

Enterobacteriales

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2022

Enterobacterales (Order)

Single family *Enterobacteriaceae* now
7 families that include the clinically relevant
genera:

- *Enterobacteriaceae*
- *Erwiniaceae*
- *Pectobacteriaceae*
- *Yersiniaceae*
- *Hafniaceae*
- *Morganellaceae*
- *Budviaceae*

Many genera & species within each family

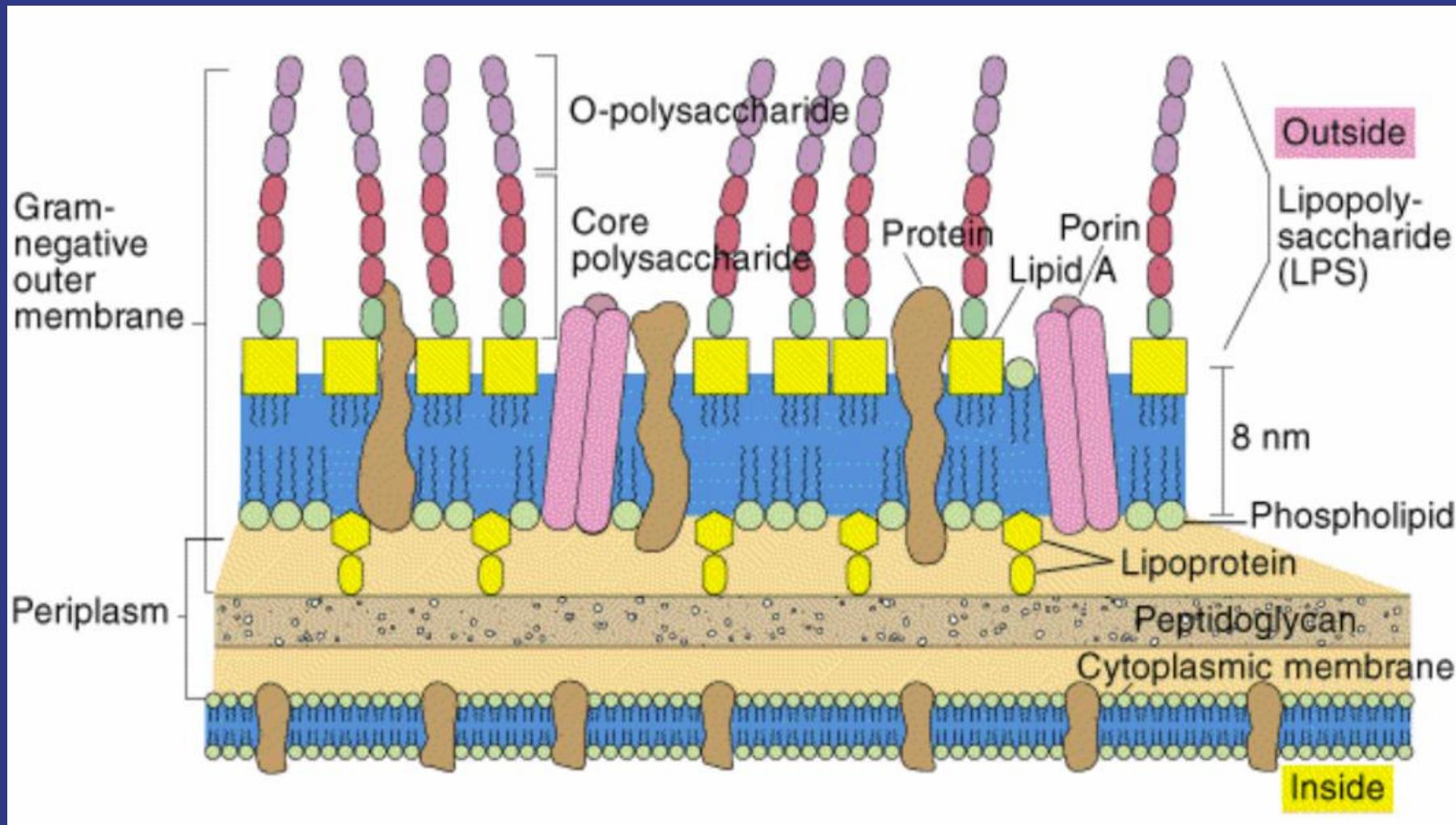
Enterobacteriales

- <20 species cause >95% of disease
- Ubiquitous
 - Soil
 - Water
 - On vegetation
 - Intestinal flora of animals

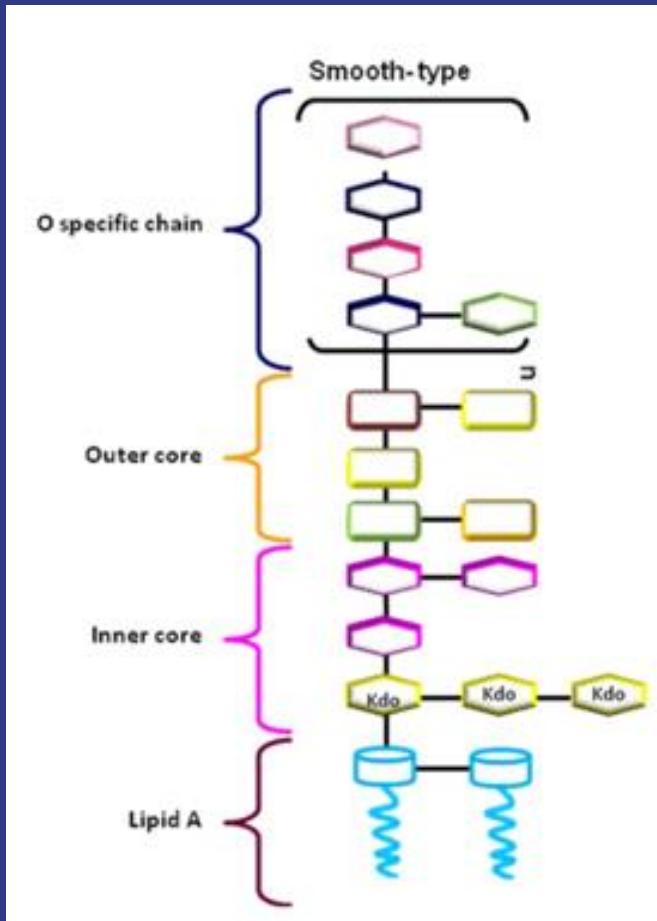
Enterobacteriales

- Gram negative rod ($0.3\text{-}1.0 \times 1\text{-}6 \mu\text{m}$)
- Non-spore forming
- Facultative anaerobes
- If motile, peritrichous flagella present
- Good growth on MacConkey (MAC) agar*
- Ferment glucose
- Catalase positive
- Oxidase negative*
- Reduction of nitrate to nitrite

Gram-negative cell wall



LPS (Endotoxin) Structure and Function



Protection & stability;
Prevents passage of low mw
molecules.

- O Ag is immunodominant; length varies
- Outer core
- Inner core
- Lipid A – toxicity; host immune activation, febrile response

Antigens - Serologic Grouping

O = somatic

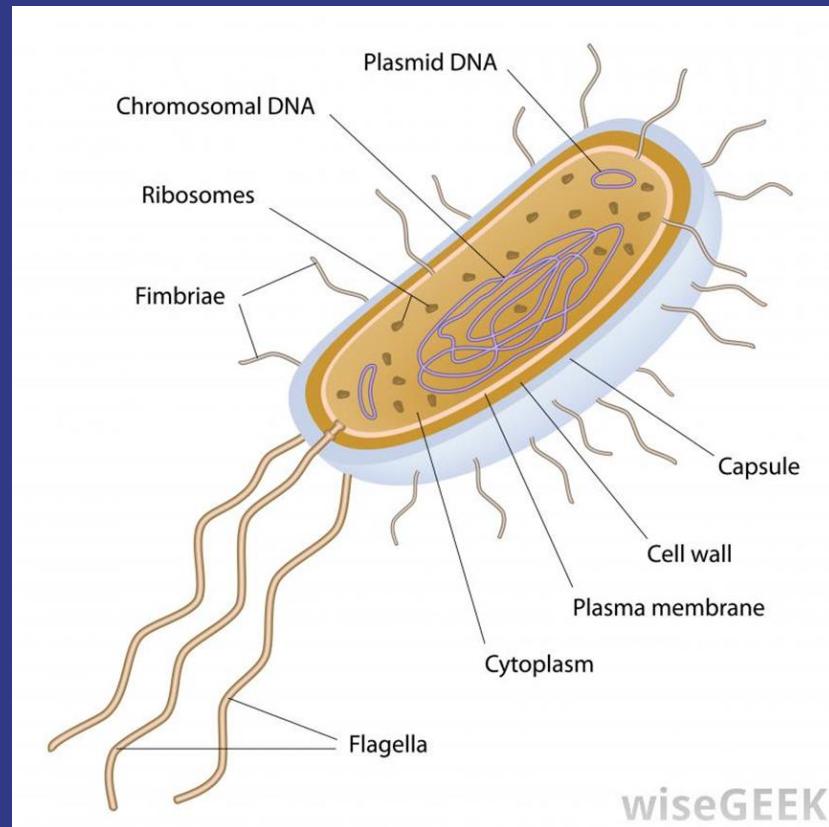
- heat-stable
- cell wall

H = flagellar

- heat-labile
- flagellar surface

K = capsular

- heat-labile polysaccharide
- certain encapsulated species



wiseGEEK

Clinical Significance

Opportunistic Infections

- Most common genera
 - *Escherichia coli*
 - *Klebsiella species*
 - *Raoultella species*
 - *Proteus species*
 - *Enterobacter species*
 - *Serratia marcesens*

Intestinal infections

- Enteric Pathogens (not commensals)
 - *Salmonella*
 - *Shigella*
 - Pathogenic *E. coli*
 - *Yersinia*

Clinical Significance

- Infections:

- abscesses
- hospital-acquired pneumonia
- meningitis
- septicemia
- wound infection
- urinary tract infection (70% of all UTIs)
- gastroenteritis

Virulence

- adherence
 - adhesins
 - fimbriae (pili)
- colonization
- invasion
- toxin production
- Lysis of immune cells
- Iron scavenging

Hospital Acquired Infections – National Data; 2003

Pathogen	Percentage of isolates, by infection type			
	Pneumonia (n = 4365)	Bloodstream infection (n = 2351)	Surgical site infection (n = 2984)	Urinary tract infection (n = 4109)
Gram negative				
<i>Escherichia coli</i>	5.0	3.3	6.5	26.0
<i>Klebsiella pneumoniae</i>	7.2	4.2	3.0	9.8
<i>Enterobacter species</i>	10.0	4.4	9.0	6.9
<i>Serratia marcescens</i>	4.7	2.3	2.0	1.6
<i>Pseudomonas aeruginosa</i>	18.1	3.4	9.5	16.3
<i>Acinetobacter species</i>	6.9	2.4	2.1	1.6
Other	14.1	3.8	9.8	10.7
Gram positive				
Coagulase-negative staphylococci	1.8	42.9	15.9	4.9
<i>Staphylococcus aureus</i>	27.8	14.3	22.5	3.6
Enterococci	1.3	14.5	13.9	17.4
Other	3.2	4.5	5.8	1.2

From: Overview of Nosocomial Infections Caused by Gram-Negative Bacilli
Clin Infect Dis. 2005;41(6):848-854. doi:10.1086/432803
Clin Infect Dis | © 2005 by the Infectious Diseases Society of America

Bacteremia – Top Ten Pathogens

	Weinstein 1975-1977	Cockerill 1984-1988	Cockerill 1996-1997	Lee 2007
1	<i>E. coli</i>	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>
2	<i>S. aureus</i>	<i>E. coli</i>	<i>E. coli</i>	Coag neg Staph
3	<i>S. pneumo</i>	<i>C. albicans</i>	Coag neg Staph	<i>Enterococcus</i>
4	<i>K. pneumo</i>	Coag neg Staph	<i>C. albicans</i>	<i>K. pneumo</i>
5	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>Enterococcus</i>	<i>Candida spp.</i>
6	<i>B. fragilis</i> grp	<i>Enterococcus</i>	<i>P. aeruginosa</i>	<i>Enterobacteriales</i>
7	<i>Enterococcus</i>	<i>K. pneumo</i>	<i>K. pneumo</i>	<i>E. coli</i>
8	<i>Group A Strep</i>	<i>S. marcescens</i>	viridans Strep	<i>P. aeruginosa</i>
9	<i>C. albicans</i>	<i>S. pneumoniae</i>	<i>E. cloacae</i>	Other gram neg
10	<i>C. glabrata</i>	<i>E. cloacae</i>	<i>S. pneumo</i>	Other Streptococci

Specimen Collection & Processing

- Extra-intestinal Infections
 - utilize normal protocols for collecting blood, respiratory, wound and urine specimens.

Media for Isolation

- Non-selective, enriched media
 - Trypticase soy blood agar
- Selective media – antibiotics, salts, dyes, etc.
 - ChromAgar for ESBL E coli or Kpn
- Selective & Differential Media
 - MacConkey & EMB agar
 - XLD & HEK agar – Enteric pathogens
 - CIN agar (Yersinia) – Enteric pathogens
 - MacConkey Sorbitol (*E. coli* O157) – Enteric pathogens

Lactose Fermentation –MacConkey Agar

- Bile salts
- Crystal violet
- Neutral red
- Lactose

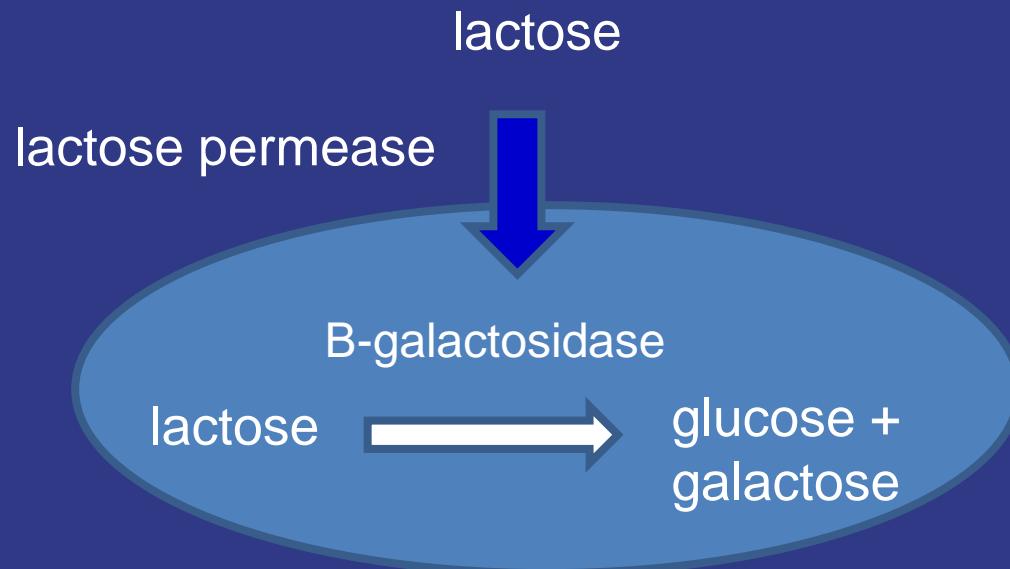


Lactose fermenters: *E. coli, Klebsiella, Enterobacter.*

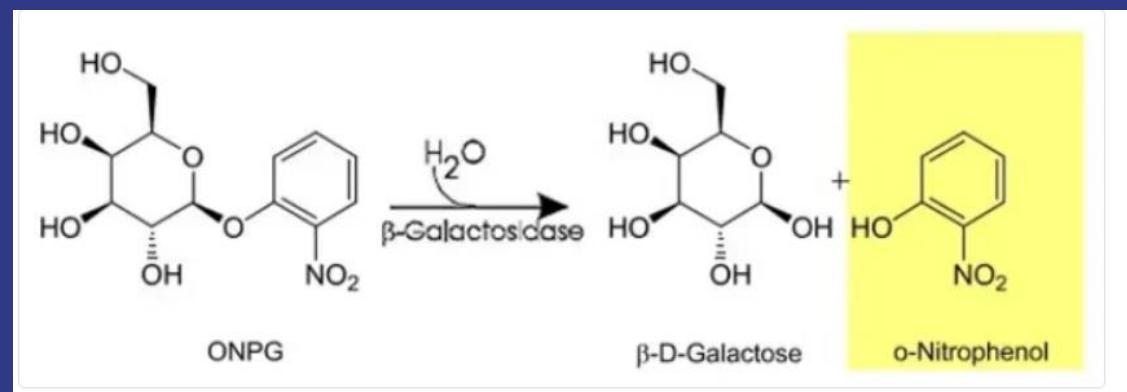
Non-lactose fermenters: *Proteus, Providencia, Morganella, Salmonella, Shigella.*

Sometimes *E. coli* and *Enterobacter*.
Citrobacter species can be either.

Lactose fermentation



ONPG Test



Eosin Methylene Blue Agar

- Contains lactose & sucrose
- Methylene blue & eosin inhibit GP
- *E. coli* – green-black metallic sheen
- Other LFs: purplish
- NLFs: transparent

E. coli



ASM MicrobeLibrary.org © Lal

Klebsiella



ASM MicrobeLibrary.org
© Cheeptham

Identification of *Enterobacterales*

TABLE 1 Biochemical reactions of the named species, biogroups, and Enteric Groups of the family Enterobacteriaceae^a

(continued on next page)

(Continued)

Identification: Biochemicals

- Standard biochemical tubes miniaturized for automated systems
- Work well for the common species.
- Newer or less common species may be problematic due to an inadequate database.
- Fastidious or nutritionally deficient organisms may present problems.

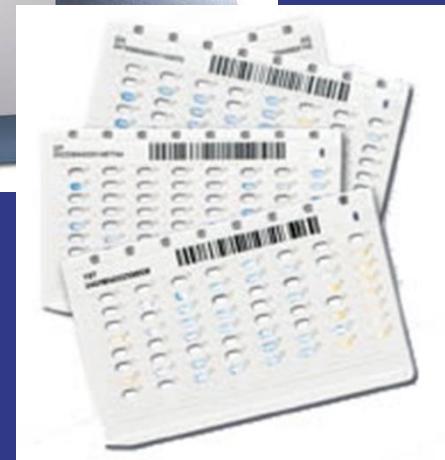
Automated ID Systems



BD
Phoenix



BioMerieux
Vitek 2



Beckman Coulter
Microscan

API 20 E Test Strips



FIGURE
5-83

API 20 E TEST STRIPS

The top strip is uninoculated; the bottom strip (with the exception of GEL) illustrates all positive results.

Useful Biochemical Tests

- Carbohydrate fermentation
- Motility
- H₂S production
- Indole from tryptophan
- Methyl red & Voges-Proskauer tests
- Citrate utilization
- Phenylalanine deaminase activity
- Urease production
- Decarboxylation of lys, arg, orn

Major Biochemical Reactions

	Lactose	Gas	H ₂ S	MR	VP	Citrate	PAD	Urea	Mot
<i>E. coli</i>	+ 90%	+	-	+	-	-	-	-	+
<i>Shigella</i>	-	-	-	+	-	-	-	-	-
<i>Salmonella</i>	-	+	+	+	-	+/-	-	-	+
<i>Citrobacter</i>	+/-	+	+/-	+		+	-	+/-	+
<i>Klebsiella</i>	+	+	-	-/+	+	+	-	+	-
<i>Enterobacter</i>	+/-	+	-	-	+	+	-	-/+	+
<i>Serratia</i>	-	+	-	-/+	+	+	-	-	+
<i>Proteus</i>	-	+/-	+	+	-	-/+	+	+	+*
<i>Morganella</i>	-	+	-	+	-	-	+	+	+
<i>Providencia</i>	-	-	-	+	-	+	+	+/-	+

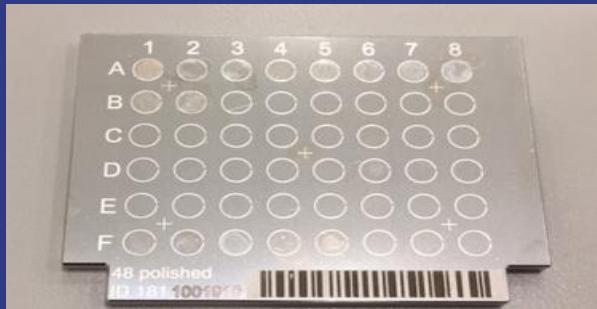
+/- either result depends on species or reaction can vary.

*Swarming motility may be observed.

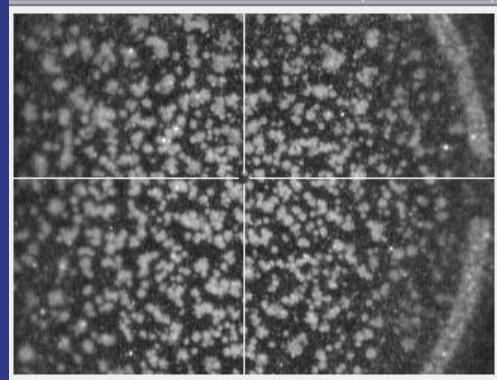
MR = methyl red VP = Voges-Proskauer PAD = phenylalanine deaminase



Tools for Identification – MALDI TOF MS

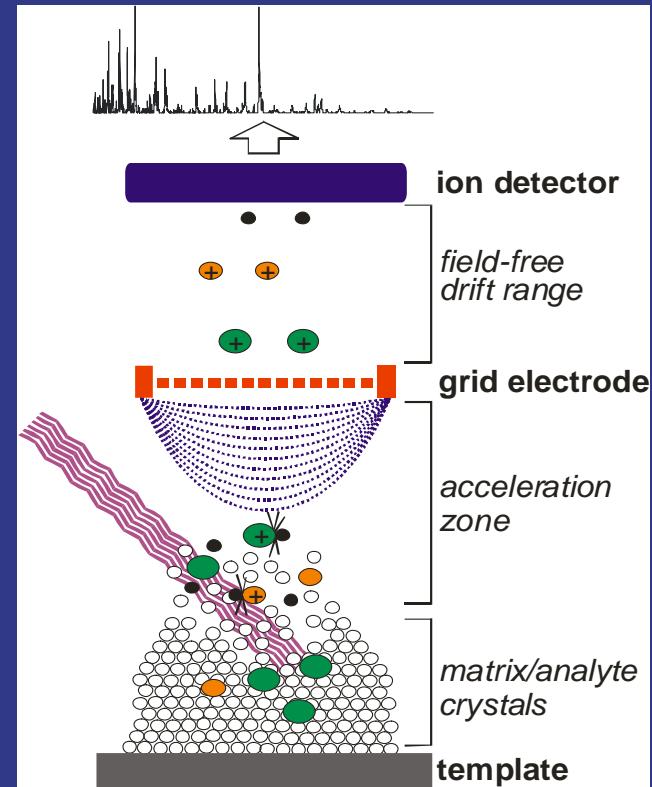


MALDI TOF
Target Plate



Camera Image of spot

detection
separation
acceleration
ionization
desorption



Courtesy bioMerieux

MALDI TOF

For *Enterobacterales* ~97% accurate compared
16S rRNA sequencing (gold standard)

- Cannot distinguish *E. coli* & *Shigella*.
- *Citrobacter* spp. are reported as “*Citrobacter freundii* complex”
- Cannot distinguish *Enterobacter cloacae* & *E. asburiae*.
Report as “*E. cloacae* complex”
- *Proteus vulgaris* and *P. penneri* not distinguished.

Common Clinically Significant *Enterobacteriales*

E. coli Opportunistic infections

- urinary tract infections
 - UPEC has specific adhesins
- wound/abscess
- septicemia
 - ascending from UTI
 - abdominal source
- meningitis
 - in newborns
 - (*E. coli* K1 capsule)



[Gastroenteritis discussed separately.]

Escherichia coli

- Usually beta-hemolytic on SBA, indole +
- 90% lactose-fermenting
- H₂S, citrate, DNase, citrate, urease, PDA all neg
- MALDI-TOF does not distinguish from *Shigella* spp.

	<i>E. coli</i>	<i>Shigella</i>
Lactose fermentation	Pos (90%)	neg
Indole	pos	pos/neg (<i>S. sonnei</i>)
Lysine decarboxylase	pos	neg
Motility	pos	neg
Gas production	pos	neg

Common opportunistic species:

*Klebsiella, Enterobacter, Serratia, Hafnia,
Pantoea, others:*

- hospital acquired & aspiration pneumonia
- UTI
- wound
- abscess, esp. liver
- septicemia
- other

Klebsiella spp.

○ *K. pneumoniae* (indole -)

- Most common pathogen
- Most virulent
- 3 subsp.
 - *pneumoniae*
 - *rhinoscleromatis*
 - *ozaenae*

○ *Raoultella* spp.

(previously *Klebsiella*)

- *R. planticola*
- *R. ornitholytica*

○ *K. oxytoca* (indole +)

○ *K. varicola*

○ *K. aerogenes* (formerly *Enterobacter*)

Klebsiella spp.

- Kpn has polysaccharide capsule
 - Hypermucoid strains esp virulent – liver abscess
- Non-motile
 - (except *K. aerogenes*)
- Lys decarboxylase +
- citrate +
- most urease +
- H₂S -



Lopez et al ID Cases 2019



Klebsiella spp.

K. granulomatis

- Genital ulcer infections –
- granuloma inguinale or Donovanosis
- tropical disease
- does not grow in culture



Enterobacter spp.

- *E. cloacae* complex - not distinguished by MALDI-TOF
 - *E. asburiae*
 - *E. cloacae*
 - 4 other species
- motile
- lactose-fermenting
- *E. cloacae* (vs. *Kpn*):
 - Lysine –
 - Arginine +
 - Ornithine +
- *Pantoea* spp.
- *P. agglomerans*
 - biochemically inert
 - formerly *Enterobacter*
 - may produce yellow pigment

Cronobacter spp.

- formerly
Enterobacter sakazakii
- uncommon
- meningitis & septicemia in neonates
- Contaminated formula



Serratia spp.

○ *S. marcescens*

- UTI
- respiratory infections
- contaminated solutions

May make red pigment – enhanced at RT



shutterstock.com · 1926227759

○ Less common opportunistic species:

- *S. rubidaea* (red)
- *S. liquifaciens*
- *S. odorifera*
- others

Serratia spp.

- late-lactose ferm
- ONPG +
- DNase +

Citrobacter spp.

C. freundii complex

- not distinguished by MALDI TOF
 - *C. freundii*
 - *C. youngae*
 - *C. braakii*
 - *C. werkmanii*
 - *C. sedlakii*

C. koseri (formerly *C. diversus*)

C. amalonaticus

Citrobacter spp.

Hospital acquired infections

- UTI
- abdominal abscess
- Septicemia
- Endocarditis (IVDU)
- nursery outbreaks –
C. koseri
 - Neonatal meningitis
 - Brain abscess

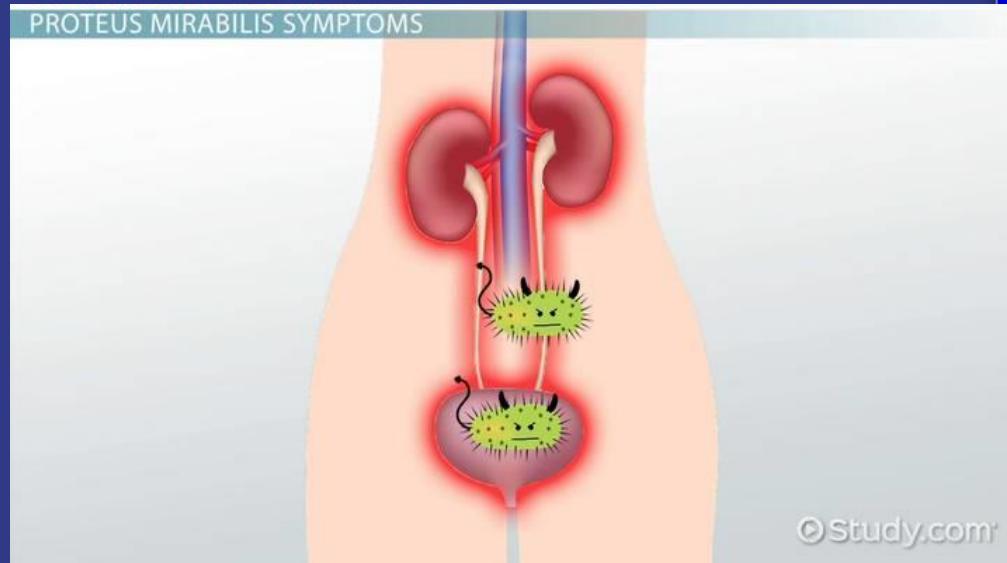
- lactose +/-
- H₂S producer (~80%)
 - Black on HE & XLD
 - Distinguish from
Salmonella
 - MALDI TOF
 - Citrobacter is lysine decarb - ; usually urease +

Proteus spp.

Infections:

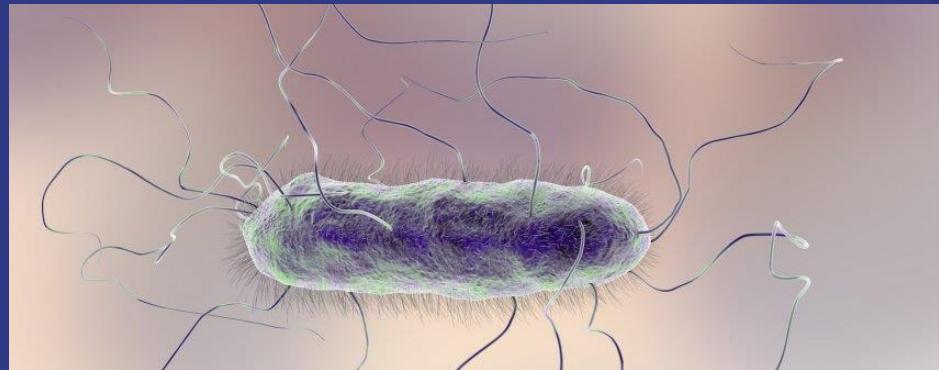
- UTI
 - assoc w/ hyper flagellated species
- kidney stones
 - assoc w/ urease pdn
- wounds
- bacteremia
- other, esp nosocomial infections

- *P. mirabilis*
- *P. vulgaris*
- *P. penneri*



Proteus spp.

- Non-lactose fermenter
- *P. mirabilis* & *P. vulgaris* – swarming colony
- Odor: chocolate cake/burned chocolate
- H₂S +; PDA +; urease +



www.hygiene-in-practice

- MALDI TOF does not distinguish *P. penneri* & *P. vulgaris*
 - *P. penneri*: indole –
 - *P. mirabilis*: indole –
 - *P. vulgaris*: indole +



Morganella & Providencia spp.

Morganella

- Mostly assoc w/UTI
- *M. morganii*
- Non-lactose fermenter
- PDA +
- Urease +



Providencia spp.

- Mostly assoc w/UTI
- *P. stuartii*
- *P. rettgeri*
- Other species
- Non-lactose fermenter
- PDA +
- Urease +/-

CDC Image library

Edwardsiella tarda

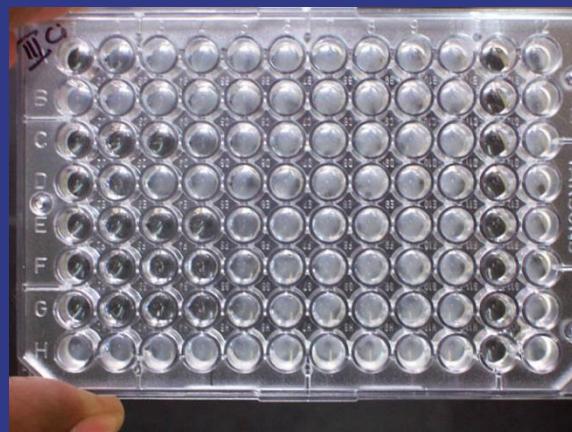
- Uncommon opportunistic species
- Wound infections
- Bacteremia

- H₂S positive
- Indole positive
- Lysine decarboxylase positive
- Citrate & urea negative

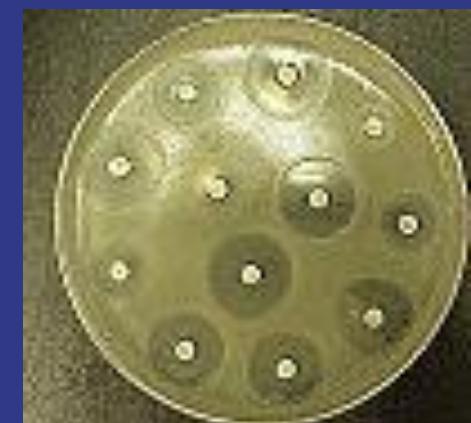
- Enterobacterales reported in cultures depending on body site, amount, presence of inflammation (WBCs), other flora
- Reported with an AST result



Vitek automated
method



Microbroth manual panels



Disk Diffusion

Antimicrobial Resistance

- Some species have intrinsic resistance to some Abx.
- Susceptibility pattern should match the identification.

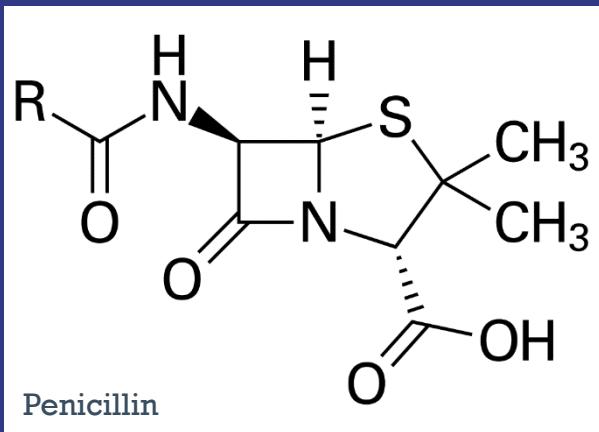
Intrinsic Resistance Summary

CLSI M100 Appendix B

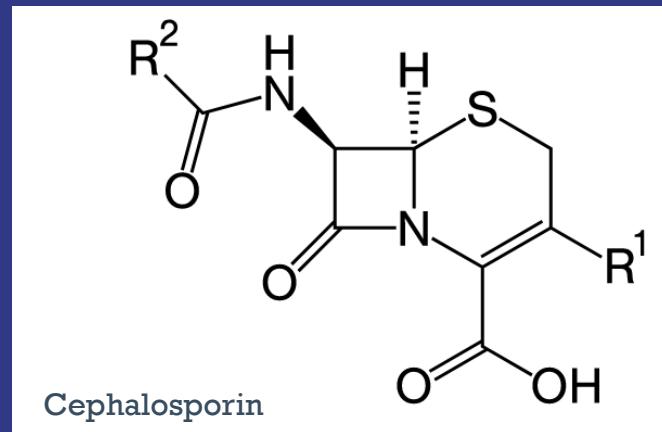
	Amp	Amox/ clav	Amp/ Sulb	Ceph 1st gen	Cepha mycins	Tetra- cycline	Tige- cycline	Nitro- furan	PolyB/ colist
<i>C. freundii</i>	R	R	R	R	R				
<i>E. cloacae cx</i>	R	R	R	R	R				
<i>E. coli</i>									
<i>K. aerogenes</i>	R	R	R	R	R				
<i>Kleb spp.</i>	R								
<i>M. morganii</i>	R	R		R			R	R	R
<i>P. mirabilis</i>						R	R	R	R
<i>P. vulg/penn</i>	R			R		R	R	R	R
<i>P. stuartii/P. rettgeri</i>	R	R		R		R	R	R	R

Additional tests for specific
resistance mechanisms or genes

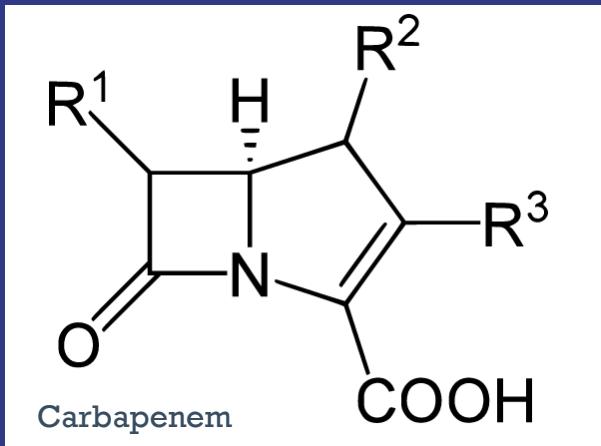
One ring to bind them all



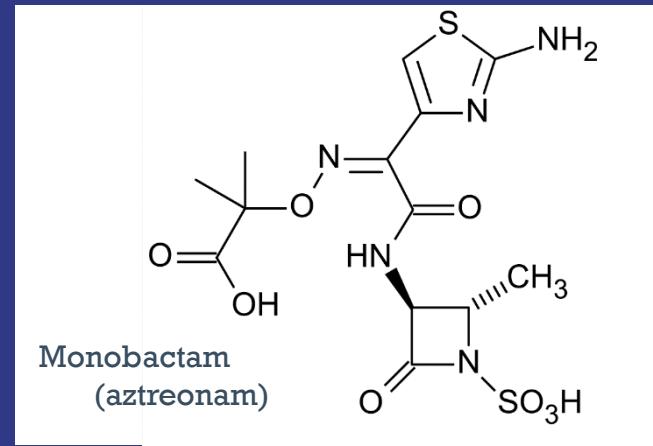
Penicillin



Cephalosporin



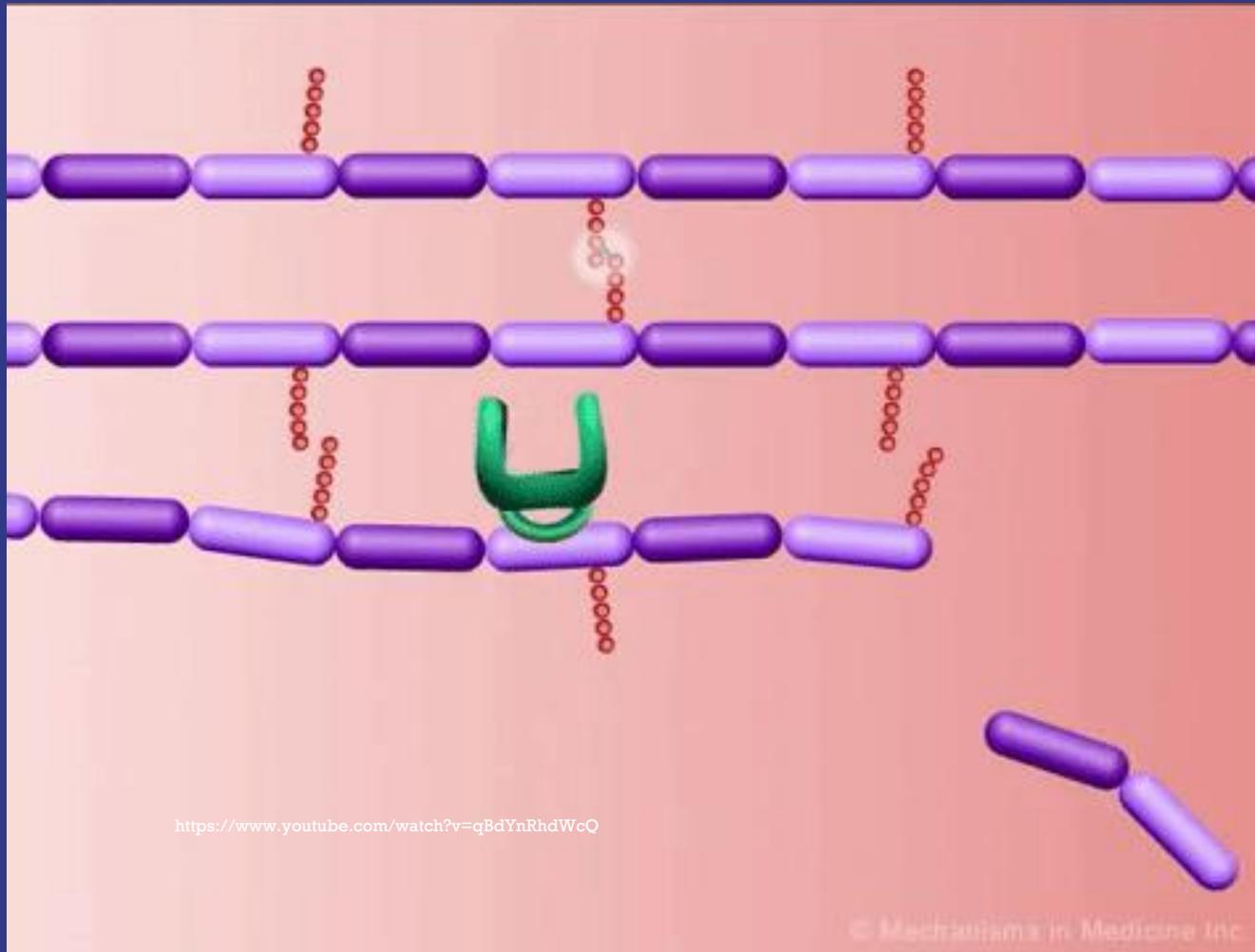
Carbapenem



Monobactam
(aztreonam)

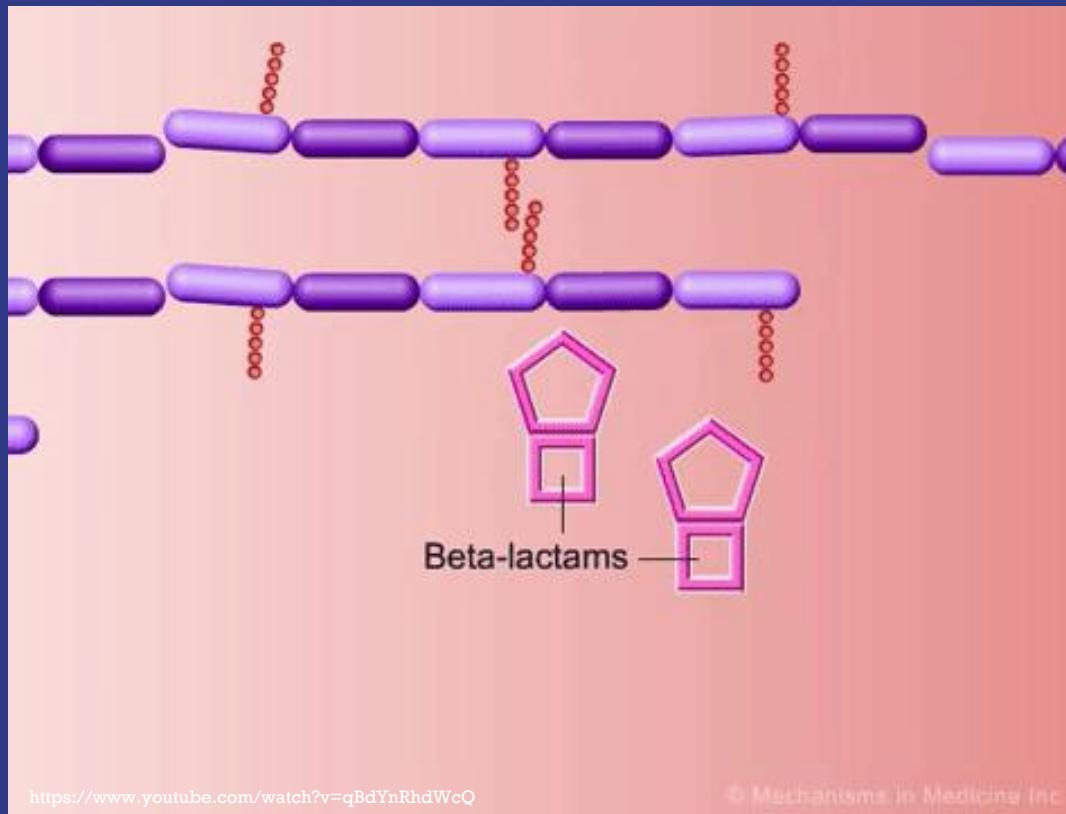
The beta lactam ring

Peptidoglycan is cross-linked by PBP.

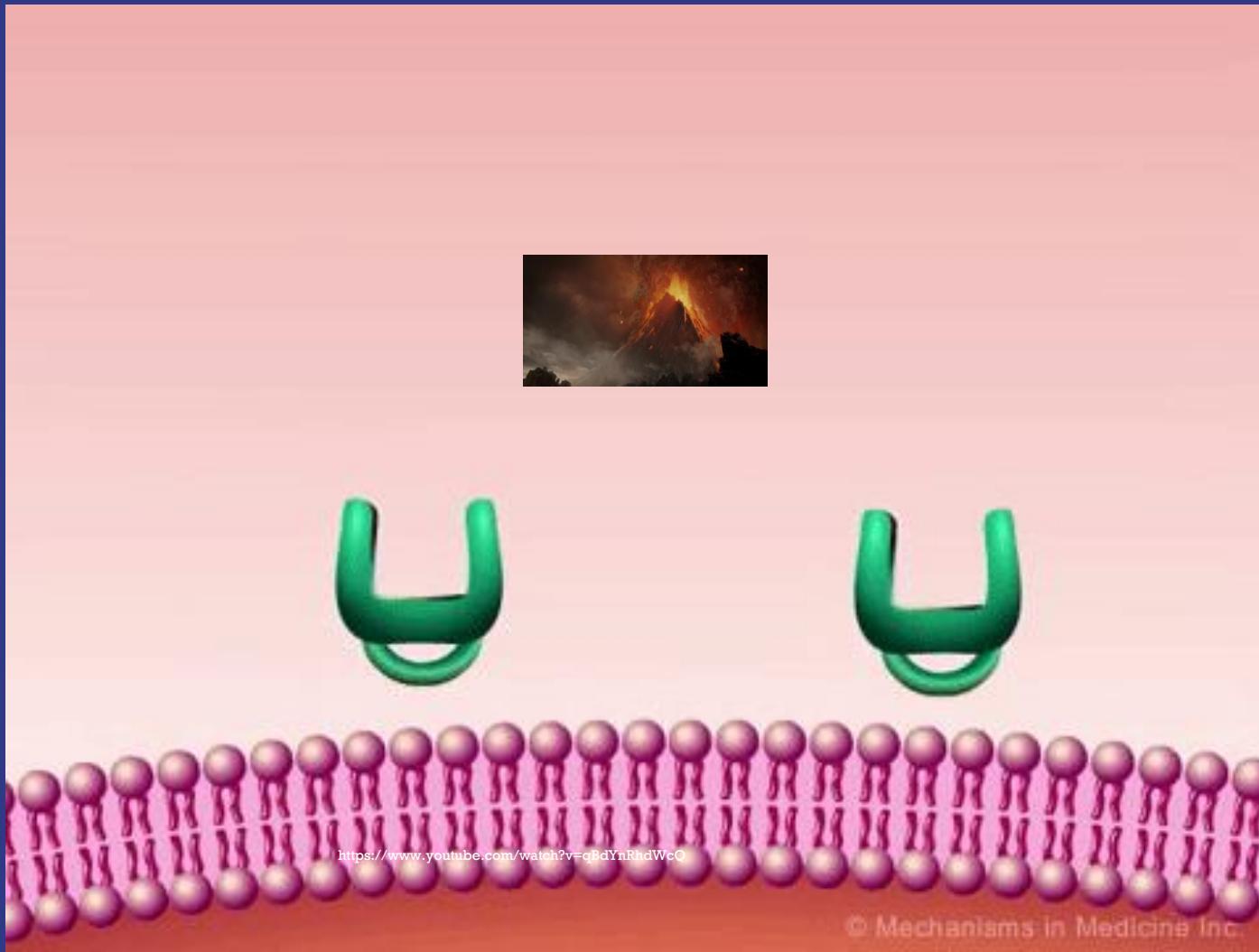


The beta lactam ring

Beta-lactams inhibit unaltered PBP.



The beta lactam ring

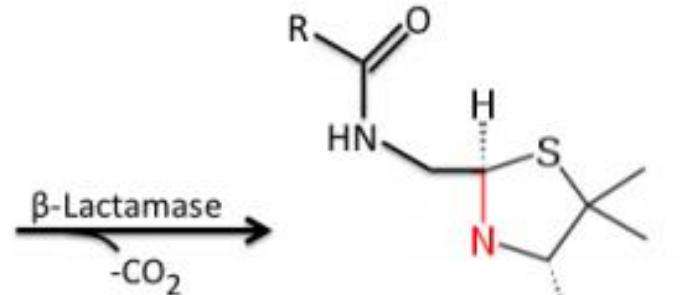


<https://www.youtube.com/watch?v=qBqYnRhWcQ>

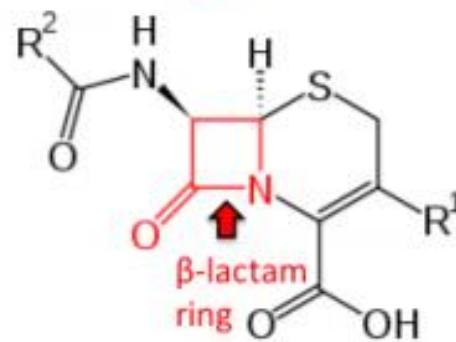
© Mechanisms in Medicine Inc.

Beta lactamase reaction

Penicillin



Cephalosporin



inactive metabolites

Beta-lactamases of *Enterobacteriales*

- Penicillinase and cephalosporinase (TEM, SHV)
- Detected with *in vitro* susceptibility testing
- Can be acquired or intrinsic resistance

Intrinsic examples:

K. pneumoniae

- Ampicillin resistance

Citrobacter, Enterobacter, Serratia

- Ampicillin, cefazolin, cephalothin resistance

ESBL – Extended Spectrum Beta-lactamase

- β -lactamases that hydrolyze extended-spectrum cephalosporins such as cefotaxime, ceftriaxone, ceftazidime and aztreonam. (often CTX-M gene)
- Resistant to all extended spectrum penicillins, cephalosporins, and monobactams.
- Commonly in *E. coli* & *Klebsiella*. Can be in any *Enterobacteriales*; plasmid-mediated
- Generally susceptible to cephemycins i.e. cefotetan and cefoxitin
- Carbapenems are drug of choice.

Extended Spectrum Beta-lactamase (ESBL) test

Screening MIC:

- If ceftazidime or ceftriaxone or other screening antibiotic MIC $\geq 2 \mu\text{g/ml}$ or $\leq 22\text{mm}$ (CTZ) or $\leq 25 \text{ mm}$ (CRO):

Do Confirmatory Test:

- Ceftazidime MIC compared to ceftazidime + clavulanate
- Cefotaxime MIC compared to cefotaxime + clavulanate

[CLSI procedure for *E. coli*, *Kpn*, *K oxy* and *P. mirabilis*]

ESBL Confirmatory test – MIC method

- ESBL confirmed if ≥ 3 fold decrease in MIC with clavulanate.

Example:

ceftazidime MIC = 8 µg/ml

ceftazidime + clavulanic acid MIC = 1 µg/ml.

= ESBL positive

ESBL Confirmatory Test -Disk Diffusion Method

ESBL confirmed if ≥ 5 mm
increase in zone diameter for

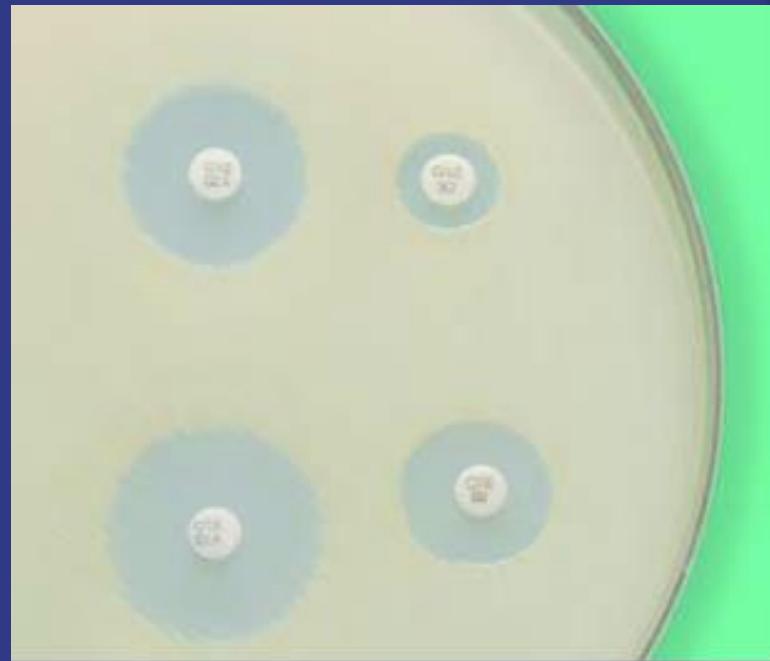
- ceftazidime-clavulanic
compared to ceftazidime OR
- cefotaxime-clavulanate
compared to cefotaxime

Example:

Ceftazidime = 15 mm

Ceftazidime + clavulanate = 22 mm

= ESBL positive



AmpC beta-lactamase

- Confers resistance to extended spectrum beta-lactams, including cephamycins (cefotetan & cefoxitin)
- Usually susceptible to:
 - Carbapenems
 - Cefepime
- *ampC* is usually chromosomal
- Mostly found in *Morganella*, *Serratia*, *Citrobacter*, *Enterobacter*
- *No specific test for this beta-lactamase.*

Carbapenemases

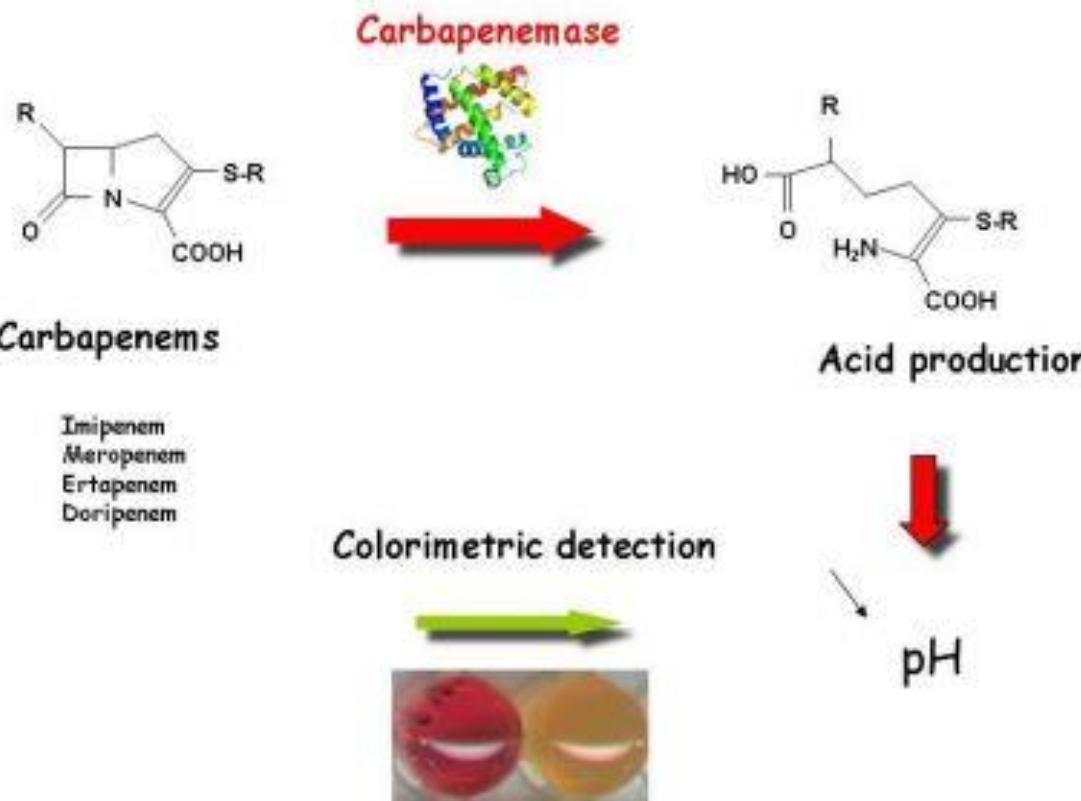
- Hydrolyze penicillins, cephalosporins, aztreonam, carbapenems
- CRE = carbapenem resistant
Enterobacteriales

Mechanisms/genes:

- *bla_{KPC}* aka KPC
(*Klebsiella pneumoniae* carbapenemase)
- NDM-1 New Delhi metallobetalactamase
- OXA-48, SME, others (less uncommon)
- IMP, VIM (unusual in Enterobacteriales)

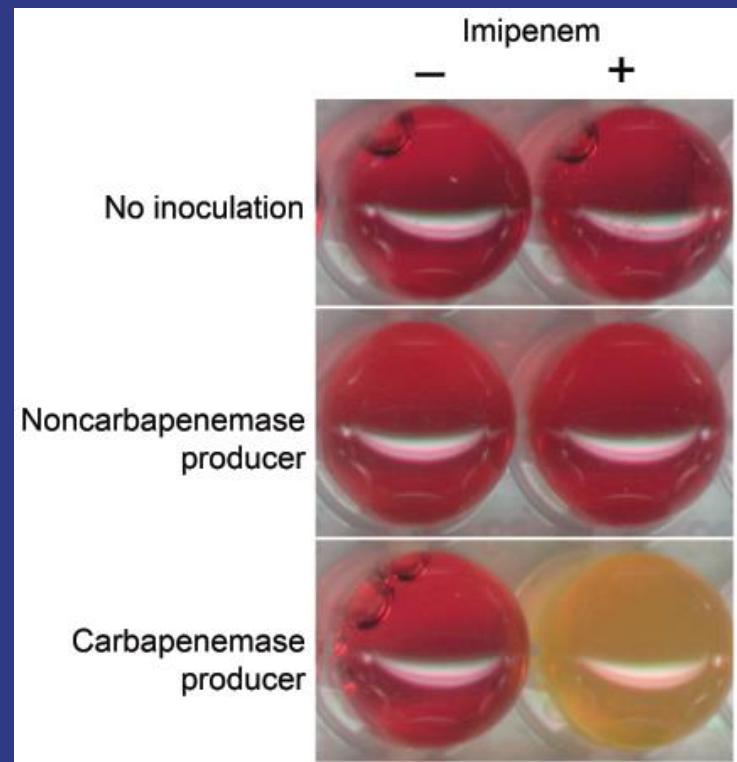
Tests for Carbapenemases from Organisms from Cultures

Carba NP Test (phenotypic)



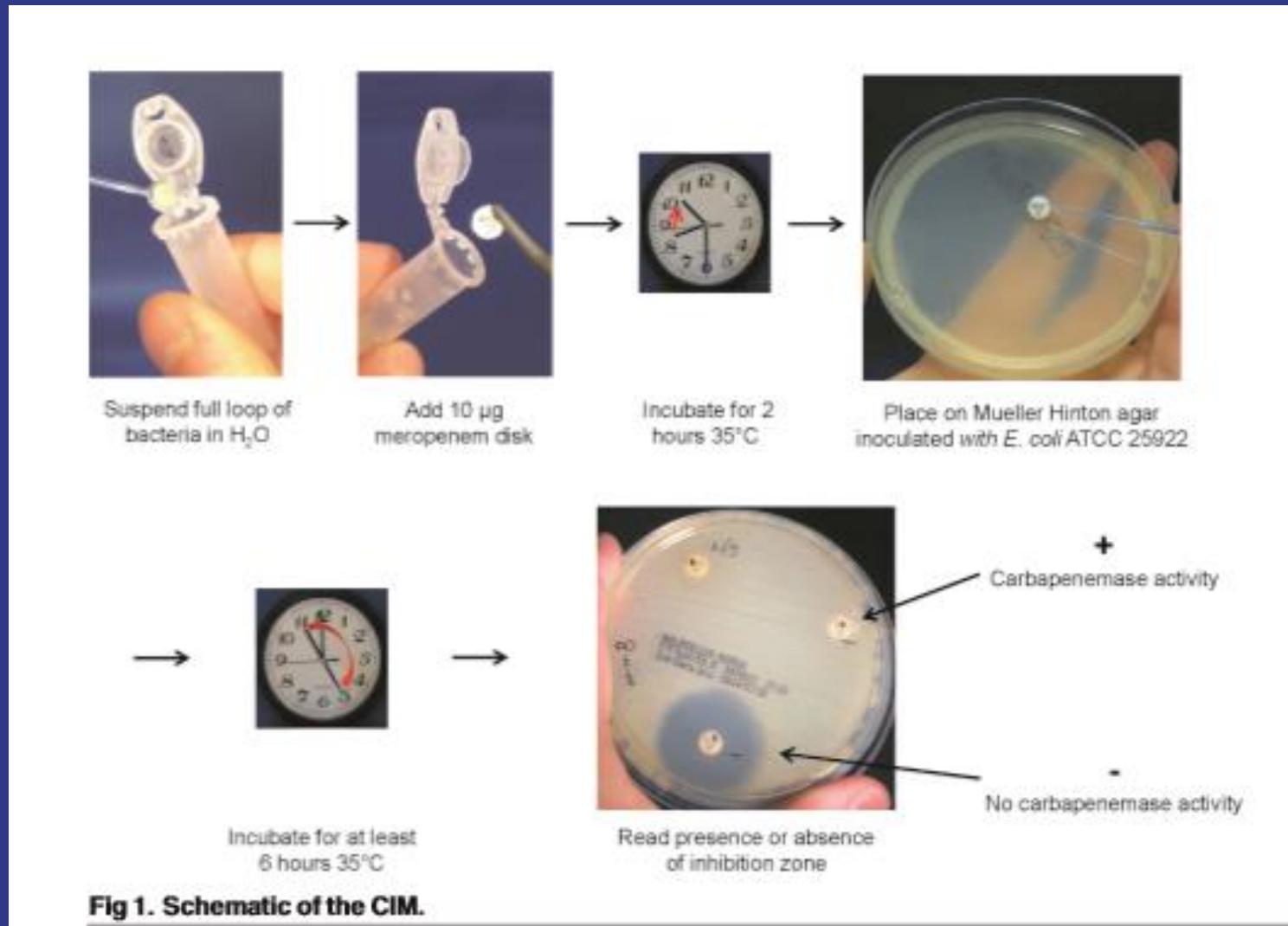
Carba NP Test - Method

- Suspend loopful of organism in protein extn reagent
- Vortex. Incubate 30 min
- Cfg 10,000g
- Mix supernatant with carbapenem soln containing phenol red indicator
- Incubate 2 hrs
- Yellow = positive

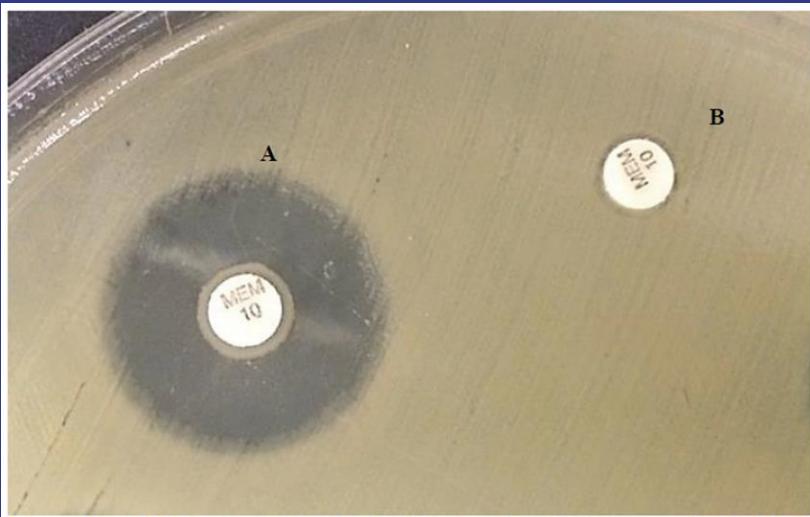


Detects: KPC, NDM, OXA, VIM, IMP, and others.

Modified Carbapenem Inactivation Phenotypic Method (mCIM) (CLSI M100-S29)



mCIM Method

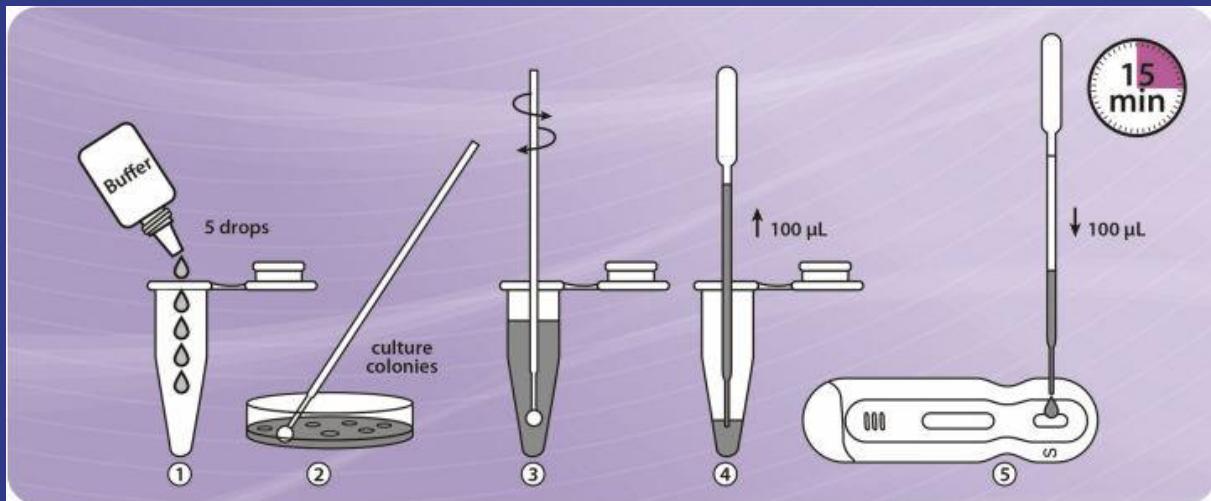


Detects: KPC, NDM,
OXA, VIM, IMP, and
others.

INTERPRETATION

- Carbapenemase Pos:
 - Zone diameter of 6-15 mm or presence of pinpoint colonies within 16-18 mm zone
 - Meropenem in the disk is hydrolyzed & *E. coli* 25922 is not inhibited
- Carbapenemase Neg:
 - Zone diameter of ≥ 19 mm
 - Meropenem in the disk is still active (not hydrolyzed) & will inhibit *E. coli* 25922
- Indeterminate:
 - zone 16-18 mm or ≥ 19 mm w/colonies in zone of inhibition

Carba5 Assay (Hardy) (phenotypic)



- Detects 5 carbapenemases
- Isolated colonies
- Immunochromatographic method



Cepheid GeneXpert CarbaR (genotypic)

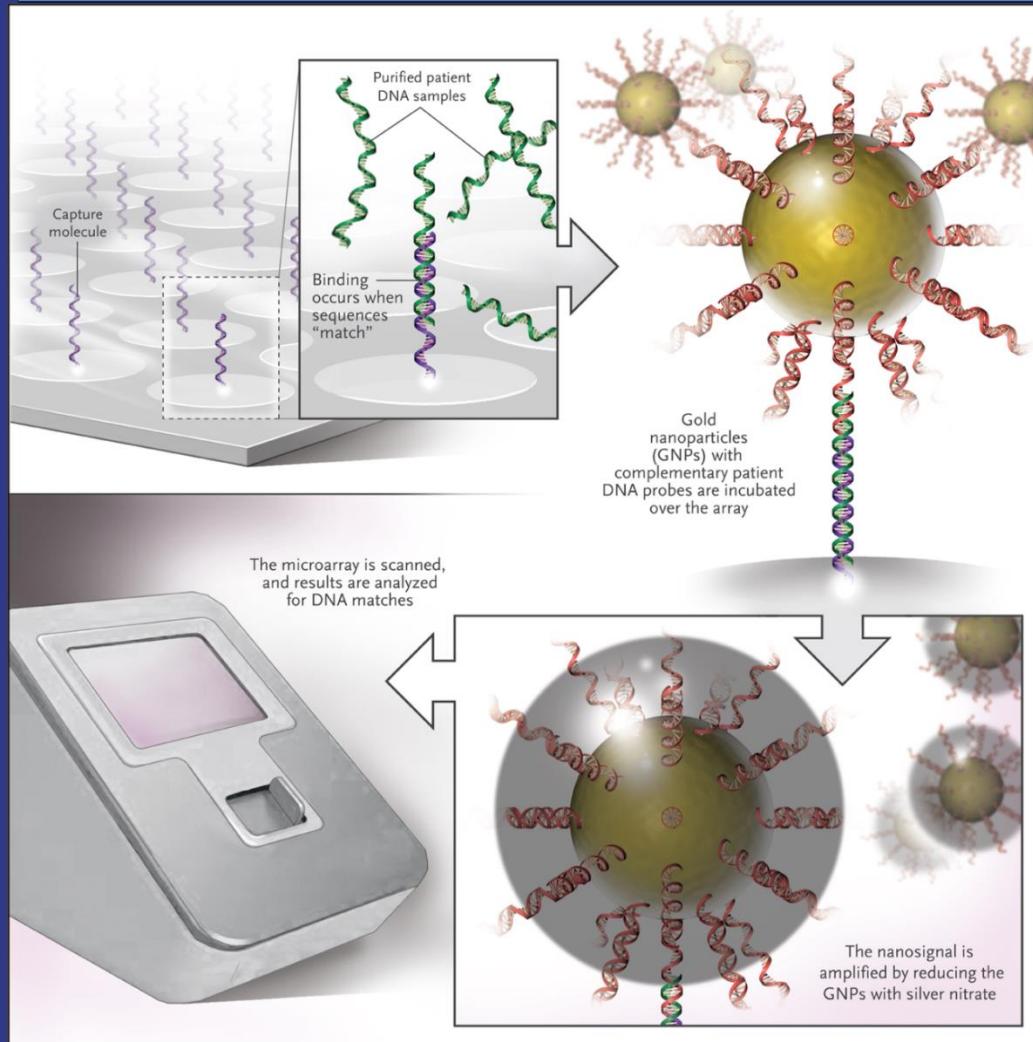


PCR

- Designed for rectal swabs to detect colonization with CRE.
- Can be lab developed/validated from colonies
- Extn, amplification, detection in cartridge
- KPC, NDM, VIM, OXA-48, IMP

Disclaimer: There are a number of PCR/NAAT/Array methods available.

Verigene: Blood Culture ID for Gram Negatives (genotypic)



Array of ID and resistance targets detected with nanoparticles

Walk-away instrument; 2.5 hrs

Verigene™ Blood Culture -GN Targets

[Can be validated to test from colonies.]

- *Acinetobacter* spp.
- *Citrobacter* spp.
- *Enterobacter* spp.
- *Proteus* spp.
- *Escherichia coli*
- *K. pneumoniae*
- *K. oxytoca*
- *P. aeruginosa*
- CTX-M (ESBL)
- KPC
- NDM
- VIM
- IMP
- OXA

β -lactams & β -lactamases

Initialisms & acronyms

- CRE: “carbapenem-resistant *Enterobacteriales*”
CRE is often inferred to possess a carbapenemase, but this is not absolutely true
- CP-CRE: “carbapenemase producing CRE”

β -lactams & β -lactamases

β -lactam

Penicillin

Cephalosporin

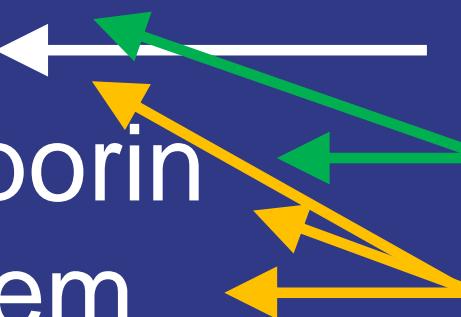
Carbapenem

β -lactamase

Penicillinase

ESBL

Carbapenemase



Questions?



And...thanks to
Dr. Rhoads for
content from
Antibiotics
Lecture and
Kaitlin Zoccola
for helpful
discussion

Extra material

Glucose Fermentation: The O/F Test



Uninoculated tubes

(Left has oil on top i.e. anaerobic; the right is open to oxygen)



Oxidative metabolism

Aerobic respiration.
Glucose metabolized to acids only in presence of oxygen (yellow).

Pseudomonas,
Stenotrophomonas,
etc

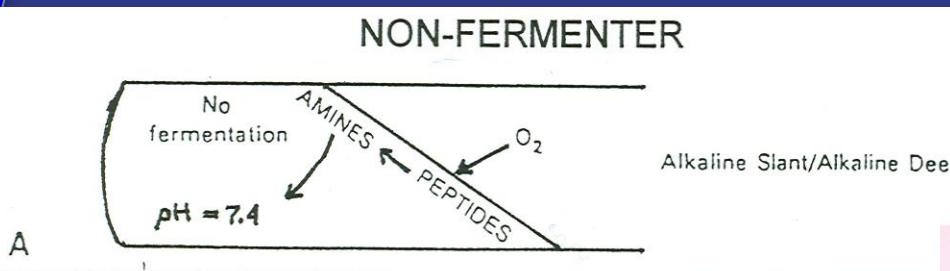


Fermentative metabolism

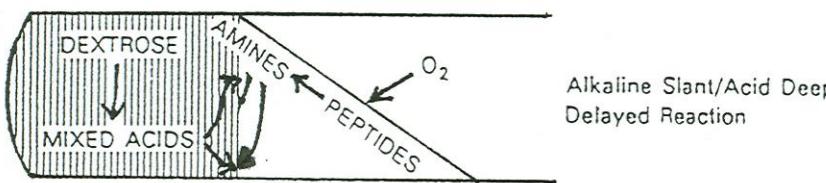
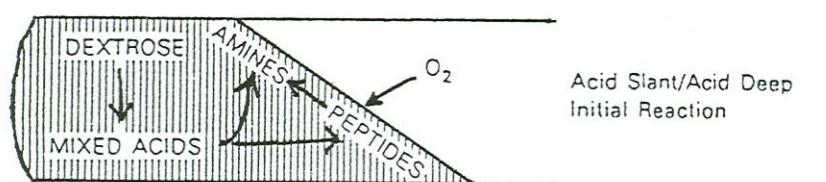
An anaerobic process.
Production of acids from glucose occurs with or without oxygen (yellow).

Enterobacteriales

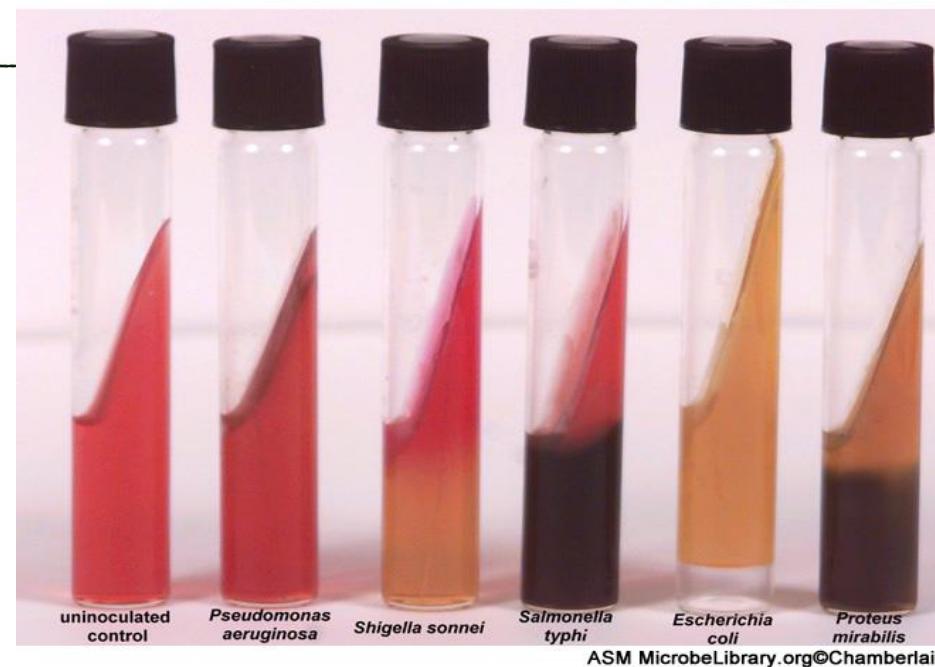
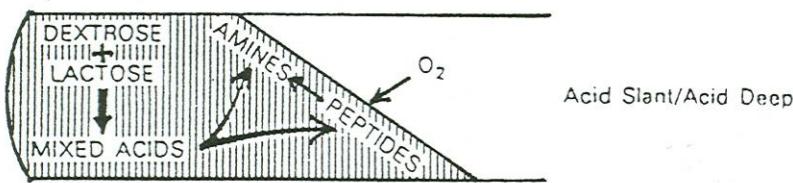
TSI Reaction



NON-LACTOSE FERMENTER



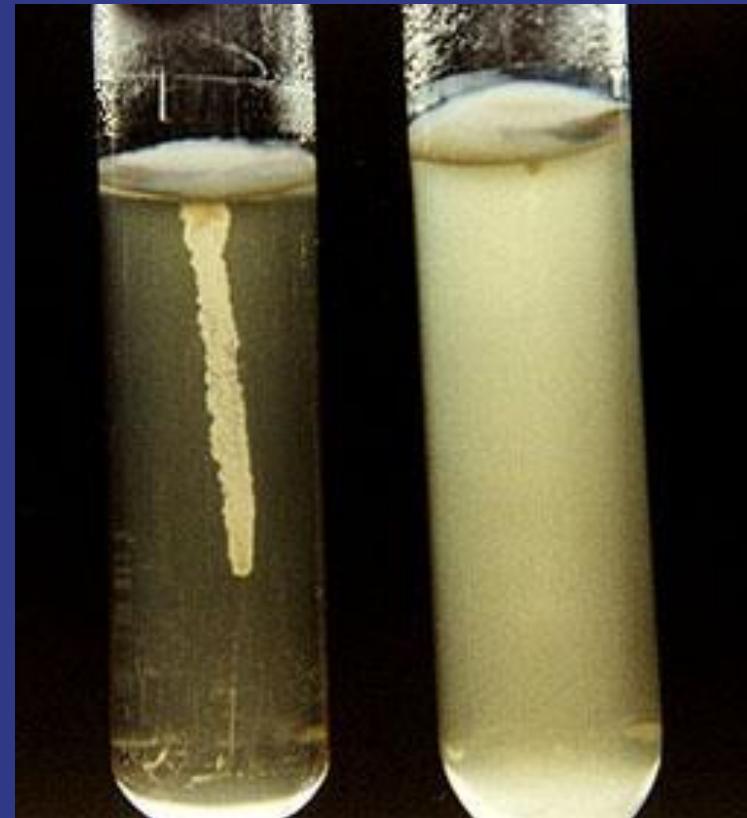
LACTOSE (SUCROSE) FERMENTER



ASM MicrobeLibrary.org@Chamberlain

Motility

- Semi-solid agar is stabbed a single time with the colony.
- After incubation observe haze of growth away from stab if motile.



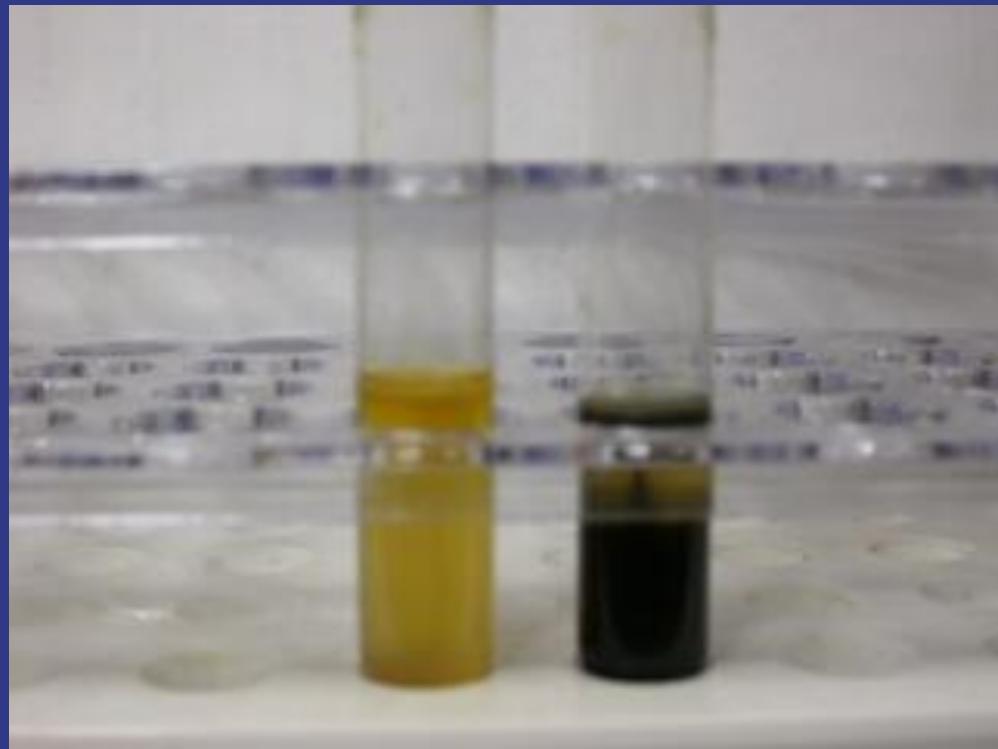
Neg

Pos

Non-motile genera:
Klebsiella, *Shigella*, *Yersinia*

H_2S Production – SIM Medium

- Sodium thiosulfate is the source of sulfur atoms.
- Iron is included to detect H_2S production.
- Black is positive



Proteus, Citrobacter, Salmonella (+)

E. coli, Klebsiella, Enterobacter, Serratia, Morganella, Providencia (-)

Fermentation pathway of glucose metabolism

- An anaerobic process
- An organic substrate is terminal electron acceptor
- “Mixed acids” – lactic, acetic, formic – detected with Methyl red test
- Pyruvate metabolized by butylene glycol pathway - lactate, fomate, alcohols – detected with Voges-Proskauer test

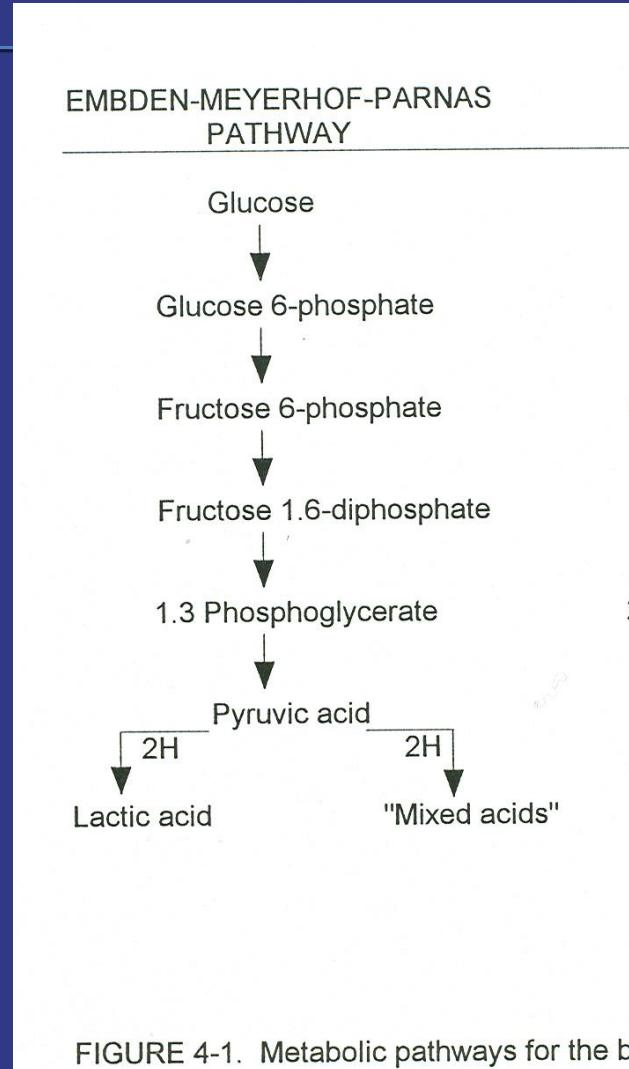
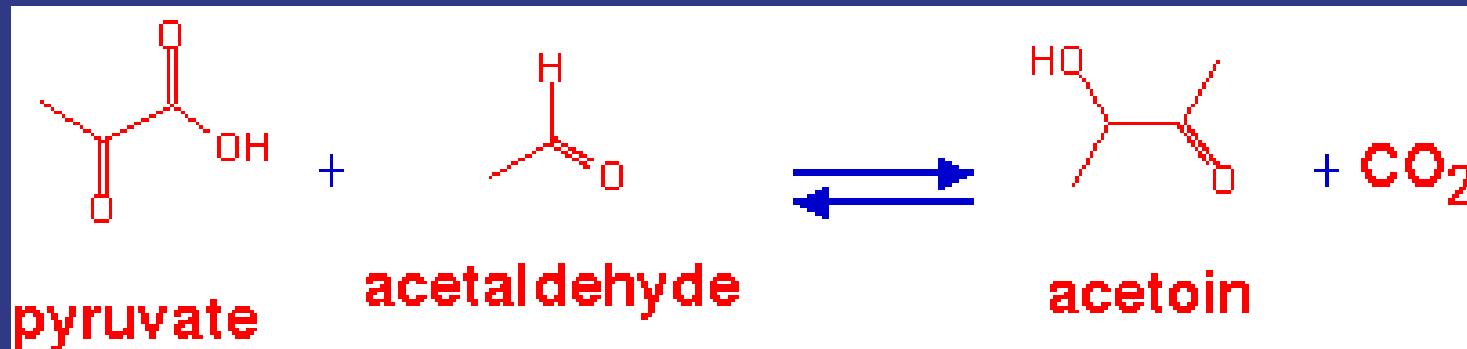


FIGURE 4-1. Metabolic pathways for the b

Voges-Proskauer Reaction



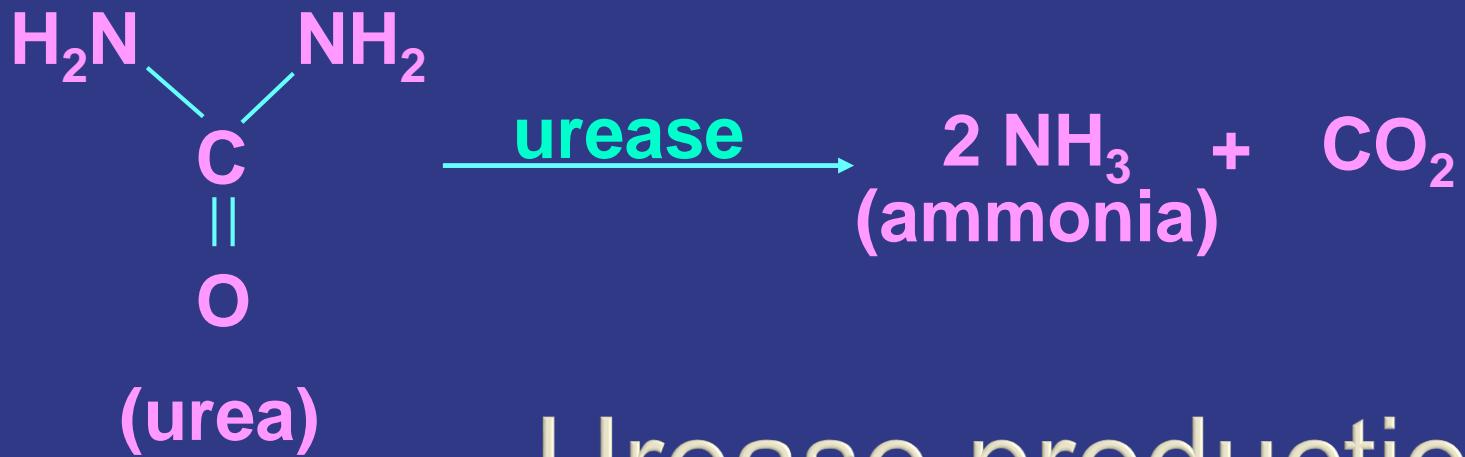
Must add KOH and α -naphthol to see result



Simmons Citrate

- Measures the ability to grow with citrate as the sole carbon source.
- Bromthymol blue is the pH indicator.
- Growth causes alkaline change (blue).

Citrobacter, Klebsiella, Enterobacter, Providencia, Salmonella (+)
E. coli (-)



Urease production



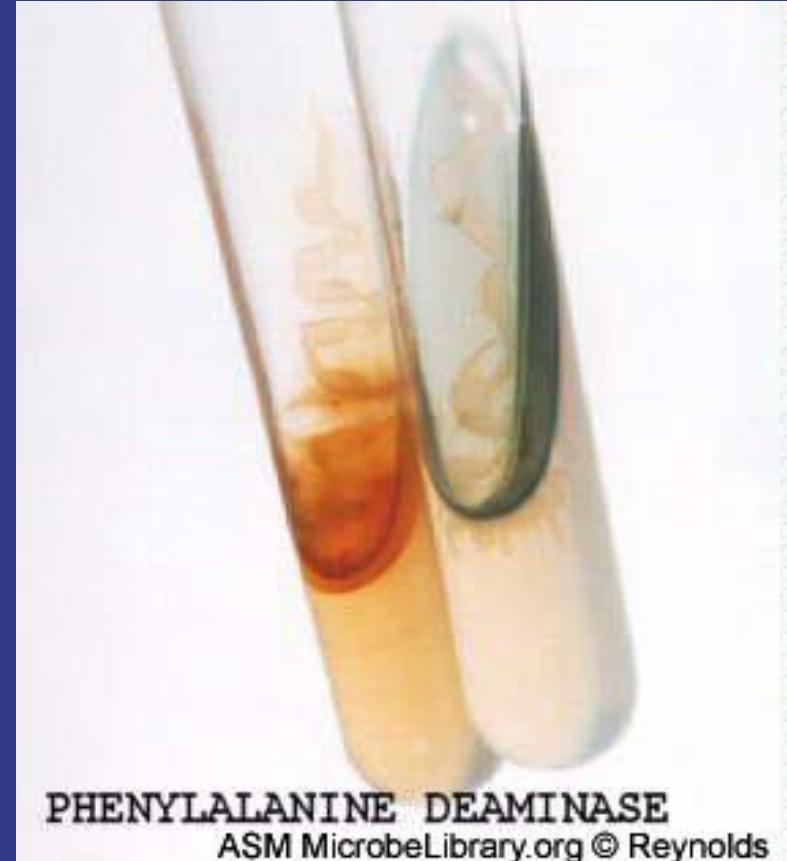
Uninoculated tube
Proteus, Klebsiella, Morganella,
some other species (+)
E coli, Salmonella, Shigella,
Serratia (-)

Phenylalanine Deaminase

- Phenylalanine deaminated to phenylpyruvate
- Ferric Chloride is added. Green color produced.

*Proteus, Providencia,
Morganella (+)*

Other genera (-)



IMVIC (% Positive reactions)

	Indole	MR	VP	Citrate
<i>E. coli</i>	98	99	0	1
<i>K. pneumoniae</i>	0	10	98	98
<i>K. oxytoca</i>	99	20	95	95
<i>E. cloacae</i>	0	5	100	100
<i>E. aerogenes</i>	0	5	98	95
<i>P. mirabilis</i>	2	97	50	65
<i>P. vulgaris</i>	98	95	0	15