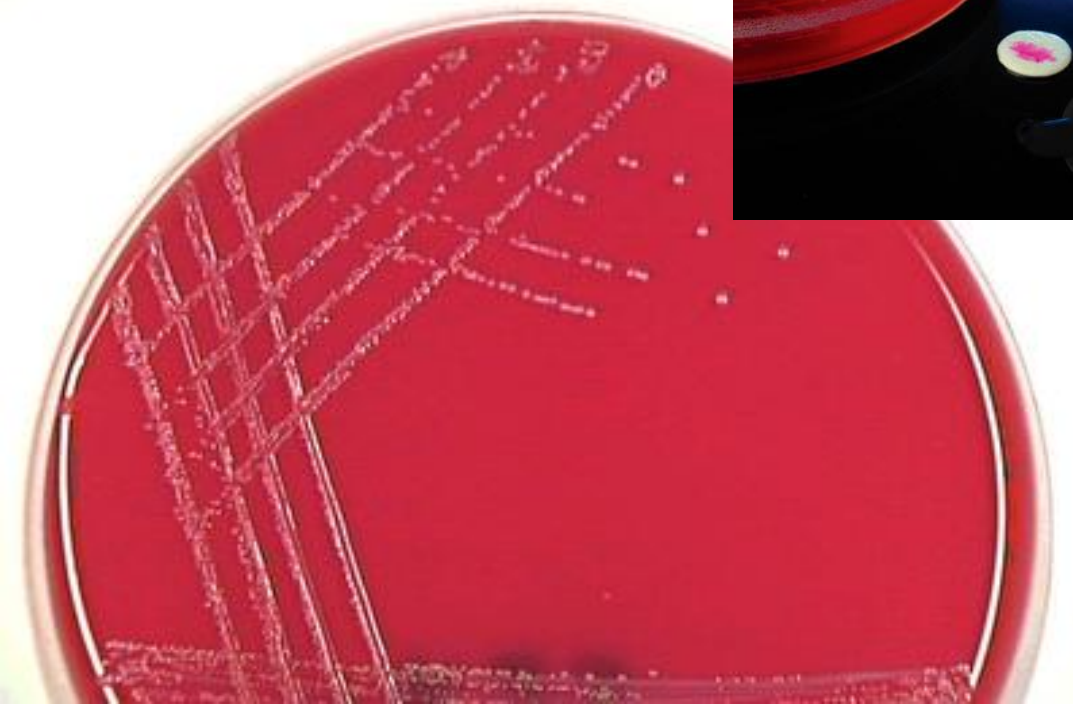
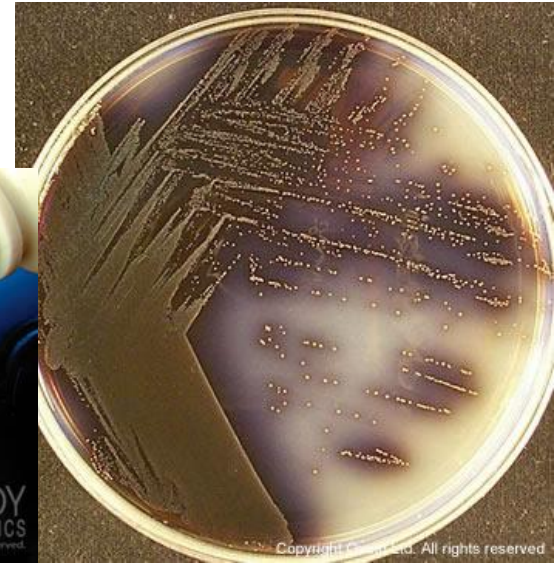
- Pnömonokok = Kapsül



- Safrada erime
- Optokin duyarlılığı



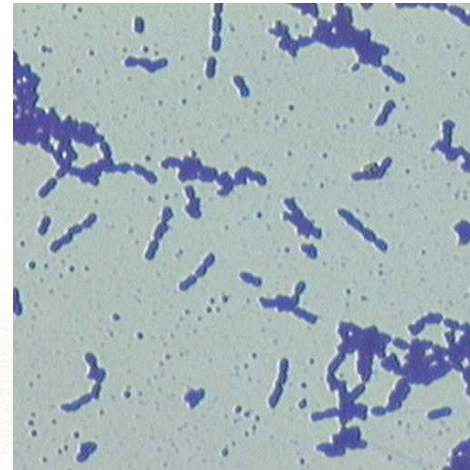
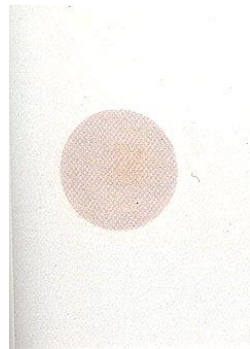
Alfa hemolitik streptokok



STREPTOCOCCUS IDENTIFICATION

Group 	Hemolysis	Bacitracin Sensitivity	CAMP Test	Bile- Esculin Agar	6.5% NaCl Broth	Optochin Sensitivity
<u>A</u> 	Beta	+	-			
<u>B</u>	Beta	-	+			
C	Beta	-	-			
D Enterococcus 	Alpha or Gamma			+	+	-
D Non-Enterococcus	Alpha or Gamma			+	-	-
Viridans	Alpha			-		-
<i>Streptococcus pneumoniae</i>	Alpha			+	-	+

VRE agar (vankomisin dirençli)



VRE

- Van C (intrensek)
- Van MİK: 2-32

E.gallinorum

E.casseliflavus hareketli olmakla
diğerlerinden ayrılırlar !

GRAM (+) ÇOMAKLAR

Sporlu (**Bacillus, Clostridium**)

Sporsuz, aerob (corynebacterium, listeria, erysipelothrix, gardnerella,...)

G (+) çomaklar

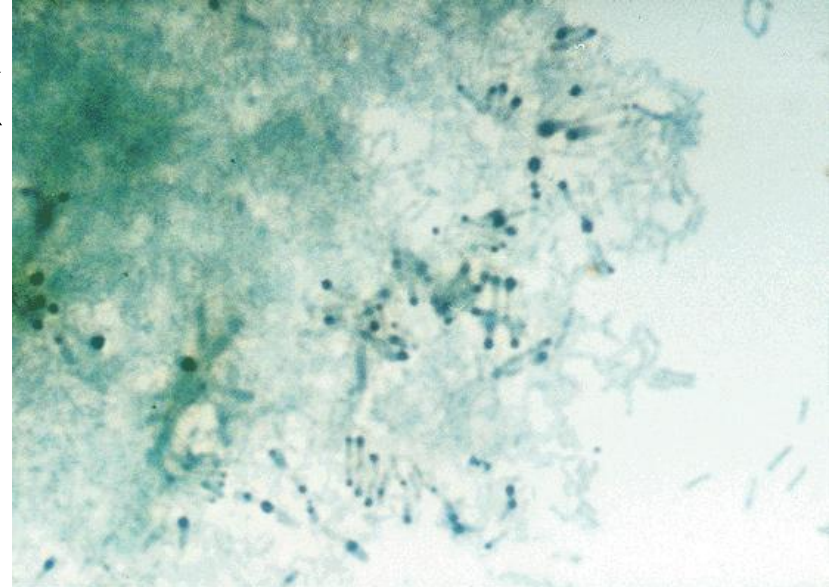
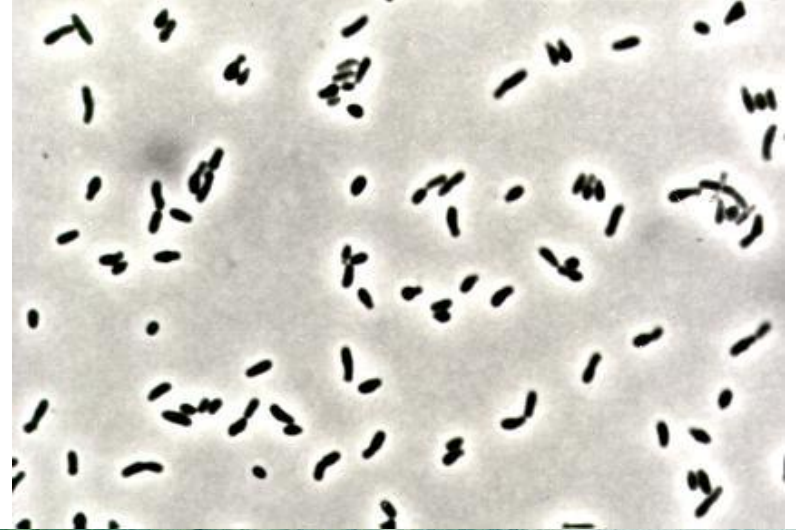
Aktinomisetler (nocardia, aktinomiset)
(**DALLANMA**)

Corynebacterium (Latince)

koryne: club- çomak

Corynebacterium

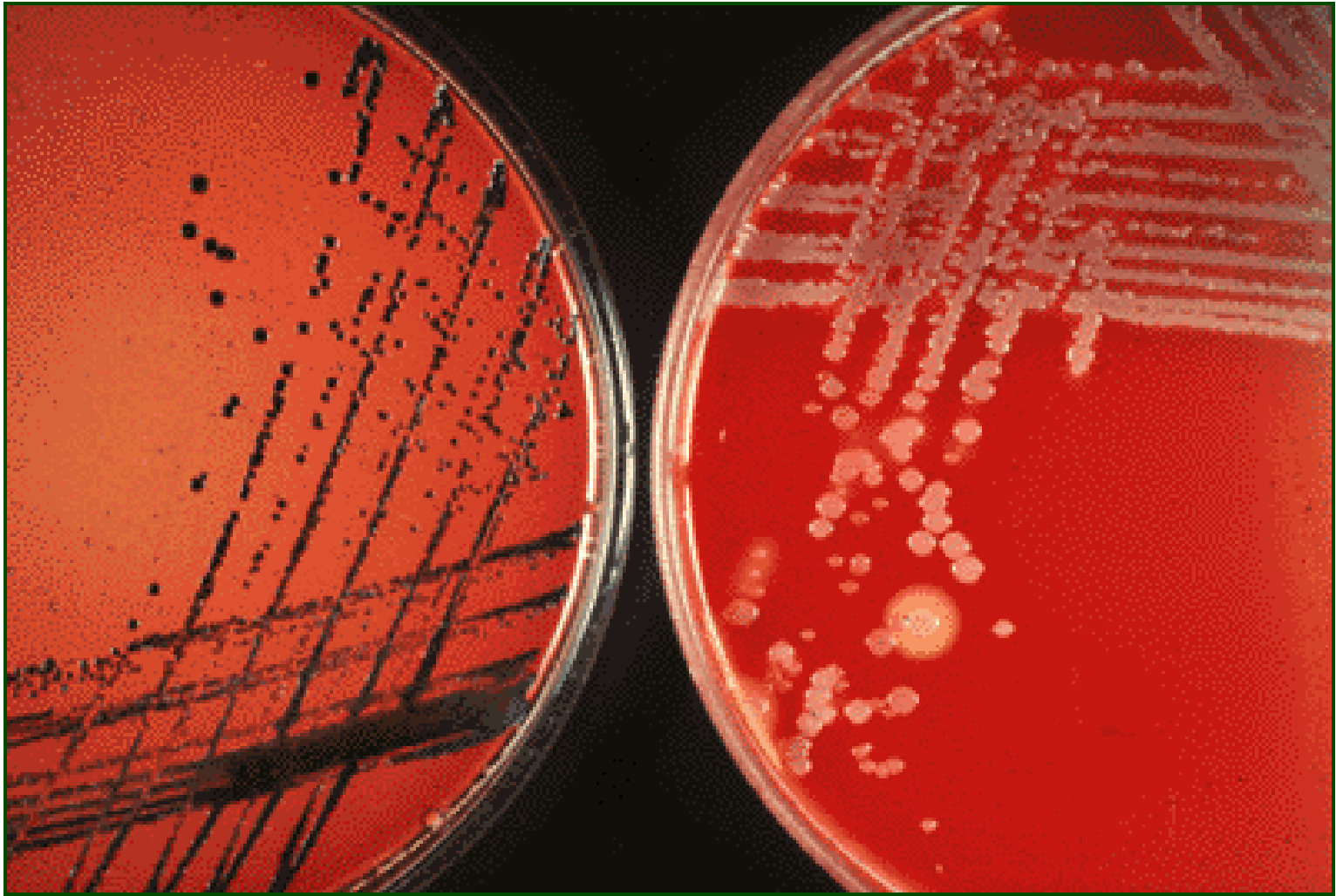
- Gram (+), sporsuz, kapsülsüz
- Volutin taneleri (Polimetafosfat)
- METAKROMATİK CİSİMCİK (NEISSER BOYAMA)



Corynebacterium diphtheriae

Tellurit agar

Kanlı agar

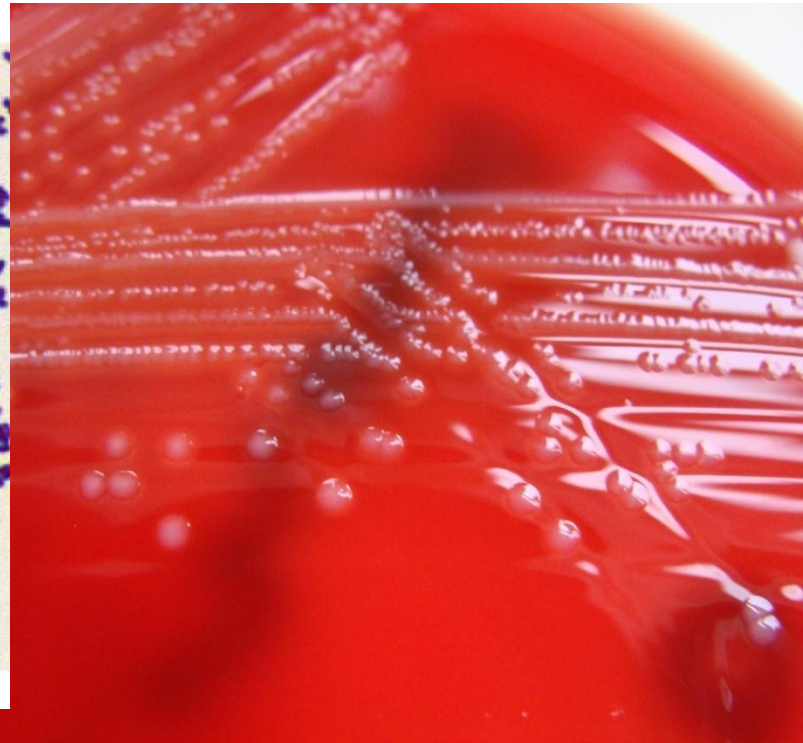
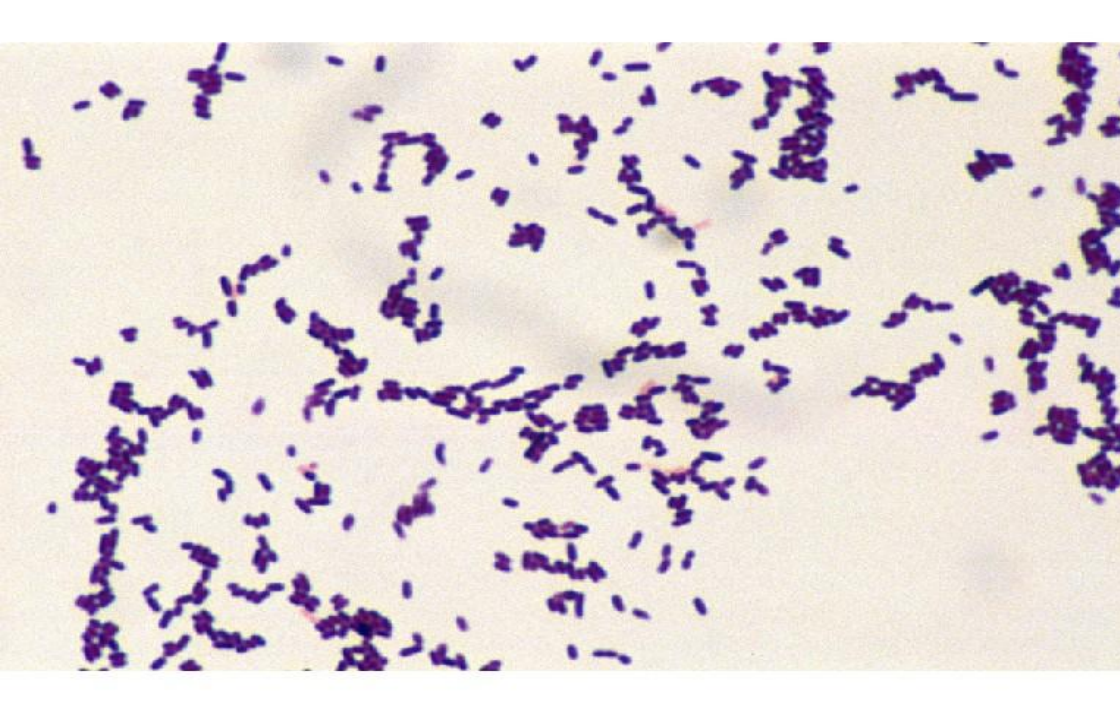


Kültür ve Toksin yapımı ??

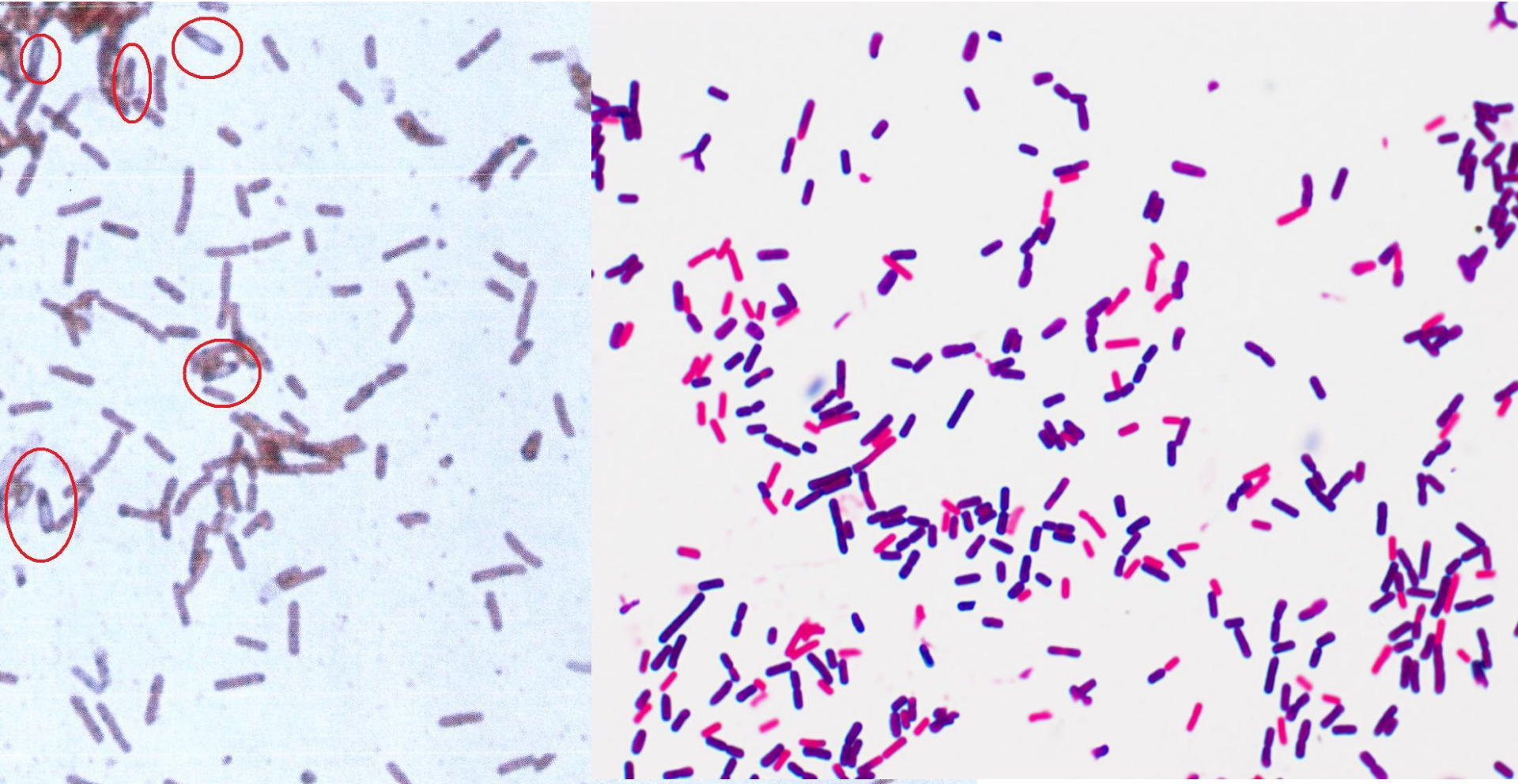
- Löffler besiyeri (Pai), tellüritli besiyeri ve kanlı agar besiyeri kültür için kullanılır
- Fare deneyleri
- ELEK testi

Listeria monocytogenes

- Soğukta zenginleştirme
- Hareket
- Eskülin (+)



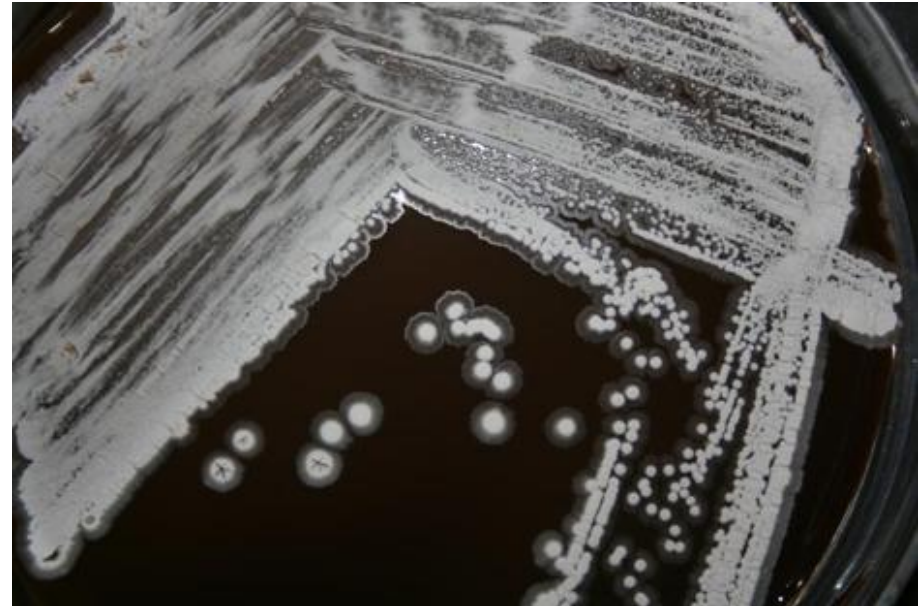
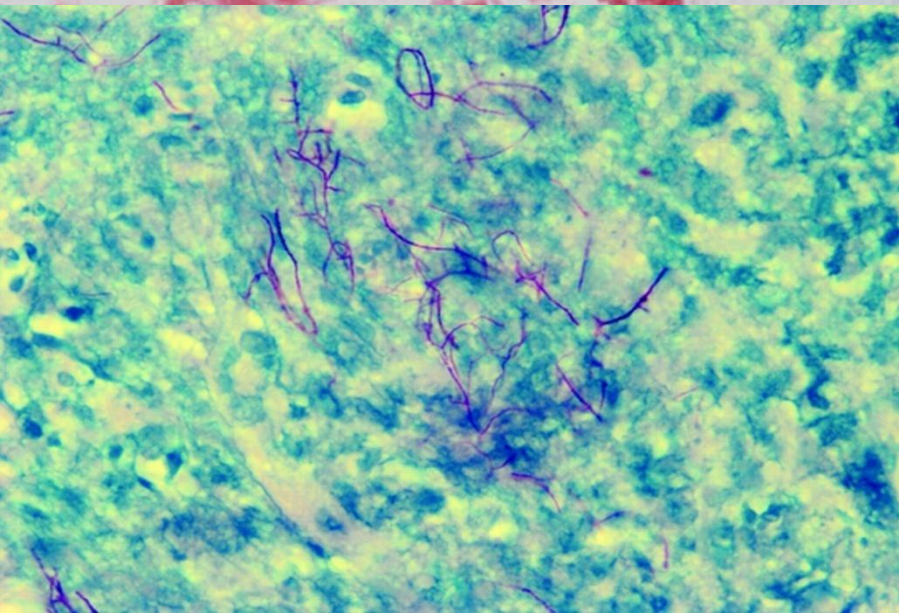
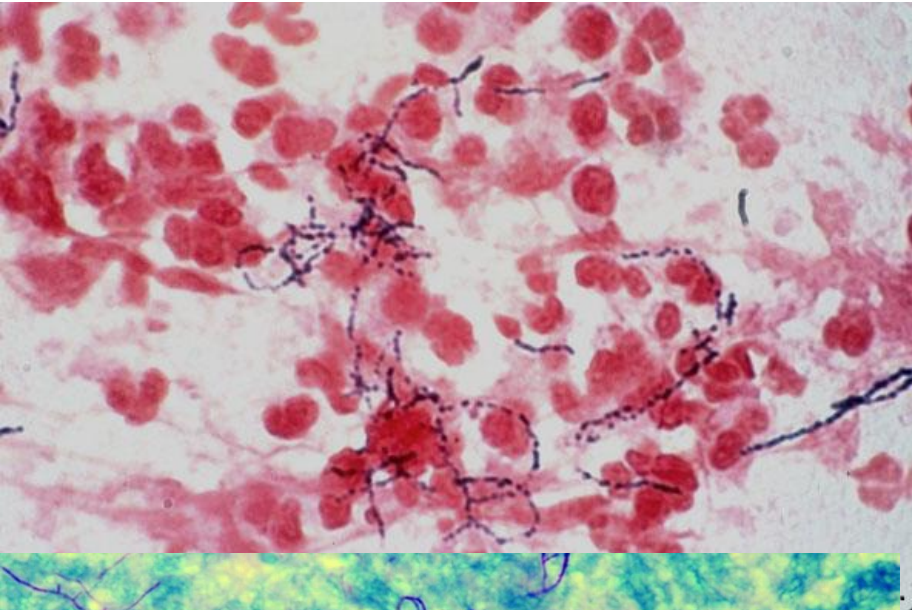
Bacillus



B.anthraxis / B.cereus ,...

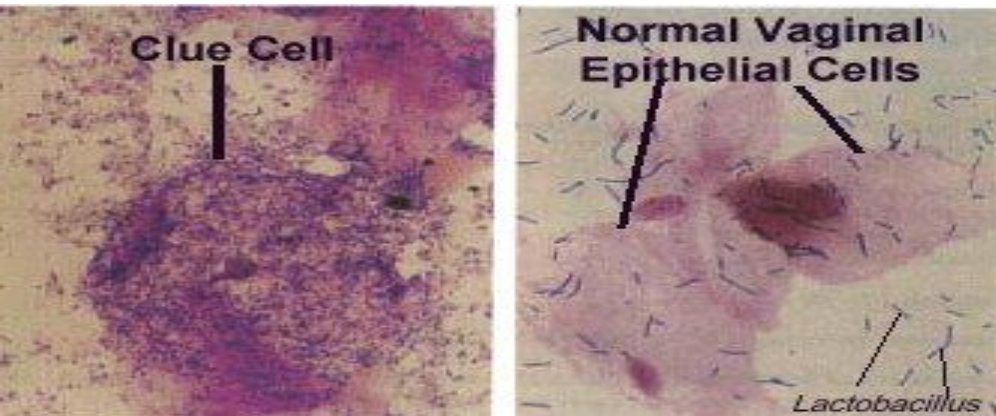


Nocardia



Gram (+) diğer çomaklar

- Erysipelothrix
- Archanobacterium
- Lactobacillus
- Gardnerella
- Dallanan Gram (+) çomaklar
 - Gordonia
 - Tsukamurella
 - Rhodococcus
 - Streptomyces



Antibiyogram tanımda faydalı olabilir

Journal of Antimicrobial Chemotherapy (2001) **48**, Suppl. S1, 87–102

JAC

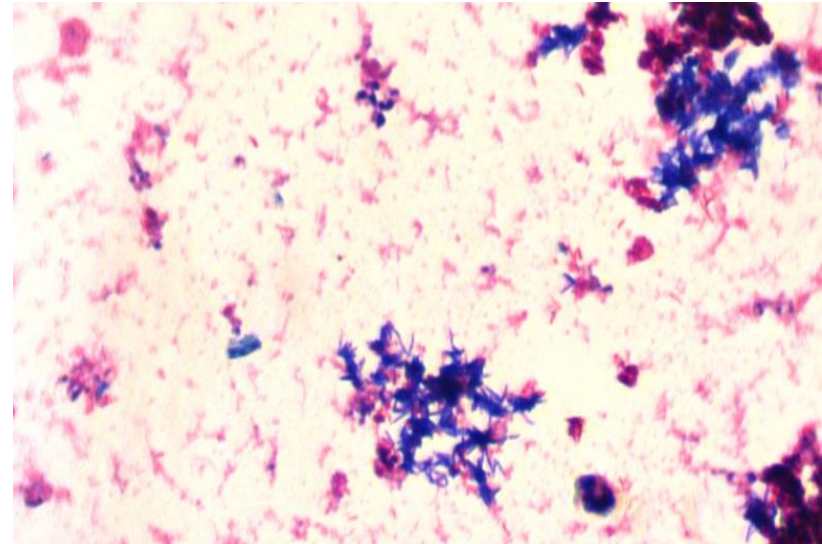
Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes

David M. Livermore^{a*}, Trevor G. Winstanley^b and Kevin P. Shannon^c

Organism	Resistances requiring confirmation
<i>S. aureus</i>	Any of: vancomycin, teicoplanin, linezolid, quinupristin/dalfopristin.
Coagulase-negative staphylococci	Any of: vancomycin, linezolid.
<i>Jeikeium coryneforms</i>	Any of: vancomycin, teicoplanin, linezolid.
<i>S. pneumoniae</i>	Any of: meropenem, vancomycin, teicoplanin, linezolid.
Group A, B, C, G β -haemolytic streptococci	Any of: penicillin, vancomycin, teicoplanin, linezolid.
Enterococci	Both ampicillin and quinupristin/dalfopristin. Linezolid.
Enterobacteriaceae	Teicoplanin but not vancomycin. Meropenem. Imipenem (except with <i>Proteus</i> spp.).
<i>H. influenzae</i>	Any third-generation cephalosporin, or carbapenem.
<i>M. catarrhalis</i>	Ciprofloxacin.
<i>Neisseria meningitidis</i>	Any of: penicillin (high level), ciprofloxacin.
<i>Neisseria gonorrhoeae</i>	Any third-generation cephalosporin.
<i>Acinetobacter</i> ; <i>P. aeruginosa</i>	Colistin.
Anaerobes in general	Metronidazole.
<i>Bacteroides</i>	Any of: metronidazole, co-amoxiclav, carbapenems.
<i>C. difficile</i>	Any of: metronidazole, vancomycin.

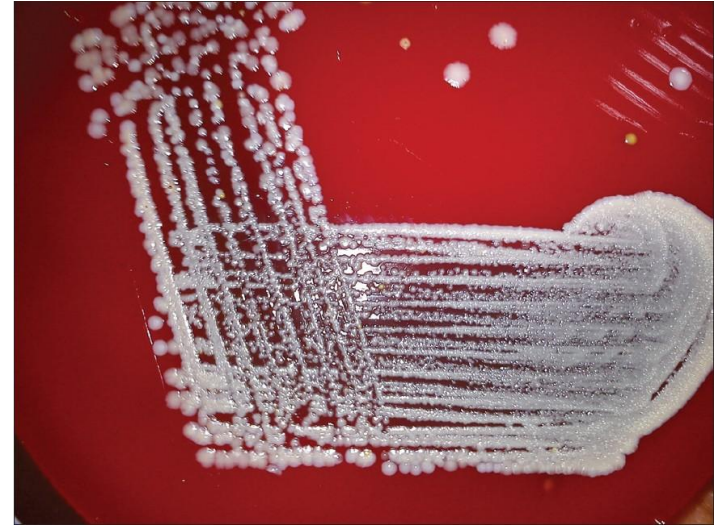
Olgu

- 26 yaşında ,K
- SLE nedeniyle uzun süredir steroid kullanıyor
- Pnömoni ön tanısıyla yatırılıyor
- Alınan HK sinyal veriyor
- Gram (+) çomak...



Olgu

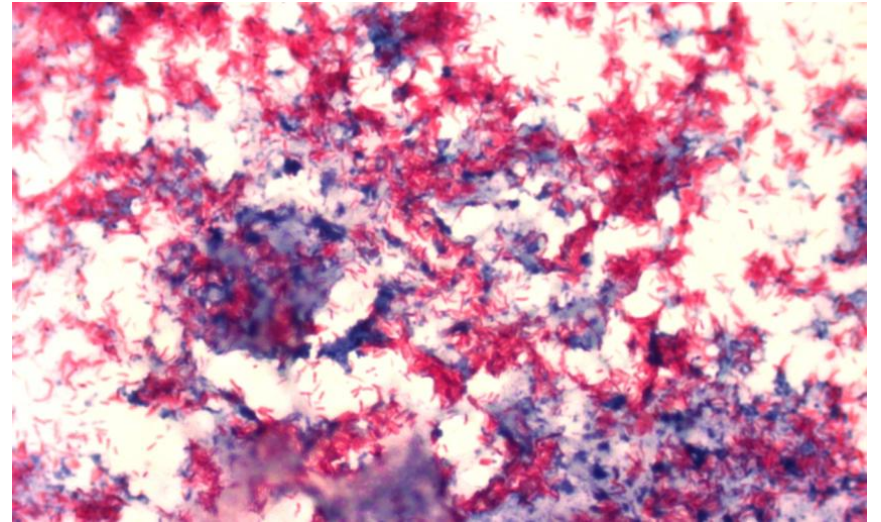
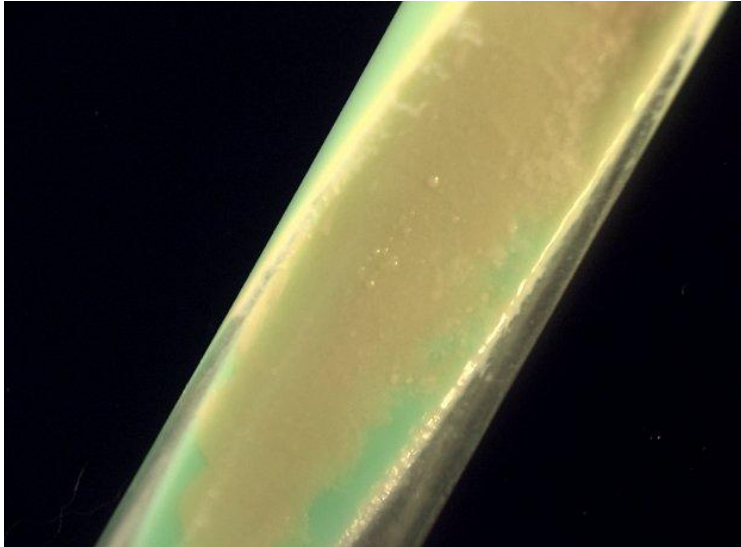
- Pasajda Çukolata, Kanlı, MC besiyerinde üreme var



- Kültür Gram : Gram (+) çomaklar
- Antibiyogram: Amikasin, Gentamisin, Linezolid, Klaritromisin duyarlı, İmipenem OD

Olgu

- Hastanın Bal öneği EZN (+)
- Üç gün sonra MGIT (+) LJ (+)
- HK üreyenden EZN
M.abcessus



Antibiyogram hatalarımızı yüzümüze vuruyor ! K.Töreci

- Bazı direnç profillerinin bazı bakterilerde saptanması beklenmez !
- Böyle bir sonuç mutlaka yeniden değerlendirilmelidir.
- Belki identifikasyon hatalıdır?
GSBL (+) Enterobacter spp ???
İBL (+) Klebsiella ???

Sonuç gibi....

- Konvansiyonel yöntemleri iyi bilen laboratuvar sorumluları ?
- Otomatize sistemlerle donanmış mikrobiyoloji laboratuvarları ?



Hastanın Adı, Soyadı :

Selma Brey

Tarih :

Prot. No :

23.3.2006

Hastanın Kurumu :

Dr. Dip. No, Adı, Soyadı :
(Varsa Kaşesi)

Tabibin Kurumu : Cerrahpaşa Tıp Fakültesi
Anabilim Dalı

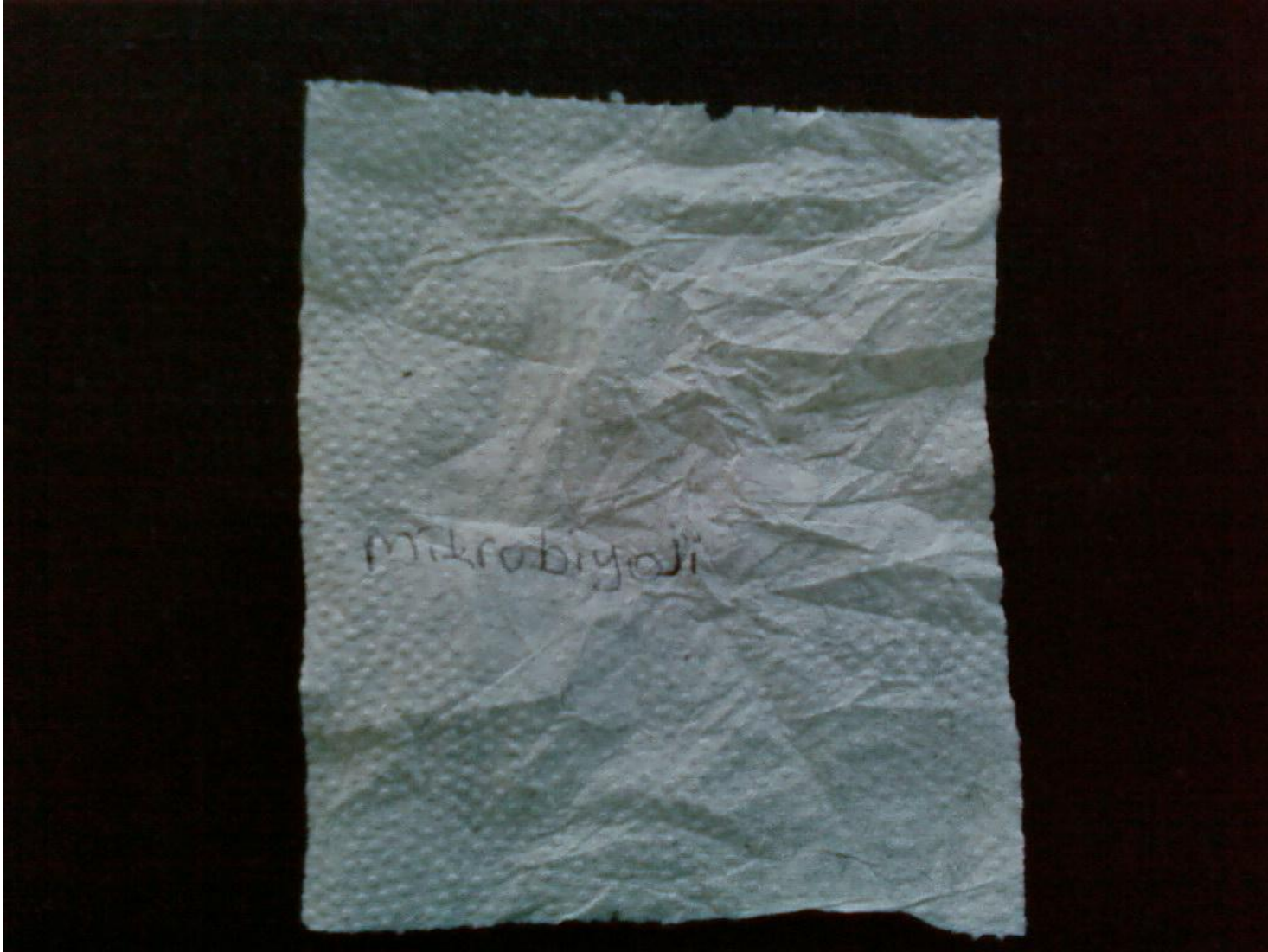
İLAÇLAR

TUTARI

Mamontyolers
Jals

— İsmail Ailtin
İürer

İletişim ?



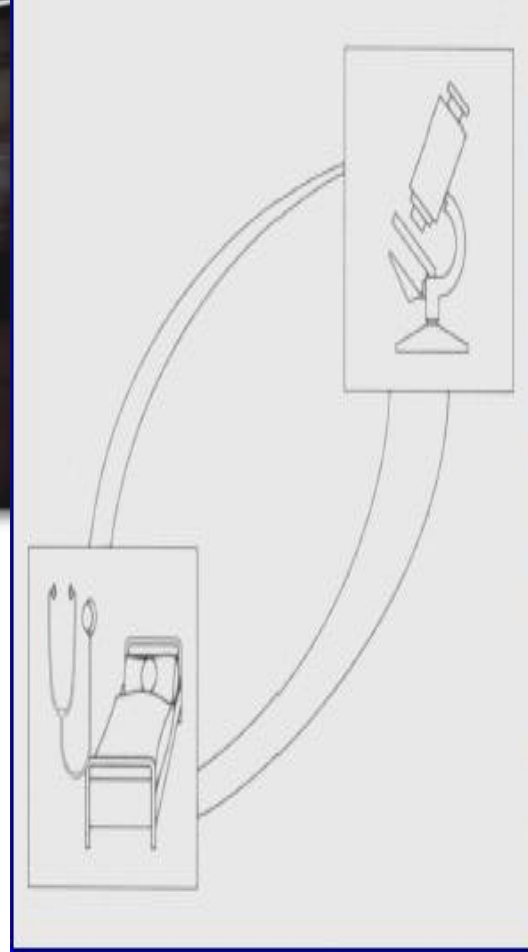
Uygun /saygılı örnek



Mikrobiyoloji Laboratuvarında teknoloji tüm sorunu çözebilir mi?

- Son derece gelişmiş teknolojiler
- Son derece azalan iletişim
- Teknoloji bağımlısı hekim/sağlık sistemi
- Daha iyi sağlık sistemi ???

Mikrobiyoloji Laboratuvarı ve Klinik



SONUÇ

- Hastayı değerlendirin ve dinleyin.
- Uygun örnek olmadan olmaz.
- Teknoloji iyi bir şeydir!
Fakat temel kurgu sağlam olmalıdır!
- En uygun sonuç iletişim ile alınır.

İlginize ve sabrınıza teşekkür ederim !

