Subota, 27. veljače 2016.

Zavod za kliničku mikrobiologiju
Klinika za infektivne bolesti "Dr. F. Mihaljević", Zagreb

11.00 - 13.00	Rad u laboratoriju / *grupa 1*
13.00 - 13.30	Kviz i zaključci / *grupa 1* (Velika predavaonica)
13.30 - 15.30	Rad u laboratoriju / *grupa 2*
15.30 - 16.00	Kviz i zaključci / *grupa 2* (Velika predavaonica)

Prijave se primaju do 20. veljače 2016. Maksimalan broj polaznika je 50. Na prijavnici molimo naznačiti preferiranu grupu za praktični dio tečaja čemu će se udovoljavati prema redoslijedu pristizanja prijava.

Službeni jezik : hrvatski
Kotizacija: 1500 kuna za liječnike mikrobiologe, 1000 kuna za specijalizante i laboratorijske inženjere;
Kotizacija se uplaćuje na Hrvatski liječnički zbor IBAN: HR7423600001101214818 poziv na broj 268-015
Potvrdnice: Za sudjelovanje na tečaju dobit će se potvrdnice s bodovima koje će odrediti Hrvatska liječnička komora.

Za sve obavijesti:
Gđa. Jasminka Blaha
Klinika za infektivne bolesti "Dr. Fran Mihaljević" Mirogojska 8, 10000 Zagreb
Mob: 091 4012 622; e-mail: jblaha@bfm.hr

Hrvatski liječnički zbor
Hrvatsko društvo za kliničku mikrobiologiju

Klinika za infektivne bolesti "Dr. Fran Mihaljević»
Referentni centar Ministarstva zdravlja za praćenje rezistencije bakterija na antibiotike

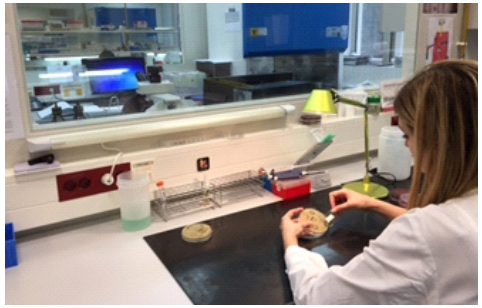
Akademija medicinskih znanosti Hrvatske
Kolegij za javno zdravstvo
Odbor za praćenje rezistencije bakterija na antibiotike
APUA Croatia

organiziraju

VIII. TEČAJ O TESTIRANJU OSJETLJIVOSTI BAKTERIJA NA ANTIBIOTIKE

Voditelj tečaja:
Prof. dr. sc. Arjana Tambić Andrašević, dr. med.

Predavači i voditelji vježbi:
Doc. dr. sc. Suzana Bukovski, dr. med.
Iva Butić, dr. med.
Deana Erceg, bacc. med. lab. dijagn.
Sanja Kuštreba, mag. med. lab. dijagn.
Sandra Lucić, mag. med. lab. dijagn.
Irina Pristaš, dr. med.
Silvija Šoprek, dr. med.
Antonio Švagelj, bacc. med. lab. dijagn.
Prof. dr. sc. Arjana Tambić Andrašević, dr. med.



Klinika za infektivne bolesti «Dr. F. Mihaljević», Zagreb
25. - 27. veljače 2016.

Poštovane kolegice i kolege

S obzirom na stalno prilagođavanje bakterija okolišu punom antibiotika u laboratoriju se susrećemo sa stalnim izazovima detekcije novih mehanizama rezistencije koji značajno utječu na interpretaciju antibiograma. Osobito je značajno uočavati prisutnost mehanizama rezistencije koji dovode do multiple rezistencije, jer širenje takvih izolata treba energično zaustaviti primjenom mjera kontaktne izolacije. Pravilno izvođenje i očitavanje testova osjetljivosti uvelike utječe na tijek liječenja pojedinog pacijenta, ali i na uspješnu kontrolu širenja rezistencije u zajednici. Kroz rad Odbora za praćenje rezistencije bakterija na antibiotike Akademije medicinskih znanosti Hrvatske i Referentnog centra Ministarstva zdravlja za praćenje rezistencije na antibiotike hrvatski mikrobiološki laboratoriji sinhronizirano uvode promjene u metodologiji, sukladno standardima koje propisuje European Committee for Antimicrobial Sensitivity Testing (EUCAST). Na ovom tečaju raspravit će se metodologija testiranja osjetljivosti na antibiotike od osnovnih postupaka do složenijih fenotipskih metoda detekcije pojedinih mehanizama rezistencije, a teoretska predavanja će biti praćena odgovarajućim vježbama u laboratoriju. Ovaj tečaj je namijenjen prvenstveno laboratorijskim djelatnicima, liječnicima i laboratorijskim inženjerima koji se u svojem rutinskom radu susreću s dilemama u interpretaciji antibiograma i uočavanju mehanizama rezistencije koji imaju veliku epidemiološku značajnost. Nadamo se da će tečaj obilovati živim diskusijama, potaknuti još bolju međulaboratorijsku suradnju te uz prijateljsku atmosferu pružiti najnovije informacije korisne za svakodnevni rad u mikrobiološkom laboratoriju.

Prof. dr. sc. Arjana Tambić Andrašević

OBAVIJESTI
Termini i mjesto održavanja:

Predavanja			
Četvrtak	25. veljače 2016.	10.00 - 12.30	Velika predavaonica Klinika za infektivne bolesti «Dr. Fran Mihaljević», Mirogojska
Petak	26. veljače 2016.	10.00 - 12.30	
Praktični rad			
Četvrtak - *grupa 1*	25. veljače 2016.	14.00 - 18.00	Zavod za kliničku mikrobiologiju, Klinika za infektivne bolesti «Dr. Fran Mihaljević», Mirogojska 8
Petak - *grupa 2*	26. veljače 2016.	14.00 - 18.00	
Subota - *grupa 1*	27. veljače 2016.	11.00 - 13.30	
Subota - *grupa 2*	27. veljače 2016.	13.30 - 16.00	

PROGRAM TEČAJA

Četvrtak, 25. veljače 2016.

Velika predavaonica
Klinika za infektivne bolesti "Dr. F. Mihaljević", Zagreb

09.00 - 10.00 registracija

10.00 - 10.15 A. Tambić Andrašević: **Uvodne riječi**
10.15 - 10.45 A. Tambić Andrašević: **Osnove izrade antibiograma**
10.45 - 11.15 I. Butić: **Gram-pozitivne bakterije: mehanizmi rezistencije i interpretacija antibiograma**
11.15 - 11.45 A. Tambić Andrašević: **Gram-negativne bakterije: mehanizmi rezistencije i interpretacija antibiograma**
11.45 - 12.15 S. Šoprek: **MDR i *C. difficile* - ruku pod ruku**
12.15 - 12.30 Diskusija

12.30 - 14.00 Pauza za ručak

Zavod za kliničku mikrobiologiju
Klinika za infektivne bolesti "Dr. F. Mihaljević", Zagreb

14.00 - 18.00 Rad u laboratoriju / *grupa 1*

Petak, 26. veljače 2016.

Velika predavaonica
Klinika za infektivne bolesti "Dr. F. Mihaljević", Zagreb

10.00 - 10.30 S. Šoprek: **Testiranje osjetljivosti na antibiotike kod izbirljivih bakterija**
10.30 - 11.00 I. Butić: **Određivanje minimalnih inhibitornih koncentracija**
11.30 - 12.00 S. Bukovski: **Kontrola kvalitete u mikrobiološkom laboratoriju**
12.00 - 12.30 Diskusija

12.30 - 14.00 Pauza za ručak

Zavod za kliničku mikrobiologiju
Klinika za infektivne bolesti "Dr. F. Mihaljević", Zagreb

14.00 - 18.00 Rad u laboratoriju / *grupa 2*

Osnove izrade antibiograma

Arjana Tambić Andrašević
Klinika za infektivne bolesti, Zagreb

EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 5.0
January 2015

Sadržaj

- Vrste medija
- Priprema inokuluma
- Inokulacija ploča
- Diskovi
- Inkubacija
- Očitavanje zona
- Interpretacija

- QC
- Analiza pogreške

Disk difuzija - vrste medija

1. Mueller-Hinton agar (MH)
2. Mueller-Hinton agar za osjetljive mikroorganizme (MH-F)

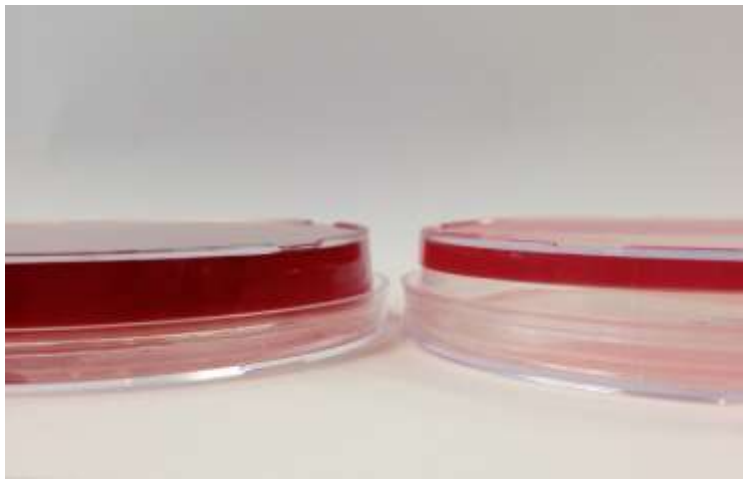
   MH + 5% defibrinirana konjska krv + 20 mg/L β-NAD (nicotinamide adenine dinucleotide)
   čistoća β-NAD mora biti ≥ 98%.

β-NAD, Sigma Aldrich, USA
MH-F, BioMerieux, France

Organisms	**Medium**
Enterobacteriaceae *Pseudomonas* spp. *Stenotrophomonas maltophilia* *Acinetobacter* spp. *Staphylococcus* spp. *Enterococcus* spp.	Mueller-Hinton agar
Streptococcus pneumoniae Streptococcus groups A, B, C and G Viridans group streptococci *Haemophilus* spp. *Moraxella catarrhalis* *Listeria monocytogenes* *Pasteurella multocida* *Campylobacter jejuni* and *coli* *Corynebacterium* spp.	Mueller-Hinton agar + 5% mechanically defibrinated horse blood + 20 mg/L β-NAD (MH-F)
Other fastidious organisms	Pending

In-house priprema medija

- Slijediti upute proizvođača
- Dobro promiješati i odmah razliti u Petrijeve ploče
- Debljina medija/agara u ploči mora biti ravnomjerna i iznositi 4.0±0.5mm
- Najčešće se koriste ploče promjera 90 mm, 100 mm (okrugle i kvadratne) ili 150 mm
- Kod pripreme MH-F dodati krv i ß-NAD kad se MH otopina ohladi na temp. 42-45°C, promiješati i odmah razlijevati !



Kontrola kvalitete Mueller Hinton agara
Pohranjivanje ploča

1. Kvaliteta svakog razlijevanja Mueller Hinton agara mora biti provjerena kontrolnim sojevima s poznatim zonama inhibicije
2. Agar ploče se čuvaju na 4-10^{0}C
3. Pohrana *in-house* pripremljenih ploča mora biti određena lokalnim protokolom
4. Pohrana komercijalno pripremljenih ploča – slijediti postupnik propisan od strane proizvođača
5. Prije upotrebe, na površini agara ne smije biti vidljivih kapljica vode, ali ploče ne smiju biti presušene
6. Agar ploče prije inokulacije moraju biti temperirane na sobnu temperaturu

Inokulum

- odabrati nekoliko morfološki sličnih kolonija
- prekonoćna kultura bakterija
- sa neselektivnog agara

Inokulum

- Sterilnom ezom ili štapićem dotaknuti nekoliko morfološki sličnih kolonija i razmutiti u 0.85% fiziološkoj otopini do gustoće suspenzije od **0.5 McFarland**
  * IZNIMKA -> *Streptococcus pneumoniae*
  a) kolonije s krvnog agara-> gustoća = 0.5 McFarland
  b) kolonije s čokoladnog agara -> gustoća = 1.0 McFarland

- Zadana gustoća suspenzije se postiže dodavanjem bakterijskih kolonija ili 0.85% fiziološke otopine

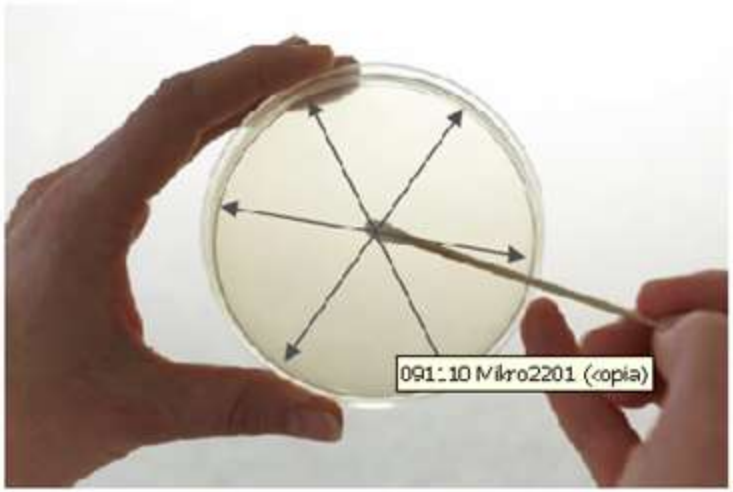
- Gustoća suspenzije se mjeri prema McFarland standardima ili fotometrijskim uređajem

McFarland standard

McFarland 0.5 = E.coli ~ 1-2 x 10^8 CFU/mL

Inokulacija ploča

- unutar 15 minuta od pripreme bakterijske suspenzije (ne dulje od 60 min!!!)
- Vateni štapić namočiti u suspenziju
- Višak tekućine odstaniti laganim okretanjem uz unutarnju stijenku epruvete (kod Gram-pozitivnih bakterija - ne prejako)
- Ravnomjerno nanijeti inokulom na površinu agara, razmazivanjem u tri smjera ili koristeći aparat za okretanje ploče (plate rotator)
- Paziti da se ne nanese prejaki inokulum !!!



Pohranjivanje antimikrobnih diskova

- Slijediti upute proizvođača
- Uvjeti pohranjivanja:

  - temperatura 4-8°C
  - zatvoreni kontejneri s desikatorom (isušivačem)
  - zaštićeni od svjetlosti
- Kako bi izbjegli kondenzaciju vlage na diskovima, kontejner s diskovima prije otvaranja mora postići sobnu temperaturu
- Ukoliko će se diskovi koristiti nekoliko puta u toku dana, preporuča se cijelo vrijeme držati ih na sobnoj temperaturi
- NE koristiti diskove kojima je istekao rok valjanosti

Aplikacija diskova

- Diskove treba nanijeti (aplicirati) na površinu agara unutar 15 minuta od inokulacije
- Disk mora jednakomjerno, cijelom površinom prianjati uz agar
- Raspored diskova mora osiguravati dobro vidljive zone inhibicije, jasne za očitavanje
- Izbjegavati preklapanja zona inhibicije !

Inkubacija ploča

- Preokrenute ploče staviti u termostat unutar 15 minuta od nanošenja diskova na ploču
- "stupci" ploča u termostatu bi trebali biti što manji, nejednoliko zagrijavanje ploča može utjecati na zone inhibicije

- Uvjeti inkubacije:

a) MH -> atmosferski zrak

b) MH-F -> atmosferski zrak s 4-6% CO_2

Incubation of plates

Organism	Incubation conditions
Enterobacteriaceae	35+/-1 ºC in air for 16-20h
Pseudomonas spp.	35+/-1 ºC in air for 16-20h
Stenotrophomonas maltophilia	35+/-1 ºC in air for 16-20h
Acinetobacter spp.	35+/-1 ºC in air for 16-20h
Staphylococcus spp.	35+/-1 ºC in air for 16-20h
Enterococcus spp.	35+/-1 ºC in air for 16-20h (24 h for glycopeptides)
Streptococcus groups A, B, C and G	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Viridans group streptococci	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Streptococcus pneumoniae	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Haemophilus spp.	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Moraxella catarrhalis	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Listeria monocytogenes	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Pasteurella multocida	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Campylobacter jejuni and *coli*	41+/-1 ºC in microaerobic environment for 24h (40-48h)
Corynebacterium spp.	35+/-1 ºC in air with 4-6% CO_2 for 16-20h (40-48h)
Other fastidious organisms	Pending

MH

MH-F

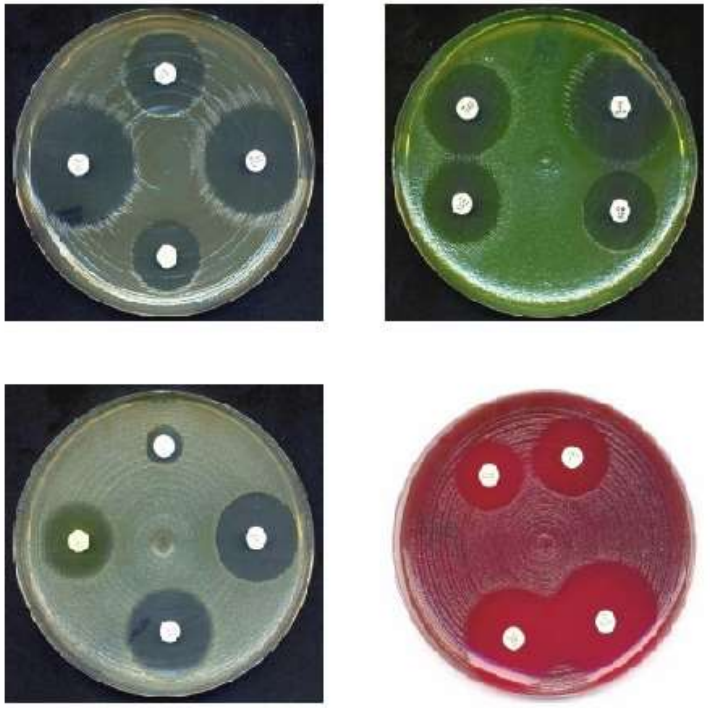
Pravilo: 15-15-15 minuta

* inokulacija ploča – **unutar 15 minuta** od pripreme bakterijske suspenzije/inokuluma (ne dulje od 60 min)

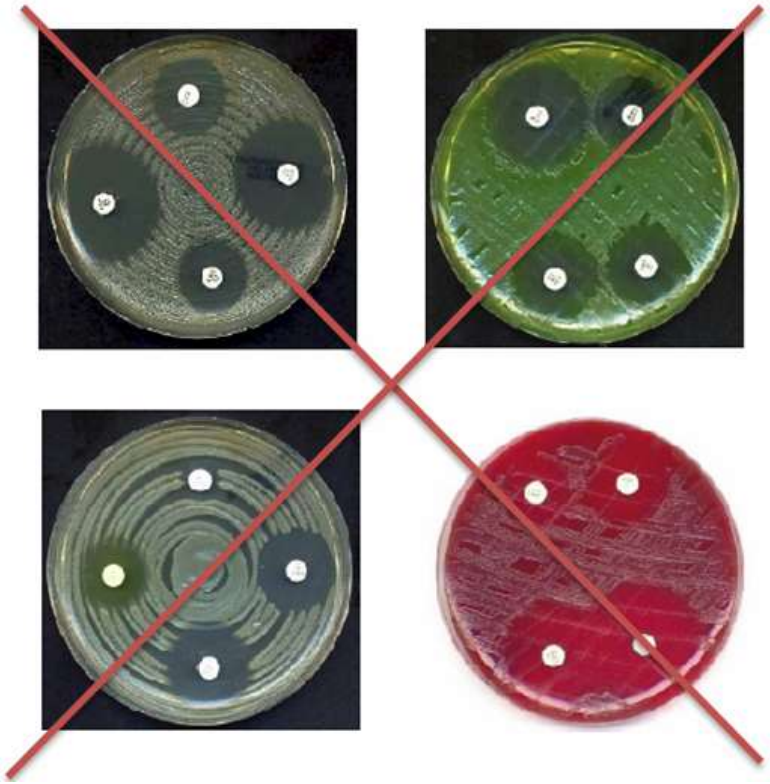
* aplikacija diskova – **unutar 15 minuta** od inokulacije ploča

* inkubacija ploča – **unutar 15 minuta** od aplikacije diskova

Porast bakterijske kulture treba biti konfluentan i ravnomjeran preko cijele ploče



Ploče bi trebale izgledati ovako…



…a ne ovako!!!

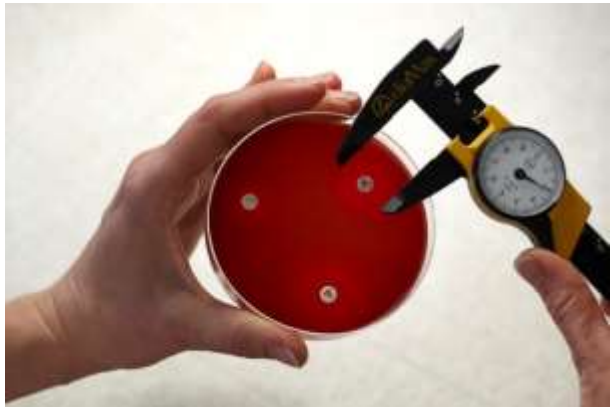
Očitavanje zona inhibicije

- Rubovi zone inhibicije se očitavaju na mjestu potpune inhibicije očitane prostim okom, **bez korištenja povećala**
- Ne očitavati s pločom okrenutom prema svjetlu, osim u posebnim prilikama, naznačenim u EUCAST Breakpoint tablici
- Zone inhibicije se mjere pomoću ravnala, kalipera ili koristeći automatski čitač

Očitavanje zone inhibicije

* **MH ploče**
  - Zonu inhibicije očitavati na poleđini ploče
  - Crna podloga kao pozadina
  - Reflektirajući izvor svjetlosti

* **MH-F ploče**
  - Zonu inhibicije očitavati s prednje strane ploče s odignutim poklopcem
  - Reflektirajući izvor svjetlosti



Očitavanje zone inhibicije

- Zone inhibicije se očitavaju na mjestu kompletne inhibicije gledajući prostim okom i držeći ploču oko 30 cm od oka
- Kod dvostrukih zona očitava se unutarnja (osim ako nije drugačije naznačeno)

Primjeri:

E. coli
Ciprofloxacin

S. aureus
Erythromycin

P.aeruginosa
Meropenem

S. pneumoniae
Rifampicin

Kolonije unutar zone inhibicije

- U slučaju jasnog porasta kolonija unutar zone inhibicije:

  -> kolonije treba subkultivirati

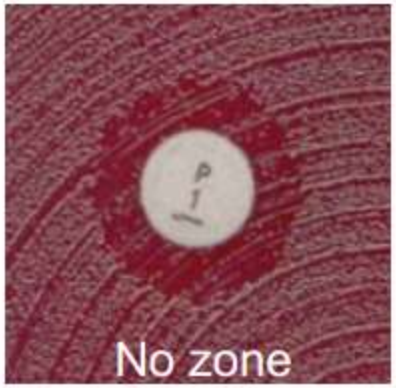
  -> provjeriti da li se radi o čistom soju

  -> po potrebi ponoviti testiranje

- Kolonije koje ne smatramo kontaminacijom treba uzeti u obzir prilikom očitavanja testa osjetljivosti tj. određivanja rubova zone inhibicije

E. coli with ESBL

H. influenzae with PBP mutations



Nejasan rub zone inhibicije
Enterobacteriaceae

- **Očitavati prostim okom!**
- **Ne koristiti pomagala za povećavanje!**
- **Ne koristiti dodatni izvor svjetlosti!**

- Ploču držimo na 30 cm udaljenosti od očiju.
- Koristimo crnu podlogu kao pozadinu kako bi lakše odredili rub zone inhibicije.

Očitavanje zona – iznimke (1)

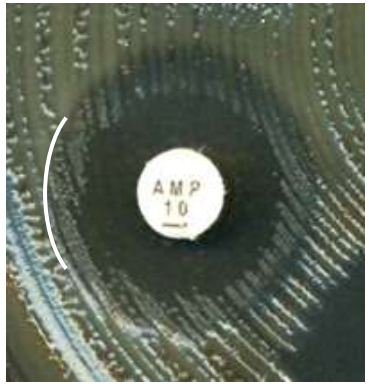
Organism	Antimicrobial agent	Reading inhibition zones
E. coli	Mecillinam	Ignore isolated colonies within the inhibition zone.
Enterobacteriaceae	Ampicillin	Ignore fine growth that may appear as an inner zone on some batches of MH agar.
Any	Trimethoprim Trimethoprim-sulfamethoxazole	Ignore a fine haze of growth up to the disk within zones with an obvious margin.
Proteus spp.	Any	Ignore swarming.

Enterobakterije i ampicilin

- Zanemariti porast kolonija koji se prikazuju kao unutarnja (dvostruka) zona

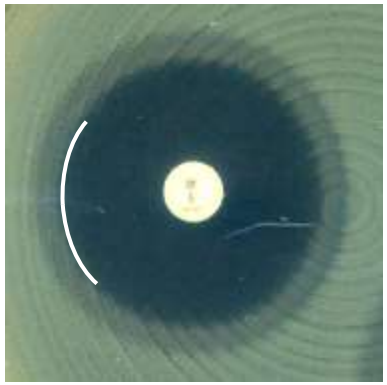
IZNIMKA!!!

- Očitavati jasan vidljiv (“gušći”) rub zone inhibicije



Trimetoprim i trimetoprim-sulfametoksazol

- Kod dvostrukih zona - očitavati unutarnju zonu inhibicije
- Kod jasno vidljivih rubova zone inhibicije - zanemariti slab porast do diska unutar zone



E. coli

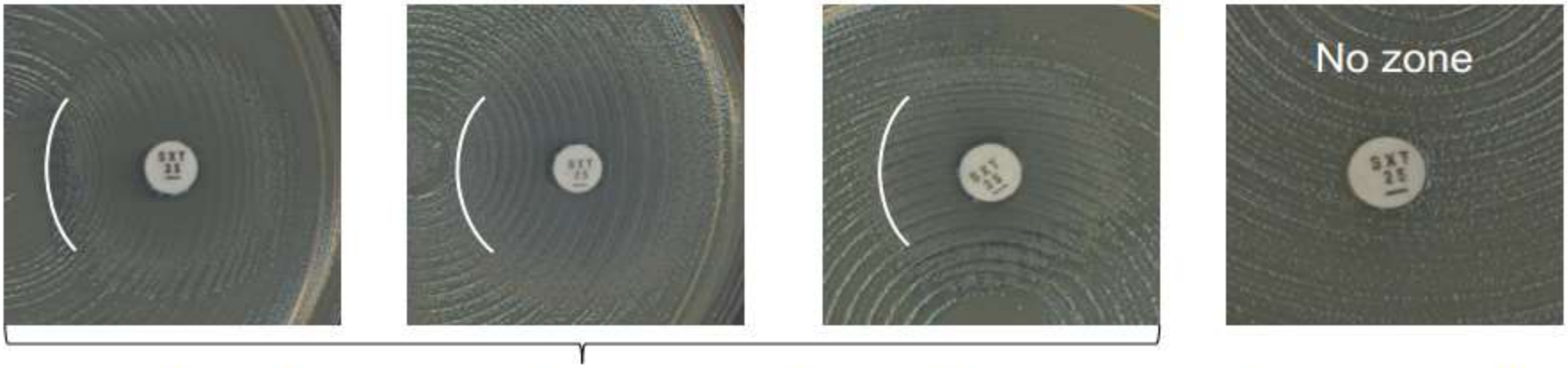
KNS

Moraxella sp.

Haemophilus sp.

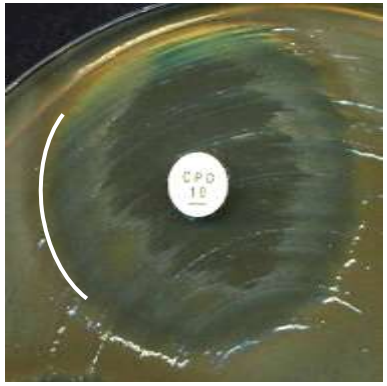
Stenotrophomonas maltophilia
i
trimetoprim-sulfametoksazol

- Očitava se vanjska zona inhibicije!
- Ignorirati porast unutar zone inhibicije = osjetljiv
- Ako vanjska zona inhibicije nije vidljiva = rezistentan



Fenomen puzanja

- Očitavamo samo zonu ihnibicije porasta bakterijske kulture.
- Ignoriramo fenomen puzanja
- Najčešće susrećemo kod *Proteus* spp.



Očitavanje zona – iznimke (2)

Organism	Antimicrobial agent	Reading inhibition zones
Streptococcus spp.	Any	Read inhibition of growth and not the zone of haemolysis.
Staphylococci	Cefoxitin	Examine zones carefully to detect colonies within the inhibition zone.
Staphylococcus spp.	Linezolid	Examine with transmitted light (plate held up to light).
S. aureus	Benzylpenicillin	Examine zone edge closely with transmitted light (plate held up to light).
Enterococcus spp.	Vancomycin	Examine with transmitted light (plate held up to light).

Porast ili hemoliza?

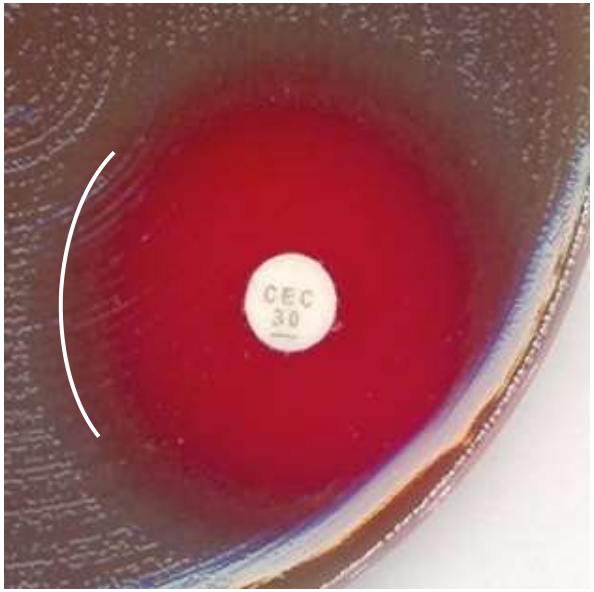
- **Očitavati samo zonu inhibicije porasta!**
- **Ne očitavati zonu hemolize!**

- Ponekad je teško razlučiti porast od hemolize:
  - β-hemolizin difundira u agar -> u zoni β-hemolize nema porasta kolonija
  - α-hemolizin ne difundira u agar ->u zoni α-hemolize često prisutan porast kolonija
  - Rub zone inhibicije i α-hemoliza se jako često pojavljuju kod *S. pneumoniae* i β-laktamskih antibiotika.

Porast ili hemoliza?

- Očitavanje -> nagib ploče pod određenim kutem
- Olakšava se očitavanje tj. bolje se može razlučiti zona hemolize od zone porasta

Često je prisutan porast kolonija duž cijele zone hemolize.

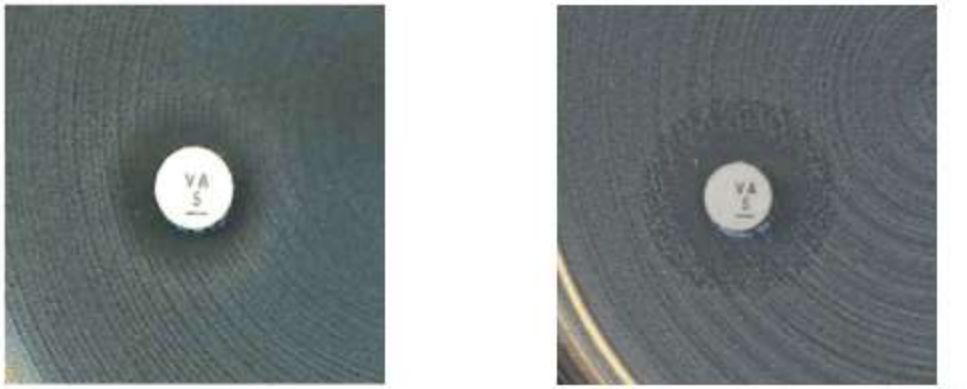


Za neke mikroorganizme na MH-F pločama karakteristična je prisutnost dodatne zone α-hemolize bez porasta. Nagibanjem ploče olakšava se razlikovanje hemolize od porasta.

Enterokoki i vankomicin

- Ploču usmjerimo prema izvoru svjetlosti.
- Nejasni rub zone inhibicije i porast kolonija unutar zone upućuju na vankomicinsku rezinstenciju.
- Obavezno provesti potvrdu detaljnijim testovima (MIK, PCR dokaz VanA, VanB…)

E. faecalis
non-VRE



E. faecium
VRE

Stafilokoki i penicilin

- Ploču usmjerimo prema izvoru svjetlosti.
- Detekcija penicilinaze:

  a) nitrocefinski disk nije pouzdan

  b) disk difuzijska metoda pouzdanija od određivanja MIK vrijednosti

S. aureus with sharp zone edge and zone diameter ≥ 26 mm = Resistant

S. aureus with fuzzy zone edge and zone diameter ≥ 26 mm = Susceptible

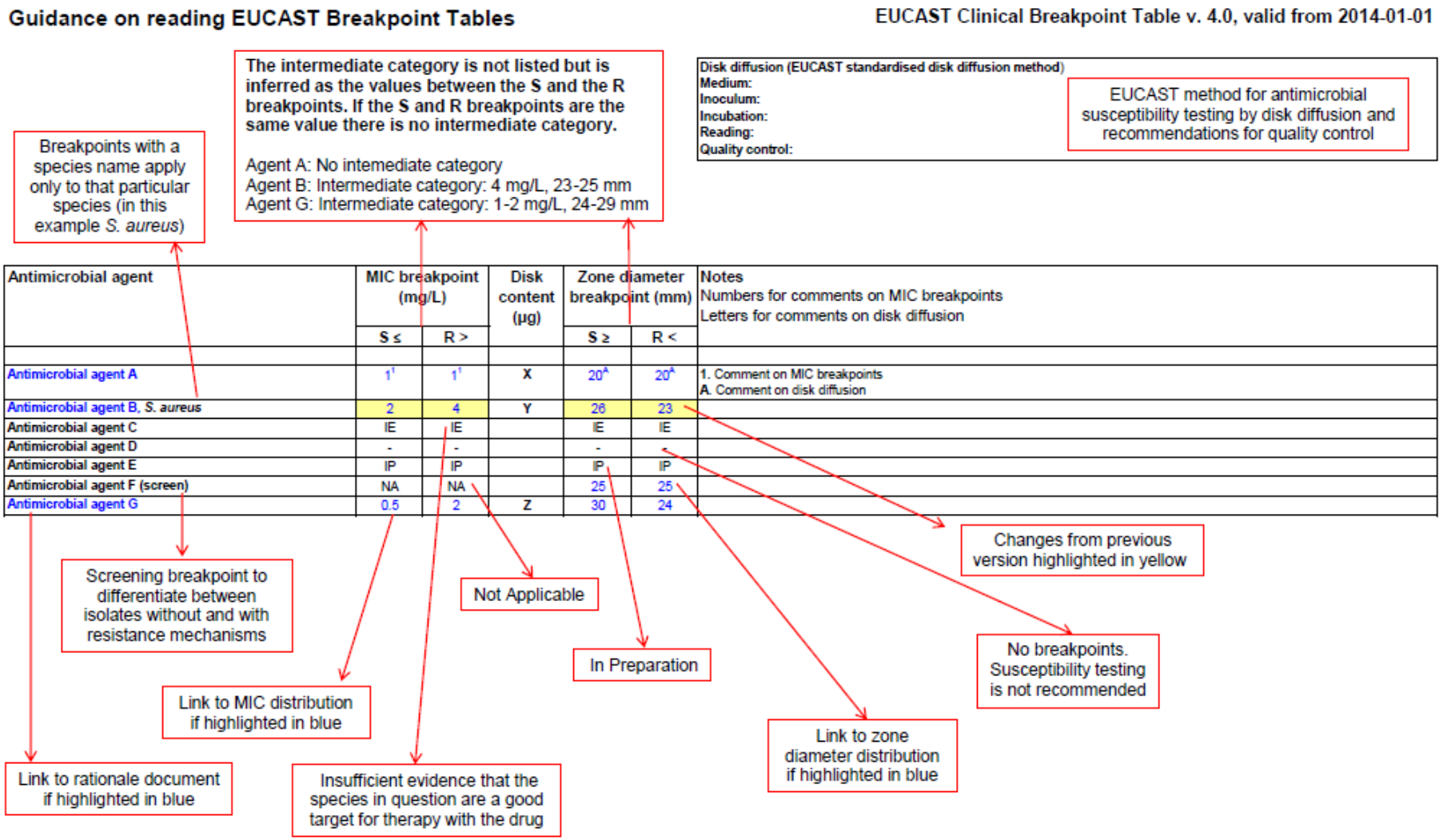
Interpretacija zona inhibicije

• Prije interpretacije testa osjetljivosti provjeriti jesu li zone inhibicije za kontrolne sojeve unutar definiranih graničnih vrijednosti

• Promjeri zona inhibicije interpretiraju se u skladu s EUCAST-ovim tablicama (www.eucast.org)

• Interpretacija testa osjetljivosti - kategorije osjetljivosti (S, I i R)

EUCAST Breakpoint tablice



Kada određivati osjetljivost izolata na antibiotike

- Čimbenici za razmotriti:
  - **Prisutnost drugih bakterija i kvaliteta uzorka**
    - Više vrsta bakterija u urinu
    - Nekvalitetan sputum
  - **Mjesto infekcije / vrsta uzorka**
    - Primarno sterilni materijali
    - Kontaminirani uzorci
  - **Stanje pacijenta**
    - Imunokompromitirani pacijent
    - Prisutnost stranog tijela

Odabir antibiotika za testiranje i izdavanje u nalazu

- panel antibiotika za testiranje ovisi o:
  - vrsti bakterije
  - mjestu infekcije
  - lokalnoj rezistenciji (NL vs. Grčka)
  - lokalnom tržištu
  - lokalnim smjernicama

- često vrsta bakterije nije poznata u vrijeme postavljanja antibiograma pa se može dogoditi da se testiraju antibiotici neprikladni za tu vrstu – u tom slučaju se ti antibiotici ne izdaju u nalazu

Odabir antibiotika za testiranje i izdavanje u nalazu

- **EUCAST smjernice**
  - Izvođenje testova osjetljivosti
  - Kategoriziranje rezultata prema graničnim vrijednostima (S/I/R)

The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016. http://www.eucast.org.

- **Dokazivanje specifičnih mehanizama rezistencije**

Giske CG, Martinez-Martinez L, Cantón R *et al.* EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0, December 2013. http://www.eucast.org.

- **Interpretacija nalaza "expert rules"**
  - Intrinzična rezistencija
  - Rijetki neočekivani fenotipovi
  - Interpretativna praavila

Leclerq R, Cantón R, Brown DFJ *et al.* EUCAST expert rules in antimicrobial susceptibility testing. *Clin Microbiol Infect* 2013; 19:141–160.

Guidance on reading EUCAST Breakpoint Tables

EUCAST Clinical Breakpoint Table v. 5.0, valid from 2015-01-01

The intermediate category is not listed but is interpreted as the values between the S and the R breakpoints. If the S and R breakpoints are the same value there is no intermediate category.

Agent A: No intermediate category
Agent B: Intermediate category: 4 mg/L, 23-25 mm
Agent G: Intermediate category: 1-2 mg/L, 24-29 mm

Disk diffusion (EUCAST standardised disk diffusion method)
Medium:
Inoculum:
Incubation:
Reading:
Quality control:

EUCAST method for antimicrobial susceptibility testing by disk diffusion and recommendations for quality control

Breakpoints with a species name apply only to that particular species (in this example *S. aureus*)

Antimicrobial agent	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
Antimicrobial agent A	1[1]	1[1]	X	20[A]	20[A]	**1.** Comment on MIC breakpoints
Antimicrobial agent B, *S. aureus*	2[2]	4	Y	26	23	**2.** New comment
Antimicrobial agent C	IE	IE		IE	IE	~~Removed comment~~
Antimicrobial agent D	-	-		-	-	
Antimicrobial agent E	IP	IP		IP	IP	**A.** Comment on disk diffusion
Antimicrobial agent F (screen)	NA	NA	Y	25	25	
Antimicrobial agent G	0.5	2	Z	30	24	

Changes from previous version highlighted in yellow

Screening breakpoint to differentiate between isolates without and with resistance mechanisms

Not Applicable

In Preparation

No breakpoints. Susceptibility testing is not recommended

MIC breakpoints in blue are linked to MIC distributions

Zone diameter breakpoints in blue are linked to zone diameter distributions

Antimicrobial names in blue are linked to EUCAST rational documents

Insufficient evidence that the organism or group is a good target for therapy with the agent

EUCAST expert rules in antimicrobial susceptibility testing

R. Leclercq[1,2], R. Cantón[2,3,4], D. F. J. Brown[4], C. G. Giske[2,4,5], P. Heisig[2,6], A. P. MacGowan[4,7], J. W. Mouton[4,8], P. Nordmann[2,9], A. C. Rodloff[4,10], G. M. Rossolini[2,11], C.-J. Soussy[4,12], M. Steinbakk[4,13], T. G. Winstanley[2,14] and G. Kahlmeter[4,15]

1) Laboratoire de Microbiologie, CHU Côte de Nacre, Caen, France, 2) EUCAST Subcommittee on Expert Rules, 3) Servicio de Microbiología and CIBER en Epidemiología y Salud Pública (CIBERESP), Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain, 4) EUCAST Steering Committee, 5) Clinical Microbiology, MTC-Karolinska Institutet, Karolinska University Hospital, Solna, Sweden, 6) Department of Pharmacy, Biology & Microbiology, University of Hamburg, Hamburg, Germany, 7) Department of Medical Microbiology, Southmead Hospital, Bristol, UK, 8) Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherland, 9) Service de Bactériologie-Virologie, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France, 10) Institut fur Medizinische Mikrobiologie der Universitat Leipzig, Leipzig, Germany, 11) Dipartimento di Biotecnologie, Sezione di Microbiologia, Siena, Italy, 12) Hôpital Henri Mondor, Service de Bactériologie, Creteil, France, 13) Department of Bacteriology and Immunology, Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway, 14) Department of Microbiology, Royal Hallamshire Hospital, Sheffield, UK and 15) Clinical Microbiology, Central Hospital, Växjö, Sweden

Abstract

EUCAST expert rules have been developed to assist clinical microbiologists and describe actions to be taken in response to specific antimicrobial susceptibility test results. They include recommendations on reporting, such as inferring susceptibility to other agents from results with one, suppression of results that may be inappropriate, and editing of results from susceptible to intermediate or resistant or from intermediate to resistant on the basis of an inferred resistance mechanism. They are based on current clinical and/or microbiological evidence. EUCAST expert rules also include intrinsic resistance phenotypes and exceptional resistance phenotypes, which have not yet been reported or are very rare. The applicability of EUCAST expert rules depends on the MIC breakpoints used to define the rules. Setting appropriate clinical breakpoints, based on treating patients and not on the detection of resistance mechanisms, may lead to modification of some expert rules in the future.

Keywords: Antimicrobial susceptibility testing, breakpoints, EUCAST, expert rules, interpretive reading
Article published online: 25 November 2011
Clin Microbiol Infect 2013; **19**: 141–160
10.1111/j.1469-0691.2011.03703.x

Enterobacteriaceae

EUCAST Clinical Breakpoint Table v. 5.0, valid from 2015-01-01

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1°C, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
Quality control: *Escherichia coli* ATCC 25922

Penicillins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin	-	-		-	-
Ampicillin	8[1]	8	10	14[A,B]	14[B]
Ampicillin-sulbactam	8[1,2]	8[2]	10-10	14[A,B]	14[B]
Amoxicillin	8[1]	8	-	Note[C]	Note[C]
Amoxicillin-clavulanic acid	8[1,3]	8[3]	20-10	19[A,B]	19[B]
Amoxicillin-clavulanic acid (uncomplicated UTI only)	32[1,3]	32[3]	20-10	16[A,B]	16[B]
Piperacillin	8	16	30	20	17
Piperacillin-tazobactam	8[4]	16[4]	30-6	20	17
Ticarcillin	8	16	75	23	23
Ticarcillin-clavulanic acid	8[3]	16[3]	75-10	23	23
Phenoxymethylpenicillin	-	-		-	-
Oxacillin	-	-		-	-
Cloxacillin	-	-		-	-
Dicloxacillin	-	-		-	-
Flucloxacillin	-	-		-	-
Mecillinam (uncomplicated UTI only)	8[5]	8[5]	10	15[D,E]	15[D,E]

Notes
Numbers for comments on MIC breakpoints
Letters for comments on disk diffusion

1/A. Wild type Enterobacteriaceae are categorised as susceptible to aminopenicillins.
Some countries prefer to categorise wild type isolates of *E. coli* and *P. mirabilis* as intermediate. When this is the case, use the MIC breakpoint S ≤ 0.5 mg/L and the corresponding zone diameter breakpoint S ≥ 50 mm.
2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
3. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
4. For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L.
5/D. Mecillinam (pivmecillinam) breakpoints relate to *E. coli*, *Klebsiella* spp. and *P. mirabilis* only.

B. Ignore growth that may appear as a thin inner zone on some batches of Mueller-Hinton agars.
C. Susceptibility inferred from ampicillin.
E. Ignore isolated colonies within the inhibition zone for *E. coli*.

Enterobacteriaceae

Cephalosporins[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Cefaclor	-	-		-	-
Cefadroxil (uncomplicated UTI only)	16	16	30	12	12
Cefalexin (uncomplicated UTI only)	16	16	30	14	14
Cefazolin	-	-		-	-
Cefepime	1	4	30	24	21
Cefixime (uncomplicated UTI only)	1	1	5	17	17
Cefotaxime	1	2	5	20	17
Cefoxitin (screen)[2]	NA	NA	30	19	19
Cefpodoxime (uncomplicated UTI only)	1	1	10	21	21
Ceftaroline	0.5	0.5	5	23	23
Ceftazidime	1	4	10	22	19
Ceftibuten (UTI only)	1	1	30	23	23
Ceftobiprole	0.25	0.25	IP	IP	IP
Ceftriaxone	1	2	30	23	20
Cefuroxime iv	8[3]	8	30	18[A]	18
Cefuroxime oral (uncomplicated UTI only)	8	8	30	18	18

Carbapenems[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Doripenem	1	2	10	24	21
Ertapenem	0.5	1	10	25	22
Imipenem[2]	2	8	10	22	16
Meropenem	2	8	10	22	16

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Aztreonam[1]	1	4	30	24	21

Izdavanje nalaza – interpretacija, ekspertna pravila

- cilj je izdati nalaz koji će kliničara usmjeriti da primijeni najmanje toksičan, uskospektralni, najučinkovitiji antibiotik

- "selective-reporting"
- dodavanje ekspertnih pravila nalazu u obliku komentara
  - automatski (LIS)
  - manualno (liječnik mikrobiolog)

Selecting antimicrobials to test and to report

CLSI, 2014

- **Group A: the primary agents - results to be reported first**

- **Group B: primarily tested but selectively reported - generally broader spectrum agents**
  - the isolate is resistant to the primary agents
  - the patient cannot tolerate drugs in Group A
  - the infection has not responded to the therapy with the primary agents
  - a secondary agent would be a better clinical choice for the particular infection or that the patient has organisms isolated from another site also
  - a secondary agent might be more appropriate for treating both organisms

Selecting antimicrobials to test and to report
CLSI, 2014

- **Group C: alternative or supplemental agents for special cases**
  - resistant strains
  - patients allergic to primary drugs
  - treatment of unusual isolates
  - epidemiological purposes (infection control)

- **Group U: agents that are used only or primarily in the treatment of urinary tract infections**

  (e.g., nitrofurantoin, norfloxacin)

Drug-bug combinations

Group	enterobacteriaceae
A – primary test and report	Ampicillin/amoxicillin Cefazolin gentamicin
B – primary test and report selectively	Amikacin Amoxicillin-clavulanate (AMC) and pip/taz Cefuroxime Ceftriaxone or cefotaxime Cefepime Ciprofloxacin Ertapenem/imipenem/meropene Co-trimoxazole
C – supplemental, report selectively	Ceftazidime Chloramphenicol Tetracycline Aztreonam Ceftaroline
U – uncomplicated UTI	Nitrofurantoin Norfloxacin Trimethoprim

CLSI. Performance Standardsfor Antimicrobial Susceptibility testing; 24th Informational Supplement. CLSI document M100-S24. Wayne, PA:CLSI;2014

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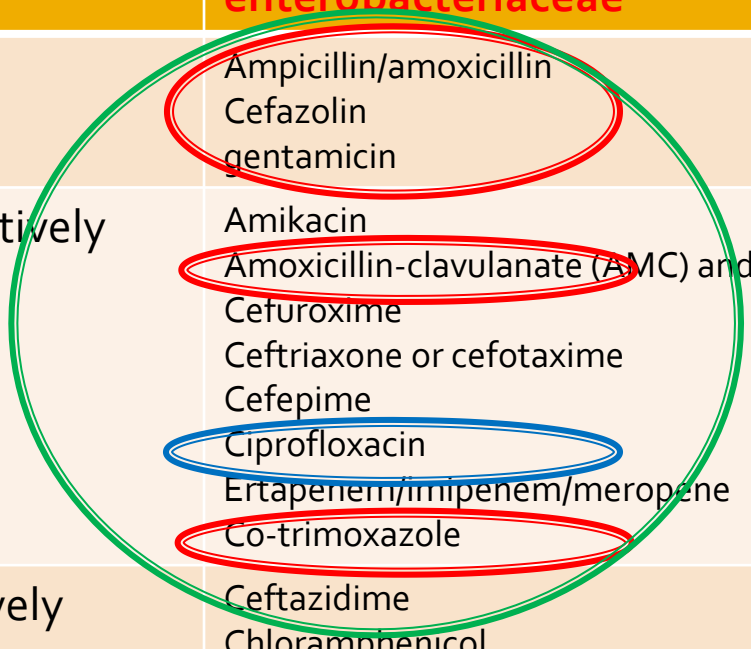


CLSI. Performance Standardsfor Antimicrobial Susceptibility testing; 24th Informational Supplement. CLSI document M100-S24. Wayne, PA:CLSI;2014

Odabir antibiotika za testiranje i izdavanje u nalazu

- **Selektivno izdavanje nalaza** ("cascade-reporting")

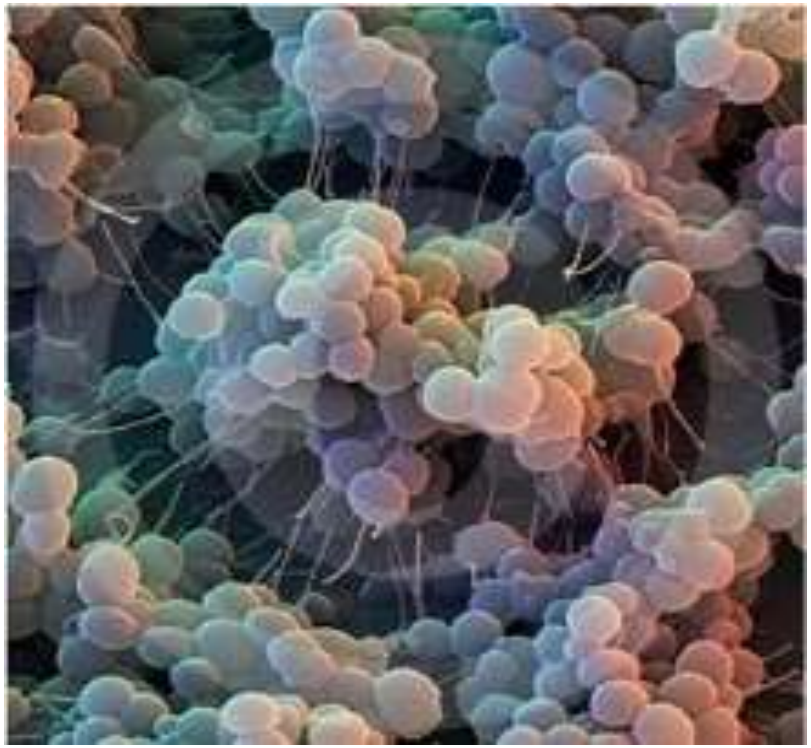
  - veći klinički značaj
  - usmjerava terapiju prema najvećem "cost – benefit" izboru
  - minimalizira selekciju multiplorezistentnih bakterija

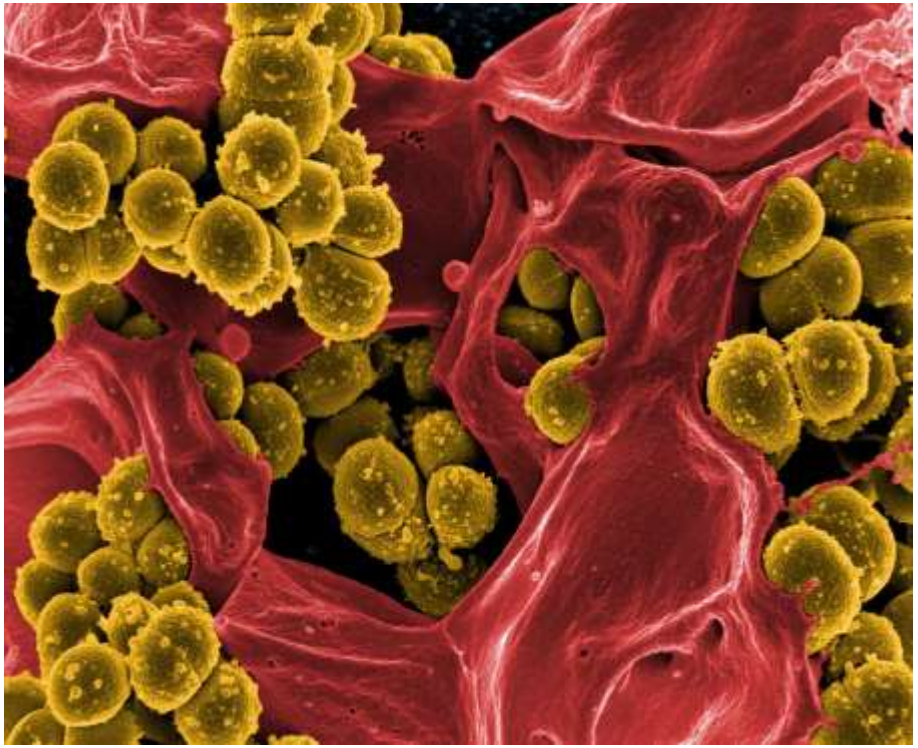
Gram-pozitivne bakterije: mehanizmi rezistencije i interpretacija antibiograma

Iva Butić

Klinika za infektivne bolesti „Dr. Fran Mihaljević”

Staphylococcus spp.

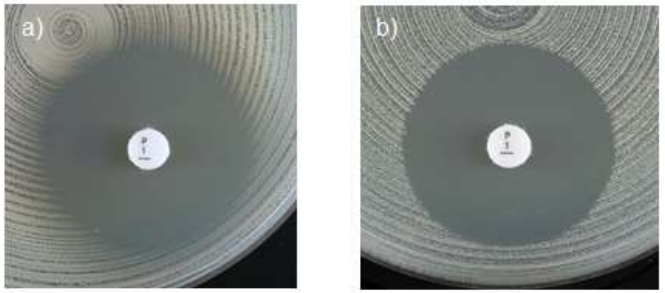




Staphylococcus spp.

osjetljivost na penicilin, EUCAST breakpoint tbl v 6.0, 2016

- *Staphylococcus aureus* - stečena rezistencija na penicillin (≈ 90%)



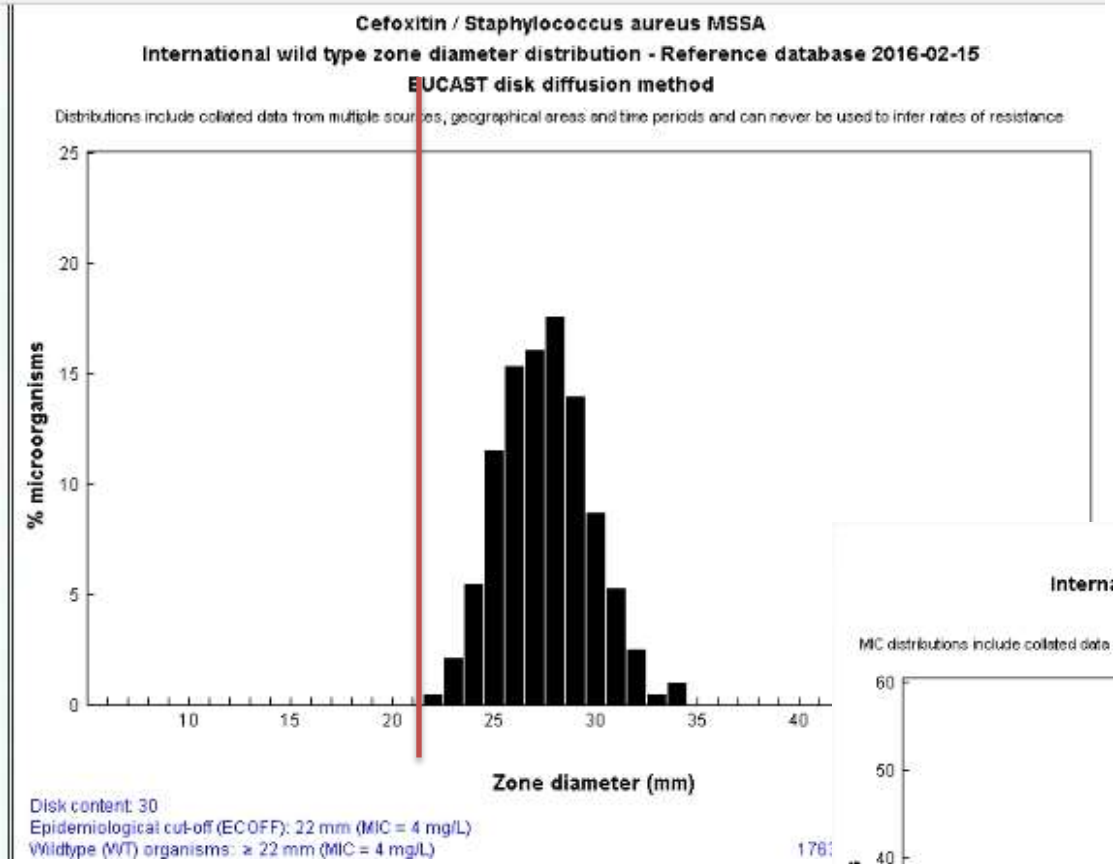
Examples of inhibition zones for *Staphylococcus aureus* with benzylpenicillin.
a) Fuzzy zone edge and zone diameter ≥ 26 mm. Report susceptible.

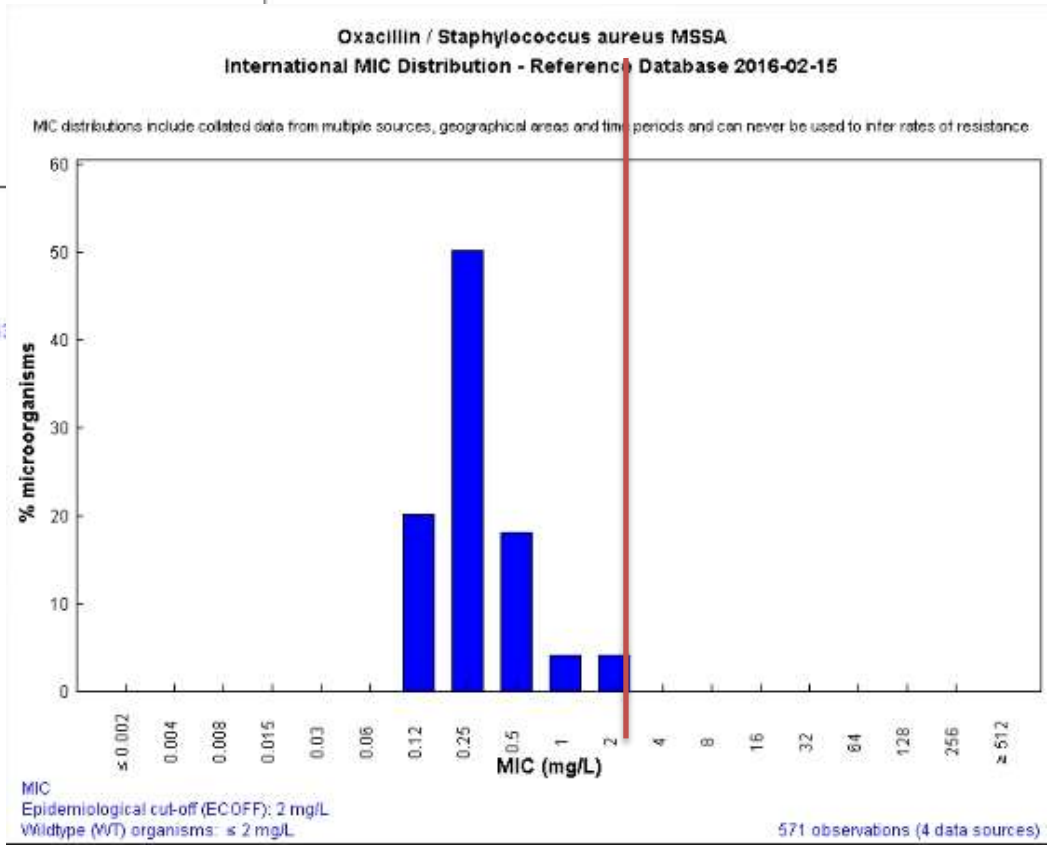
- Detekcija penicilinaze – disk difuzija **najbolja** metoda (eng. *„cliff effect"*)
- KNS - ne postoji pouzdana metoda detekcije produkcije penicilinaze

Penicilini	MIK (mg/L)		Zona inhibicije (mm)	
	OSJETLJIV (S ≤)	REZISTENTAN (R >)	OSJETLJIV (S ≥)	REZISTENTAN (R <)
Benzilpenicilin *S.aureus*	**0.125**	**0.125**	**26**	**26**
Benzilpenicilin *S.lugdunensis*	**0.125**	**0.125**	**26**	**26**

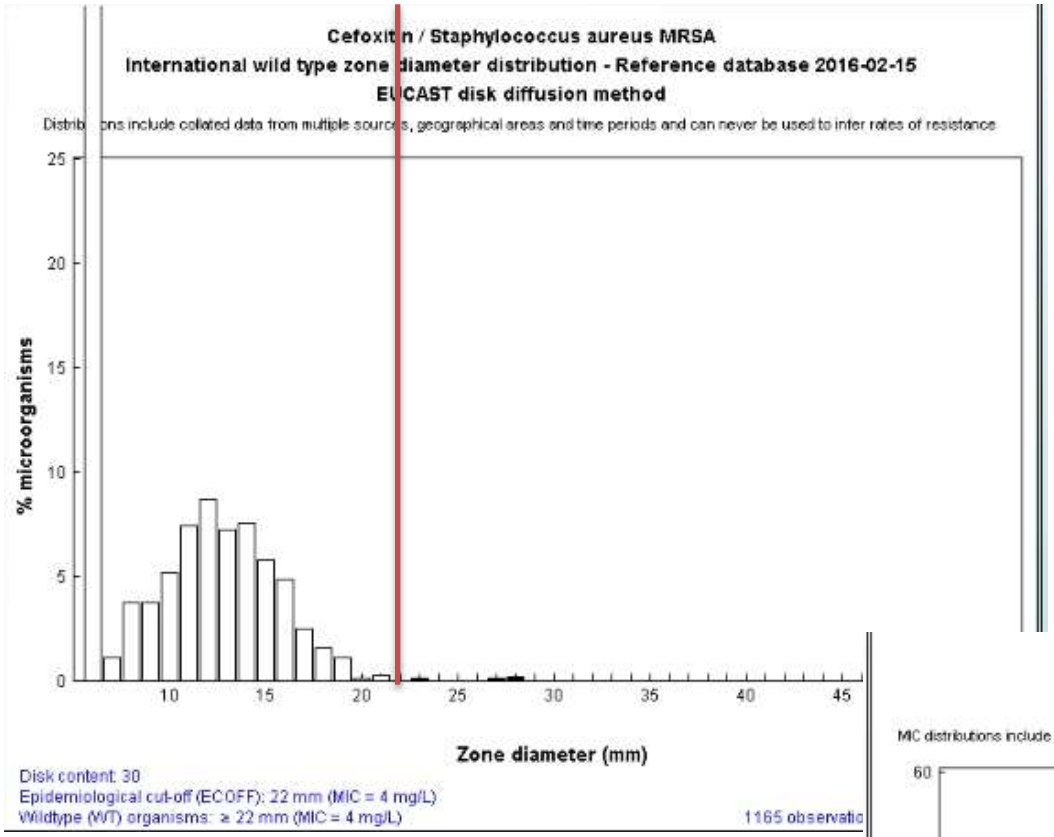
Meticilin rezistentni *Staphylococcus aureus* (MRSA)

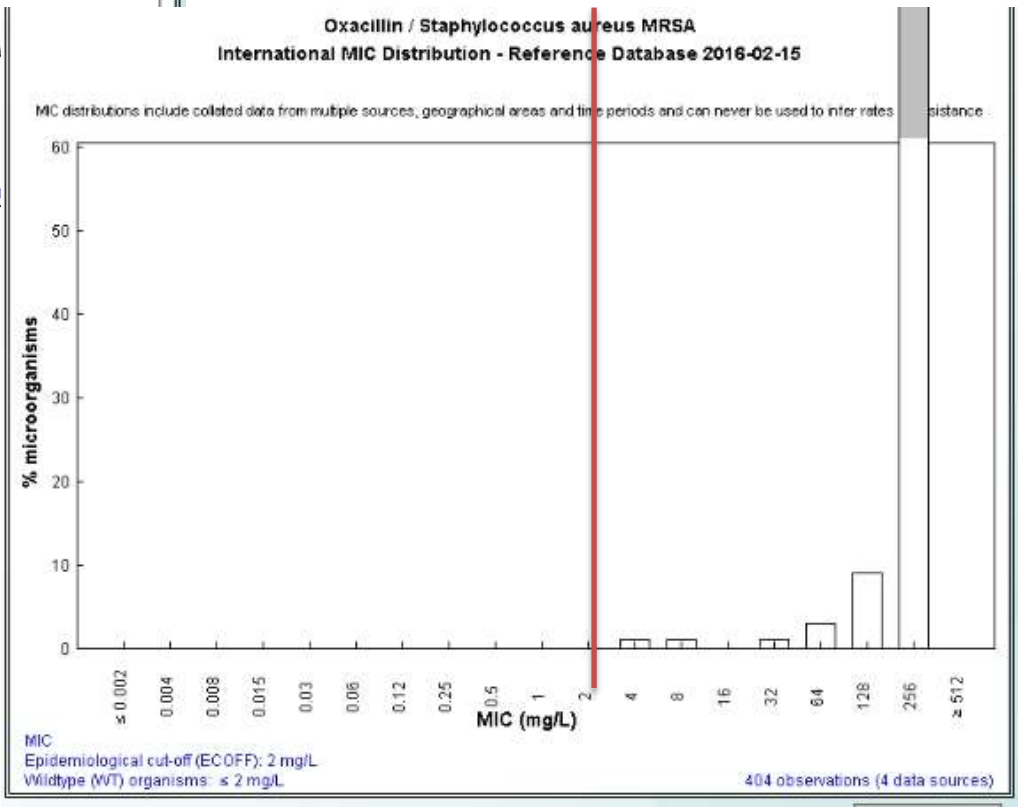
BAKTERIJA	CEFOKSITIN (zona inhibicije-mm)		CEFOKSITIN MIK (mg/L)		OKSACILIN MIK (mg/L)	
	OSJETLJIV (S ≥)	REZISTENTAN (R <)	OSJETLJIV (S ≥)	REZISTENTAN (R <)	OSJETLJIV (S ≤)	REZISTENTAN (R >)
S.aureus, S.lugdunensis	22	22	4	4	2	2
S.saprophyticus	22	22	8	8	2	2
KNS	25	25	-	-	0.25	0.25





http://www.eucast.org/mic_distributions_and_ecoffs/





http://www.eucast.org/mic_distributions_and_ecoffs/

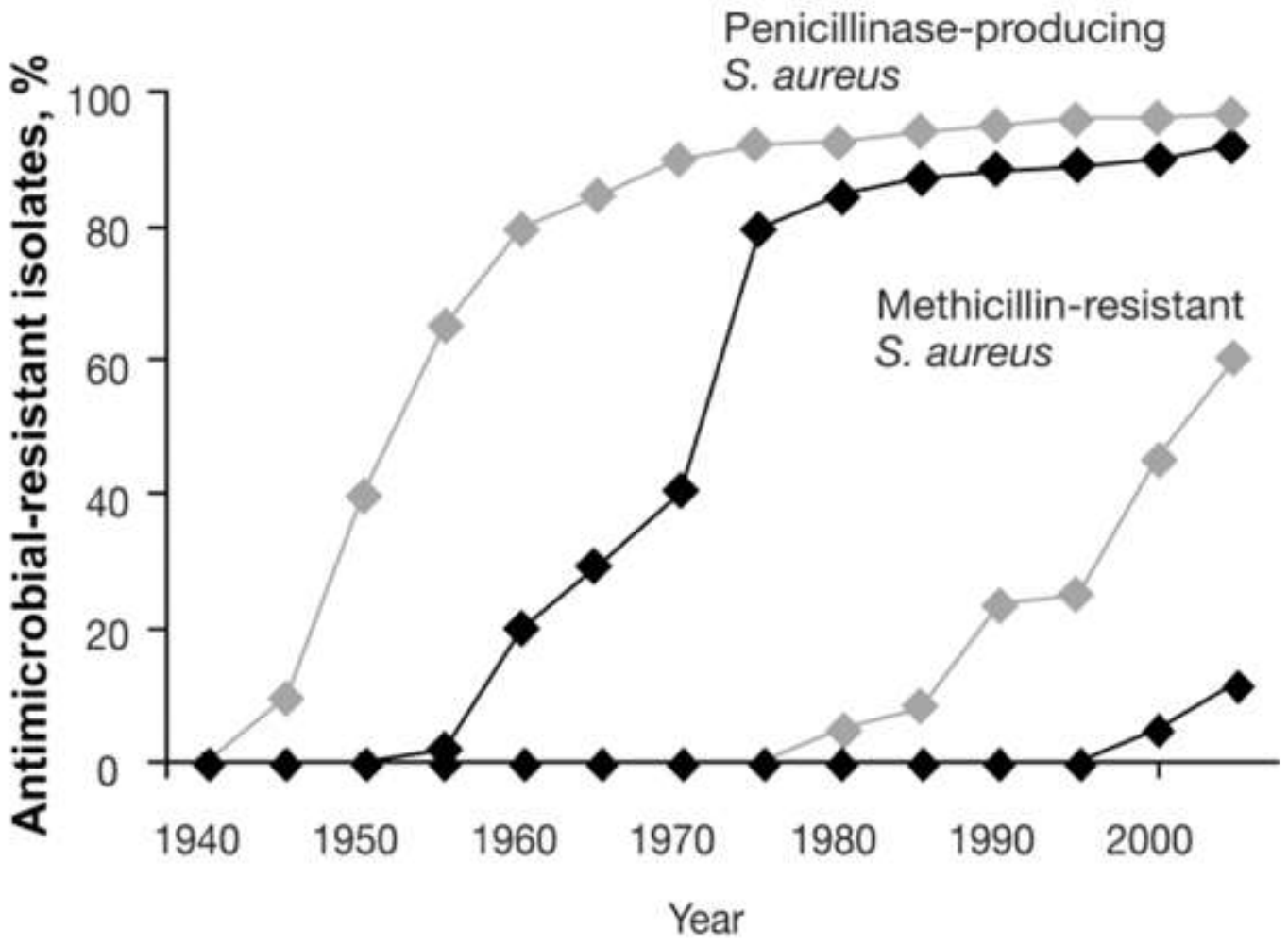
Meticilin rezistentni *Staphylococcus aureus* (MRSA)

Table 1. Interpretation when oxacillin and cefoxitin results are discrepant.

		Cefoxitin result by MIC or disk diffusion	
		S	R
Oxacillin result by MIC	S	Report as oxacillin S	Report as oxacillin R
	R	Report as oxacillin R	Report as oxacillin R

- **Detekcija meticilinske rezistencije:**
  a) disk difuzija (cefoksitin)
  b) određivanje MIK-a cefoksitina (ili oxacilina)
  c) lateks aglutinacija – PBP2a protein
  d) PCR – *mecA* i *mecC* geni

Giske CG, Martinez-Martinez L, Cantón R *et al.* **EUCAST guidelines for detection of resistance mechanisms** and specific resistances of clinical and/or epidemiological importance. Version 1.0, December 2013. http://www.eucast.org.



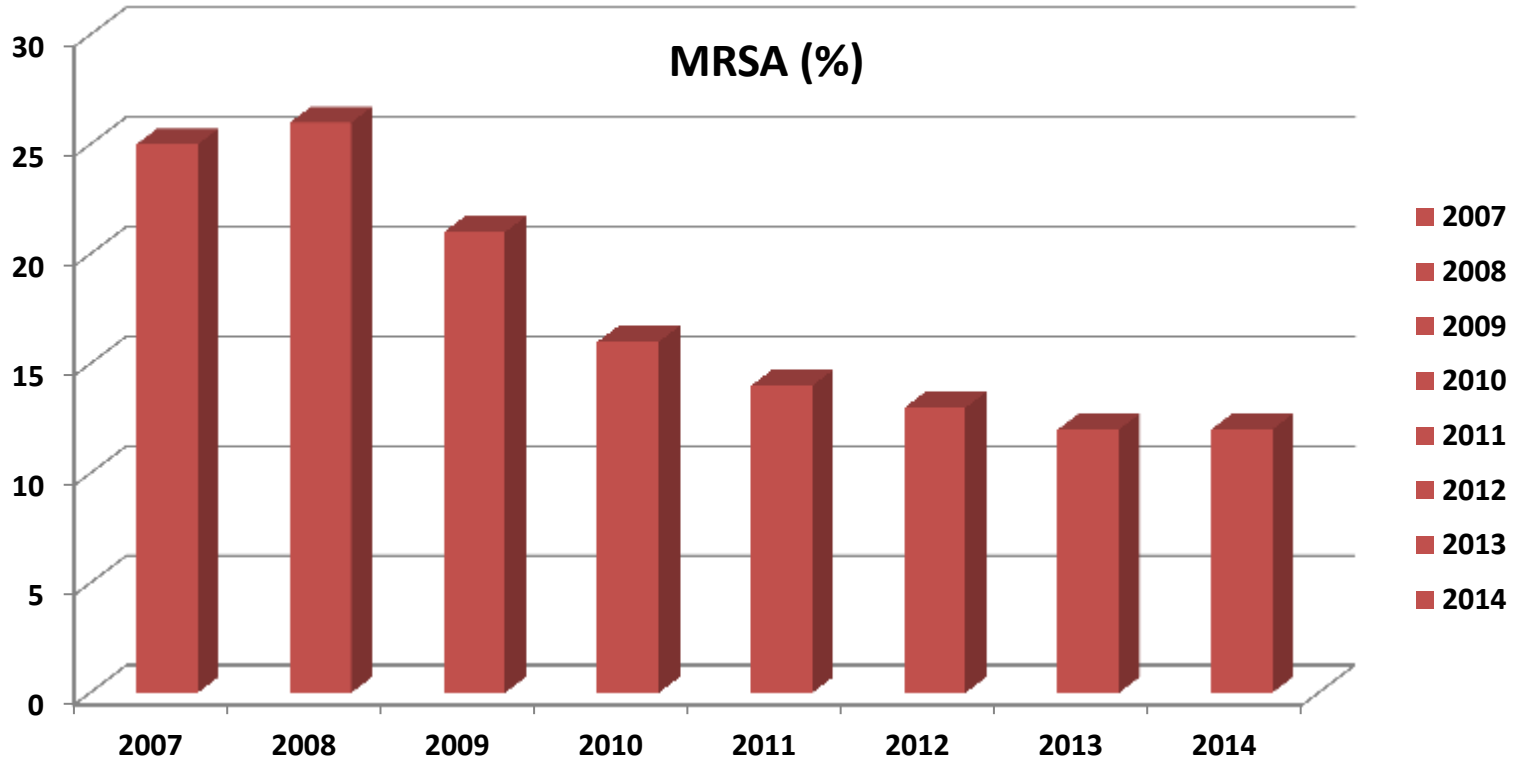
Evolution of antimicrobial-resistant *Staphylococcus aureus* as a cause of nosocomial and, then, community-acquired infections.

Black squares, nosocomial infection; *gray squares*, community-acquired infection.

Staphylococcus aureus

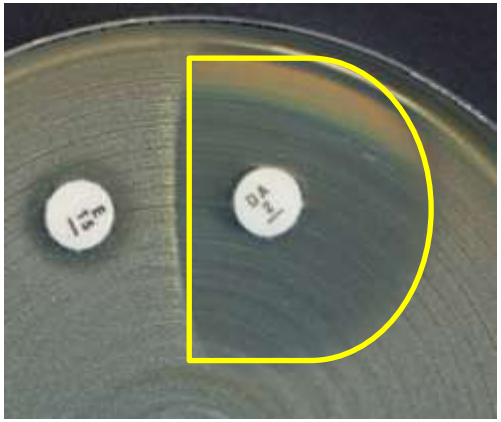
rezistencija na meticilin, 2007 – 2014, Hrvatska

Odbor za praćenje rezistencije bakterija na antibiotike, AMZH



Staphylococcus aureus
rezistencija na klindamicin

- Metoda disk difuzije: udaljenost između diskova 10-12mm od ruba do ruba



- Očitavanje testa osjetljivosti: (S/I/R)

			S ≥	R <
Eritromicin:	10 mm	**Rezistentan**	21 mm	18 mm
Klindamicin:	23 mm	**Osjetljiv**	22 mm	19 mm

- Interpretacija rezultata:
  Inducibilna rezistencija na klindamicin (D-zona) **iMLS$_B$ rezistencija** (*ermA, ermC*)

- Klindamicin izdati rezistentan uz komentar: *Klindamicin još uvijek može biti klinički djelotvoran, ali duljom primjenom se može inducirati rezistencija.*
- **Konstitutivna rezistencija na klindamicin** cMLS$_B$ – makrolidi **R**, klindamicin **R**
- 2014 HR – 87% klindamicin R (konstitutivna 71%, inducibilna 16%)*

***Odbor za praćenje rezistencije bakterija na antibiotike, AMZH**

Staphylococcus aureus

osjetljivost na glikopeptide

- **Samo određivanjem MIK vrijednosti:** gradient test, dilucija u bujonu, agar dilucija ili automatizirane metode
- **NEMA** disk difuzije!!!

Glikopeptidi	MIK (mg/L)		Zona inhibicije (mm)	
	OSJETLJIV (S ≤)	REZISTENTAN (R >)	OSJETLJIV (S ≥)	REZISTENTAN (R <)
Vankomicin *S.aureus*	2	2	-	-
Vankomicin KNS	4	4	-	-
Teikoplanin *S.aureus*	2	2	-	-
Teikoplanin KNS	4	4	-	-

Nema VISA sojeva!

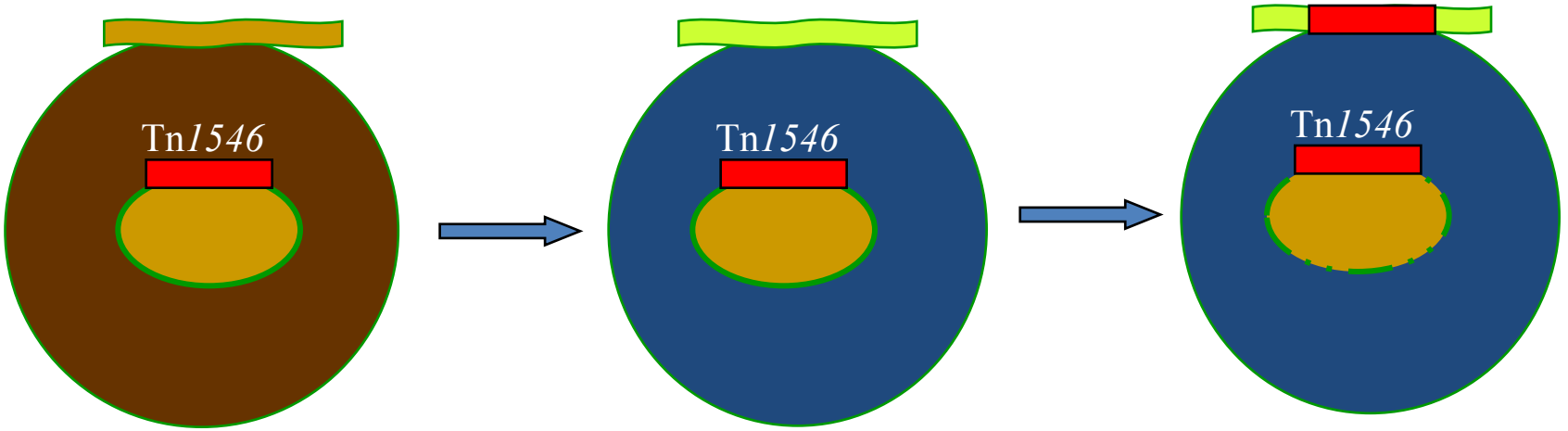
Staphylococcus aureus

rezistencija na glikopeptide

Mehanizam rezistencije:

a) *vanA* gen (stečen od enterokoka)

GRSA: *S. aureus* rezistentan na glikopeptide: MIK vankomicina >8 mg/L



P Courvalin, 2004

- 2002 – prvi VRSA soj, USA

  CDC - *Staphylococcus aureus* resistant to vancomycin – United States, 2002. Morb Mortal Wkly Rep MMWR. 2002;51:565–567
- 2013 – prvi VRSA soj u Europi (Portugal)

  José Melo-Cristino, Cristina Resina, Viviana Manuel, Luís Lito, Mário Ramirez Lancet. 2013. Vol 382

Staphylococcus spp.
rezistencija na glikopeptide

b) zadebljanje stanične stijenke:

- GISA: *S. aureus* intermedijaran na glikopeptide*:*
  - MIK vankomicina 4 - 8 mg/L, ≤1% u Europi

- hGISA: *S. aureus* heterorezistentan na glikopeptide
  - MIK vankomicina ≤2mg/L, ali pojedine bakterijske kolonije (1 od 10^6)
    imaju MIK vankomicina >2 mg/L
  - ≤2% u Europi

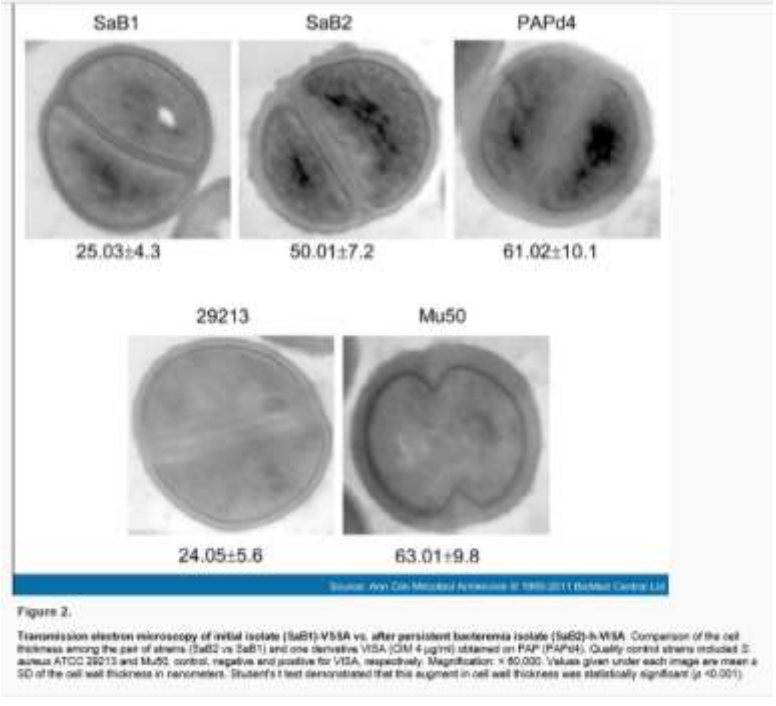
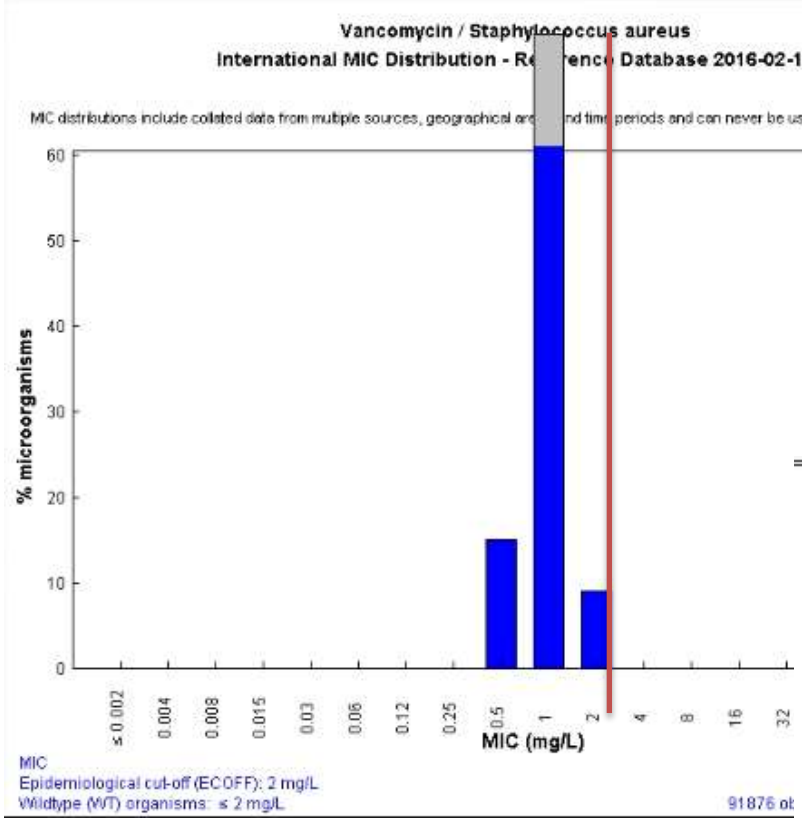


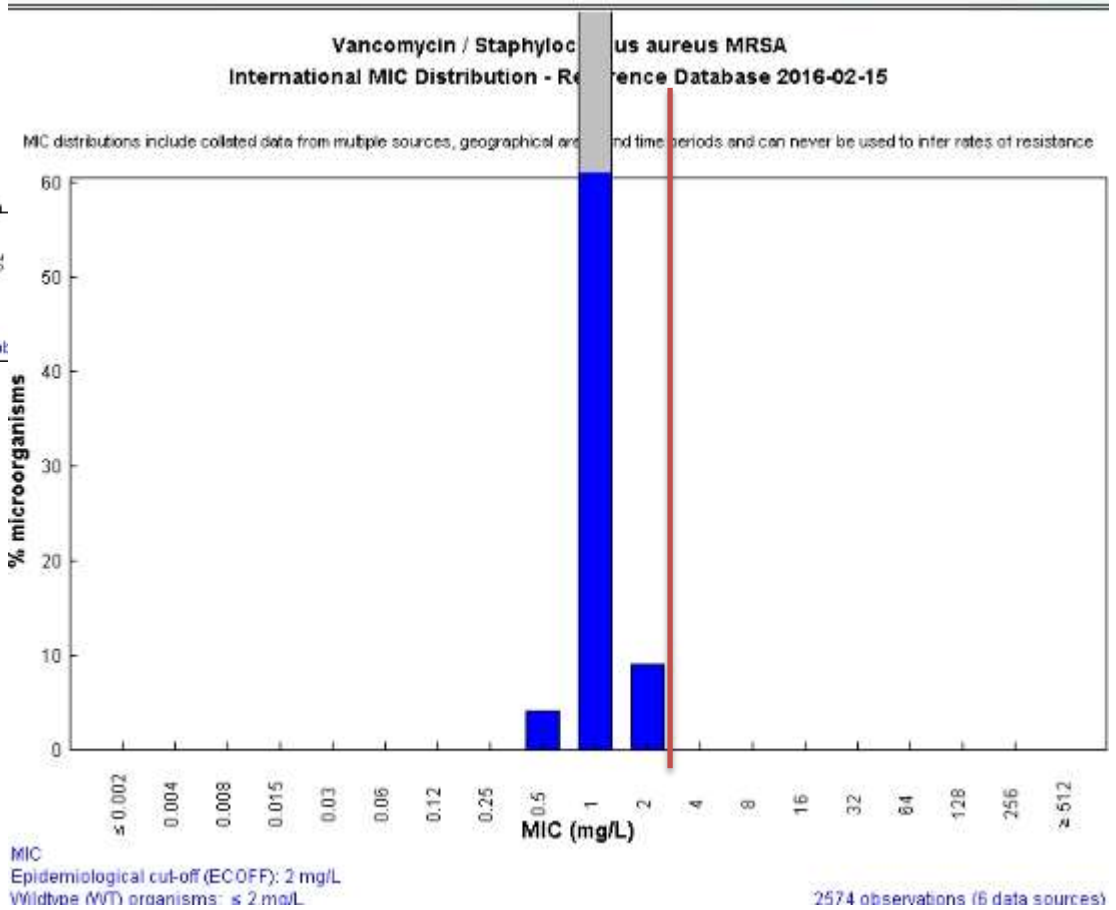
Figure 2.

Transmission electron microscopy of initial isolate (SaB1)-VSSA vs. after persistent bacteremia isolate (SaB2)-h-VISA. Comparison of the cell thickness among the pair of strains (SaB2 vs SaB1) and one derivative VISA (OM 4 µg/ml) obtained on PAP (PAPd4). Quality control strains included S. aureus ATCC 29213 and Mu50, control, negative and positive for VISA, respectively. Magnification: × 60,000. Values given under each image are mean ± SD of the cell wall thickness in nanometers. Student's t test demonstrated that this augment in cell wall thickness was statistically significant ($p < 0.001$).

Hiramatsu, Keiichi et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet. 1997 Dec 6;350(9092):1670-3

Giske CG, Martinez-Martinez L, Cantón R *et al.* **EUCAST guidelines for detection of resistance mechanisms** and specific resistances of clinical and/or epidemiological importance. Version 1.0, December 2013. http://www.eucast.org.



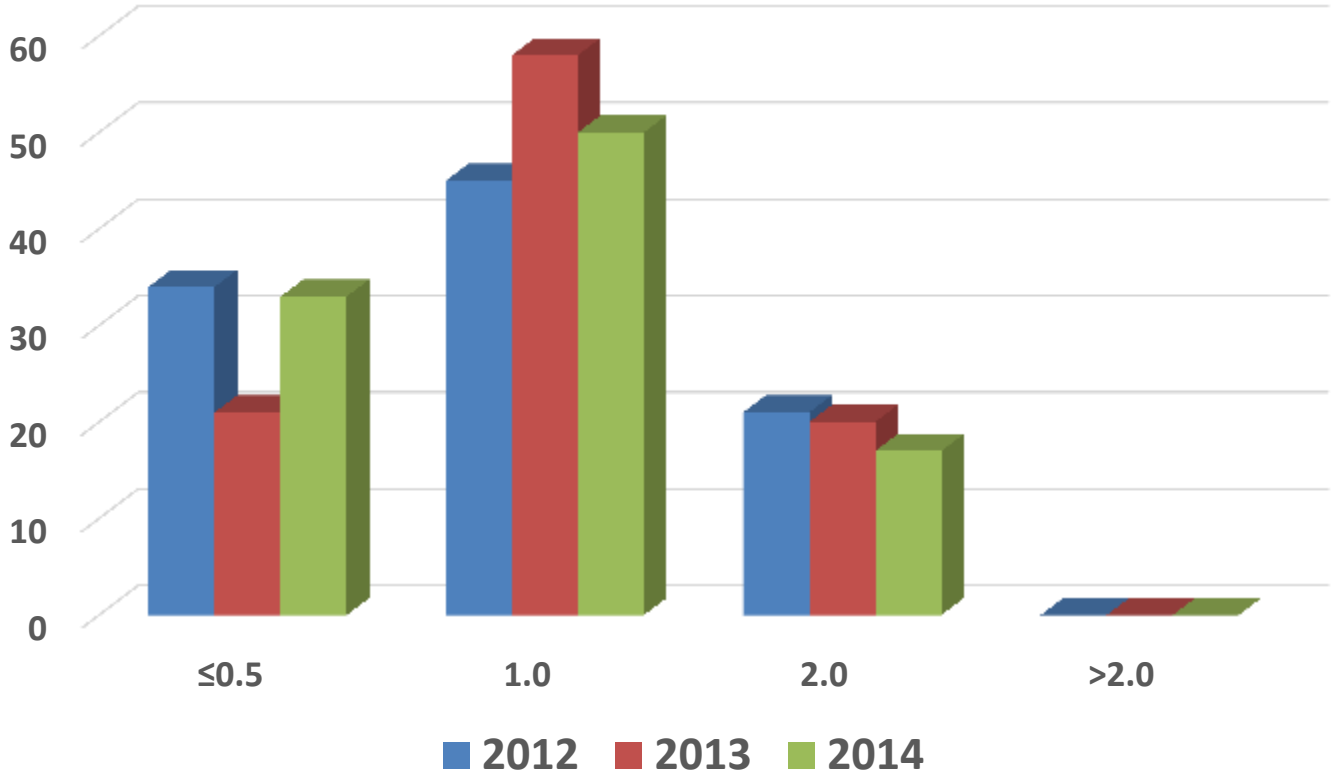


http://www.eucast.org/mic_distributions_and_ecoffs/

MRSA

distribucija MIK-ova vankomicina, 2012-2014

Odbor za praćenje rezistencije bakterija na antibiotike, AMZH



Vankomicin MIK "creep"[1,2,3]

- MRSA izolati s MIK vrijednostima blizu gornje granice osjetljivosti
- MIK vankomicina = 1.0 – 2.0 mg/L
- Dugotrajna terapija vankomicinom te liječenje visokim dozama
- Klonalno širenje?!
- „greška" metode određivanja MIK vrijednosti?!
- Mnogo studija s različitim (oprečnim) rezultatima
- Neuspjeh u liječenju?! Veći mortalitet?! Promjena terapije?!

[1] Sader H.S. and al. Evaluation of Vancomycin and Daptomycin Potency Trends (MIC Creep) against Methicillin-Resistant *Staphylococcus aureus Isolates* Collected in Nine U.S. Medical Centers from 2002 to 2006.AAC 2009; 4127-4132
[2] Wang G. and al.Increased Vancomycin MICs for *Staphylococcus aureus Clinical Isolates* from a University Hospital during a 5-Year Period. JCM 2006; 3883-3886
[3]Alos JAC 2008;62:773-5; Holmes AAC 2008;52:757-60; Jones CID 2006;42:S13-24

E-test

In summary, with the exception of the Vitek Legacy system, the performance characteristics of all the MIC susceptibility testing methods, when they were measured by essential agreement, were excellent. However, the Phoenix system, Etest, and the Micro-Scan system tended to yield MIC results 1 dilution higher than those obtained by the broth reference method; and agar dilution, the Sensititre system, and the Vitek 2 system yielded results that were 1 dilution lower than those obtained by the broth reference method. The Vitek Legacy system gave no MIC results of 4 or 8 μg/ml, and thus, it is difficult to compare the results obtained with the Vitek Legacy system with those obtained by the reference method. The disk diffusion test did not distinguish vancomycin-intermediate strains from vancomycin-susceptible strains, and the vancomycin agar screen lacked sensitivity for strains with MICs of 4 μg/ml. Clinical laboratories may enhance their ability to detect *S. aureus* isolates with reduced susceptibility to vancomycin by performing further testing (e.g., by the vancomycin Etest) with isolates for which the MICs are 2 μg/ml with one of the commercial systems evaluated in the present study.

VITEK 2

*Swenson J.M. and all. Accuracy of Commercial and Reference Susceptibility Testing Methods for Detecting Vancomycin Intermediate *Staphylococcus aureus*. JCM 2009, 2013–2017

Testovi za dokazivanje GRSA, GISA i hGISA

Probirni (screening) testovi:

1. Makro gradient test (Makro E-test)
2. Detekcija glikopeptidne rezistencije gradient testom
   (eng. Glycopeptide Resistance Detection (GRD) gradient test)
3. Teikoplanin probirni (screening) agar

Potvrdni test za hGISA/GISA:

PAP metoda (eng. the **p**opulation **a**nalysis **p**rofile)

Giske CG, Martinez-Martinez L, Cantón R *et al.* **EUCAST guidelines for detection of resistance mechanisms** and specific resistances of clinical and/or epidemiological importance. Version 1.0, December 2013. http://www.eucast.org.

Makro gradient test (Makro E-test)

- Ispitivanje smanjene osjetljivosti na vankomicin
  (NIJE vrijednost MIK-a vankomicina !!!)
- **Ne može** razlučiti hGISA I GISA
- Bakterijska suspenzija 2.0 McFarland na BHI agar

Očitavanje testa:
a) Teikoplanin ≥ 12 mg/L: GISA or hGISA

b) Teikoplanin = 8 mg/L ⇒ odrediti MIK vankomicina.

1. vankomicin ≥ 8 mg/L: GISA ili hGISA **pozitivno**
2. vankomicin < 8 mg/L: GISA ili hGISA **negativno**

c) Teikoplanin < 8 mg/L: GISA i hGISA **negativno**

Detekcija glikopeptidne rezistencije gradient testom (GRD test)

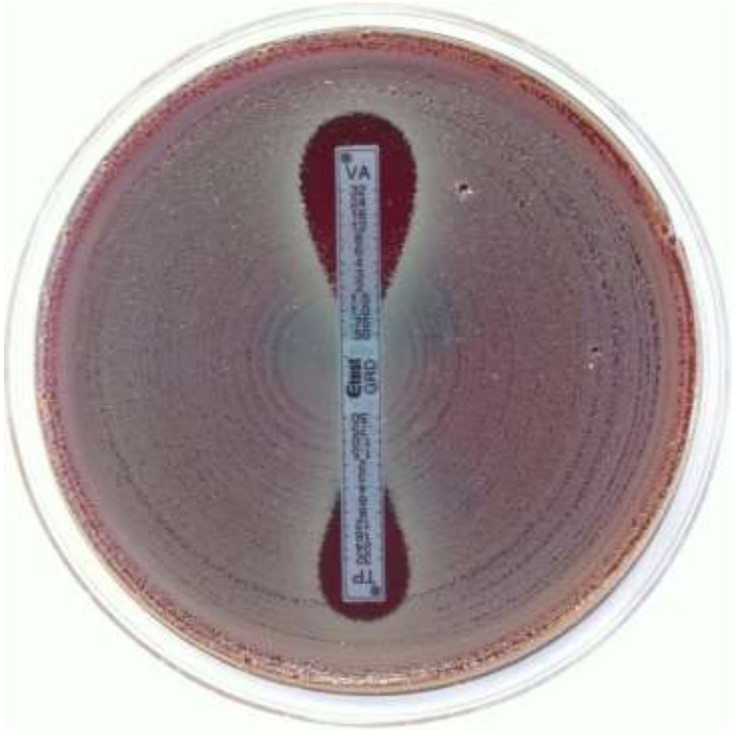
- **Probirni (screening) test za hGISA/GISA (Etest GRD, Biomerieux)**

  Bakterijska suspenzija 0.5 McFarland na MH-F agaru

  E-test GRD pozitivan: VA ili TP ≥ 8mg/L => hGISA/GISA:

  a) hGISA: E-test GRD pozitivan i MIK vankomicina <4mg/L

  b) GISA: E-test GRD pozitivan i MIK vankomicina ≥4mg/L



Teikoplanin probirni (screening) agar

- Mueller Hinton agar + 5 mg/L teikoplanin
- Bakterijska suspenzija = 2.0 McFarlanda
- 10 µL bakterijske suspenzije se inokulira na površinu MH
- Inkubacija 24-48 h na temp. 35°C
- Očitavanje testa:
  porast >2 kolonije = <u>sumnja</u> na smanjenu osjetljivost na glikopeptide

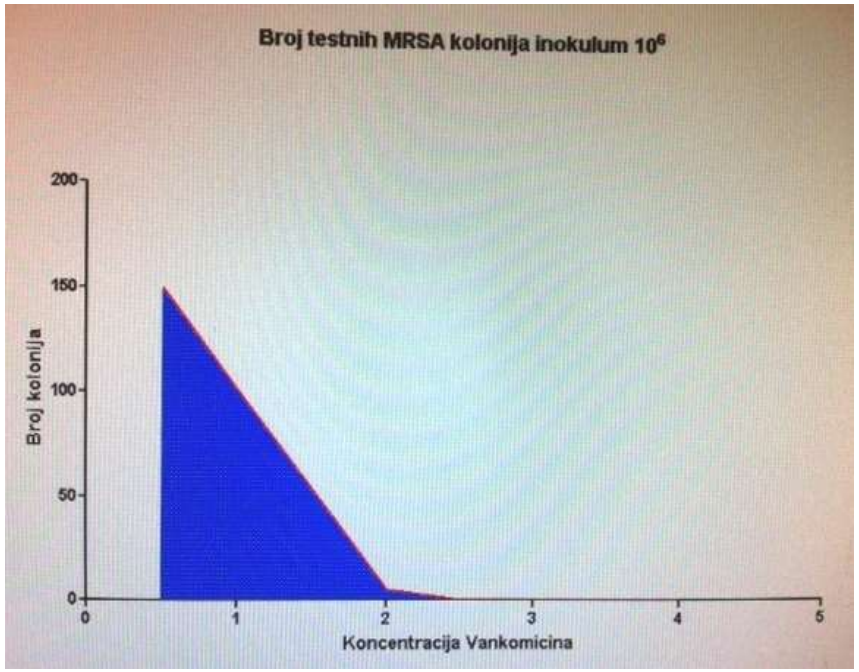
Potvrdni test za hGISA/GISA (PAP test)

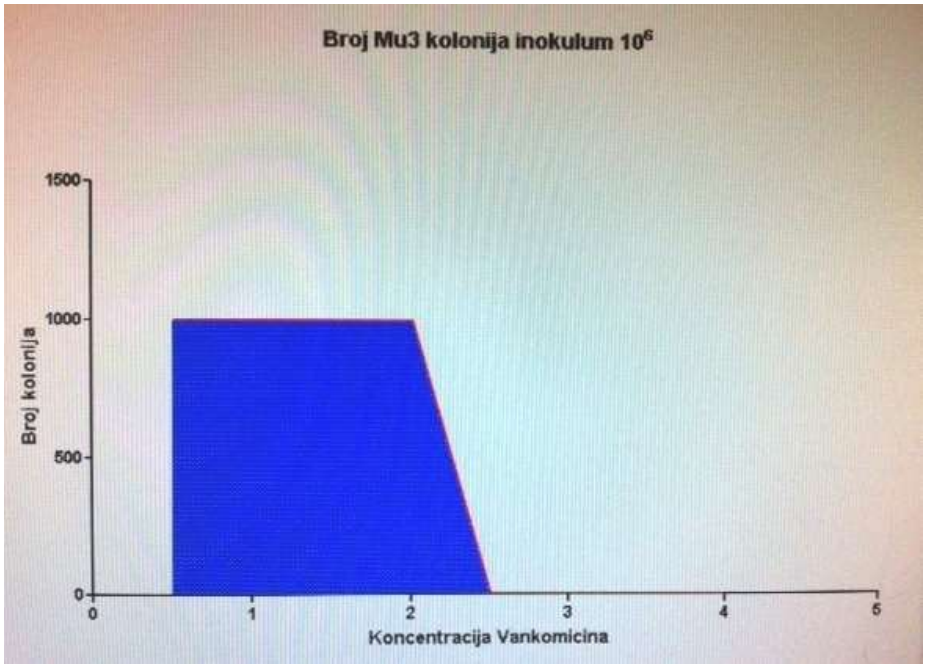
- Vremenski zahtjevna pretraga
- Nije rutinska mikrobiološka pretraga (educirani lab. djelatnici)
- 24h inkubacija u TSB (10^8cfu/mL)
- Pripremiti bakterijske suspenzije od 10^{-3}cfu/mL i 10^{-6}cfu/mL
- Inokulacija na ploče Brain-Heart Infusion Agara (BHIA) s vankomicinom u koncentracijama 0.5, 1, 2, 2.5 i 4 mg/L
- 48h inkubacija/37°C

*Wooton M and al., A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus auresu* in a UK hospitals. JAC 2001

PAP metoda

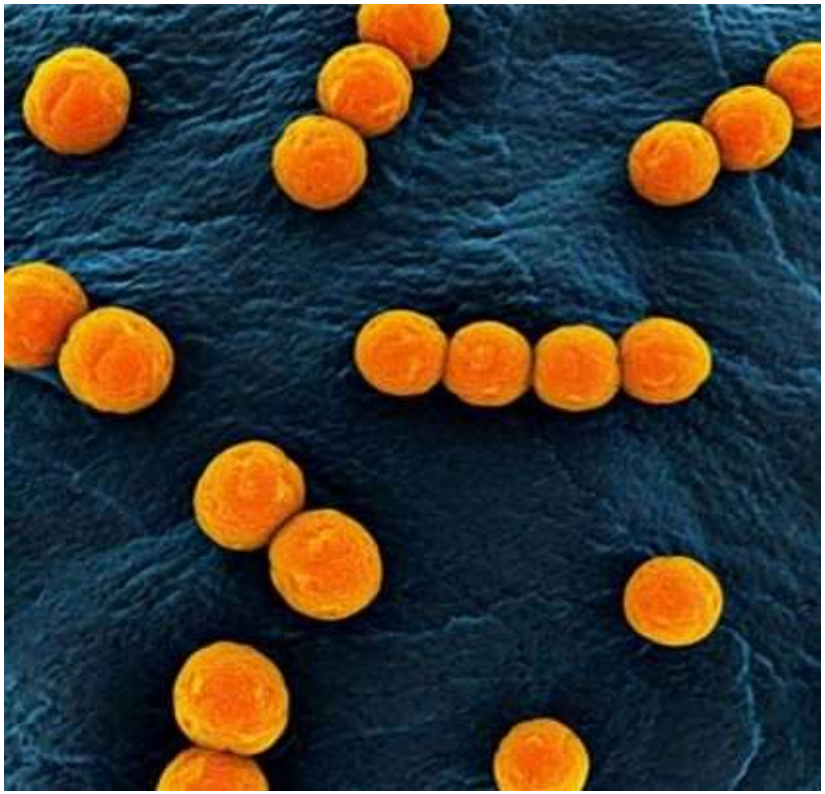
Interpretacija rezultata

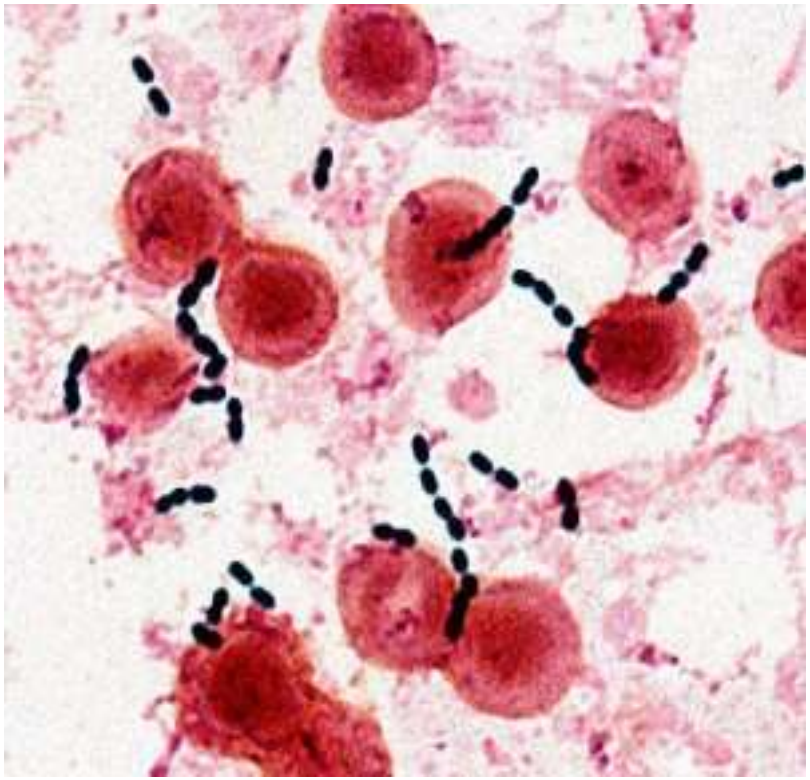




- Prism software
- AUC MRSA ispitivani soj / AUC Mu3 = <0.9 => **MRSA**
- AUC MRSA ispitivani soj / AUC Mu3 = ≥ 0.9 and ≤ 1.3 => **hVISA**
- AUC MRSA ispitivani soj / AUC Mu3 = > 1.3 => **VISA**

Enterococcus spp.





Enterococcus spp.

Intrinzična rezistencija

TABLE 4. Intrinsic resistance in Gram-positive bacteria; Gram-positive bacteria are also intrinsically resistant to aztreonam, temocillin, polymyxin B/colistin, and nalidixic acid

Rule no.	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Erythromycin	Clindamycin	Quinupristin–dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulphonamides
4.1	*Staphylococcus saprophyticus*	R	R	–	–	–	–	–	–	–	R	R	–
4.2	*Staphylococcus cohnii*, *Staphylococcus xylosus*	–	R	–	–	–	–	–	–	–	–	R	–
4.3	*Staphylococcus capitis*	–	R	–	–	–	–	–	–	–	R	–	–
4.4	Other coagulase-negative staphylococci and *Staphylococcus aureus*	–	R	–	–	–	–	–	–	–	–	–	–
4.5	*Streptococcus* spp.	R	–	–	R[a]	–	–	–	–	–	–	–	–
4.6	*Enterococcus faecalis*	R	R	R	R[a]	R	R	R	–	–	–	–	R
4.7	*Enterococcus gallinarum*, *Enterococcus casseliflavus*	R	R	R	R[a]	R	R	R	R	–	–	–	R
4.8	*Enterococcus faecium*	R	R	R	R[a,b]	R	–	–	–	–	–	–	R
4.9	*Corynebacterium* spp.	–	–	–	–	–	–	–	–	–	R	–	–
4.10	*Listeria monocytogenes*	–	R	R	–	–	–	–	–	–	–	–	–
4.11	*Leuconostoc* spp., *Pediococcus* spp.	–	–	–	–	–	–	–	R	R	–	–	–
4.12	*Lactobacillus* spp. (some species)	–	–	–	–	–	–	–	R	R	–	–	–
4.13	*Clostridium ramosum*, *Clostridium innocuum*	–	–	–	–	–	–	–	R	–	–	–	–

R, resistant.

[a]Low-level resistance to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides.

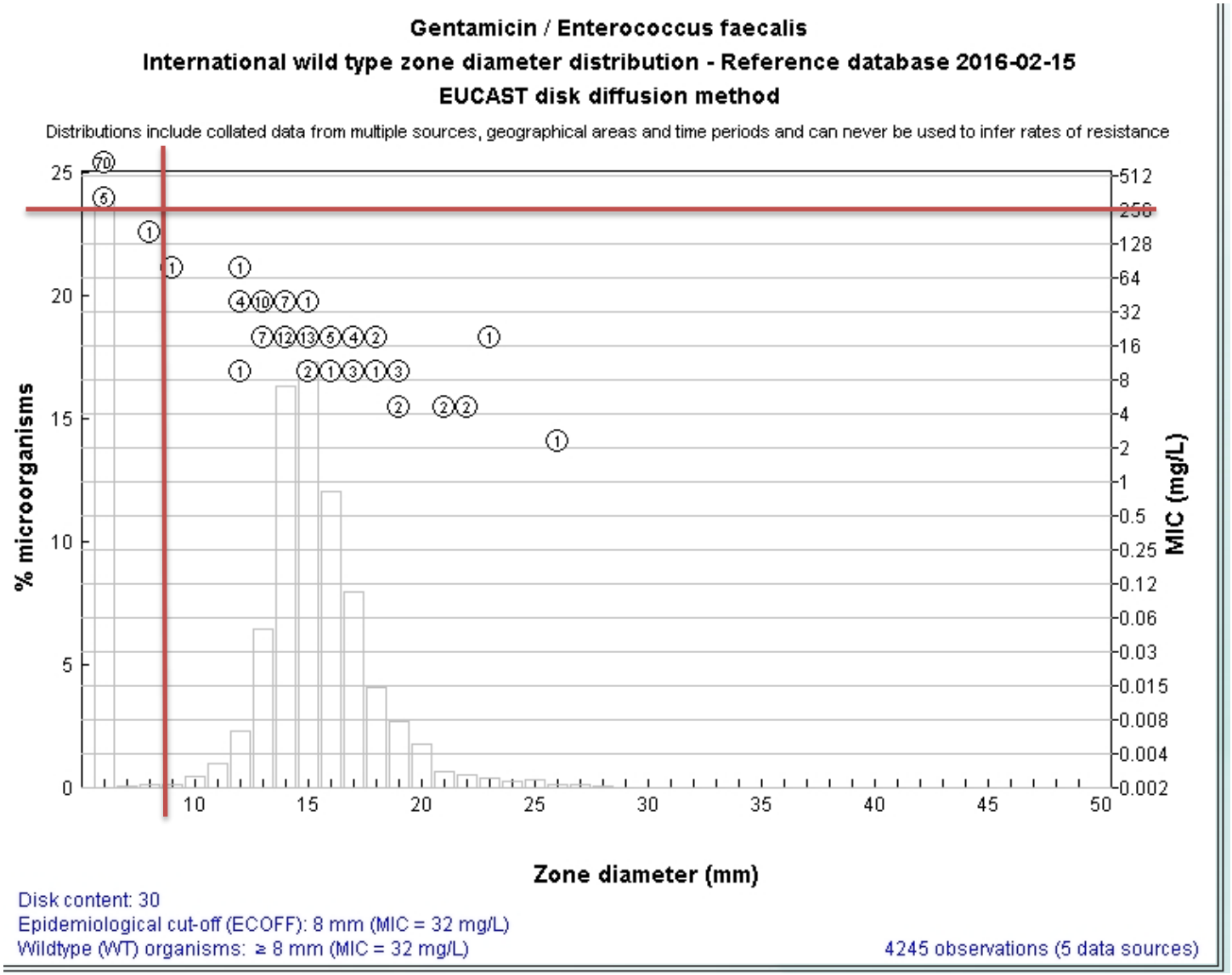
[b]In addition to low-level resistance to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6′) enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin, arbekacin, and streptomycin) and penicillins or glycopeptides.

E.feacalis i *E.feacium*
visoka rezistencija na aminoglikozide, HLAR

- Intrinzična rezistencija na aminoglikozide
- Visoka doza aminoglikozida + β-laktamski antibiotik -> **sinergističko djelovanje**
- Razlučiti intrinzičnu od visoke rezistencije na aminoglikozide (*eng.high-level aminoglikozide resistance*, **HLAR**)
- Detekcija HLAR:
  a) disk difuzija – disk gentamicina 30µg
  b) MIK gentamicina (i streptomicina)

Enterococcus spp.	MIK (mg/L)		Zona inhibicije (mm)	
	Intrinzična rezistencija	HLAR	Intrinzička rezistencija	HLAR
Gentamicin	≤128	>128	≥8	<8
Streptomicin	≤512	>512		

- HLAR gentamicin = svi aminozikozidi HLAR osim streptomicina
- HLAR streptomicin = samo HLAR streptomicin



http://www.eucast.org/mic_distributions_and_ecoffs/

Enterococcus spp.
rezistencija na vankomicin

- *E.gallinarum* i *E.casseliflavus* - intrinzična rezistencija, vanC fenotip (MIK vankomicina 4-16 mg/L)

- *E.faecalis* i *E.faecium* – stečena rezistencija, vanA i vanB fenotipovi

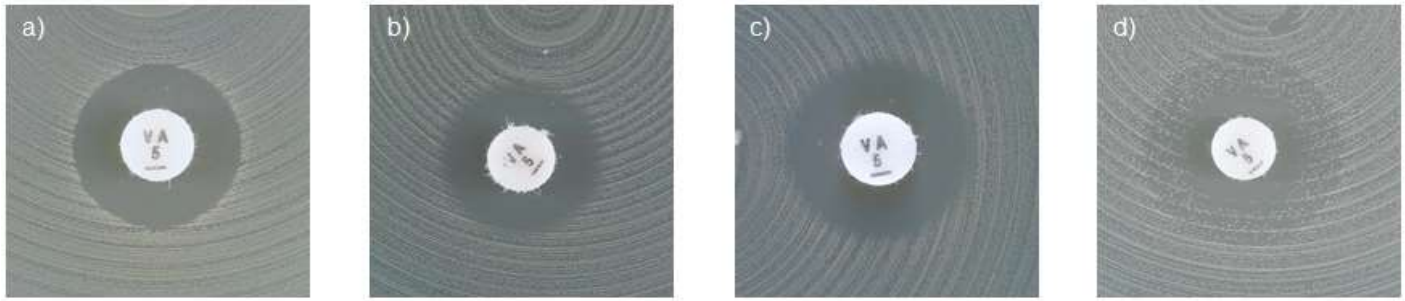
Enterococcus spp.	MIK (mg/L)		Zona inhibicije (mm)	
	OSJETLJIV (S ≤)	REZISTENTAN (R >)	OSJETLJIV (S ≥)	REZISTENTAN (R <)
Vankomicin	4	4	12	12
Teikoplanin	2	2	15	15

Enterococcus spp.

rezistencija na vankomicin

Detekcija mehanizma rezistencije:

1. Disk difuzija

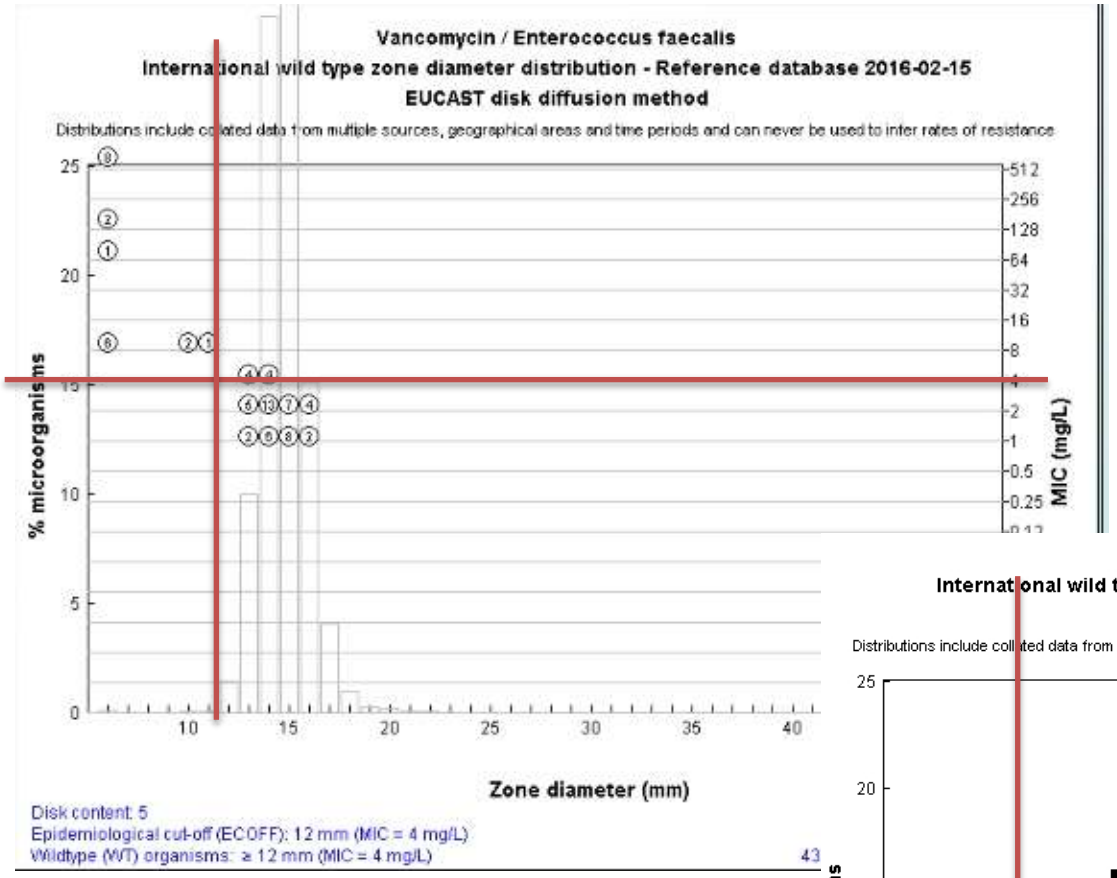


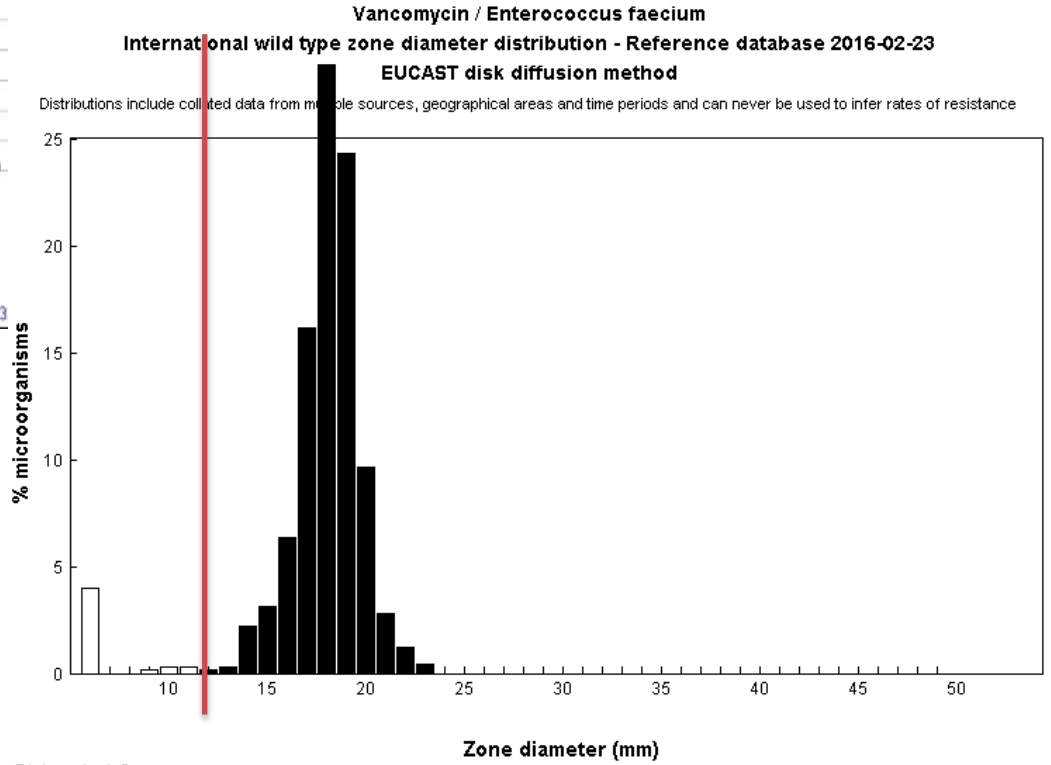
Examples of inhibition zones for *Enterococcus* spp. with vancomycin.
a) Sharp zone edge **and** zone diameter ≥ 12 mm. Report susceptible.
b-d) Fuzzy zone edge or colonies within zone. Perform confirmatory testing with PCR or report resistant even if the zone diameter ≥ 12 mm.

2. MIK vankomicina i teikoplanina

Glycopeptide	MIC (mg/L)	
	VanA	VanB
Vancomycin	64-1024	4-1024
Teicoplanin	8-512	0.06-1

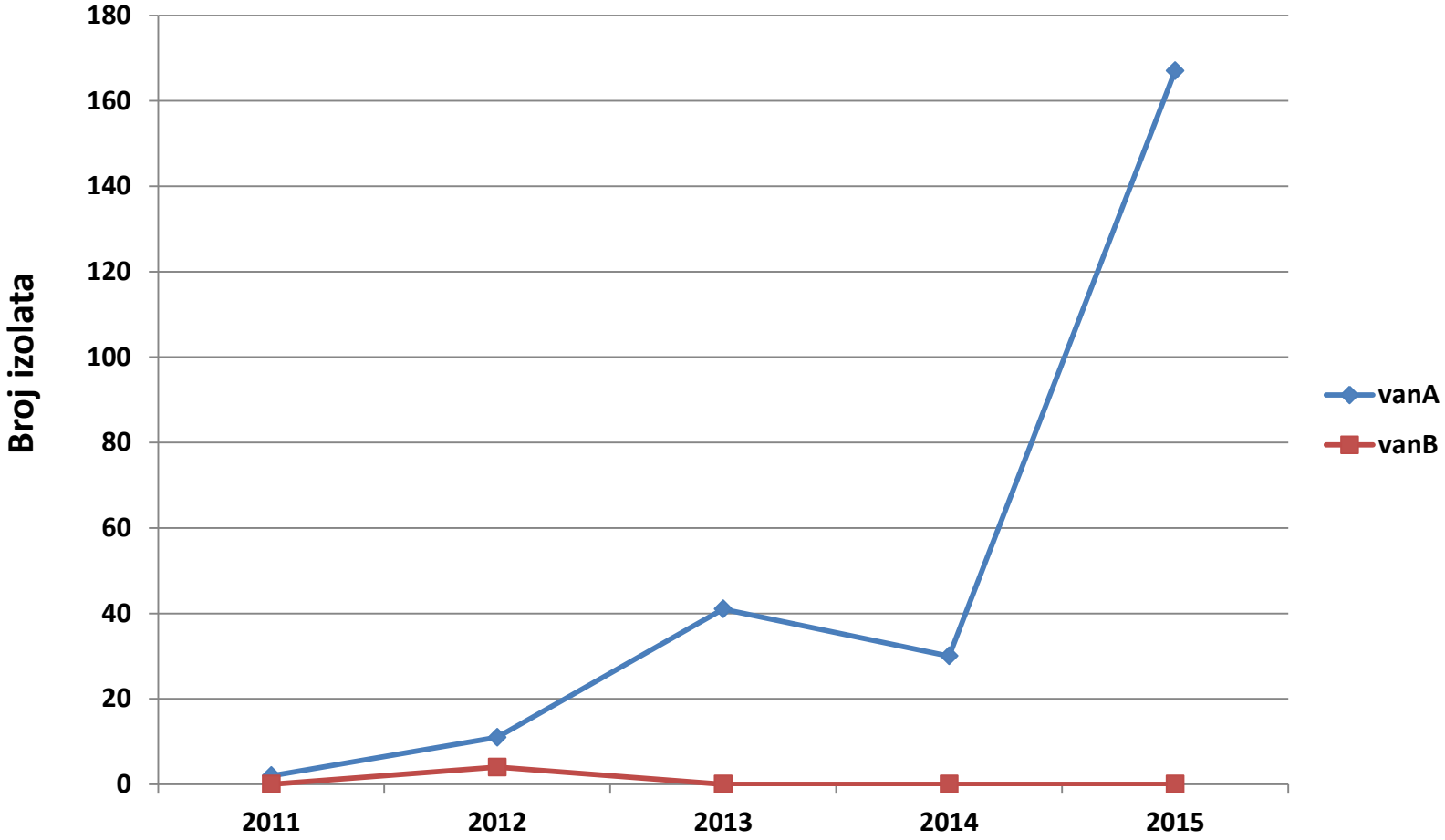
3. PCR – *vanA* i *vanB* geni





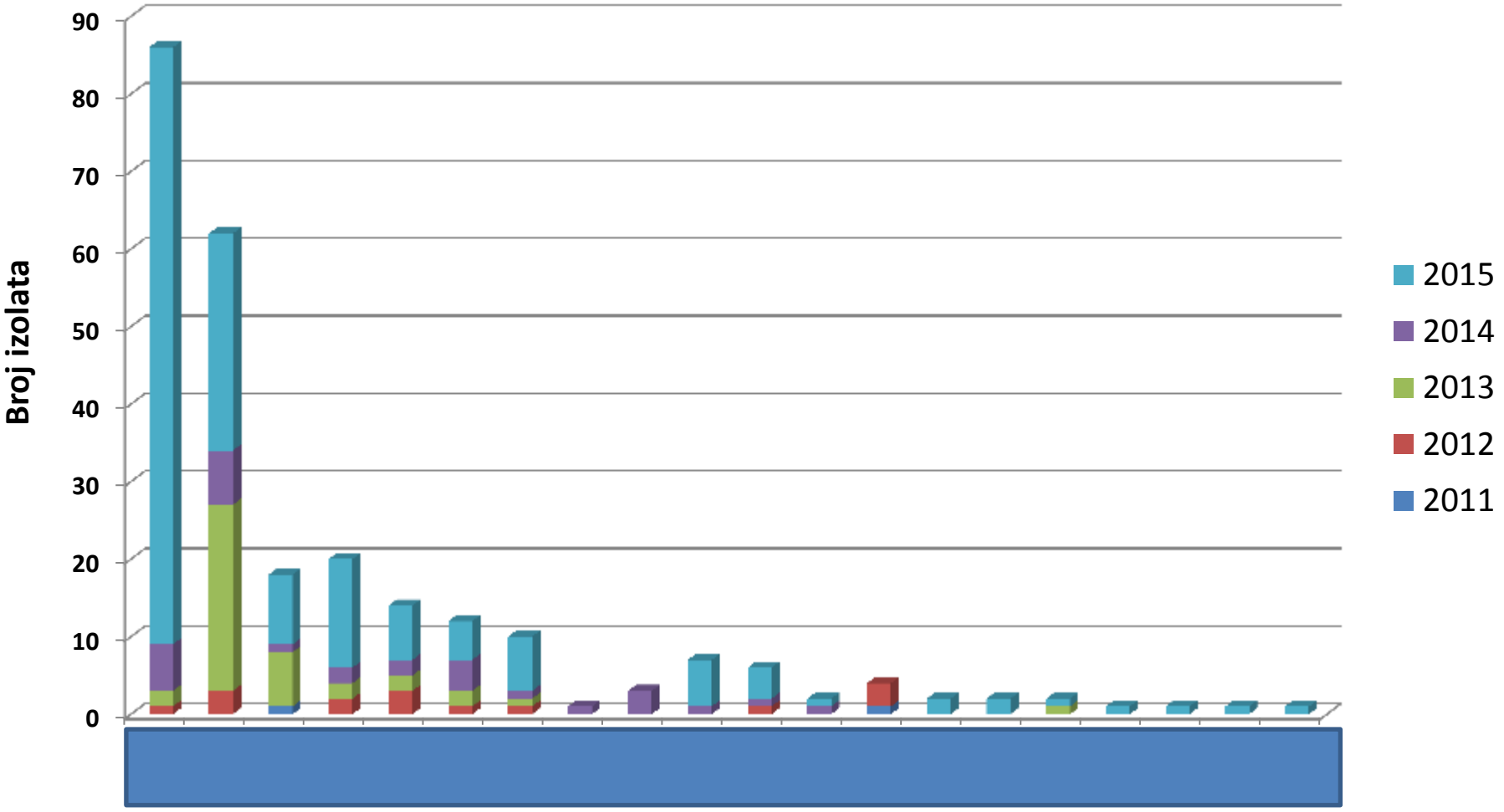
http://www.eucast.org/mic_distributions_and_ecoffs/

Enterococcus feacium VRE
distribucija fenotipova, Hrvatska 2011-2015



Enterococcus faecium VRE

Hrvatska, 2011-2015



Enterococcus feacalis i *Enterococcus feacium* rezistencija na kinolone

- Norfloksacin - probirni („screening”) disk
- Izdavanje nalaza:

a) AKO je norfloksacin S ONDA izdati ciprofloksacin S i levofloksacin S

b) AKO je norfloksacin R ONDA izdati ciprofloksacin R i levofloksacin R

Enterococcus spp.	MIK (mg/L)		Zona inhibicije (mm)	
	OSJETLJIV (S ≤)	REZISTENTAN (R >)	OSJETLJIV (S ≥)	REZISTENTAN (R <)
Norfloksacin *„screen”*	-	-	12	12
Ciprofloksacin (nekomplicirane IMS)	4	4	15	15
Levofloksacin (nekomplicirane IMS)	4	4	15	15

- 2014 HR – *E.faecalis* 21%, *E.faecium* 80%*

*Odbor za praćenje rezistencije bakterija na antibiotike, AMZH

Iznimke:

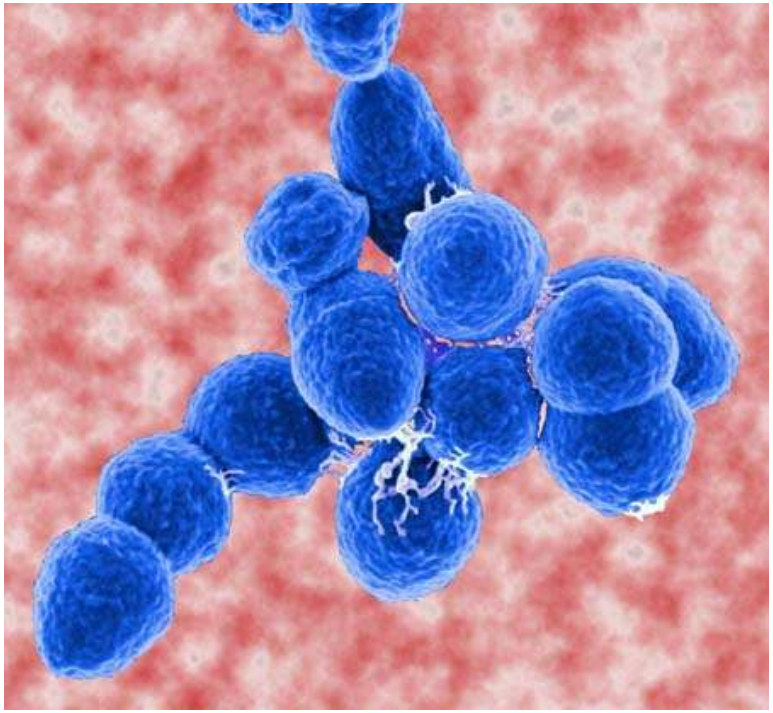
1. Kvinupristin-dalfopristin –> samo *E.faecium*

- *E.faecalis* – rezistentan (uvijek) - *lsa* gen – efluks pumpa
- *E.faecium* – nizak % rezistencije - *vatD* i *vatE* geni

Hershberger E.,Donabedian S.,Konstantinou K., Zervos MJQuinupristin-Dalfopristin Resistance in Gram-Positive Bacteria: Mechanism of Resistance and Epidemiology. CID 2004:38

2. Nitrofurantoin –> samo *E.faecalis* (nekomplicirane IMS)

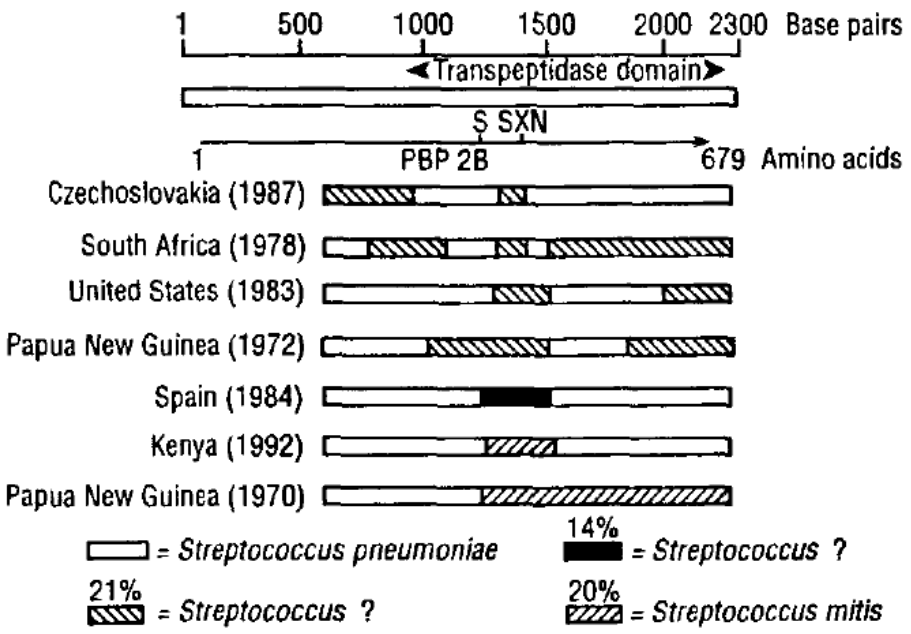
Streptococcus pneumoniae



Streptococcus pneumoniae
smanjena osjetljivost na penicilin

- Promjena PBP-ova (mozaični geni - *Streptococcus viridans* ?!)
- Rezistencija na β-laktamske atb.

Fig. 2. Mosaic PBP 2B genes in penicillin-resistant pneumococci. The divergent regions in the PBP 2B genes of seven resistant pneumococci from different countries are shown. These regions have been introduced from at least three sources, one of which appears to be *S. mitis*. The approximate percent sequence divergence of the divergent regions from the PBP 2B genes of susceptible pneumococci is shown. The figure was drawn from data in (*20, 21*).



Spratt BG, RESISTANCE TO ANTIBIOTICS MEDIATED BY TARGET ALTERATIONS *Science*, 1994. Vol: 264, Pages: 388-393, ISSN: 0036-8075

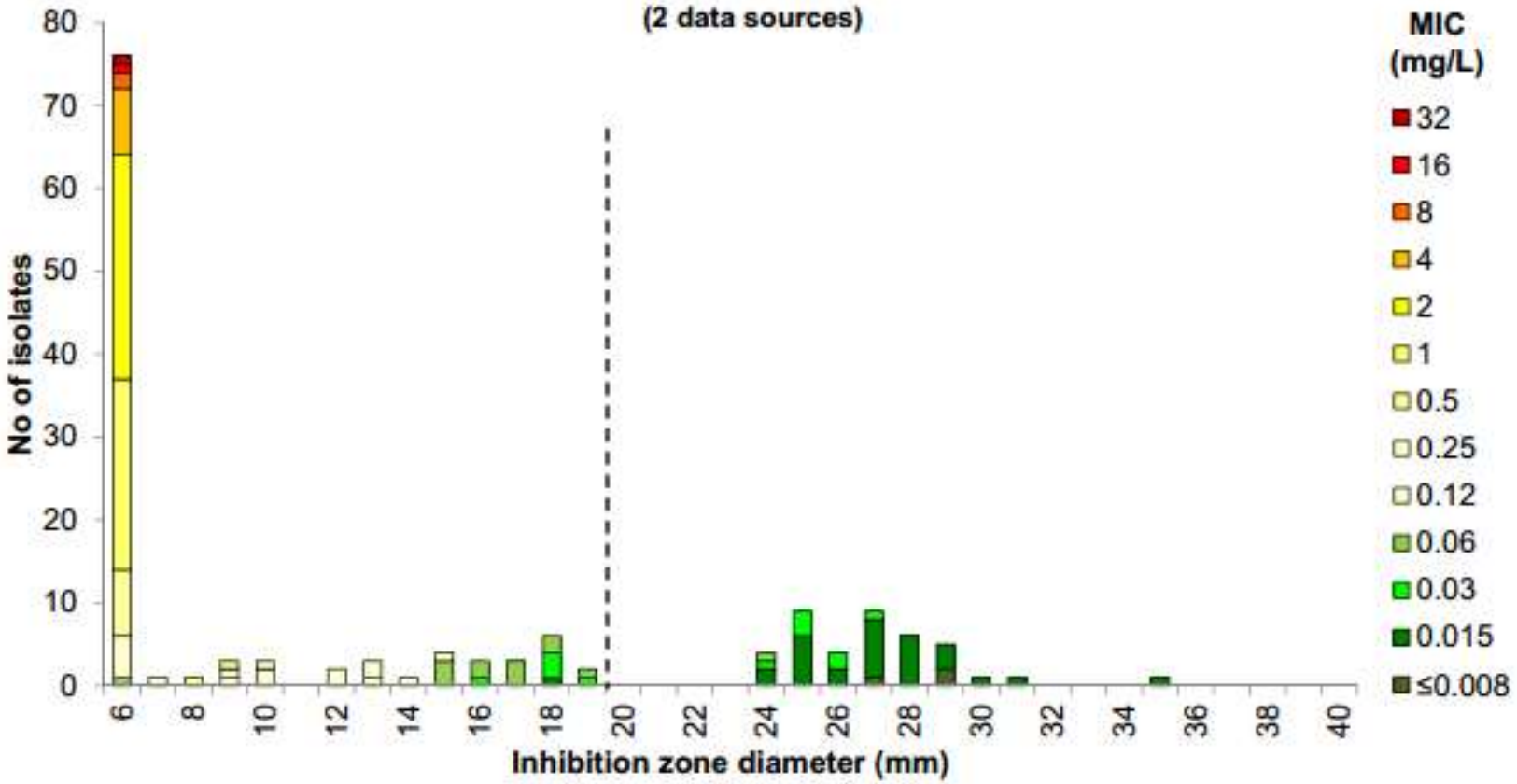
Streptococcus pneumoniae
rezistencija na β-laktamske antibiotike – probirni test

- Oksacilin 1µg disk – probirni disk

Oksacilin (OX) 1µg Zona inhibicije (mm)	Antibiotik	Dodatna testiranja i interpretacija rezultata
≥20	svi β-laktamski atb.	osjetljiv
<20	penicilin (meningitis) i oralni penicilni (sve indikacije)	rezistentan
	Ampicilin, amoksicilin i piperacilin (sa i bez inhibitora), cefotaksim, ceftriakson, ceftaroline i cefepime	oksacilin ≥8mm – izdati osjetljiv Meningitis – odrediti MIK
		oksacilin<8mm – odrediti MIK za atb (terapijski izbor)
	Ostali β-laktamski atb. (uključujući parenteralni penicilin – sve indikacije osim meningitisa)	odrediti MIK-ove za atb (terapijski izbor)

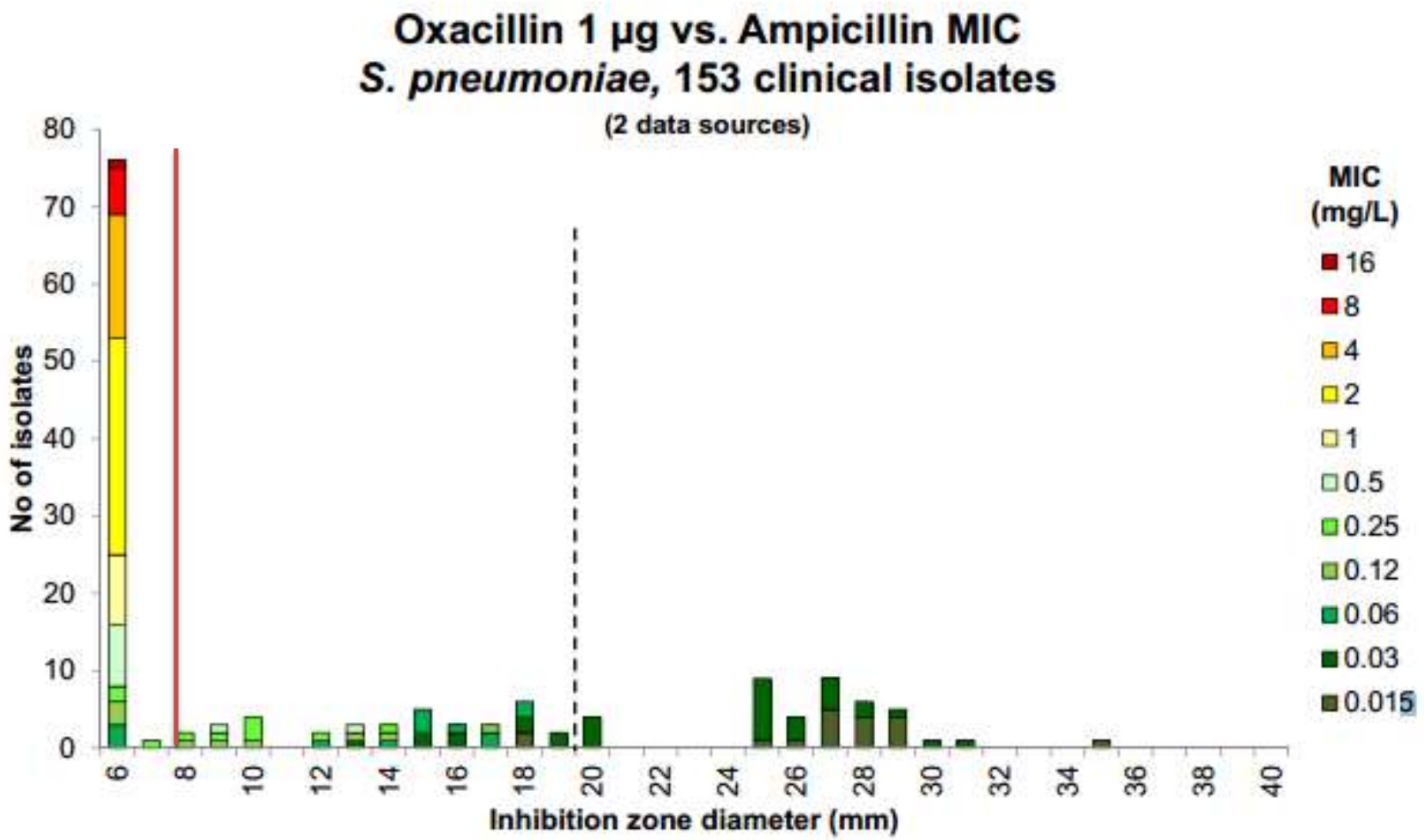
Giske CG, Martinez-Martinez L, Cantón R *et al.* **EUCAST guidelines for detection of resistance mechanisms** and specific resistances of clinical and/or epidemiological importance. Version 1.0, December 2013. http://www.eucast.org.

Oxacillin 1 µg vs. Benzylpenicillin MIC
S. pneumoniae, 148 clinical isolates



Breakpoints		**ECOFF**
Benzylpenicillin (non-meningitis) MIC	S≤0.06, R>2 mg/L	WT≤0.06 mg/L
Oxacillin zone diameter (screen)	S≥20 mm	

http://www.eucast.org/mic_distributions_and_ecoffs/



Breakpoints		**ECOFF**
Ampicillin MIC	S≤0.5, R>2 mg/L	WT≤0.06 mg/L
Oxacillin zone diameter (screen)	S≥20 mm	

http://www.eucast.orgributions_and_ecoffs/

Penicilin

Interpretacija rezultata osjetljivosti

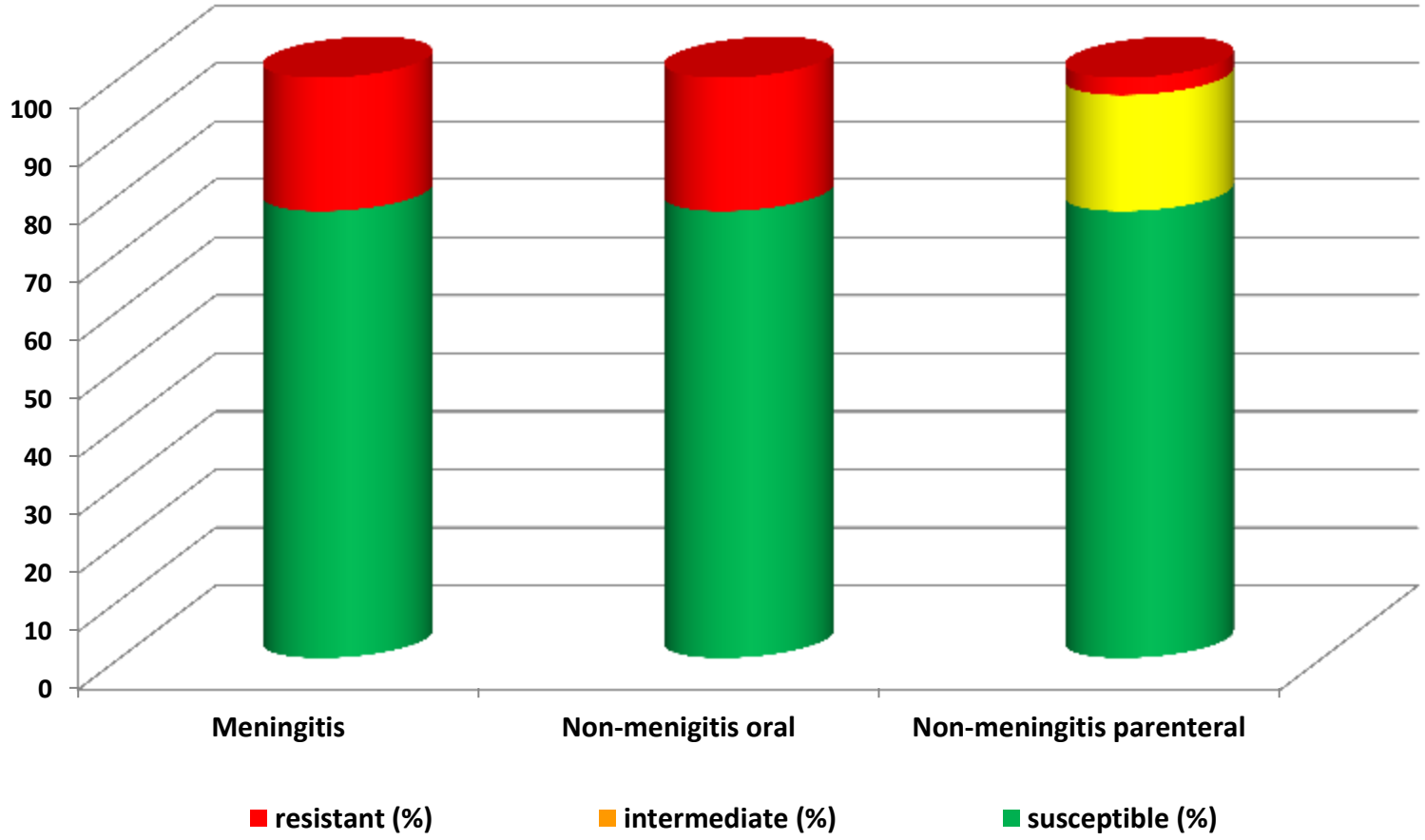
Table 2. Reporting of benzylpenicillin susceptibility in meningitis and non-meningitis.

Indications	MIC breakpoint (mg/L)		Notes
	S ≤	R >	
Benzylpenicillin (non-meningitis)	0.06 I 0.125 - 2	2	**In pneumonia**, when a dose of 1.2 g x 4 is used, isolates with **MIC ≤0.5 mg/L** should be regarded as susceptible to benzylpenicillin. **In pneumonia**, when a dose of 2.4 g x 4 or 1.2 g x 6 is used, isolates with **MIC ≤1 mg/L** should be regarded as susceptible to benzylpenicillin. **In pneumonia**, when a dose of 2.4 g x 6 is used, isolates with **MIC ≤2 mg/L** should be regarded as susceptible.
Benzylpenicillin (meningitis)	0.06	0.06	

Note: 1.2 g of benzylpenicillin is equal to 2 MU (million units) of benzylpenicillin

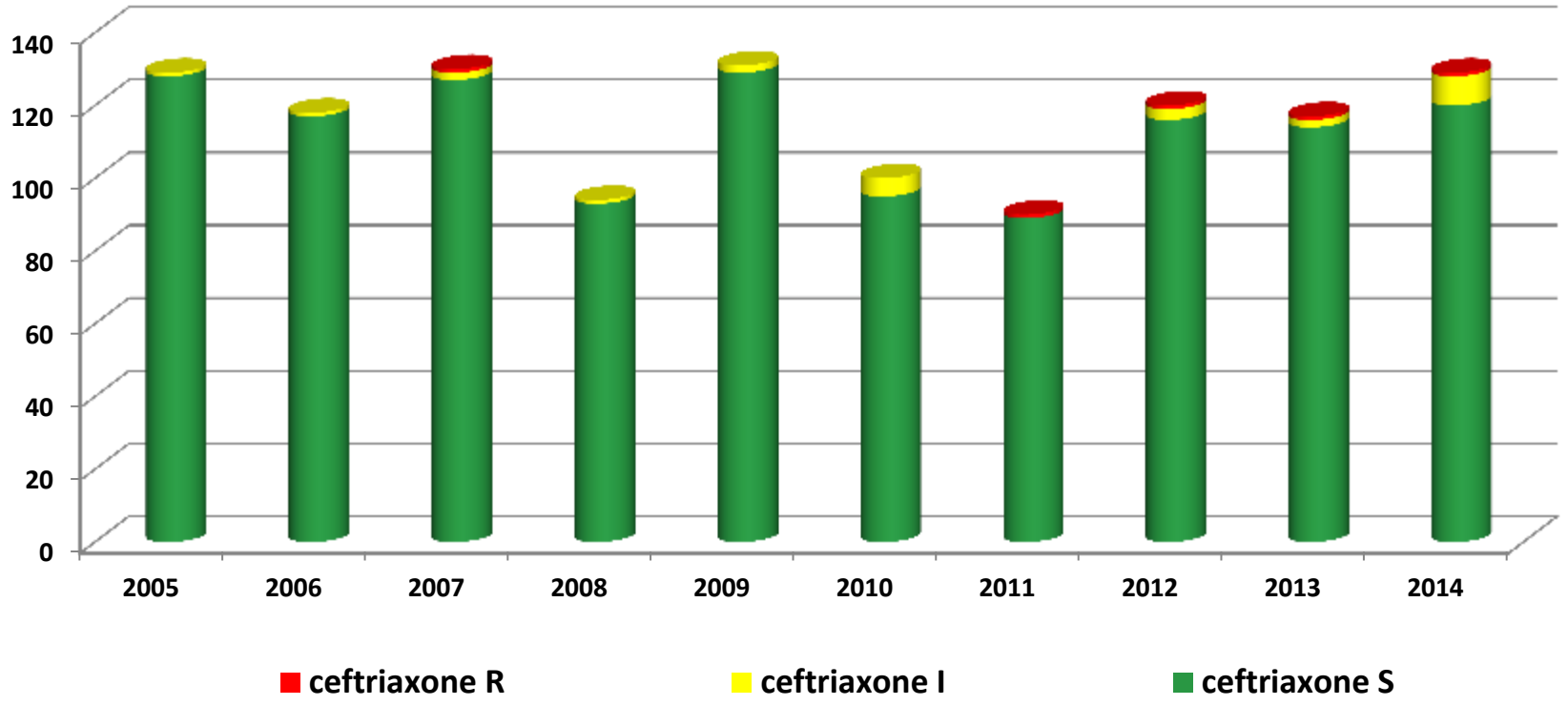
S. pneumoniae – osjetljivost na penicilin

Odbor za praćenje rezistencije bakterija na antibiotike, 2014



Invazivni *Streptococcus pneumoniae*

rezistencija na ceftriakson, EARS-Net 2005-2014



Streptococcus pneumoniae

rezistencija na makrolide i klindamicin

- **Efluks pumpa:**

  a) *mef (A)* geni - rezistencija na makrolide ->**M fenotip**

  b) eritromicin R = azitromicin R, klaritromicin R… ostali makrolidi R

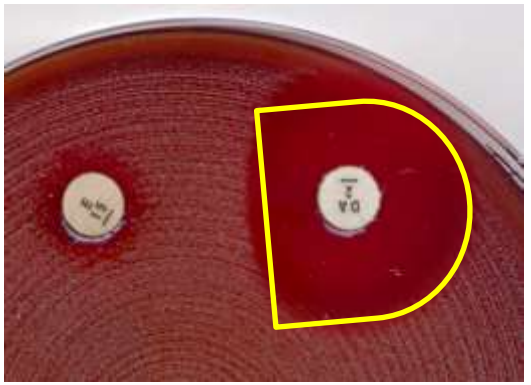
- **MLS_B rezistencija:** rezistencija na makrolide, linkozamide i streptogramin B

  a) Konstitutivna ($cMLS_B$) – makrolidi, klindamicin i streptogramin B **rezistentni**

  b) Inducibilna ($iMLS_B$) : *erm(B)* geni

  - stečena rezistencija (promijena ciljnog mjesta-ribosom)
  - detekcija: udaljenost između diskova 12-16mm od ruba do ruba diska
  - očitavanje osjetljjivosti: makrolidi rezistentni, a klindamicin osjetljiv
  - izdavanje nalaza: klindamicin **rezistentan** uz komentar:

Klindamicin još uvijek može biti klinički djelotvoran, ali duljom primjenom može se inducirati rezistencija.

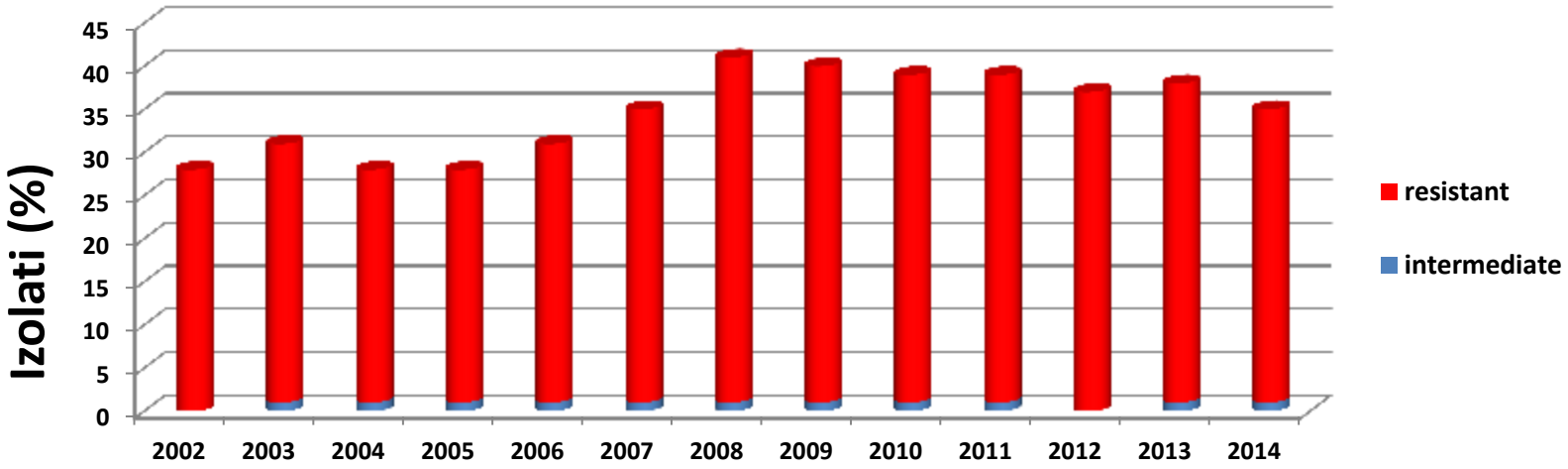


Streptococcus pneumoniae
osjetljivost na makrolide i klindamicin

Streptococcus pneumoniae	MIK (mg/L)		Zona inhibicije (mm)	
	OSJETLJIV (S ≤)	REZISTENTAN (R >)	OSJETLJIV (S ≥)	REZISTENTAN (R <)
Eritromicin	0.25	0.5	22	19
Azitromicin	0.25	0.5	-	-
Klaritromicin	0.25	0.5	-	-
Klindamicin	0.5	0.5	19	19

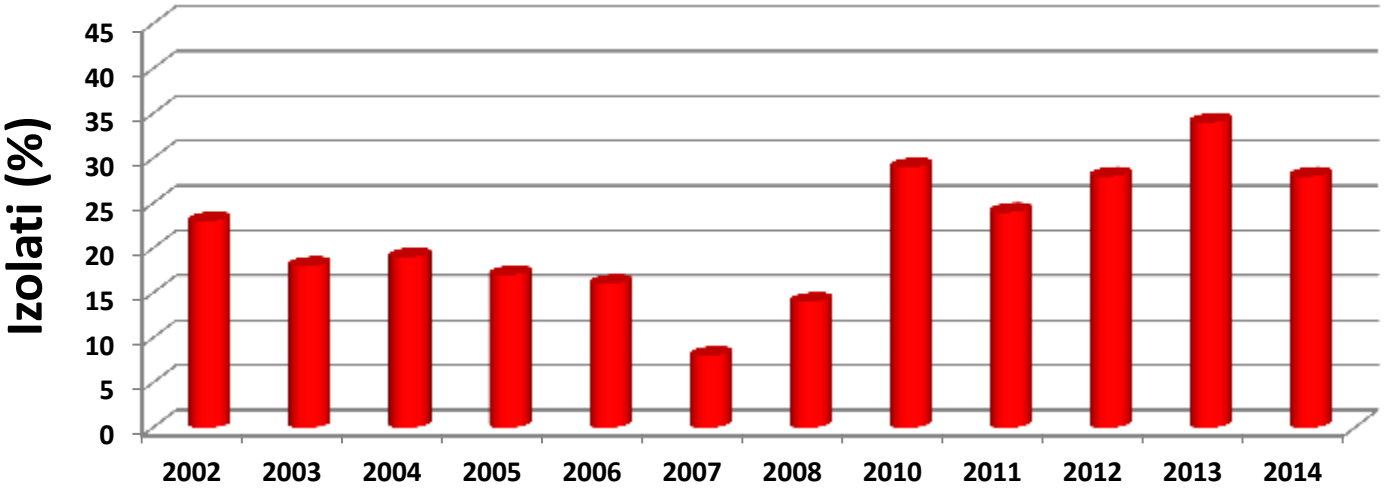
S. pneumoniae – rezistencija na makrolide

Odbor za praćenje rezistencije bakterija na antibiotike, 2002-2014



Invasivni *S. pneumoniae* – rezistencija na makrolide

EARS-Net, 2002-2014



Streptococcus pneumoniae
rezistencija na kinolone

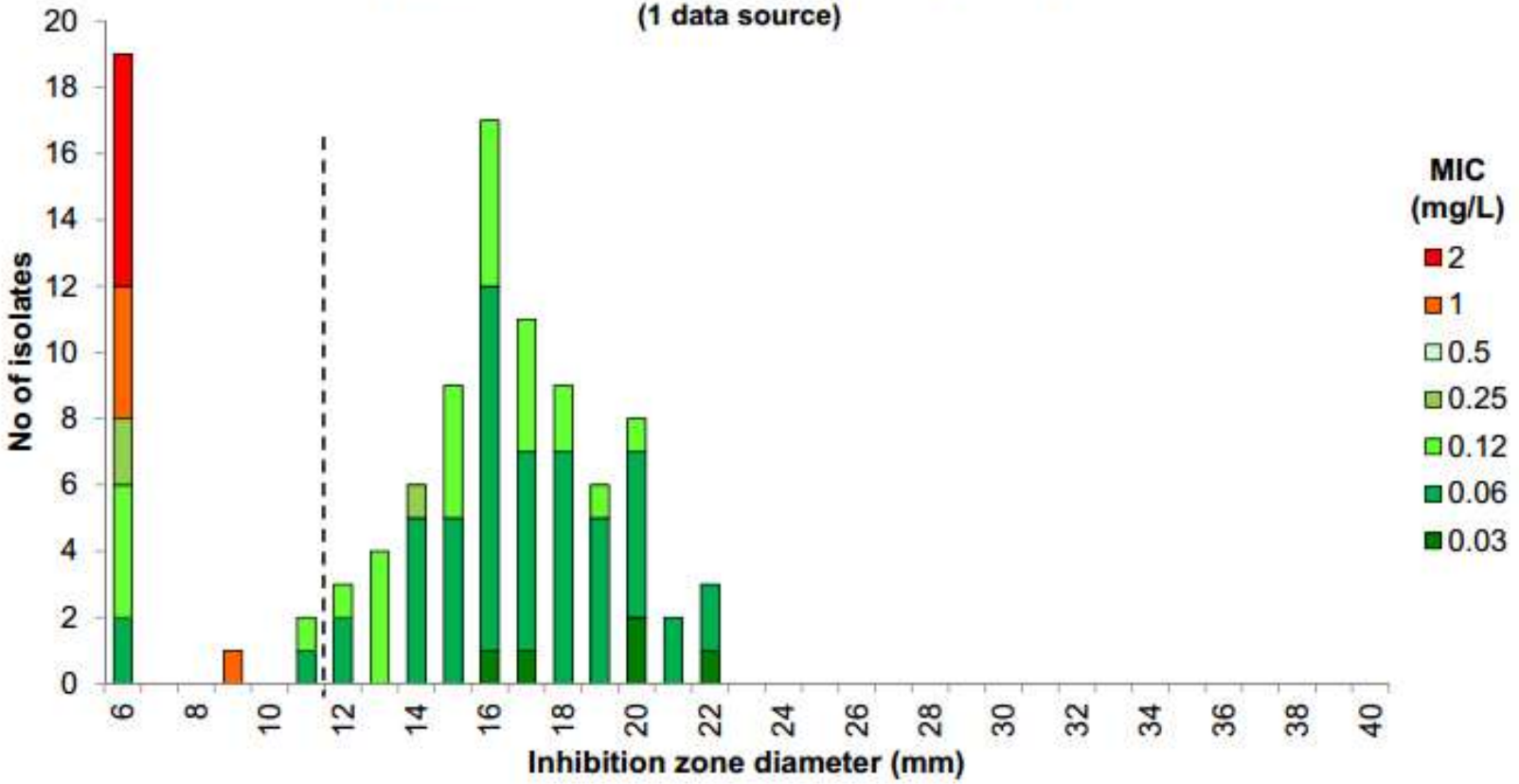
- *parC i gyrA* geni

Streptococcus pneumoniae	MIK (mg/L)		Zona inhibicije (mm)	
	OSJETLJIV (S ≤)	REZISTENTAN (R >)	OSJETLJIV (S ≥)	REZISTENTAN (R <)
Norfloksacin *„screening"*	-	-	12	12
Moksifloksacin	0.5	0.5	22	22
Levofloksacin	2	2	17	17
Ciprofloksacin	0.125	2	50	16

- Interpretacija rezultata:
  a) AKO je norfloksacin S ONDA izdati moksifloksacin S i levofloksacin S
  b) AKO je norfloksacin R ONDA treba odrediti osjetljivost za svaki kinolon
  c) AKO su levofloksacin R i moksifloksacin R ONDA su svi kinoloni R

Norfloxacin 10 µg vs. Moxifloxacin MIC
S. pneumoniae, 100 clinical isolates

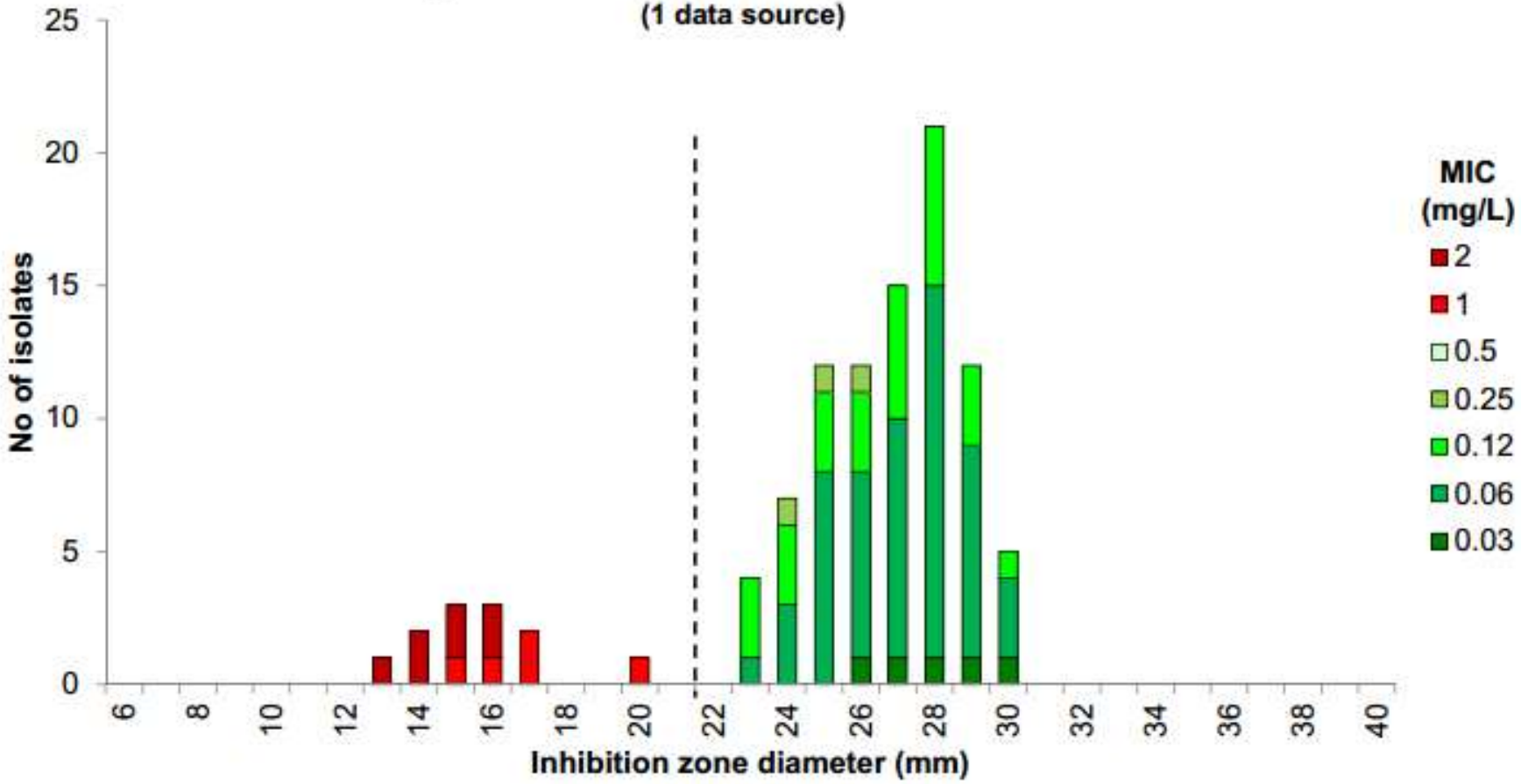
(1 data source)



Breakpoints		**ECOFF**
Moxifloxacin MIC	S≤0.5, R>0.5 mg/L	WT≤0.5 mg/L
Norfloxacin zone diameter (screen)	S≥12 mm	

Moxifloxacin 5 µg vs. MIC
S. pneumoniae, 100 clinical isolates

(1 data source)



Breakpoints		**ECOFF**
MIC	S≤0.5, R>0.5 mg/L	WT≤0.5 mg/L
Zone diameter	S≥22, R<22 mm	

Special resistance mechanism	Importance of detection of resistance mechanism		
	Required for antimicrobial susceptibility categorization	Infection control	Public health
Carbapenemase-producing Enterobacteriaceae	No	Yes	Yes
Extended-spectrum β-lactamase-producing Enterobacteriaceae	No	Yes	Yes
Acquired AmpC β-lactamase-producing Enterobacteriaceae	No	Yes	Yes
Methicillin resistant *Staphylococcus aureus*	Yes	Yes	Yes
Glycopeptide non-susceptible *Staphylococcus aureus*	Yes	Yes	Yes
Vancomycin resistant *Enterococcus faecium* and *Enterococcus faecalis*	Yes	Yes	Yes
Penicillin non-susceptible *Streptococcus pneumoniae*	Yes	No	Yes

Giske CG, Martinez-Martinez L, Cantón R *et al.* **EUCAST guidelines for detection of resistance mechanisms** and specific resistances of clinical and/or epidemiological importance. Version 1.0, December 2013. http://www.eucast.org.

BHS grupe A, B, C i G

1. **Penicilin** – zasad nije opisana rezistencija osim kod BHS grupa B (rijetko)

- Penicilin S = aminopenicilini S, cefalosporini S i karbapenemi S
- BHS grupa B – samo parenteralni penicilin

2. **Makrolidi i klindamicin:**

a) M-fenotip - rezistencija na makrolide

Eritromicin R = azitromicin R, klaritromicin R… ostali makrolidi R

b) MLS_B fenotip: rezistencija na makrolide, linkozamide i streptogramin B

1. Konstitutivna (makrolidi R i klindamicin R)
2. Inducibilna (makrolidi R i klindamicin S)

- Klindamicin je klinički djelotovoran, ali NIJE izbor za duže atb. liječenje
- Klindamicin izdati **rezistentan** uz komentar!

BHS grupe A, B, C i G

- Kinoloni

BHS grupa A, B, C i G	MIK (mg/L)		Zona inhibicije (mm)	
	OSJETLJIV (S ≤)	REZISTENTAN (R >)	OSJETLJIV (S ≥)	REZISTENTAN (R <)
Norfloksacin *„screening”*	-	-	12	12
Moksifloksacin	0.5	1	18	15
Levofloksacin	1	2	18	15
Ciprofloksacin	-	-	-	-

- Nitrofurantoin: samo za BHS grupa B (nekomplicirane IMS)

Streptococcus viridans

intrinzična rezistencija

TABLE 4. Intrinsic resistance in Gram-positive bacteria; Gram-positive bacteria are also intrinsically resistant to aztreonam, temocillin, polymyxin B/colistin, and nalidixic acid

Rule no.	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Erythromycin	Clindamycin	Quinupristin–dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulphonamides
4.1	*Staphylococcus saprophyticus*	R	R	–	–	–	–	–	–	–	R	R	–
4.2	*Staphylococcus cohnii*, *Staphylococcus xylosus*	–	R	–	–	–	–	–	–	–	–	R	–
4.3	*Staphylococcus capitis*	–	R	–	–	–	–	–	–	–	R	–	–
4.4	Other coagulase-negative staphylococci and *Staphylococcus aureus*	–	R	–	–	–	–	–	–	–	–	–	–
4.5	*Streptococcus* spp.	R	–	–	R[a]	–	–	–	–	–	–	–	–
4.6	*Enterococcus faecalis*	R	R	R	R	R	R	R	–	–	–	–	R
4.7	*Enterococcus gallinarum*, *Enterococcus casseliflavus*	R	R	R	R[a]	R	R	R	R	–	–	–	R
4.8	*Enterococcus faecium*	R	R	R	R[a,b]	R	–	–	–	–	–	–	R
4.9	*Corynebacterium* spp.	–	–	–	–	–	–	–	–	–	R	–	–
4.10	*Listeria monocytogenes*	–	R	R	–	–	–	–	–	–	–	–	–
4.11	*Leuconostoc* spp., *Pediococcus* spp.	–	–	–	–	–	–	–	R	R	–	–	–
4.12	*Lactobacillus* spp. (some species)	–	–	–	–	–	–	–	R	R	–	–	–
4.13	*Clostridium ramosum*, *Clostridium innocuum*	–	–	–	–	–	–	–	R	–	–	–	–

R, resistant.

[a]Low-level resistance to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides.

[b]In addition to low-level resistance to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6′) enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin, arbekacin, and streptomycin) and penicillins or glycopeptides.

Streptococcus viridans

1. Penicilin:

- Promjena PBP-ova – mozaični geni
- AKO je penicillin R ONDA je potrebno odrediti MIK ampicilina (amoksicilina) i cefotaksima (ili ceftriaksona)

Streptococcus viridans	MIK (mg/L)		Zona inhibicije (mm)	
	OSJETLJIV (S ≤)	REZISTENTAN (R >)	OSJETLJIV (S ≥)	REZISTENTAN (R <)
Benzilpenicilin	0.25	2	18	12
Benzilpenicilin "screen"	0.25	2	18	18
Ampicilin	0.5	2	21	15
Cefotaksim	0.5	0.5	23	23
Ceftriakson	0.5	0.5	27	27

Streptococcus viridans

2. Aminoglikozidi:

- HLAR – detekcija mehanizma rezistencije:

  a) disk difuzija – gentamicin 30µg - <8mm

  b) MIK gentamicina >128 mg/L

3. Klindamicin

- Konstitutivna rezistencija
- Inducibilna rezistencija (eritromicin i klindamicin, 12-16 mm, od ruba do ruba diska)
- AKO je klindamicin S ONDA u nalazu izdati R uz komentar…..


Hvala

Gram-negativne bakterije:
mehanizmi rezistencije i interpretacija antibiograma

Prof. Arjana Tambic Andrasevic, MD

Klinika za infektivne bolesti “Dr. Fran Mihaljević”

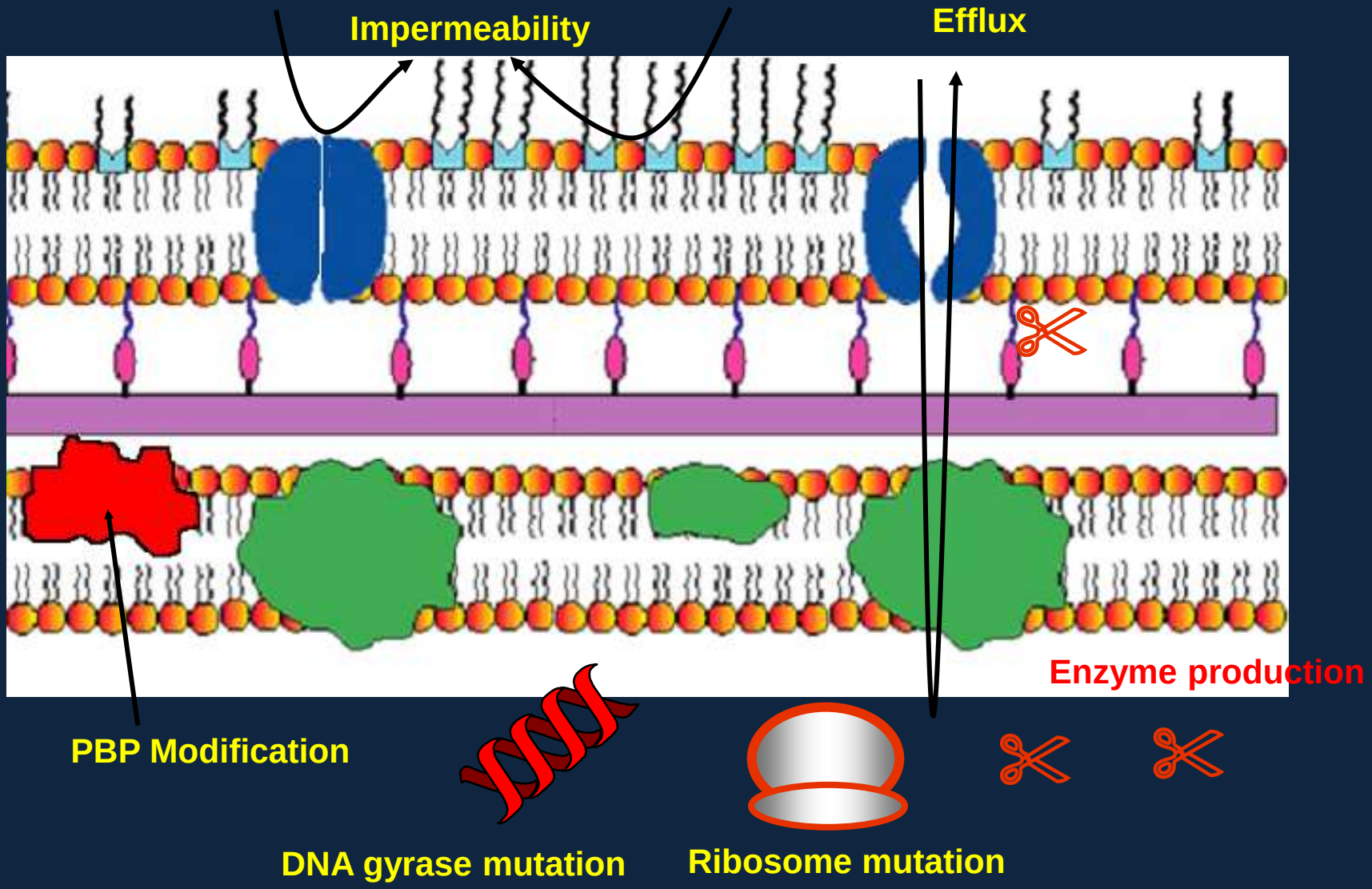
Rezistencija na antibiotike

- **Urođena rezistencija**
  - karakteristika vrste
- **Stečena rezistencija**
  - često prisutno istovremeno više mehanizama rezistencije
  - uočavanje novih fenotipova, novih mehanizama rezistencije
  - slanje u referentne laboratorije
  - spriječavanje širenja rezistentnih sojeva

Mehanizmi rezistencije u gram-negativnih bakterija

- **PROMJENA CILJNOG MJESTA**
  - Penicillin binding proteins (PBP) za beta-laktame
  - DNA gyrase za kinolone
- **EFFLUX**
  - Rezistencija na tetracikline
  - Rezistencija na kinolone
- **PROIZVODNJA ENZIMA**
  - Beta-laktamaze
  - Enzimi koji inaktiviraju aminoglikozide
- **NEPROPUSNOST STIJENKE**
  - rezistencija na beta-laktame

Resistance mechanisms in gram-negative bacteria



BETA-LAKTAMSKI ANTIBIOTICI

- Beta-laktamaze glavni mehanizam rezistencije enterobakterija na beta-laktamske antibiotike
- široko rasprostranjene
- učinkovitost ovisi o:
  - načinu ekspresije (konstitutivne, inducibilne)
  - količini
  - jačini

BETA-LAKTAMAZE

- ~ 500 različitih vrsta beta-laktamaza s varijabilnom razinom količine, jačine i ekspresije
- Različiti supstrati:
  - **Penicilinaze**
    - Penicillinase
    - Broad spectrum beta-lactamases (TEM, SHV, OXA)
    - Extended Spectrum β lactamase (ESβL)
    - Inhibitor Resistant TEM (IRT)
  - **Cefalosporinaze**
    - Cefuroximase
    - Inducible ampC cefalosporinase
  - **Karbapenemaze**

•TEM => penicillinase named after the patient (TEMoneira) providing the first sample
•SHV => SulfHydril reagent Variable
•OXA => active on OXAcillin, cloxacillin

FUNKCIONALNE KLASIFIKACIJE BETA-LAKTAMAZA

- 1968.g. Sawai *et al*
- 1973.g. Richmond & Sykes

  - beta-laktamaze gram-neg. bakterija u 5 grupa
- 1976.g. Sykes & Matthew
- 1981.g. Mitsuhashi & Inone
- **1989.g. Bush**

  - supstrat / osjetljivost na inhibitore / molekularna struktura
- **1995.g. Bush-Jacoby-Medeiros**

CLASSIFICATION OF BETA-LACTAMASES

Ambler classification / molecular

molecular sequence

- A: Penicillinase
- B: Metallo beta-lactamase
- C: Cephalosporinase
- D: Oxacillinase

Bush classification / functional

supstrate / sensitivity to inhibitors

- **group 1** : cephalosporinases / no inhibition by clavulanic acid (class C)
- **group 2** : beta-lactamases inhibited by clav. acid (class A and D)
  - 2a: penicillinase in staph
  - 2b: acquired penicillinase
  - 2be: ESBLs
  - 2br: inhibitor resistant TEM
  - 2c: carbenicillinase
  - **2d**: Oxacillinases
  - 2e: cefuroximase
  - 2f: carbapenemases
- **group 3** : metallo beta-lactamases / inhibited by EDTA (mol. class B)
- **group 4** : penicillinases not inhibited by clav. acid

BETA-LAKTAMAZE

A) SPECIES-SPECIFIČNE BETA-LAKTAMAZE

- prirođene, izražene u svih pripadnika vrste
- određene kromosomskim genima
- evolucijski “stare”
  (prisutne prije uvođenja beta-laktama)
- fiziološka uloga :
  - sinteza staničnog zida (strukturno sliče PBP)
  - obrana od prirodnih beta-laktama
  - nutritivna uloga
- sve enterobakterije osim salmonela
- velike razlike u:
  - količini
  - načinu ekspresije (konstitutivne / inducibilne)

Urođene beta-laktamaze

- ***E. coli, Shigella* spp.**

  - male, beznačajne količine AmpC beta-laktamaza
  - dobra osjetljivost na ampicilin i ostale beta-laktame

- ***Enterobacer* spp., *Citrobacter freundii, Serratia* spp., *Morganella morganii*, *Providencia stuartii, P. rettgeri***

  - male količine AmpC beta-laktamaza, ali u prisutnosti antibiotika dolazi do hiperprodukcije **(inducibilne AmpC beta-laktamaze)**
  - **rezistencija na ampicilin i cefalosporine I gen.** (jaki induktori i labilni supstrati)
  - osjetljivost na cefalosporine III gen. (labilni supstrati, ali slabi induktori) i karbapeneme (jaki induktori, ali stabilni supstrati)
  - ne inhibiraju ih inhibitori beta-laktamaza

Urođene beta-laktamaze

- ***Klebsiella* spp.**

  - male količine kromosomskih beta-laktamaza klase A
  - dovoljno za **rezistenciju na ampicilin, karbenicilin, tikarcilin**
  - *K. pneumoniae* (kromosomska SHV-1 beta-laktamaza) *K. oxytoca* (K1, KOXY)
  - osjetljivost na cefalosporine I gen. i piperacilin *in vitro* +++, ali MIK ipak povišen, pogotovo uz veći inokulum (PIP/TAZ uvijek veća zona nego oko PIP) (samo za UTI ?)
  - osjetljivost na inhibitore beta-laktamaza

Urođene beta-laktamaze

- ***Proteus vulgaris, Citrobacter koseri (diversus)***
  - kromosomske inducibilne beta-laktamaze klase A
  - **rezistencija na ampicilin, kod *P. vulgaris* i na cefalosporine I i II gen.**
  - osjetljivost na inhibitore beta-laktamaza
- ***Proteus mirabilis***
  - zanemariva ekspresija kromosomskih beta-laktamaza
  - odlična osjetljivost na sve beta-laktame

TABLE I. Intrinsic resistance in *Enterobacteriaceae*; *Enterobacteriaceae* are also intrinsically resistant to benzylpenicillin, glycopeptides, fusidic acid, macrolides (with some exceptions[a]), lincosamides, streptogramins, rifampicin, daptomycin, and linezolid

Rule no.	Organisms	Ampicillin	Amoxycillin–clavulanate	Ticarcillin	Piperacillin	Cefazolin	Cefoxitin	Cefamandole	Cefuroxime	Aminoglycosides	Tetracyclines/ tigecycline	Polymyxin B/colistin	Nitrofurantoin
1.1	*Citrobacter koseri*	R	–	R	R	–	–	–	–	–	–	–	–
1.2	*Citrobacter freundii*	R	R	–	–	R	R	–	–	–	–	–	–
1.3	*Enterobacter cloacae*	R	R	–	–	R	R	–	–	–	–	–	–
1.4	*Enterobacter aerogenes*	R	R	–	–	R	R	–	–	–	–	–	–
1.5	*Escherichia hermannii*	R	–	R	–	–	–	–	–	–	–	–	–
1.6	*Hafnia alvei*	R	R	–	–	R	–	–	–	–	–	–	–
1.7	*Klebsiella* spp.	R	–	R	–	–	–	–	–	–	–	–	–
1.8	*Morganella morganii*	R	R	–	–	R	–	–	R	–	R	R	R
1.9	*Proteus mirabilis*	–	–	–	–	–	–	–	–	–	R	R	R
1.10	*Proteus vulgaris*	R	–	–	–	R	–	R	R	–	R	R	R
1.11	*Proteus penneri*	R	–	–	–	R	–	R	R	–	R	R	R
1.12	*Providencia rettgeri*	R	R	–	–	R	–	–	–	–	R	R	R
1.13	*Providencia stuartii*	R	R	–	–	R	–	–	–	Note[b]	R	R	R
1.14	*Serratia marcescens*	R	R	–	–	R	–	R	R	Note[c]	–	R	R
1.15	*Yersinia enterocolitica*	R	R	R	–	R	R	R	–	–	–	–	–
1.16	*Yersinia pseudotuberculosis*	–	–	–	–	–	–	–	–	–	–	R	–

R, resistant.

[a]Azithromycin is effective *in vivo* for the treatment of typhoid fever, and erythromycin may be used to treat travellers' diarrhoea.

[b]*Providencia stuartii* produces a chromosomal AAC(2′)-Ia enzyme and should be considered to be resistant to clinically available aminoglycosides, except amikacin, arbekacin, and streptomycin. Some isolates express the enzyme poorly and can appear to be susceptible to netilmicin *in vitro*, but should be reported as resistant, as mutation can result in overproduction of this enzyme.

[c]All *Serratia marcescens* isolates produce a chromosomal AAC(6′)-Ic enzyme that affects the activity of clinically available aminoglycosides, except streptomycin, gentamicin, and arbekacin.

EUCAST expert rules, version 2.0, CMI 2011

Enterobacteriaceae: innate resistance to antibiotics

	Ampicillin	Amoxicillinc lavulanate	Cefazolin	Cefoxitin	Cefuroxime	Tetracycline Tigecycline	Colistin	Nitrofurant oin
E.coli								
K.pneumoniae	R							
Enterobacter spp.	R	R	R	R				
Citrobacter koseri	R							
Citrobacter freundii	R	R	R	R				
Serratia marcescens	R	R	R		R		R	R
Proteus mirabilis						R	R	R
Proteus vulgaris	R		R		R	R	R	R
Morganella morganii	R	R	R		R	R	R	R
Providencia spp.	R	R	R			R	R	R
***Salmonella* spp.**								
***Shigella* spp.**								

BETA-LAKTAMAZE

B) SEKUNDARNE BETA-LAKTAMAZE

- stečene, evolucijski “nove”
  - pojavile se nakon uvođenja penicilina
  - potječu od species-specifičnih
- najčešće određene plazmidima
- jedina fiziološka uloga:
  - zaštititi bakteriju od antibiotika

BETA-LAKTAMAZE
stečena rezistencija

- **beta-laktamaze širokog spektra**
  (TEM-1, TEM-2, SHV-1)
- **beta-laktamaze proširenog spektra**
  (extended spectrum beta-lactamases, ESBLs)
- **derepresija inducibilnih AmpC beta-laktamaza**
- **karbapenemaze**

Beta-laktamaze širokog spektra

- 1965.g. TEM-1 (najraširenija)
- TEM-2, SHV-1, OXA-1, PSE-1,4

➢ **Rezistencija na:**

- ampicilin, tikarcilin, cefalosporine I gen., piperacilin

(niska razina beta-laktamaza - *in vitro* cef. I gen. +++, PIP +++)

➢ **Ovisno o količini beta-laktamaza (varijacije do 150x) rezistencija na:**

- kombinacije s inhibitorima beta-laktamaza
- cefoperazon

➢ **Osjetljivost na:**

- cefalosporine III g. (osim CFP), aztreonam, karbapeneme

BETA-LAKTAMAZE
stečena rezistencija

- **beta-laktamaze širokog spektra**
  (TEM-1, TEM-2, SHV-1)
- **beta-laktamaze proširenog spektra**
  (extended spectrum beta-lactamases, ESBLs)
- **derepresija inducibilnih AmpC beta-laktamaza**
- **karbapenemaze**

BETA-LAKTAMAZE PROŠIRENOG SPEKTRA (ESBL)

- molekularna klasa A i D, Bush grupa 2
- rezistencija na sve beta-laktame osim karbapenema (temocilina, cefamicina)
- osjetljive na inhibitore beta-laktamaza
- sekundarne, evolucijski “nove” (od penicilinaza širokog spektra TEM-1, SHV-1, OXA-2, -10)
- plazmidne, konstitutivne
- najraširenije, najefikasnije
- razl. jačina enzimske aktivnosti (TEM-12 slaba, TEM-24,-26 jaka)
- razlike ovisno o supstratu / ceftazidimaze (TEM-5,-9,-26), cefotaksimaze (TEM-3,-4, SHV-2), oba cef. (SHV-5)

ESBL – kliničko značenje

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European Society of Clinical Microbiology and Infectious Diseases

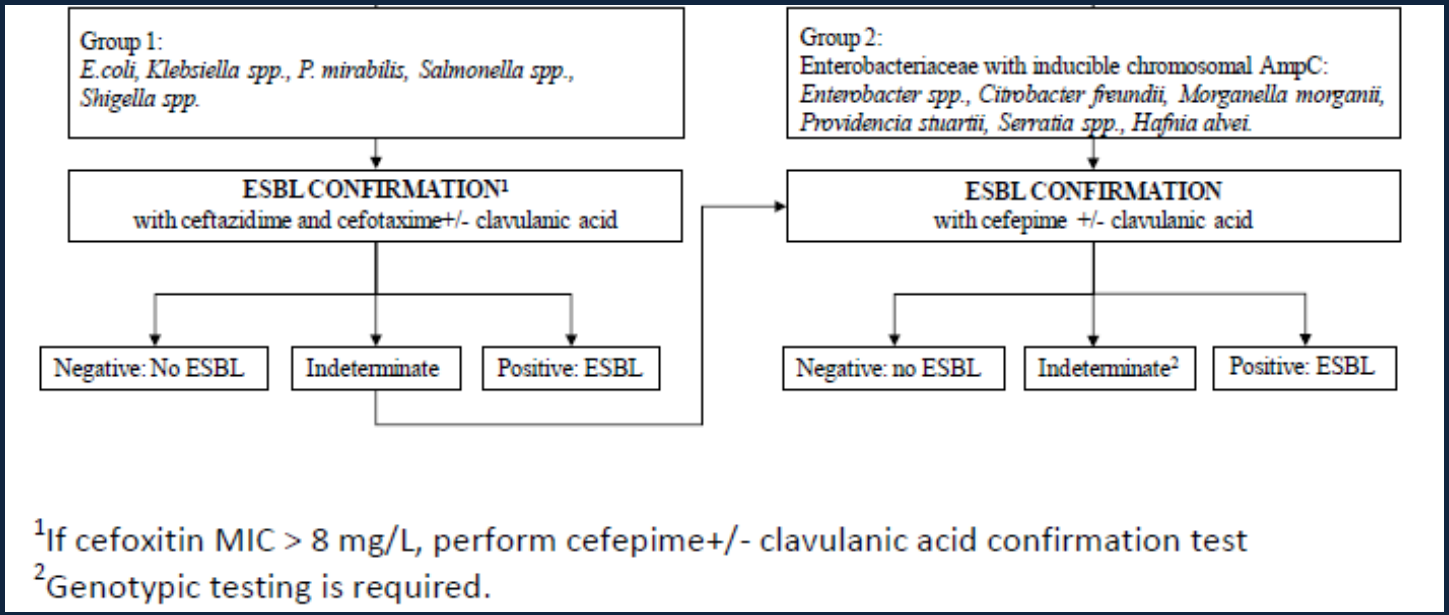
EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance

3. Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae

Importance of detection of resistance mechanism	
Required for antimicrobial susceptibility categorization	No
Infection control	Yes
Public health	Yes

Dokazivanje ESBL

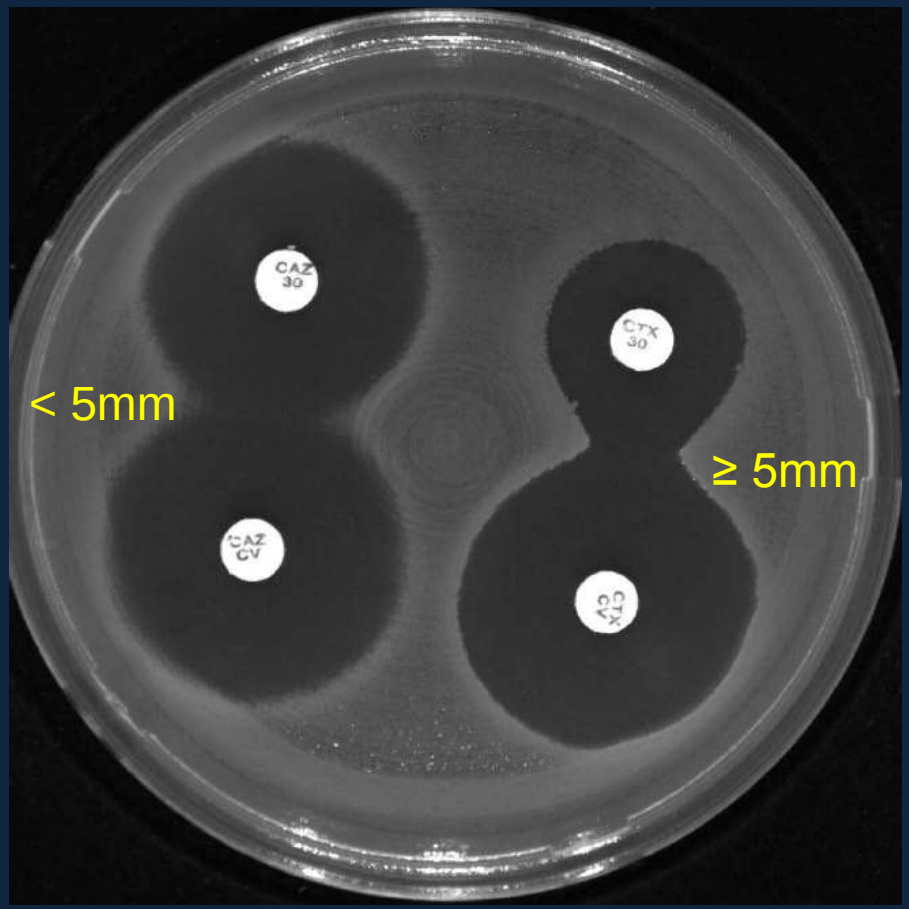
- **Probir**
  - **NS na** cefotaksim / ceftriakson i/ili ceftazidim
- **Potvrda**
  - fenotipske metode (the combination disk test (CDT), the double-disk synergy test (DDST), the Etest ESBL, the broth microdilution testovi prilagođeni bakterijskoj vrsti)



  - genotipske metode (PCR, ESBL gene sequencing)

ESBL

combination disk test, CDT

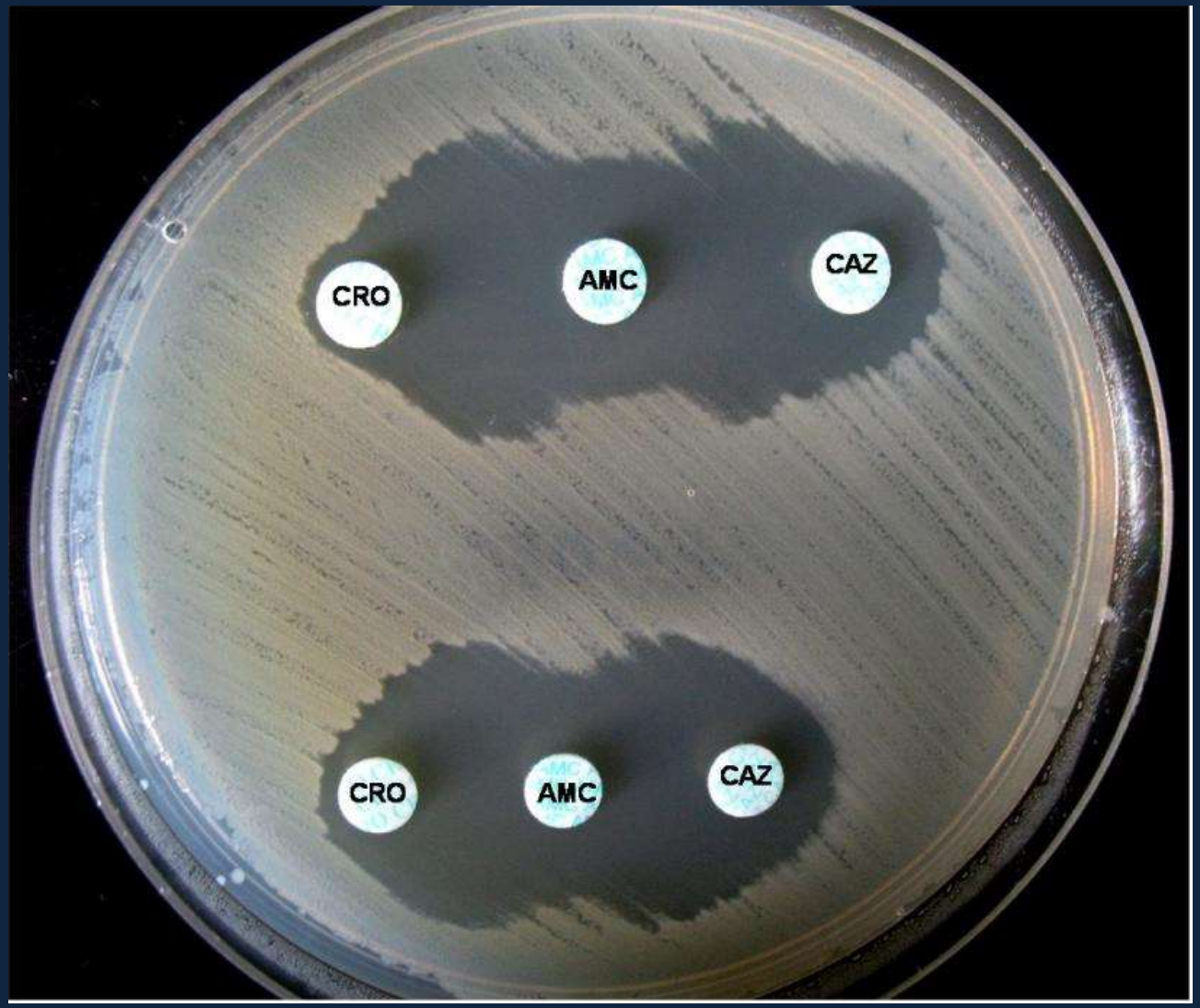


ESBL negative: CAZ CV – CAZ < 5mm and CTX CV - CTX < 5mm

ESBL positive: CAZ CV – CAZ ≥ 5mm and/or CTX CV - CTX ≥ 5mm

ESBL

double-disk synergy test, DDST



BETA-LAKTAMAZE
stečena rezistencija

- **beta-laktamaze širokog spektra**
  (TEM-1, TEM-2, SHV-1)
- **beta-laktamaze proširenog spektra**
  (extended spectrum beta-lactamases, ESBLs)
- **derepresija inducibilnih AmpC beta-laktamaza**
- **karbapenemaze**

INDUCIBLE AmpC CEPHALOSPORINASES

- strukturna klasa C, Bush grupa 1
- rezistentne na inhibitore beta-laktamaza
- široki spektar supstrata (penicilini, cefalosporini, monobaktami)
- kromosomski geni
- evolucijski “stare”, species specifične (prirodno ih luče svi gram-neg. bacili, osim *Salmonella* spp.)
- Hiperprodukciju moguće inducirati u vrsta *Enterobacter* spp., *Citrobacter freundii*, *Serratia* spp.

INDUCIBILNE AmpC CEFALOSPORINAZE

a) pojačana ekspresija AmpC gena u prisutnosti induktora

	INDUKTOR	SUPSTRAT	ATB
ureidopenicilini	-	+	S*
cefalosporini I./II.gen.	+	+	R
cefalosporini III.gen.	-	+	S*
karbapenemi	+	-	S

INDUCIBILNE AmpC CEFALOSPORINAZE

a) pojačana ekspresija AmpC gena u prisutnosti induktora

	INDUKTOR	SUPSTRAT	ATB
ureidopenicilini	-	+	**R**
cefalosporini I./II.gen.	+	+	R
cefalosporini III.gen.	-	+	**R**
karbapenemi	+	-	S

b) stabilna derepresija AmpC gena - stalna (konstitutivna) hiperprodukcija beta-laktamaza

- spontanom mutacijom (*Enterobacter* spp. 10^{-4}, *P. aerug.* 10^{-9})
  (u tijeku terapije - slabi induktor / dobar substrat)

INDUCIBILNE AmpC CEFALOSPORINAZE

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	INDUKTOR	SUPSTRAT	ATB
ureidopenicilini	-	+	**R**
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cefalosporini III.gen.	-	+	**R**
karbapenemi	+	-	S

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- spontanom mutacijom (*Enterobacter* spp. 10^{-4}, *P. aerug.* 10^{-9}) (u tijeku terapije - slabi induktor / dobar substrat)

c) sekundarne AmpC beta-laktamaze

- plazmidne, konstitutivne (*E. coli, Klebsiella* spp.)

SEKUNDARNE plazmidne AmpC CEFALOSPORINAZE

1988: prvo opisane u *E. Coli* i *K. pneumoniae*

MIR-1, ACT-1	*E. cloacae*
BIL-1, LAT-1, LAT-2, CMY-2	*C. freundii*
MOX-1, FOX-1, FOX-2, CMY-1	*P. aeruginosa*

Plazmidne AmpC
kliničko značenje

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3. Acquired AmpC β-lactamase-producing Enterobacteriaceae

Importance of detection of resistance mechanism	
Required for antimicrobial susceptibility categorization	No
Infection control	Yes
Public health	Yes

Stečene (plazmidne) AmpC beta-lactamase

- **Probir**
  - **Groupa 1 enterobacterija** (*E.coli, K.pneumoniae, P.mirabilis, Salmonella* spp., *Shigella* spp.)
  - **NS na** cefotaksim / ceftriakson i/ili ceftazidim (MIC >1mg/L)
  - cefoksitin MIC >8 mg/L
    - lažno neg: sve AmpC ne uzrokuju nužno rezistenciju na cefoksitin
    - lažno poz: moguća rezistencija na cefoksitin zbog gubitka porina
- **Potvrda**
  - Fenotipske metode (inhibicija AmpC kloksacilinom ili boroničnom kiselinom)
  - Genotipske metode (PCR)

AmpC

BETA-LAKTAMAZE
stečena rezistencija

- **beta-laktamaze širokog spektra**
  (TEM-1, TEM-2, SHV-1)
- **beta-laktamaze proširenog spektra**
  (extended spectrum beta-lactamases, ESBLs)
- **derepresija inducibilnih AmpC beta-laktamaza**
- **karbapenemaze**

Karbapenemaze

Class of beta-lactamase	ENZYME	BACTERIA
Class A (serin)	SHV-38 SME (1-3) IMI (1-2) NMC-A **KPC (1-10)** GES (2,4,5,6,8)	Enterobacteriaceae (rare in *P.aeruginosa*)
Class B (Zn) (metallo-beta-lactamase)	Kromosomske IMP, **VIM**, GIM SPM SIM **NDM**	*Bacteroides, S.maltophilia, Aeromonas* *P.aeruginosa* Enterobacteriaceae *Acinetobacter* spp. Enterobacteriaceae
Class D (serin)	OXA **OXA-48**	*Acinetobacter* spp. *K.pneumoniae*

Carbapenem breakpoints for enterobacteriaceae

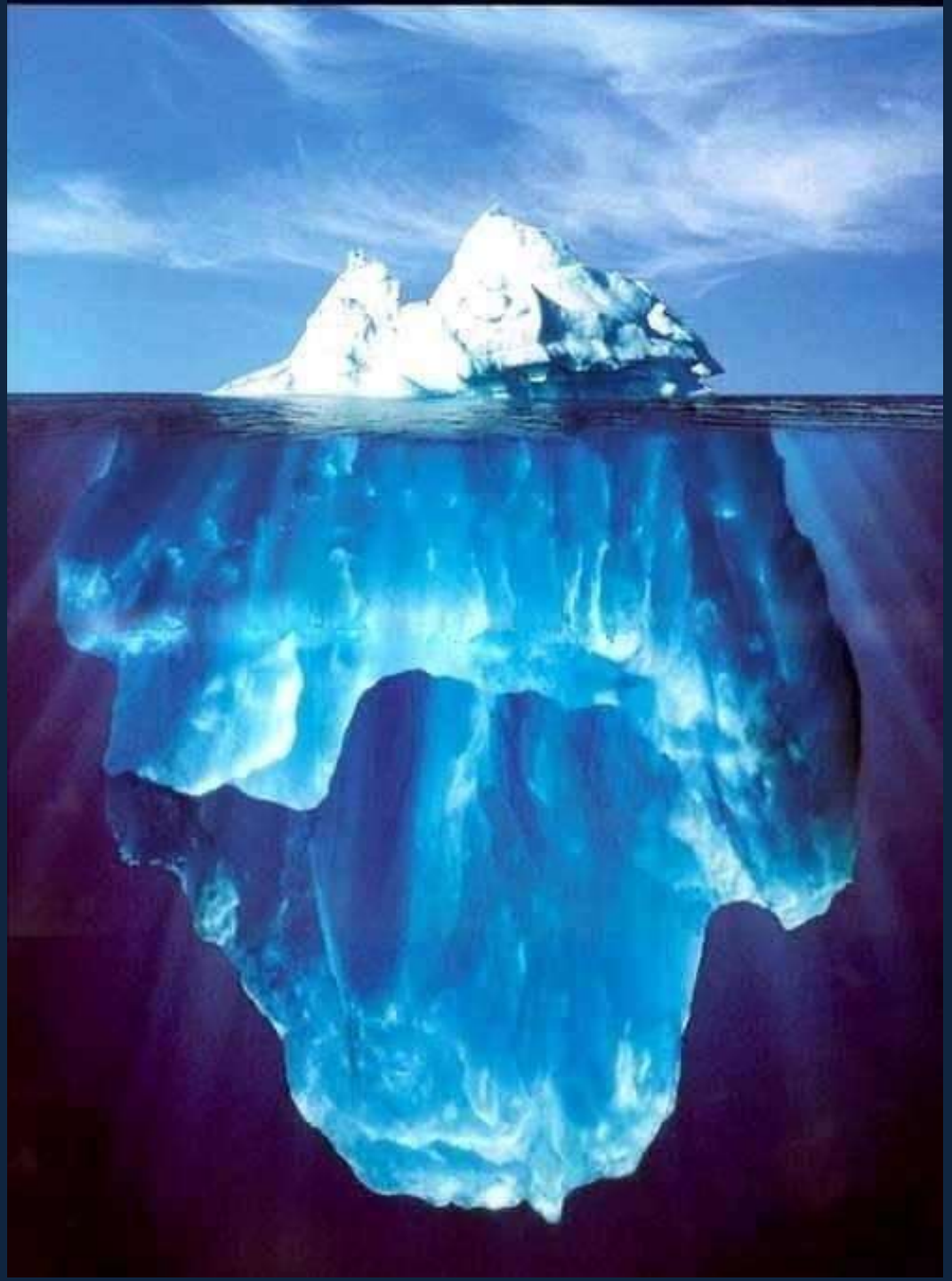
Carbapenem nonsusceptibility not readily detected *in vitro*

	FDA	CLSI (2010)		EUCAST (EMA) (2010)		
	S	S	R	S	R	ECOFF
Imipenem	≤4	≤4 (≤1)*	≥16 (≥4)	≤2	>8	≤0.5; ≤1**
Meropenem	≤4	≤4 (≤1)*	≥16 (≥4)	≤2	>8	≤0.125
Ertapenem	≤2	≤2 (≤0.25)*	≥8 (≥1)	≤0.5	>1	≤0.06
Doripenem	≤0.5	ND (≤1)*	ND (≥4)	≤1	>4	≤0.12

*Approved June 2010; ***E. coli* and *K. pneumoniae;* ND: not defined

EUCAST breakpoints higher than new CLSI breakpoints !

Because most of these carbapenemases confer not resistance but only reduced susceptibility to carbapenems in *Enterobacteriaceae*, they may remain underestimated as a consequence of the lack of their detection.



G. Cornaglia, 2005

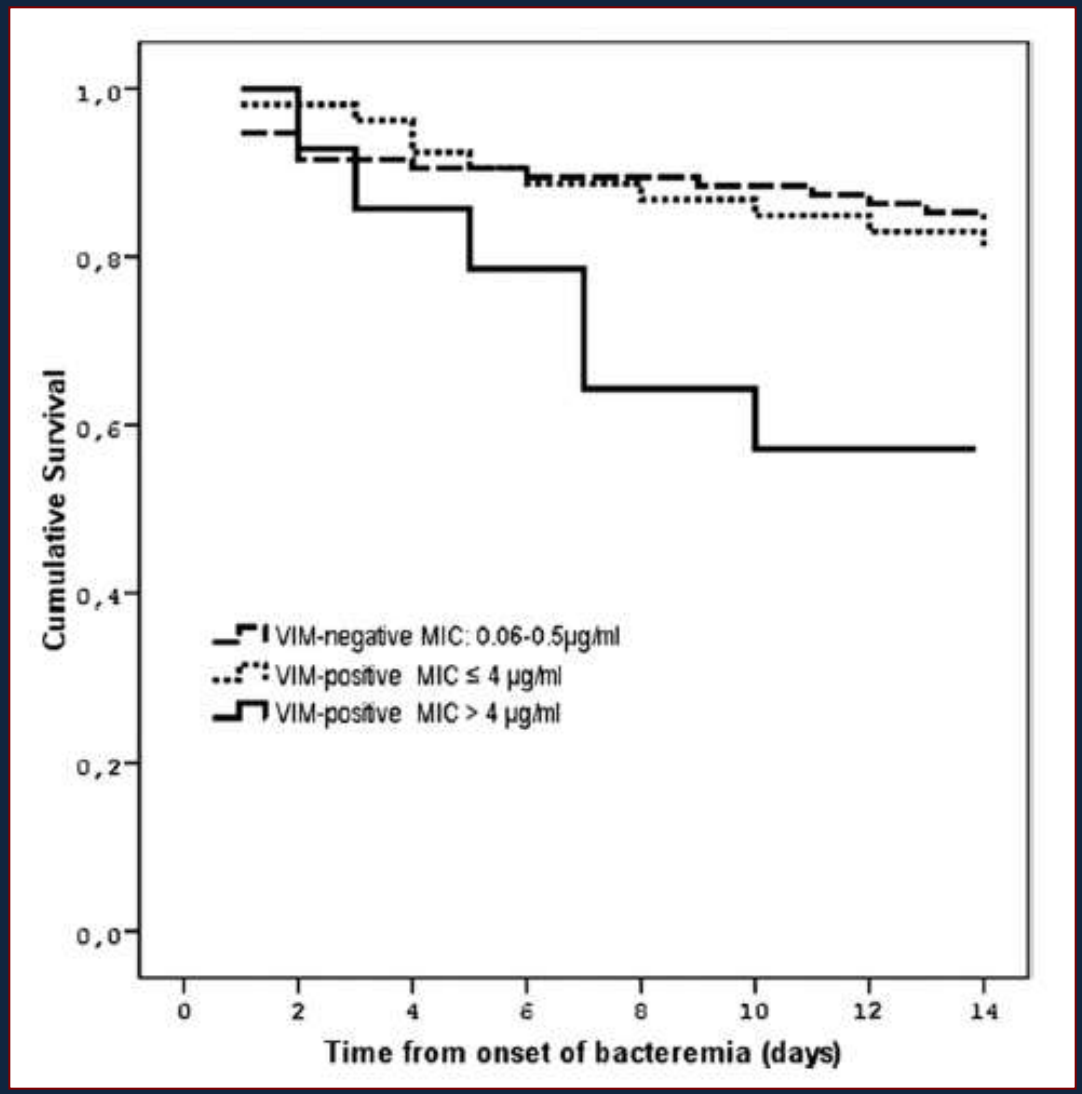
Because most of these carbapenemases confer not resistance but only reduced susceptibility to carbapenems in *Enterobacteriaceae*, they may remain underestimated as a consequence of the lack of their detection.

G. Cornaglia, 2005

Imipenem / meropenem and metalo-β-lactamase (VIM)

Kaplan-Meier curves of survival probability of patients with VIM-producing *K. pneumoniae* bloodstream infections according with susceptibility to carbapenems (either imipenem or meropenem):

Patients infected with a VIM-(+) organism for which the MICs of both imipenem and meropenem were >4 mg/L were more likely to die than those infected with a VIM-(+) carbapenem-susceptible of VIM-negative organisms (P 0.044)



Daikos et al. Antimicrob Agents Chemother 2009; 53: 1868-73

Carbapenemases

- clinical significance

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2. Carbapenemase-producing Enterobacteriaceae

Importance of detection of resistance mechanism	
Required for antimicrobial susceptibility categorization	No
Infection control	Yes
Public health	Yes

Dokazivanje karbapenemaza u enterobakterija

- **Probir**

[1] best balance sens / spec
[2] for OXA larger zones
[3] high sens / low spec

Carbapenem	MIC (mg/L)		Disk diffusion zone diameter (mm)	
	S/I breakpoint	Screening cut-off	S/I breakpoint	Screening cut-off
Meropenem[1]	≤2	>0.125	≥22	<25[2]
Imipenem	≤2	>1	≥22	<23
Ertapenem[3]	≤0.5	>0.125	≥25	<25

- **Potvrda**
  - Dokaz hidrolize karbapenema (MALDI-TOF ili Carba NP test)
  - PCR metode (dokazuju samo ono što se traži)
  - fenotipske metode (double-disk synergy test, combination disk test)

β-lactamase	Synergy observed as increase in meropenem zone diameter (mm) with 10µg disk				Temocillin MIC > 32 mg/L or DD ≤11mm
	DPA/EDTA	APBA/PBA	DPA+APBA	CLX	
MBL	≥5	-	-	-	NA[1]
KPC	-	≥4	-	-	NA[1]
MBL+KPC[2]	Variable	Variable	≥5	-	NA[1]
OXA-48-like[3]	-	-	-	-	Yes
AmpC + porin loss	-	≥4	-	≥5	NA[1]
ESBL + porin loss	-	-	-	-	No

MBL

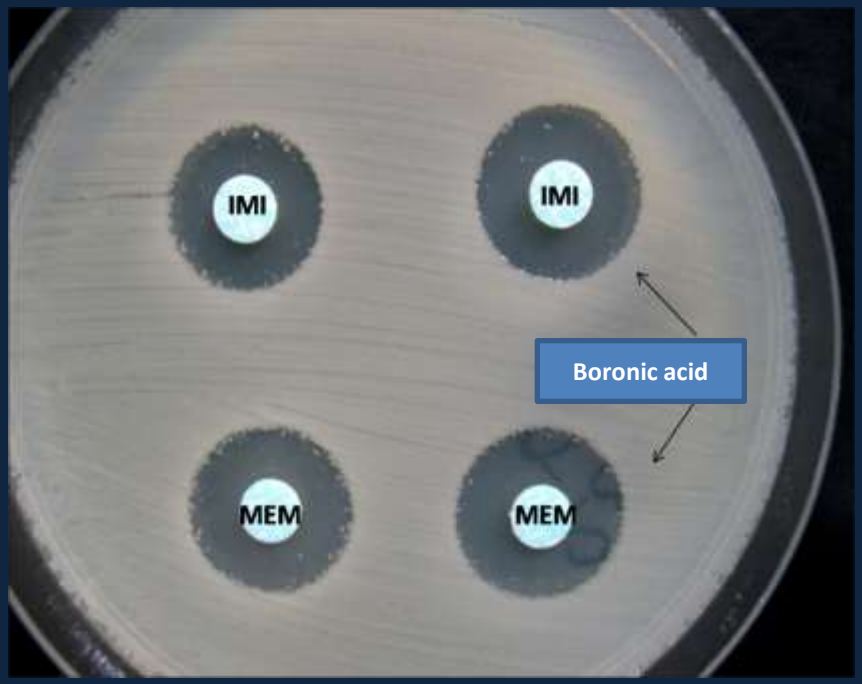
double disk synergy test, DDST

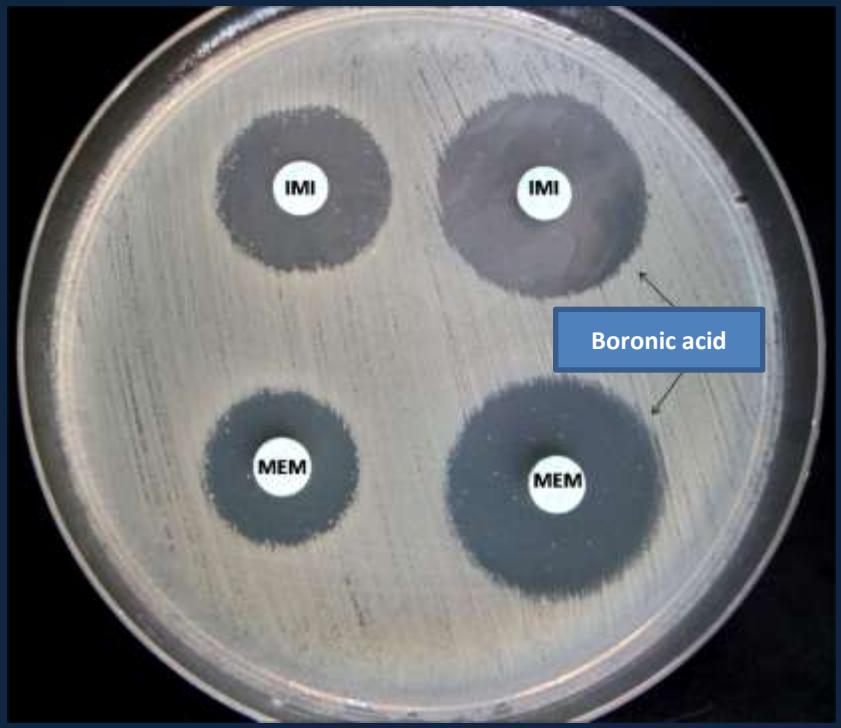
KPC

double disk synergy test, DDST

KPC

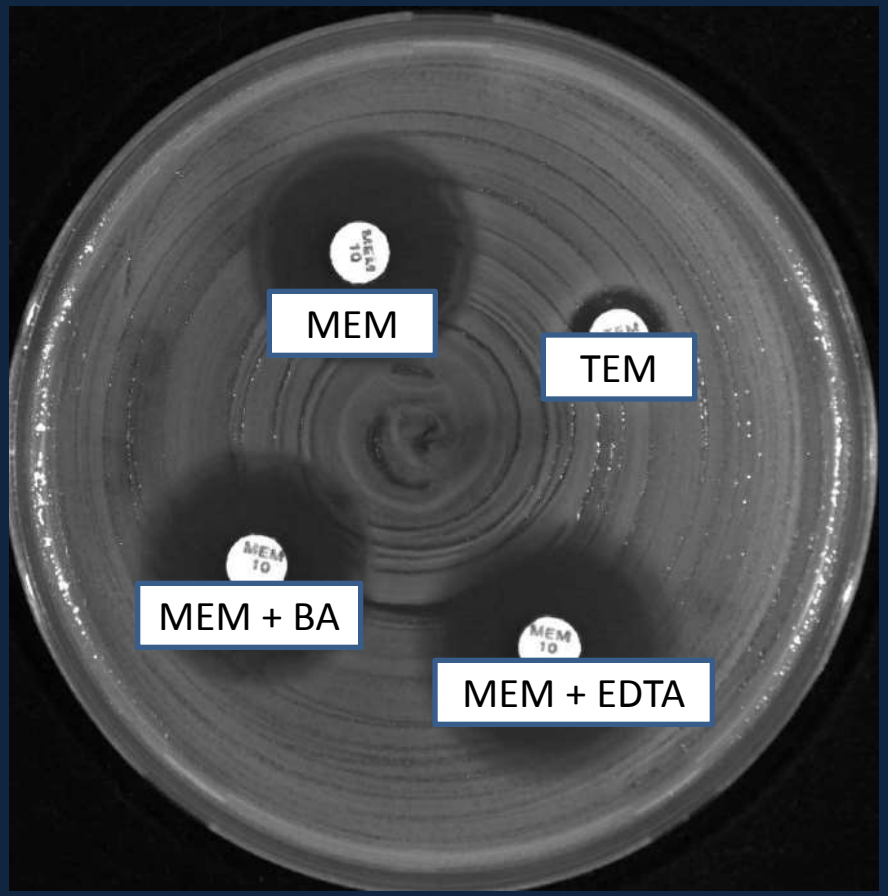
combination disk test, CDT

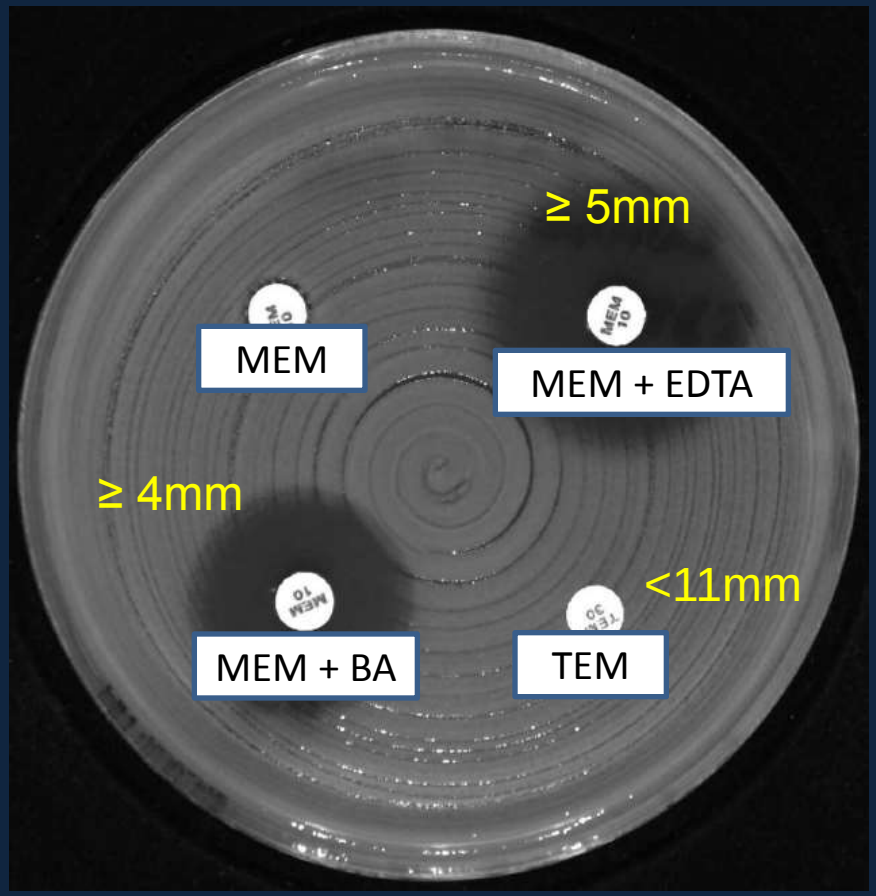




CARBAPENEMASE(S)

combination disk test, CDT



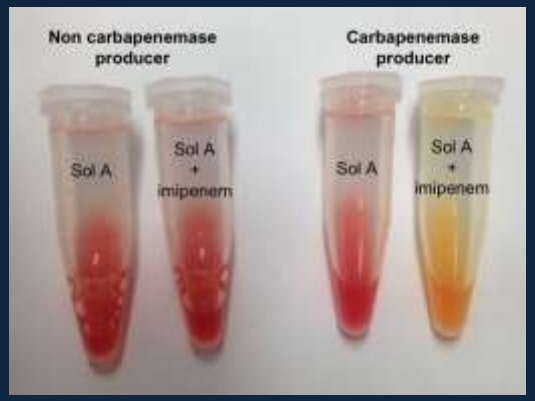


MBL negative: MEM+EDTA - MEM <5mm
KPC negative: MEM+boronic acid - MEM <4mm
OXA-48 negative: TEMOCILLIN >11mm

MBL positive: MEM+EDTA - MEM ≥5mm
KPC positive: MEM+boronic acid - MEM ≥4mm
OXA-48 positive: TEMOCILLIN < 11mm

CARBA NP test*

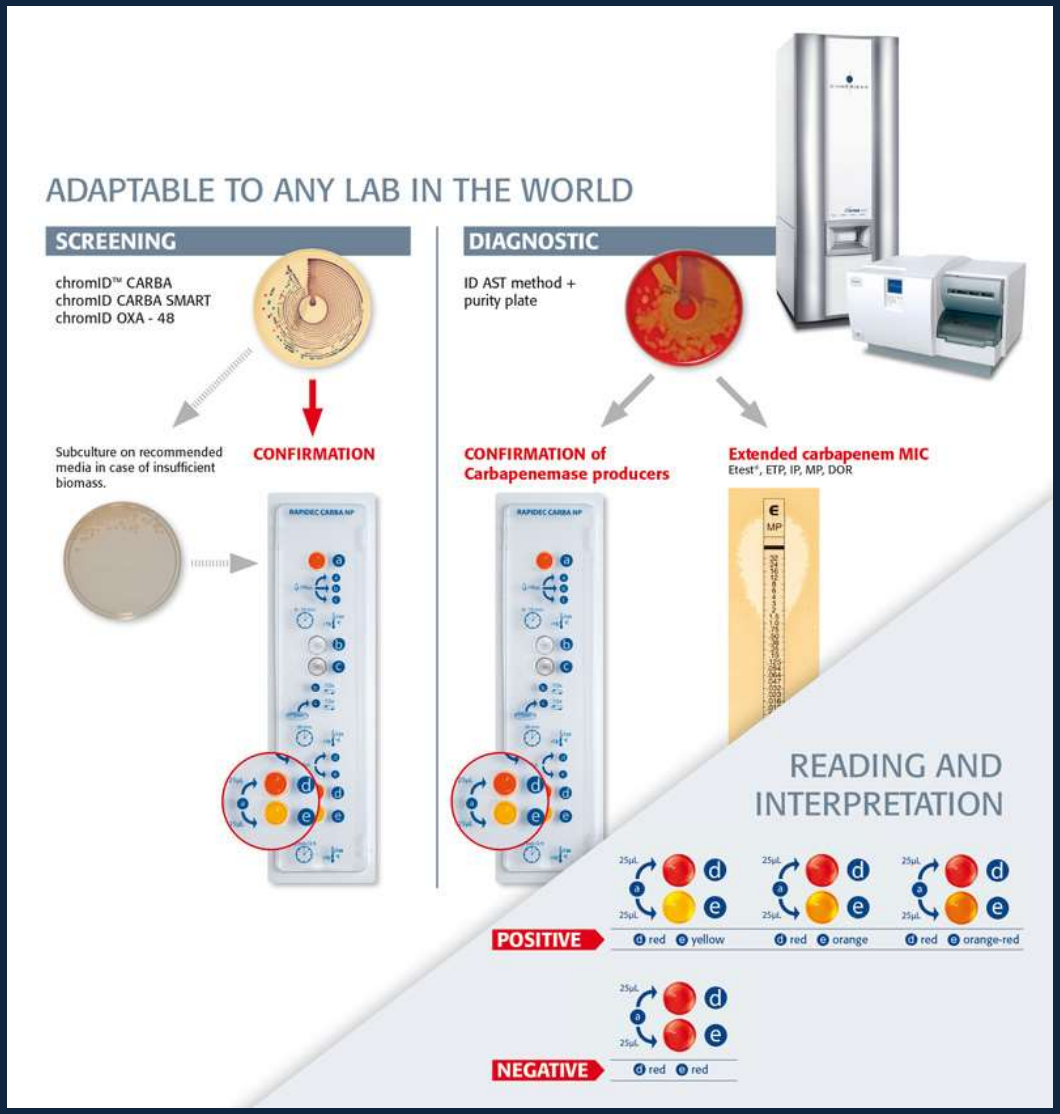
- Brzo dokazivanje karbapenemaza u enterobakterija
- Zasniva se na hidrolizi imipenema
- Visoka osjetljivost i specifičnost
- Niska cijena, prilagođen rutinskoj uporabi
- Rezultati u max 2h
- Rana adekvatna terapija i primjena mjera kontrole bolničkih infekcija



	No antibiotic (tube A)	Imipenem (tube B)	Imipenem + tazobatam (tube C)	Imipenem + EDTA (tube D)
No carbapenemase	Red	Red	Red	Red
Ambler class A carbapenemase	Red	Orange/Yellow	Red	Orange/Yellow
Ambler class B carbapenemase	Red	Orange/Yellow	Orange/Yellow	Red
Ambler class D carbapenemase	Red	Orange/Yellow	Orange/Yellow	Orange/Yellow
No interpretable	Yellow	Yellow	Yellow	Yellow

* **Nordmann P** *et al.* Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2012; 18: 1503-7.

CARBA NP test

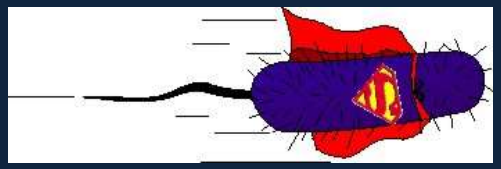


1) **Phenol red**: pH indicator

2) A **carbapenem**: imipenem (carbapenemase substrate) **+ Zinc**, required for the detection of metallodependent carbapenemase-producing strains

MDR
gram-negativne bakterije
kontrola bolničkih infekcija

- Standardne mjere predostrožnosti
- Kontaktna izolacija
- Poslati izolat u Referentni Centar
- Lokalno i nacionalno praćenje rezistencije



KPC *K. pneumoniae*

Ministry of Health

ISKRA
INTERDISCIPLINARNA SEKCIJA ZA KONTROLU REZISTENCIJE NA ANTIBIOTIKE

IC Committee, MH

AMR
RC

IC
RC

Manager
Microbiologist
IC Team

Odbor za praćenje rezistencije bakterija na antibiotike

Akademija medicinskih znanosti Hrvatske

- **Odbor za praćenje rezistencije bakterija na antibiotike** osnovan 1996.g. pri Akademiji medicinskih znanosti Hrvatske
- Na početku 17 laboratorija pozvano na sudjelovanje u praćenju / voditelji laboratorija / infektolozi / klinički farmakolozi
- Danas u radu Odbora sudjeluju 34 mikrobiološka laboratorija
- pokrivenost podacima >90%

Odbor za praćenje rezistencije bakterija na antibiotike

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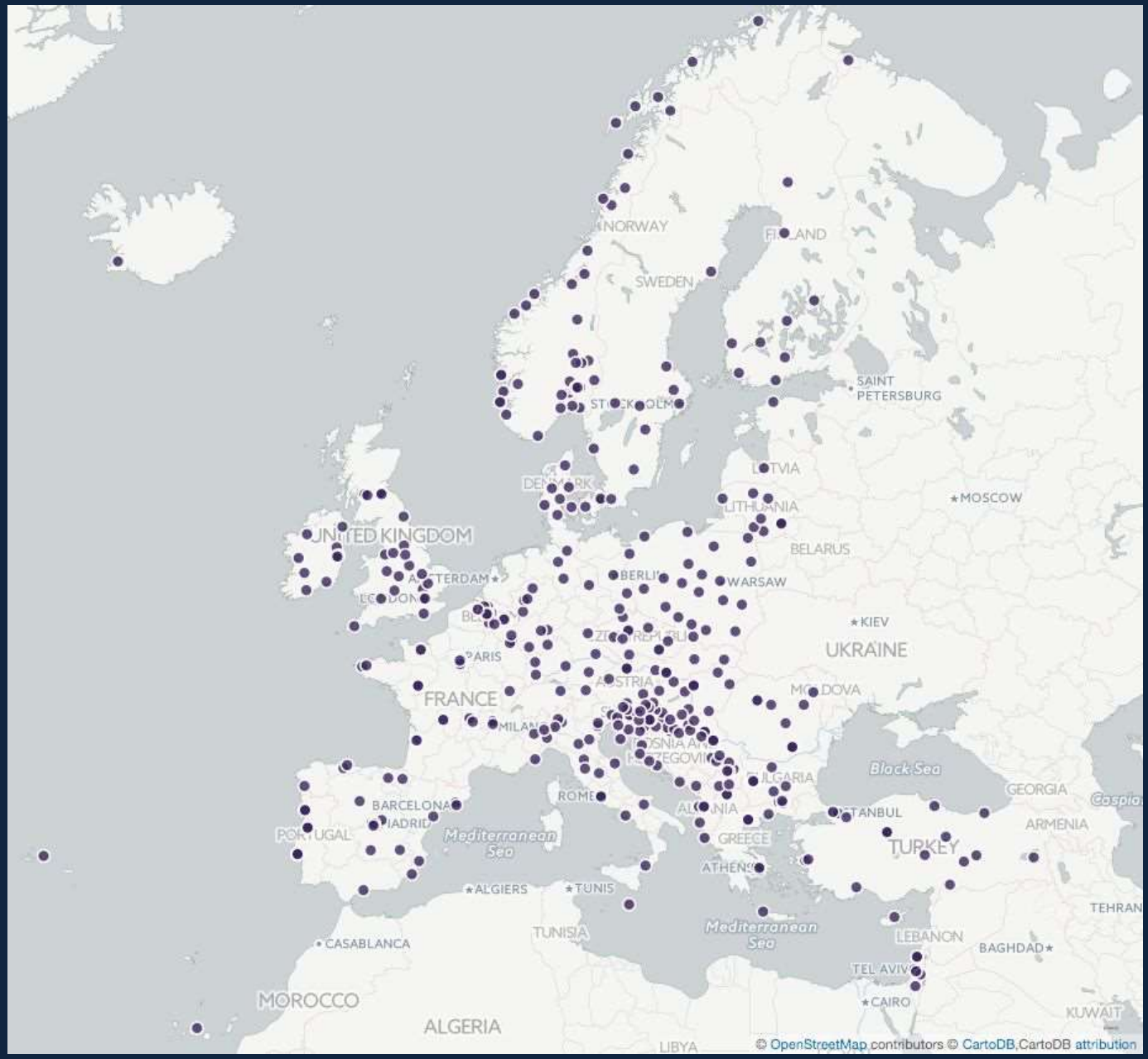
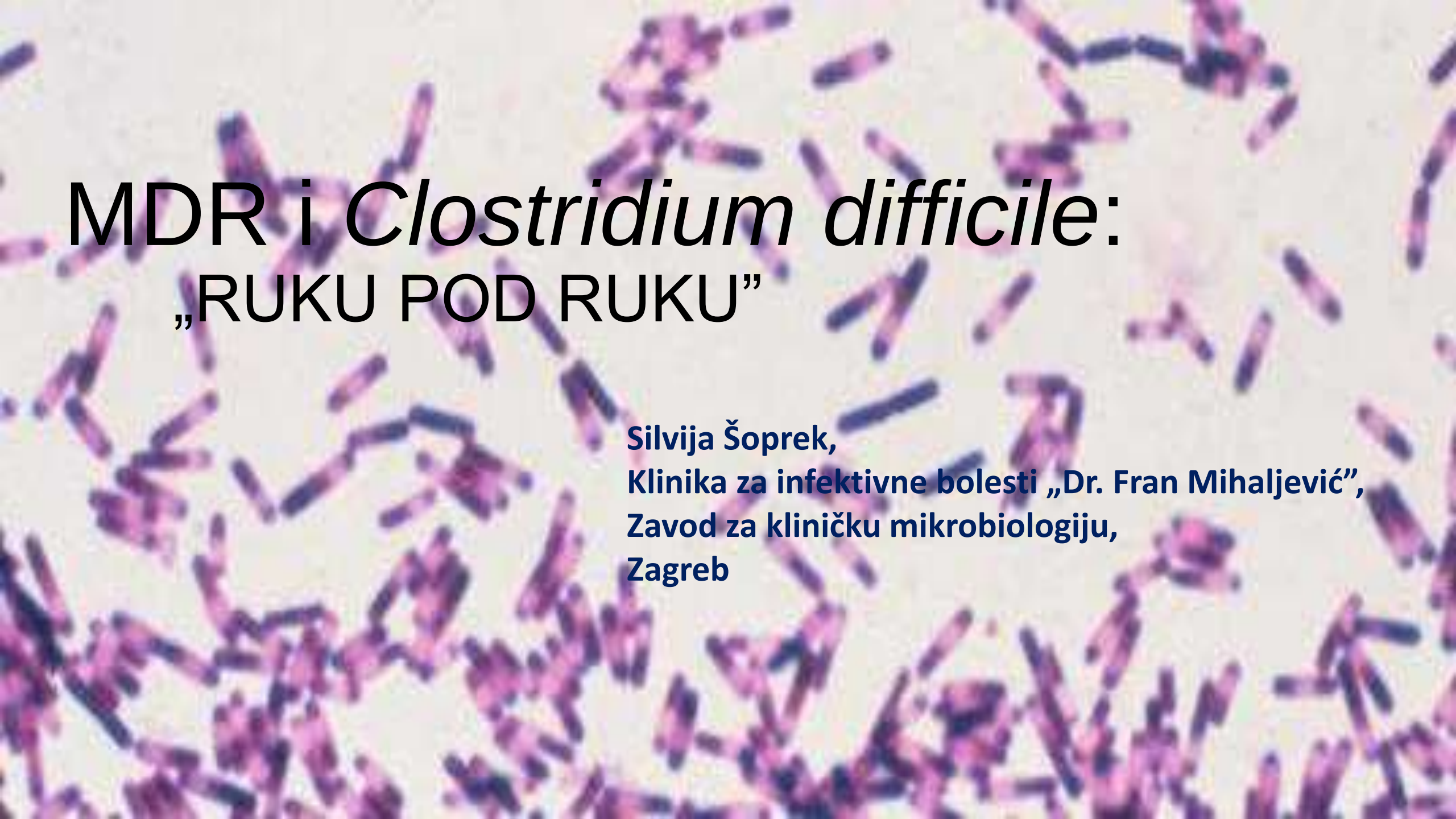
- Danas u radu Odbora sudjeluju 34 mikrobiološka laboratorija
- pokrivenost podacima >90%

Map of Europe displaying the locations of the sentinel hospitals of the EuSCAPE project





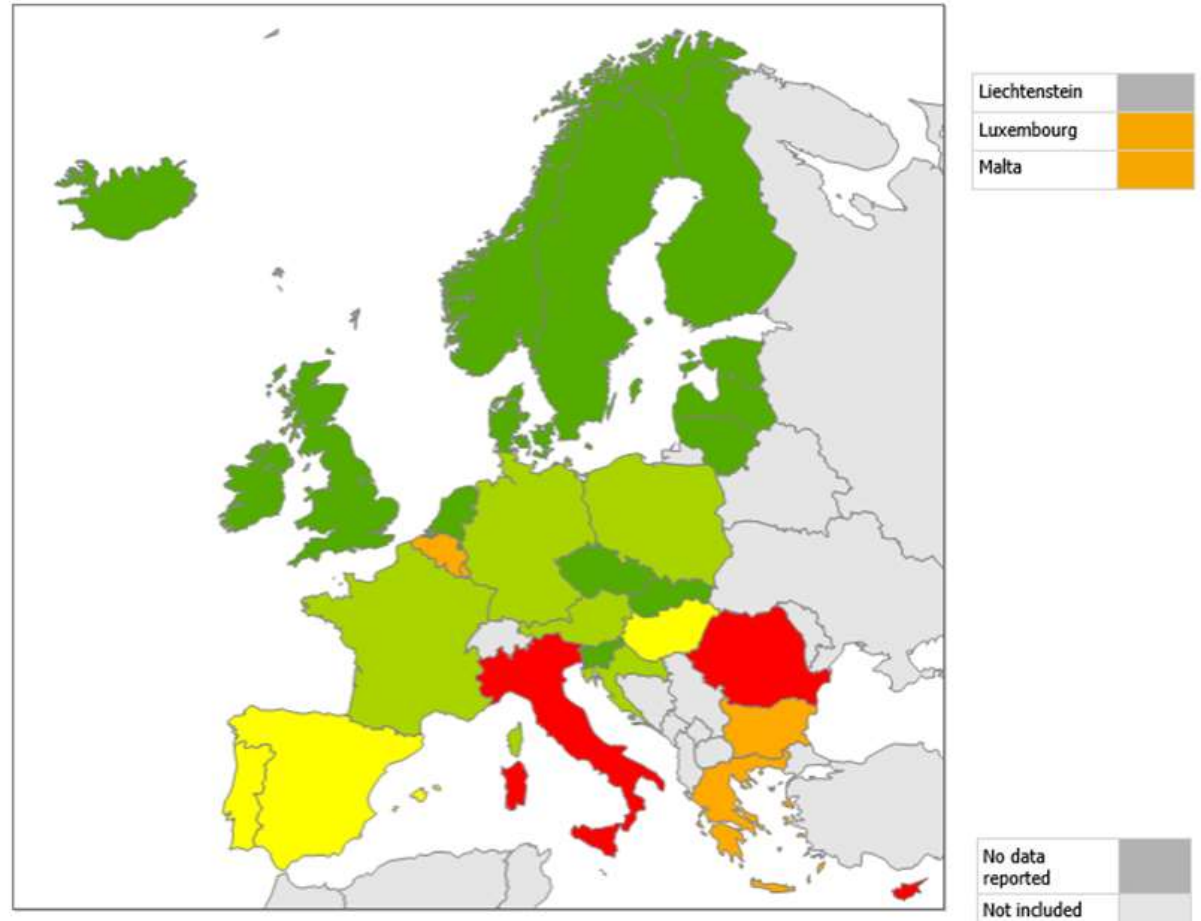
MDR i *Clostridium difficile*:
„RUKU POD RUKU”

Silvija Šoprek,
Klinika za infektivne bolesti „Dr. Fran Mihaljević”,
Zavod za kliničku mikrobiologiju,
Zagreb

Geographical distribution of the consumptio Third-Generation Cephalosporins (ATC grou J01DD) in the community (primary care sec Europe, reporting year 2014

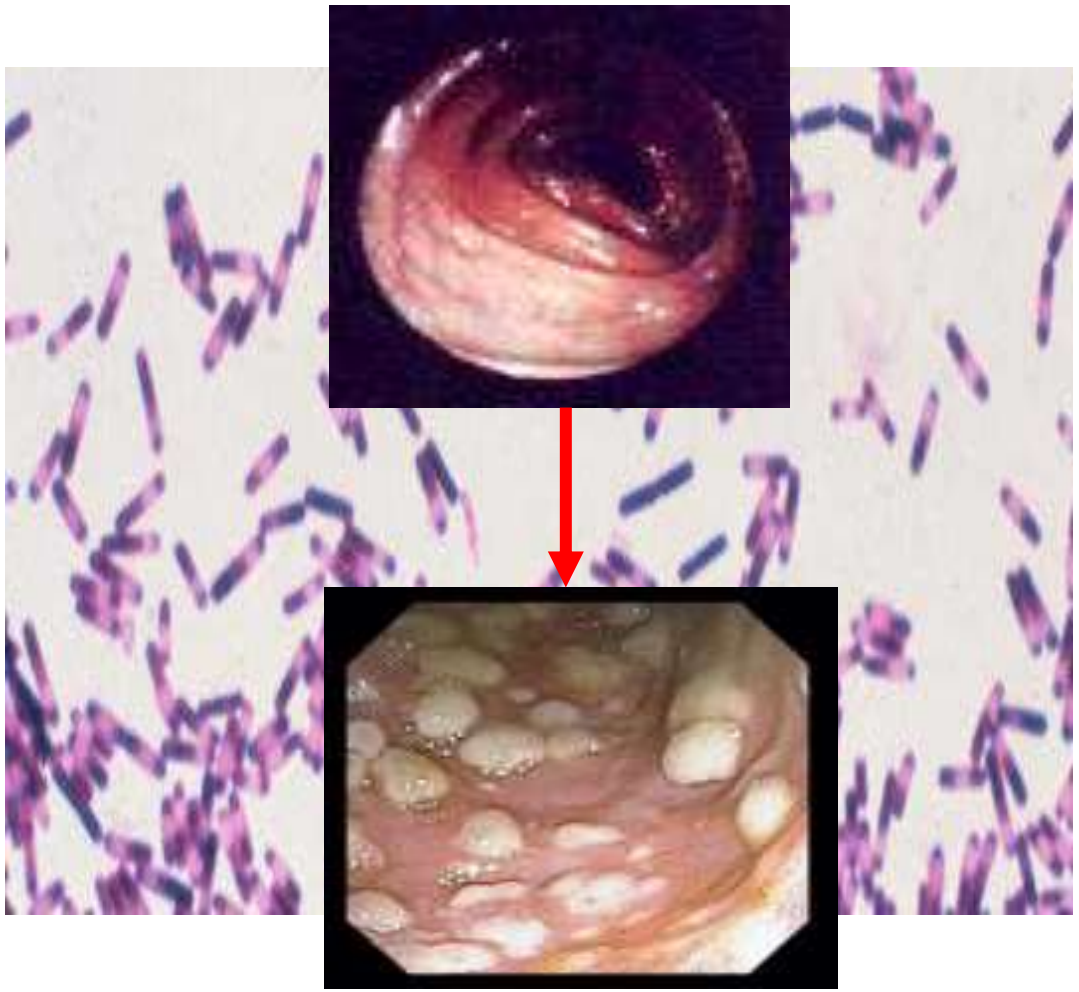
Geographical distribution of the consumption of Quinolone Antibacterials (ATC group J01M) in the community (primary care sector) in Europe, reporting year 2014

Consumption of Quinolone Antibacterials (ATC group J01M) in the community (primary care sector) in Europe, reporting year 2014



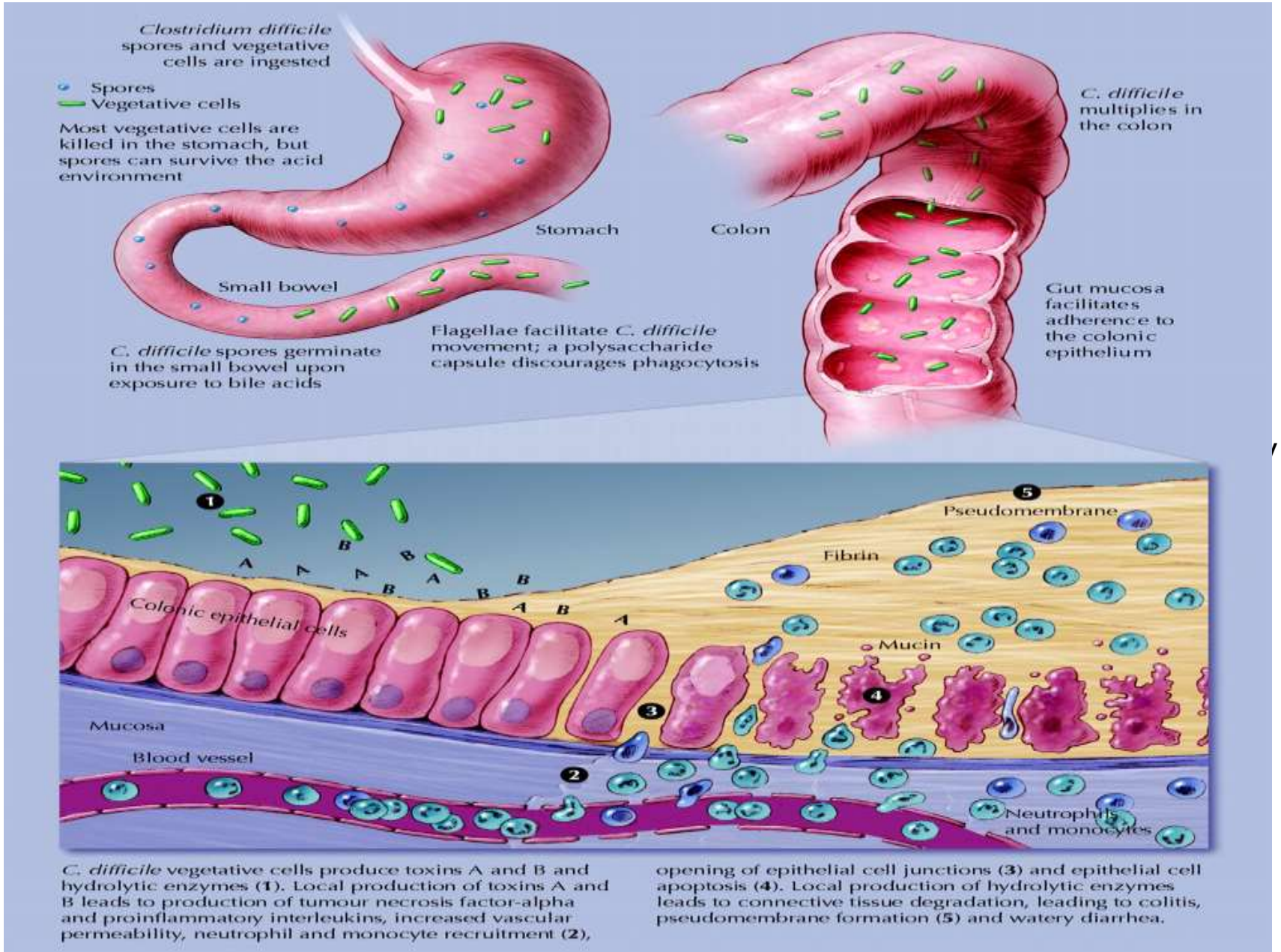
C.difficile-činjenice...

- Sporogeni, anaerobni, gram-pozitivni štapić
- 2% kliconoša u općoj populaciji (novorođenčad čak do 85%)*
- *Clostridium difficile*-associated disease (CDAD)
  - Blaga klinička slika
  - Pseudomembranozni kolitis, toksični megakolon, smrt
- Prijenos
  - Fekalno oralnim putem
  - Direktni / indirektni kontakt
  - Zoonoza?
  - Hrana/voda?
- Nozokomijalna infekcija!!!
- Rizični čimbenici
  - Izlaganje samom mikroorganizmu
  - Izlaganje antibioticima – glavni rizik oboljevanja
    - Supresija fiziološke mikrobiote crijeva
    - Akviriranje i rast *C. difficile*
    - Klindamicin, penicilini, cefalosporini (II, III), KINOLONI
  - Ostali čimbenici rizika
    - Starija životna dob
    - Produljeni boravak u bolnici
    - Težina osnovne bolesti
    - Antacidi ?



*Surawicz CM, Brandt LJ, Binion DG et al. Guidelines for Diagnosis, Treatment, and Prevention of Clostridium difficile Infections. Am. J. Gastroenterol. 108(4), 478–498 (2013).

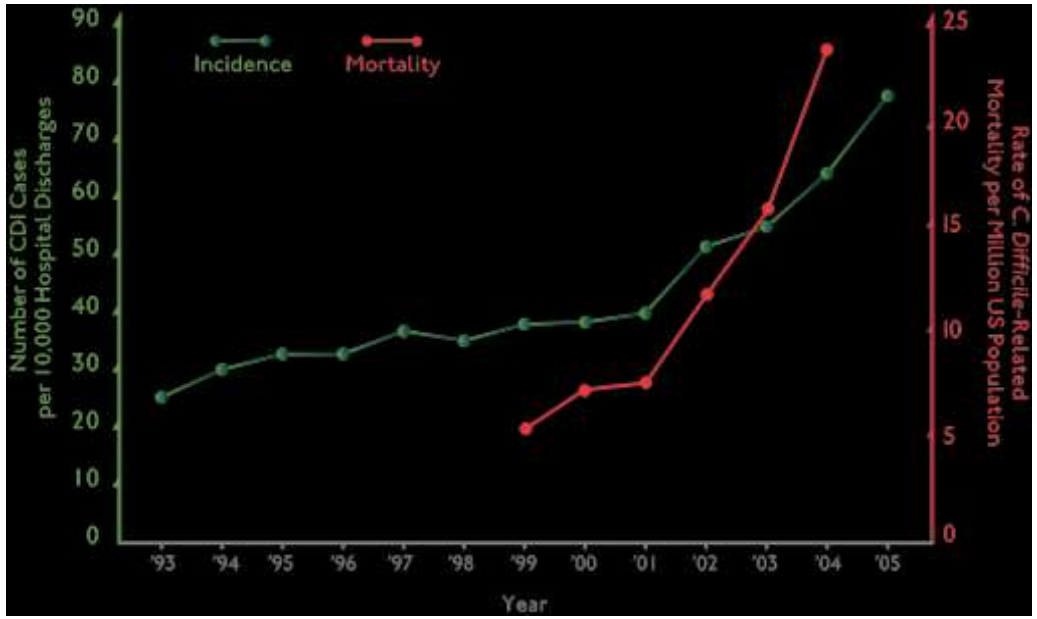
Patogeneza…

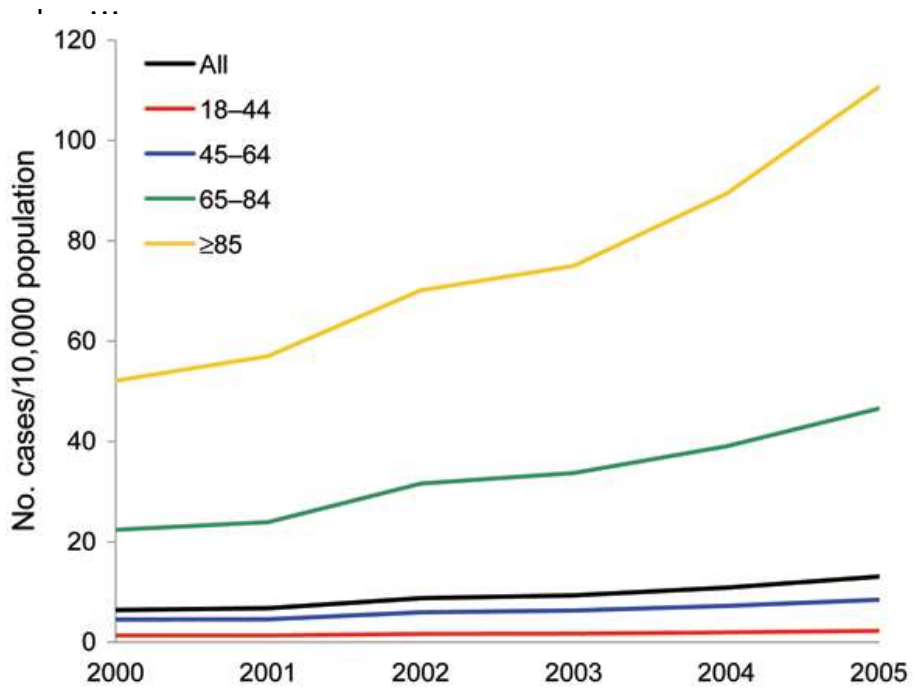


C. difficile vegetative cells produce toxins A and B and hydrolytic enzymes (**1**). Local production of toxins A and B leads to production of tumour necrosis factor-alpha and proinflammatory interleukins, increased vascular permeability, neutrophil and monocyte recruitment (**2**), opening of epithelial cell junctions (**3**) and epithelial cell apoptosis (**4**). Local production of hydrolytic enzymes leads to connective tissue degradation, leading to colitis, pseudomembrane formation (**5**) and watery diarrhea.

„NOVO LICE”...

- Clostridium difficile associated disease (CDAD)-životno ugrožavajuća bolest
  -pridružen 30dnevni mortalitet 14% do 30%
- Povećana incidencija/teže kliničke slike povezane s pojavom i širenjem opasnijih epidemijskih sojeva (PCR ribotipovi 027 & 078)
- Broj hospitalizacija zbog CDAD povećan 3 PUTA, a stope mortaliteta 400% (zadnjih 10 godina)
- Pojava recidiva u **12-24%** pacijenata
- Stariji i imunokompromitirani pacijenti-povećan rizik za razvijanje komplikacija
- Kliconoštvo s toksigenim sojevima *C difficile* u stolici varira- 2% u općoj po
  -7–15% kod pac
  -85% novorođe





„NOVO LICE”…epidemiološka perspektiva

- Promjene u osjetljivosti domaćina
  - Starija životna dob
  - Imunokompromitirani pacijenti
- Promjene u propisivanju antibiotika
  - Povećana uporaba kinolona
- Novi soj povećane virulencije
  - NAP1 / BI / toxinotype III / ribotype 027
- Promjene u mjerama kontrole bolničkih infekcija
  - Uvođenje alkoholnih preparata

- Seli se u izvanbolničku sredinu
- Zahvaća atipičnu populaciju -djeca/trudnice
  -osobe bez prijašnjeg kontakta s bolničkom okolinom
  -bez prijašnjeg konzumiranja antibiotika i ostalih predisponirajućih čimbenika
- Teže kliničke slike/češći relapsi/povećana smrtnost
- Teže CDAD povezane s pojavom patogenijih epidemijskih sojeva (PCR ribotipovi 027 i 078)
- *C. difficile* ribotip 027 izazvao epidemije u skoro svim zemljama Europe
- Bolničke epidemije velikih razmjera
  - Prva izvješća: Kanada, USA, 2003
  - Kanada, USA, Japan, Europa
  - Bolnice, domovi za starije osobe
- Do 2007 O27 soj zabilježen samo u USA i Europi
- 2008 interkontinentalno širenje!!!

“Lethal hospital bug cases rocket”
Velika Britanija

- Potencijalno letalni slučajevi *C. difficile* infekcija “rocketed” od 1990tih do 2004
- Slučajevi porasli od 1,000 u 1990.g. Na preko 35,000 u 2003.g.
- 44,488 slučajeva *C. difficile* infekcije u osoba ≥ 65 g. U 2004.

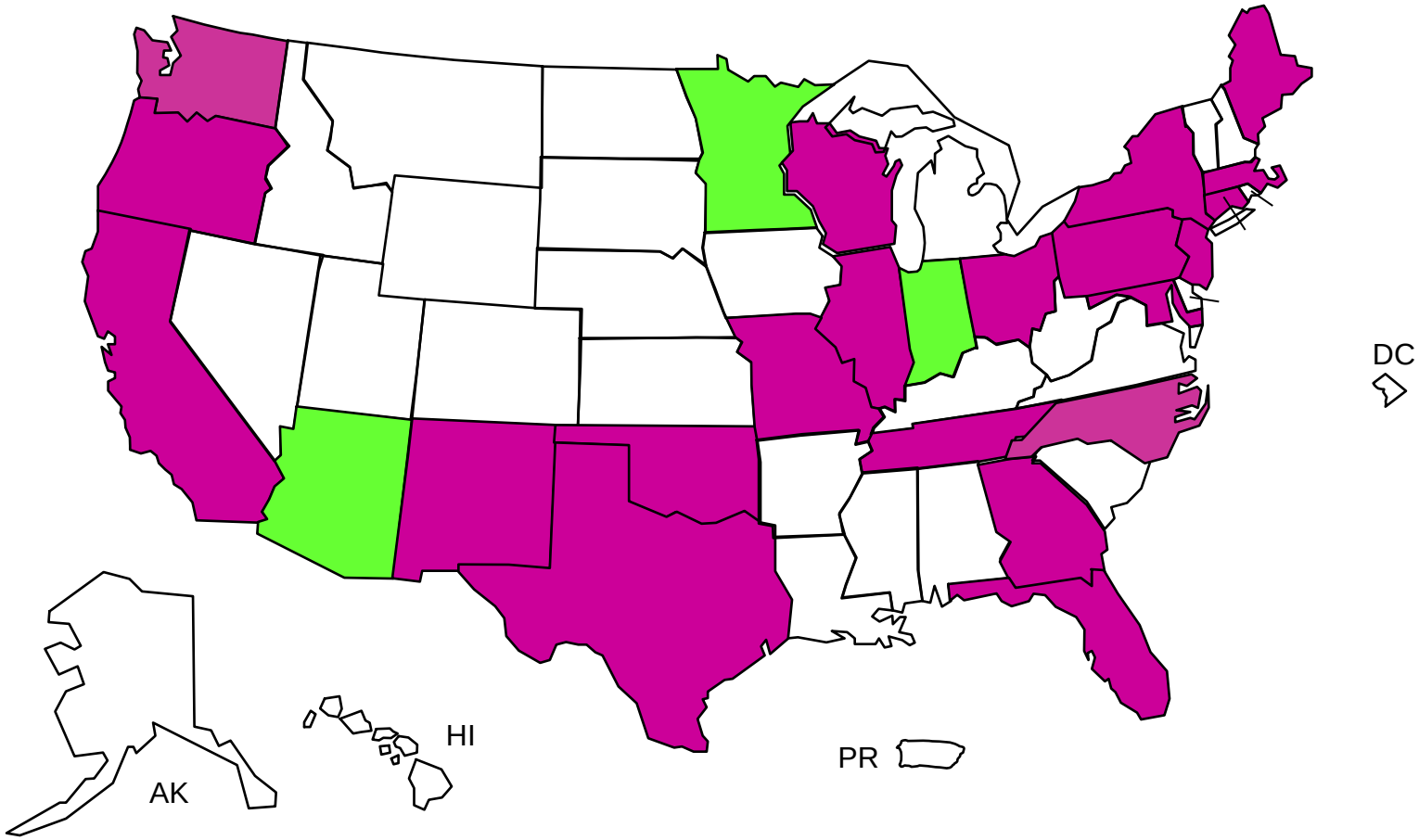
BBC News. http://news.bbc.co.uk/2/hi/health/4186834.stm

BI/NAP1 u Nizozemskoj, 2006

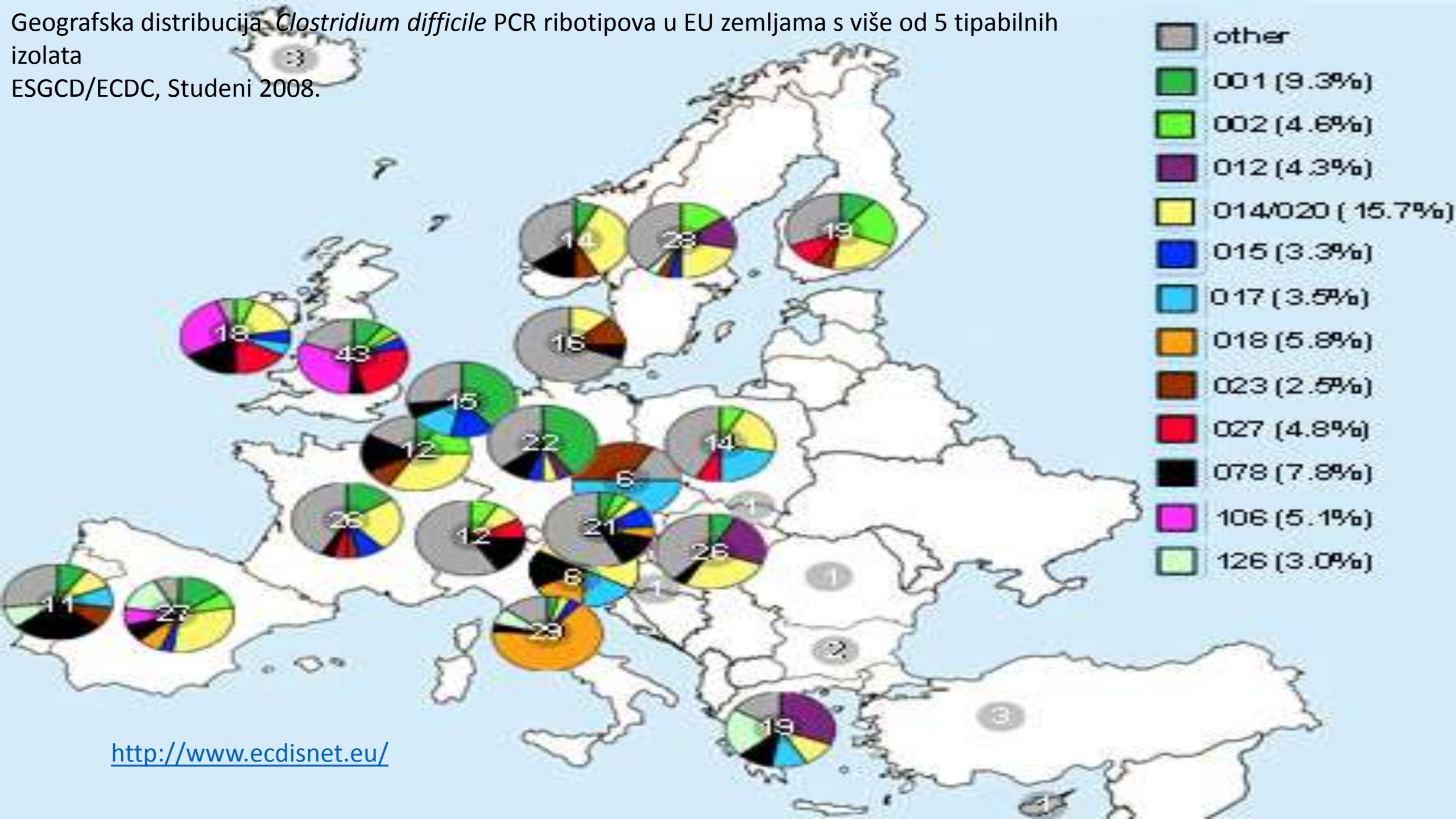
Kuiper EJ et al. Emerg Infect Dis 2006;12(5):827-830.

Amerika: epidemijski soj *C. difficile*

Potvrđeno-CDC u Hines VA laboratoriju (N=23),
2/9/2007



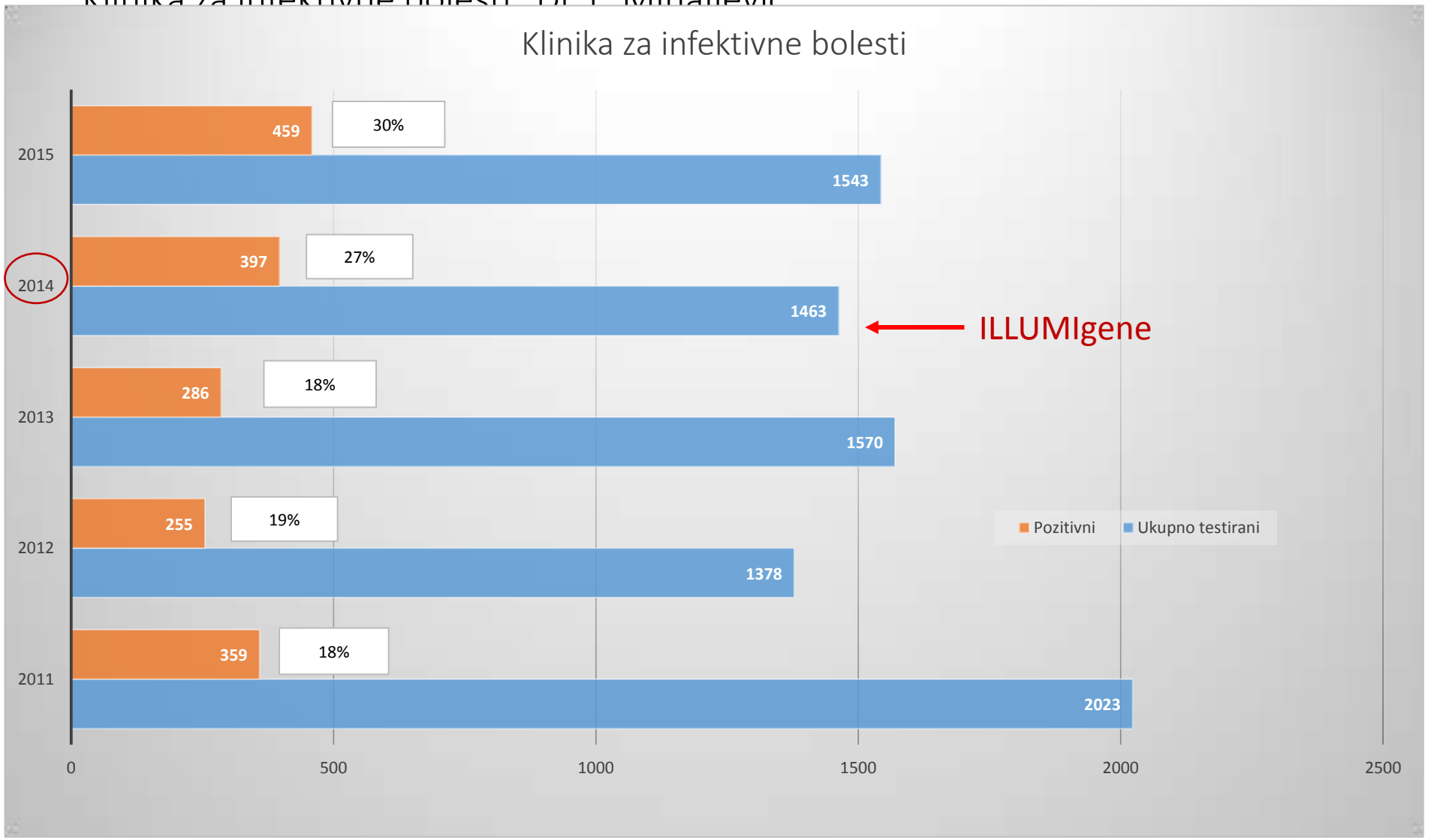
Geografska distribucija *Clostridium difficile* PCR ribotipova u EU zemljama s više od 5 tipabilnih izolata
ESGCD/ECDC, Studeni 2008.



http://www.ecdisnet.eu/

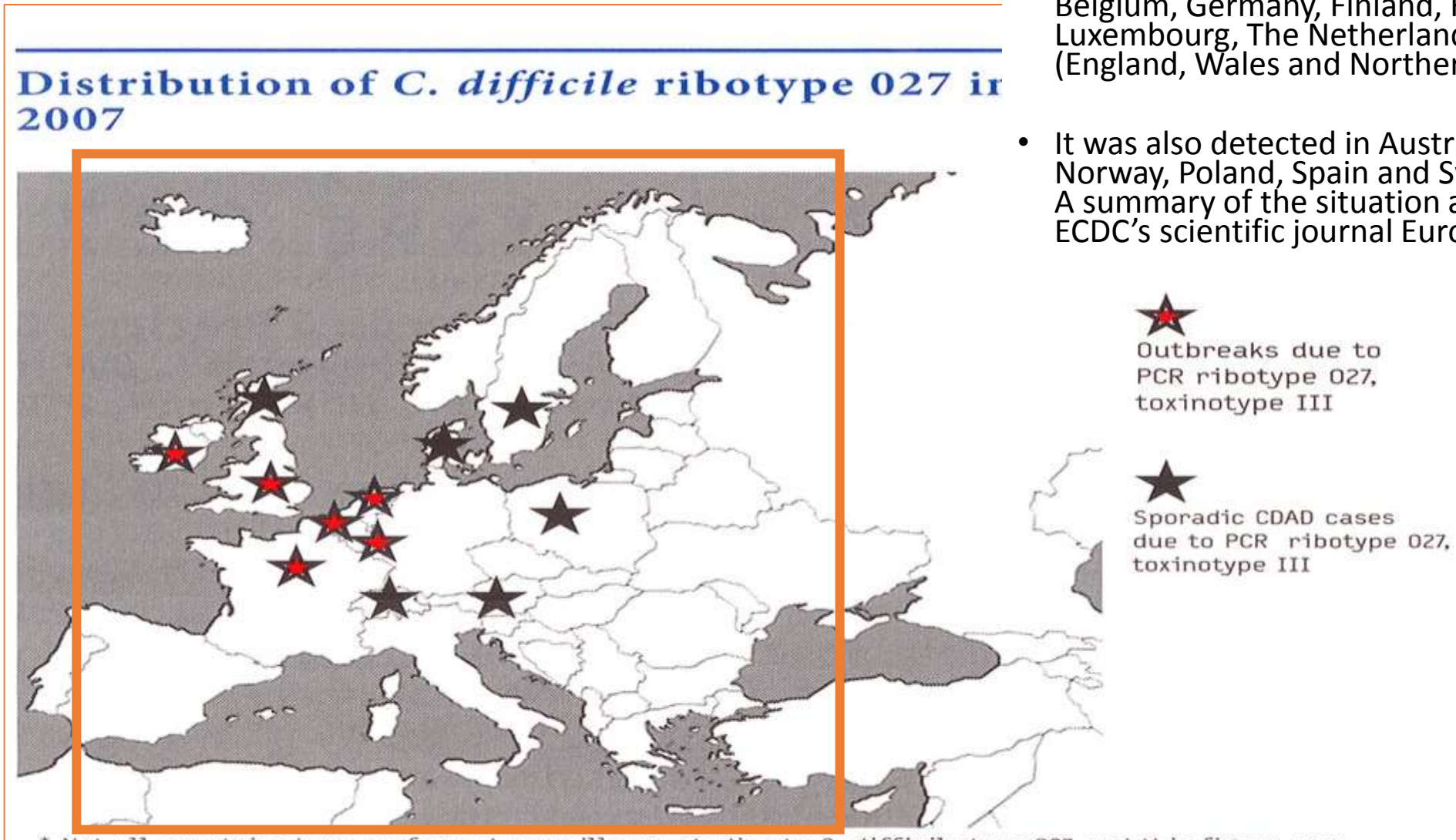
Clostridium difficile

Klinika za infektivne bolesti "Dr. F. Mihaljević"



ECDC izvješće

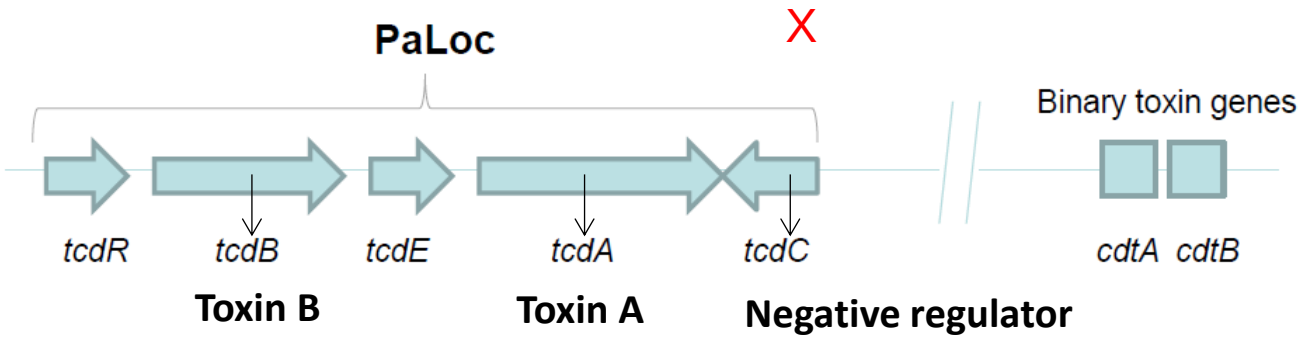
- As of June 2008, C. difficile PCR ribotype 027 had been reported by healthcare facilities in 16 European countries.
- Outbreaks were reported in 9 countries including Belgium, Germany, Finland, France, Ireland, Luxembourg, The Netherlands, Switzerland and the UK (England, Wales and Northern Ireland).
- It was also detected in Austria, Denmark, Hungary, Norway, Poland, Spain and Sweden, and in Scotland. A summary of the situation across Europe is published in ECDC's scientific journal Eurosurveillance



Hipervirulentni epidemijski sojevi

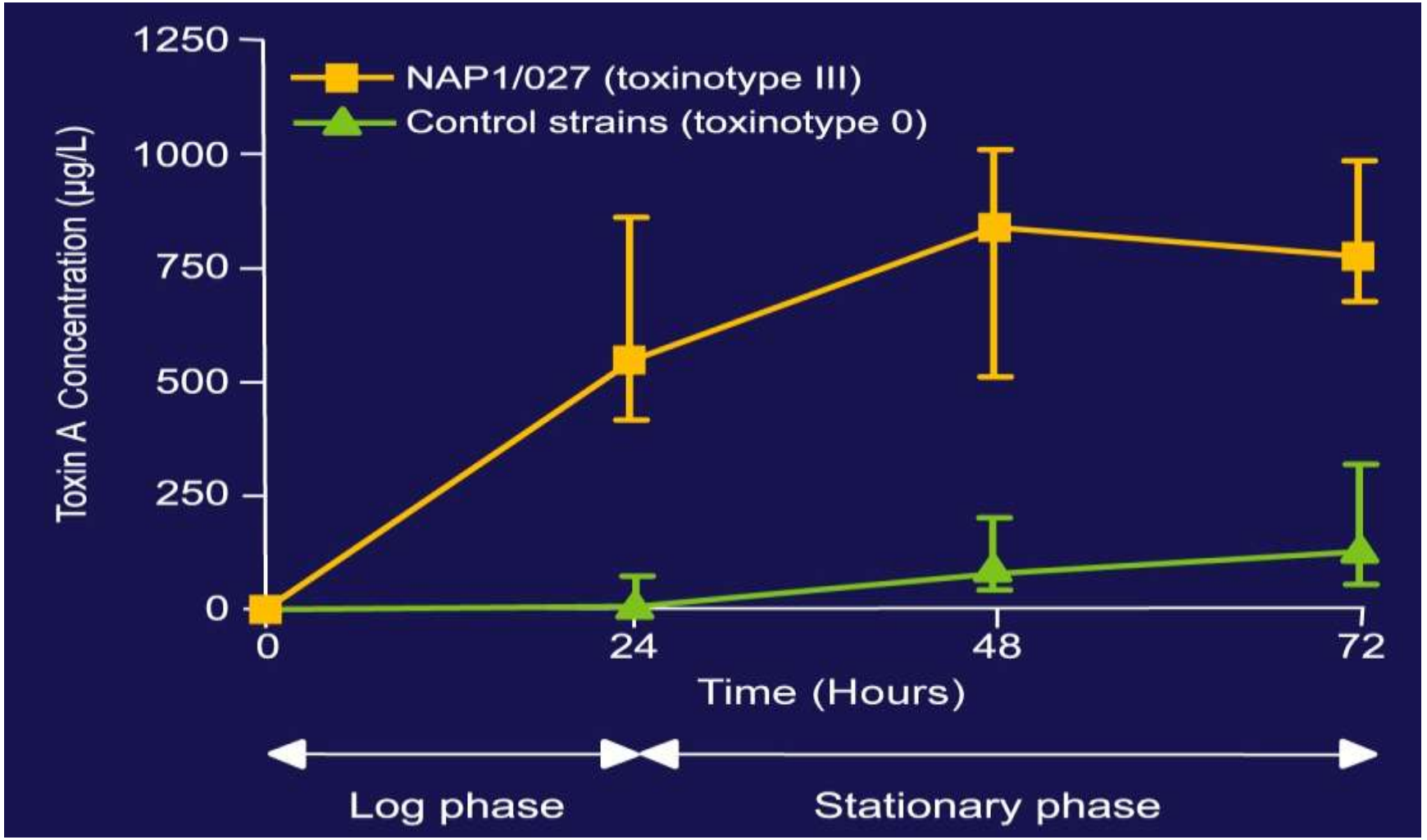
Različiti od “J” epidemijskog soja / 1989-1992 Johnson S, et al. *N Engl J Med.* 1999;341:1645-1651

- Sadrže delecije tcdC gena
- Dokazana povećana produkcija toksina A i toksina B *in vitro* Warny M, et al. *Lancet*. 2005;366:1079-1084.
- Visoka rezistencija na fluorokinolone
- Povećana sposobnost sporulacije
- Produkcija binarnog toksina



Microbiology and molecular biology reviews, USA
Journals ASMorg, Jonathan .J Hunt, Jimmy D. Ballard, Deceber 2013, Volume 77, No.4

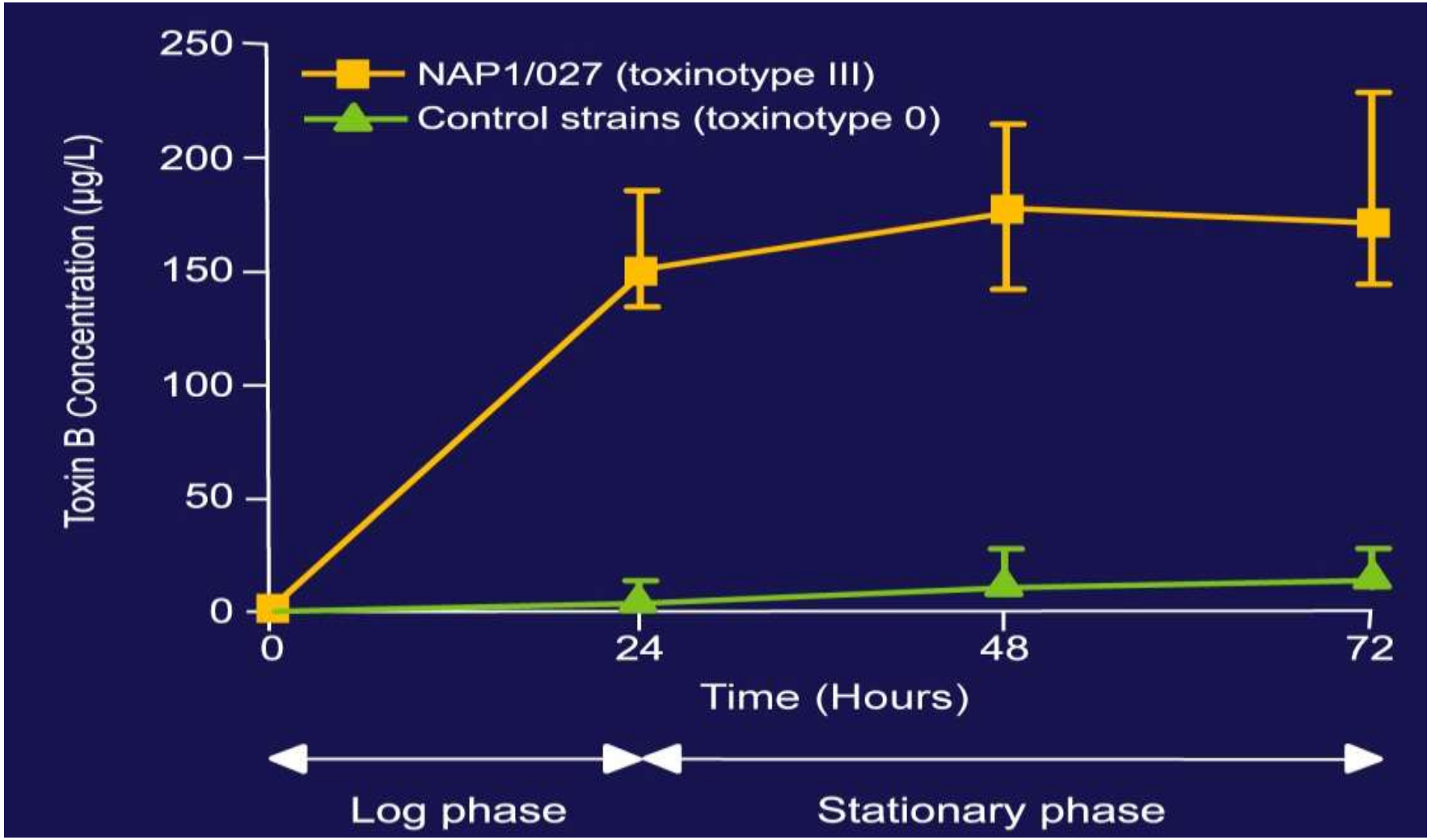
Povećana produkcija Toksin A *in vitro*



***In vitro* production of toxins A and B by *C. difficile* isolates. Median concentration and IQRs are shown. *C. difficile* strains included 25 toxinotype 0 and 15 NAP1/027 strains (toxinotype III) from various locations.**

From Warny M, et al. *Lancet.* 2005;366:1079-1084.

Povećana produkcija Toxin B *in vitro*



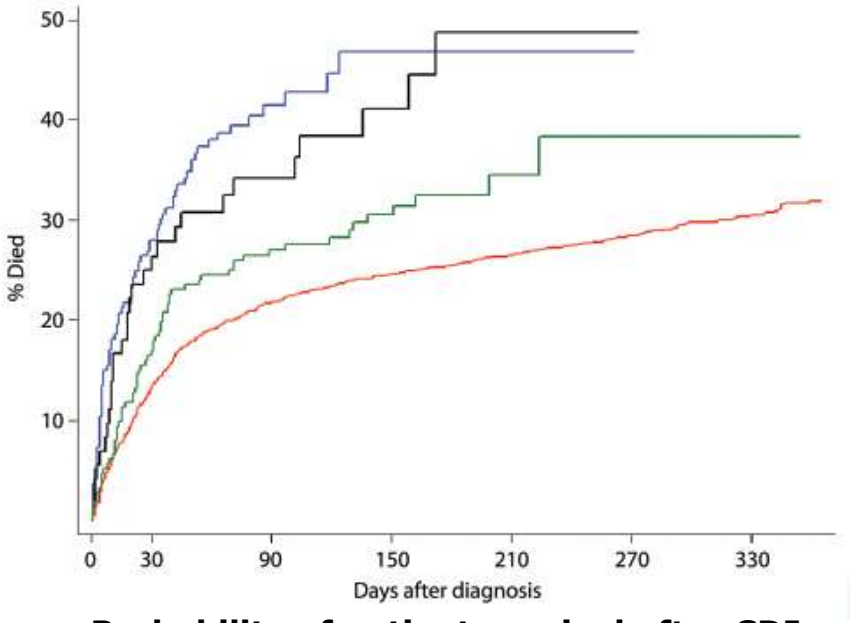
***In vitro* production of toxins A and B by *C. difficile* isolates. Median concentration and IQRs are shown. *C. difficile* strains included 25 toxinotype 0 and 15 NAP1/027 strains (toxinotype III) from various locations.**

From Warny M, et al. *Lancet.* 2005;366:1079-1084.

- **Binarni toksin:** Mortalitet i rizik za recidiv

- **Binarni toksin**

-marker virulentnosti *C. difficile* soja/
-direktan čimbenik virulecije



Probability of patient survival after CDI diagnosis in 4 different groups

- ***C. difficile* PCR ribotype 027**
- **Binary toxin positiv, not 027**
- **Toxin A+B+, binary toxin negativ**
- **unselected strains not referred for typing**

Bacci et al., Emerging Infectious Diseases Vol. 17, No. 6, June 2011

- Prisutnost gena za binarni toksin je nezavisan čimbenik virulencije povezan s pojavom **recidiva.**

Virulence factor	Incidence within study population (n=69)	Association with recurrence (p value)	Association with admission for RCDC (p value)
Toxin A	61 (88 %)	0.56	0.78
Toxin B	66 (96 %)	0.73	0.60
Binary toxin gene (CDT)	42 (61 %)	0.02	0.02
tcdC mutation	39 (56 %)	0.18	0.04
Ribotype 027	26 (38 %)	0.32	0.02

- **The presence of a binary toxin gene was the single virulence factor independently associated with recurrence (p00.02).**
- **The combination of a tcdC mutation with binary toxin gene resulted in the highest odds of recurrence (OR, 5.3; 95 % CI, 3.52–6.09).**
- ***C. difficile* isolates which produce binary toxin may require longer antibiotic regimens.**

Stewart et al., J Gastrointest Surg, DOI 10.1007/s11605-012-2056-6

Binary toxin

K-158

Incidence and Clinical Impact of Binary Toxin in Hospitalized Patients with *Clostridium difficile* Infection

W. Wei[1], J. O. Ikwuagwu[1], M. J. Alam[2], D. N. Shah[2], W. L. Musick[1], K. W. Garey[2];
[1]The Methodist Hosp., Houston, TX, [2]Univ. of Houston Coll. of Pharmacy, Houston, TX

Background: The purpose of this study was to assess the incidence of the binary toxin in hospitalized patients with CDI and to assess clinical outcomes stratified by binary toxin.

Results: A total of 185 patients were assessed of whom 49 (26.5%) were positive for binary toxin. The majority (65%) were initially treated with metronidazole.
Patients infected with binary toxin C. difficile strains were more likely to experience treatment failure (40.8% vs. 22%), requirement for colectomy (6% vs. 0%), and had a higher mortality rate (29% vs. 15%). Patients with binary toxin strains had significantly higher white blood cell counts and lower albumin.

Conclusions: Patients with binary toxin positive CDI were more likely to experience treatment failure and had higher rates of mortality. Further study on effective management of patients with CDI infected with binary toxin positive strains is needed.

http://www.icaaconline.com/php/icaac2013abstracts/data/papers/2013/K/2013_K-158.htm

C. difficile - terapija

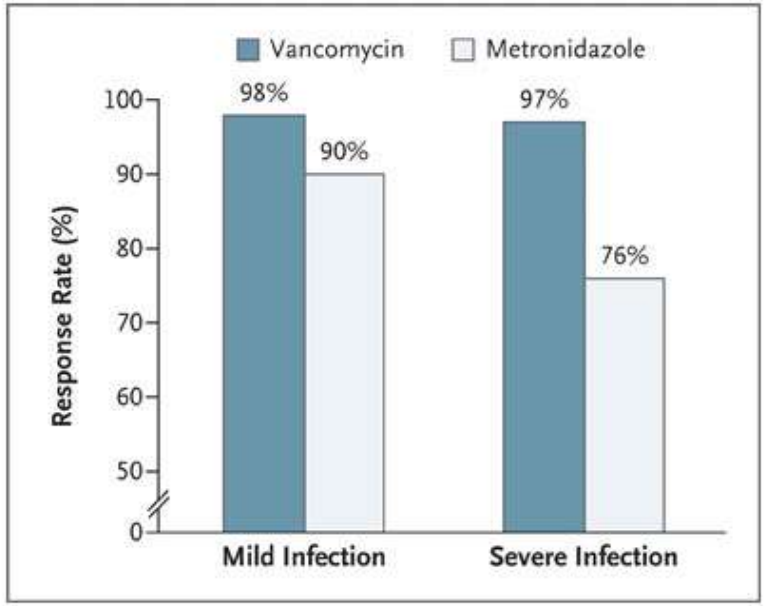
Surawicz CM, Brandt LJ, Binion DG et al. Guidelines for Diagnosis, Treatment, and Prevention of Clostridium difficile Infections. Am. J. Gastroenterol. 108(4), 478–498 (2013).

- Kod sumnje na CDAD **što prije početi** s terapijom (smanjenje stope smrtnosti i mogućnost recidiva infekcije)
- Isključiti dotadašnju antibiotsku terapiju (ako postoji i ako je moguće)
- **Vankomicin** dokazano bolji terapijski izbor u odnosu na Metronidazol kod pacijenata s **teškim oblicima CDAD**
- Fidaksomicin (od 2011.)- za sada se pokazao kao dobra terapija, manja pojava recidiva, pojava rezistencije

TABLE 3. Recommendations for the Treatment of *Clostridium difficile* Infection (CDI)

Clinical definition	Supportive clinical data	Recommended treatment	Strength of recommendation
Initial episode, mild or moderate	Leukocytosis with a white blood cell count of 15,000 cells/μL or lower and a serum creatinine level less than 1.5 times the premorbid level	Metronidazole, 500 mg 3 times per day by mouth for 10–14 days	A-I
Initial episode, severe*	Leukocytosis with a white blood cell count of 15,000 cells/μL or higher or a serum creatinine level greater than or equal to 1.5 times the premorbid level	Vancomycin, 125 mg 4 times per day by mouth for 10–14 days	B-I
Initial episode, severe, complicated	Hypotension or shock, ileus, megacolon	Vancomycin, 500 mg 4 times per day by mouth or by nasogastric tube, plus metronidazole, 500 mg every 8 hours intravenously. If complete ileus, consider adding rectal instillation of vancomycin	C-III
First recurrence	…	Same as for initial episode	A-II
Second recurrence	…	Vancomycin in a tapered and/or pulsed regimen	B-III

* The criteria proposed for defining severe or complicated CDI are based on expert opinion. These may need to be reviewed in the future upon publication of prospectively validated severity scores for patients with CDI.



Zar et al., CID 2007, 45, 302-307, Engberg Okt. 2011, Kelly and LaMont NEJM, 2008
Clinical Practice Guidelines for Clostridium difficile Infection in Adults. Infection control and hospital epidemiology, may 2010, vol. 31, no. 5

Uzorkovanje i BRZA dijagnostika

- Aktivno traženje (screening) kliconoša među asimptomatskim pacijentima se **NE** preporuča / **pretragu izvoditi samo na proljevastim stolicama**
  - Asimptomatski kliconoše – potencijalni rezervoar, ali manje kontaminiraju okoliš
  - Asimptomatski kliconoše – manji rizik od razvoja CDAD
- Sve uzorke stolice bolesnika s nozokomijalnom dijareom promptno testirati na CD toksin u stolici
  - Test citotoksičnosti
  - EIA, imunokromatografski testovi, PCR
- Opetovano retestiranje kod negativnog nalaza se NE preporuča (<od 5 % ih bude pozitivno, a povećava se mogućnost dobivanja lažno pozivitivnog nalaza)
- Ne treba uzimati kontrolne uzorke nakon izlječenja!!!!!
- U slučaju teže kliničke slike ili epidemije iz toksin pozitivnih stolica izolirati *C. difficile* te spremiti sojeve (ribotipizacija....)
- Bris rektuma također može biti uzorak za dijagnostiku (pacijenti s ileusom...)

CDAD-dijagnostika

Ciljevi:

- dokaz uzročnika bolesti
- ciljana terapijska preporuka (anti CD algoritam)

Važno!!!:

- pouzdanost dijagnostičkog algoritma
  (visoka osjetljivost & specifičnost pretraga)
- brzina (time to diagnosis)
- cijena
- jednostavnost

- Kliničarima i laboratoriju je nužan: brz, te visoko osjetljiv i specifičan dijagnostički algoritam koji će omogućiti :
  - brzu i fokusiranu terapiju
  - kontrola daljeg širenja infekcije (specifične mjere kontaktne izolacije)
- Mogućnost otkrivanja hipervirulentnih sojeva

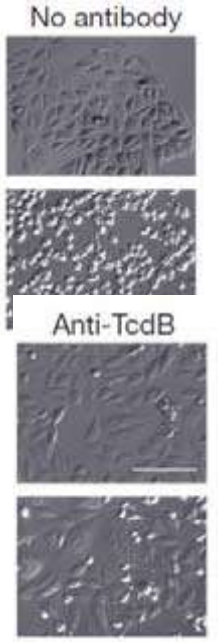
Metode dostupne za detekciju CD

detekcija *C difficile*:

1. Dokaz *C. difficile* produkata:
   glutamat-dehidrogenaza
   toksin A i/ili B (enzim imunoesej (EIA) ili cell culture neutralization assay (CCNA)

2. Kultivacija
3. Dokaz gena (dokaz toksičnih gena/dokaz bakterije: 16s, PCR geni za toksin, za GDH)

Referentna metoda za dijagnostiku



Postoje 2 referentne metode za dijagnozu CDI: **test neutralizacije i toksigena kultura**.

Test neutralizacije (TN)

- Inkubacija filtriranog uzorka stolice na staničnoj kulturi
- Ako je toksin B prisutan doći će do stvaranja citopatskog efekta (stanice se zaokružuju)
  stanična kultura se promatra nakon 24 i 48 h, kada se izdaje nalaz

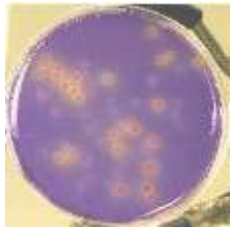
Toksigena kultura (TC)

- Inokulacija uzorka stolice na selektivni medij
- Inkubacija u anaerobnim uvjetima kroz 48h
- Izolat se testira na produkciju toksina

Ovisno o izboru referentne metode dobijamo **RAZLIČITE INFORMACIJE!** *interpretacija*

- **+ Test neutralizacije** potvrđuje prisutnost toksina u dobivenom zorku stolice,
  dok **+TC** procjenjuje sposobnost *C. difficile* izolata da producira toksin u *in vitro* uvjetima.

Klinička značajnost? Kolonizacija s C.difficile?



Zlatni standard: TN ili TC?

- osjetljivost TN je samo **67–86%** u usporedbi s TC
- Studije koje koriste različite referentne metode (TC/TN) pružaju podatke koji nisu kompatibilni za usporedbu

Nekoliko studija (npr. Dubberke i suradnici) evaluirale **TN i TC** s obzirom na referentni standard **klinički značajne dijareje:**

TN je imala manju osjetljivost od TC (62.9% naspram 100%) ali sličnih rezultata gledajući specifičnost testa (93.9% i 92.2%)

- **TN nije dovoljno osjetljiva za postavljanje dijagnoze CDAD: skoro sve studije koriste TC kao referentni standard**

ALI:

Planche i suradnici., uspoređivali referentne metode sa kliničkim ishodom i ZAKLJUČILI:

- **TN najbolje korelira sa stvarnim slučajevim CDAD**

Dubberke ER, Han Z, Bobo L et al. Impact of clinical symptoms on interpretation of diagnostic assays for Clostridium difficile infections. J. Clin. Microbiol. 49(8), 2887–2893 (2011).

Timothy D Planche et al. Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnosticvalidation study of *C difficile* infection. www.thelancet.com/infection Published online September 3, 2013

Rutinska dijagnostika

- TN
  - potrebna 2 dana za završetak nalaza
  - zahtjeva rad sa staničnim kulturama
- TC
  - Anaerobna kultura uzorka stolice
  - 2-5 dana za završetak nalaza

- **BRZA detekcija toksigenih sojeva** *C. difficile* je ključna za početak optimalne terapije i sprečavanje daljnjeg širenja *C. difficile*

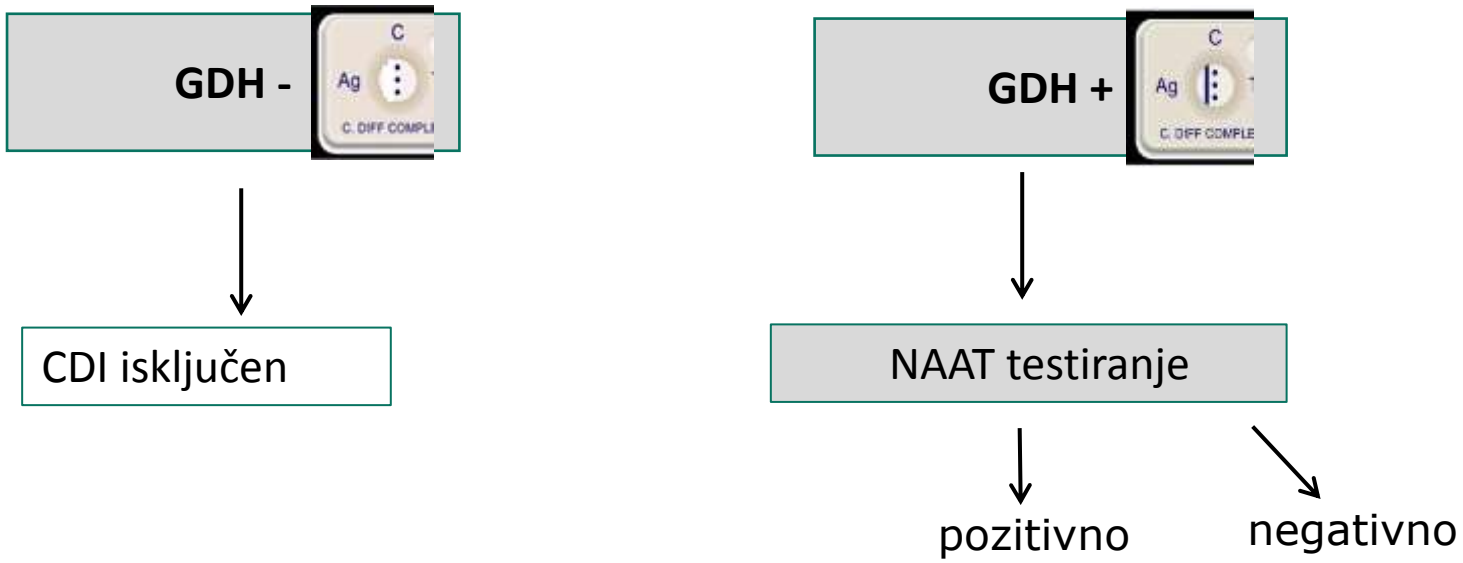
- **EIA** kao dokaz *C. difficile* **toksina** (TcdA i TcdB) najčešće je upotrebljavan test. Najveći manjak je **nedovoljna osjetljivost** (40 do 60% u usporedbi s TC, ali i s kliničkom dijagnozom).
- Usporedbom s TC kao referentnog standarda, sve analizirane EIA metode su imale neprihvatljivo nisku osjetljivost*
- **ZAKLJUČNO**: EIA detekcija toksina **NE MOŽE se koristiti kao** samostalan test u dokazivanju CDAD.

* ESCMID: Data review and recommendations for diagnosing Clostridium difficile infection (CDI)
Clinical Microbiology and Infection, Volume 15 Number 12, December 2009

Timothy D Planche et al. Differences in outcome according to *Clostridium difficile* testing method: a prospective multicentre diagnosticvalidation study of *C difficile* infection. www.thelancet.com/infection Published online September 3, 2013

„Two-steps” dijagnostički algoritam

* ESCMID: Data review and recommendations for diagnosing Clostridium difficile infection (CDI) Clinical Microbiology and Infection, Volume 15 Number 12, December 2009



GDH sensitivity is lower than NAATs, and some positive cases will be missed by the diagnostic algorithms.

Swindells et al.

Glutamat dehidrogenaza antigen esej

- Detektira antigen (GDH enzm) koji se u velikoj količini nalazi kod SVIH sojeva *C. difficile* (uključujući toksin producirajuće i neproducirajuće sojeve)
- **NE MOŽE** se koristiti kao **SAMOSTALAN test** u dokazivanju CDI (potreban je dokaz toksinogenosti soja)
- **Pozitivni GDH sojevi se MORAJU testirati na prisunost toksina!**
- koristi se kao screening test (1. korak u dijagnostičkim algoritmima)
- GDH osjetljivost ovisi o *C. difficile* PCR ribotipu
- Negativna prediktivna vrijednost GDH je izrazito visoka (>98%)

Study	Sens.	Spec.
Eastwood 2009 J.Clin.Micro.	87.6%	94.3%
Peterson 2009 J.Clin.Micro.	83%	89%
Gilligan 2008 J.Clin.Micro.	84.1%	89.8%
Reyes 2007 Diag.Micro.Inf.Dis.	94%	97%
Novak 2010 J.Clin.Micro.	86.1%	96.1%
Crist 2010 CVS Daytona poster	91.7%	96%

- NAATS: komercijalni eseji

Kit	Method	Target	DNA extraction	TAT (min)	Pooled Sensitivity (%)	Number of studies
BD GeneOhm	RT-PCR	tcdB	Manual	120	93% (95% CI: 89–95%)	8
Illumigene C. difficile	LAMP	tcdA	Manual	60	92% (95% CI, 88–94%)	5
Xpert C. difficile	Multiplex RT-PCR	tcdB, tcdCD117 binary toxin gene	Automated	50	99% (95% CI, 97–100%).	8
BD Max Cdiff	RT-PCR	tcdB	Automated	100	?	2
ProGastro Cd	RT-PCR	tcdB	Automated (EasyMAG)	180	?	2

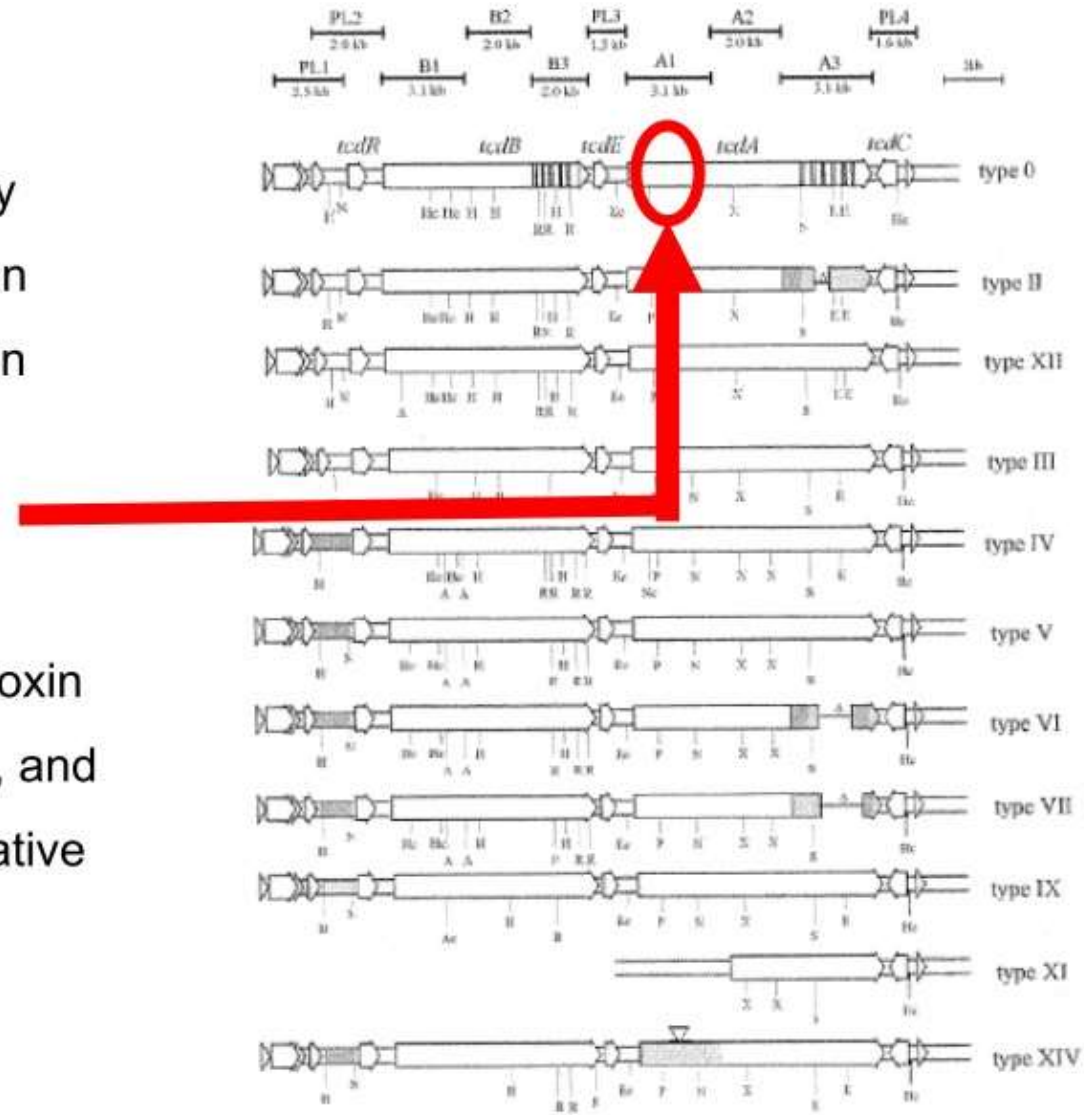
Remi Le Guern, Stephanie Herwegh, Rene Courcol and Frederic Wallet.
Expert Rev. Mol. Diagn. 13(7), 681–692 (2013)

illumigene™ Product Review:

Target Sequence

The MBI Molecular C diff assay contains primers that amplify an ~204 base pair sequence within the 5’ region of the Toxin A (tdcA) gene.

This region is found in all cytotoxin positive (A+/B+, A-/B+) strains, and is not present in cytotoxin negative strains (A-/B-).



illumigene™ Product Review:

Target Sequence

Will the ILLUMIgene toxin A primer detect different *C. difficile* A/B phenotypes?

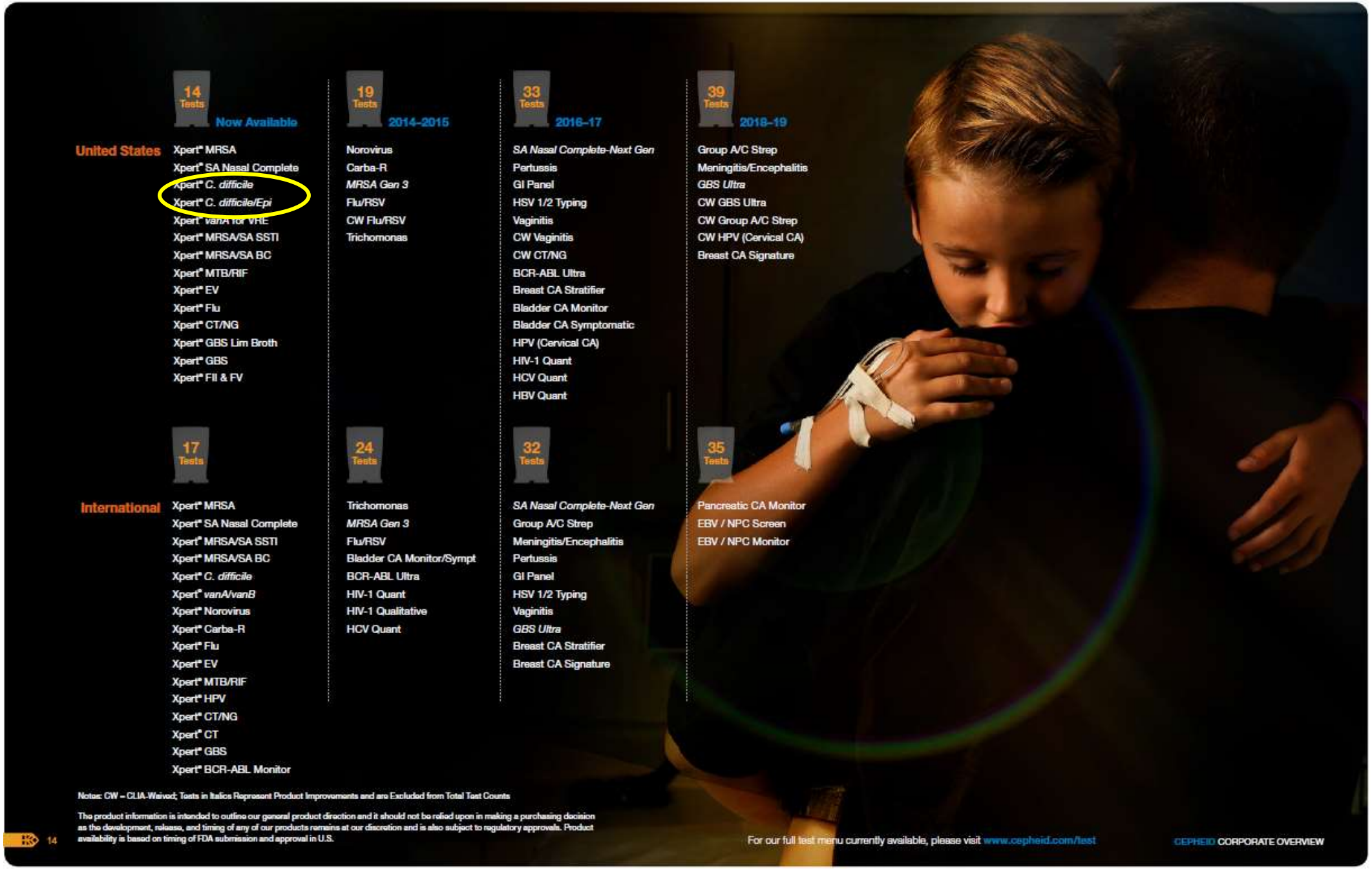
Phenotype	MBI C diff Molecular Assay Target Sequence	
	Expected Result	IG Result
A- B+	Positive	Positive
A- B-	Negative	Negative
A+ B+	Positive	Positive
A+ B+ binary+	Positive	Positive
A- B- binary +	Negative	Negative

GeneXpert

- Multiplex RT-PCR
- „Catridge based”
- Potpuno automatizirani sustav
- Razvijen za vojne svrhe (antraks)
- Target tcdB, tcdCD117 gen za binarni toksin
- Visoka osjetljivost i specifičnost
- Brz test (45min)

Planche et al.

The optimum algorithm compared with cytotoxigenic culture was glutamate dehydrogenase enzyme immunoassay–nucleic acid
amplification test (Xpert C.difficile), with 94·6% sensitivity, and specificity around 99%



	14 Tests — Now Available	19 Tests — 2014–2015	33 Tests — 2016–17	39 Tests — 2018–19
United States	Xpert® MRSA Xpert® SA Nasal Complete Xpert® *C. difficile* Xpert® *C. difficile/Epi* Xpert® *vanA* for VRE Xpert® MRSA/SA SSTI Xpert® MRSA/SA BC Xpert® MTB/RIF Xpert® EV Xpert® Flu Xpert® CT/NG Xpert® GBS Lim Broth Xpert® GBS Xpert® FII & FV	Norovirus Carba-R *MRSA Gen 3* Flu/RSV CW Flu/RSV Trichomonas	*SA Nasal Complete-Next Gen* Pertussis GI Panel HSV 1/2 Typing Vaginitis CW Vaginitis CW CT/NG BCR-ABL Ultra Breast CA Stratifier Bladder CA Monitor Bladder CA Symptomatic HPV (Cervical CA) HIV-1 Quant HCV Quant HBV Quant	Group A/C Strep Meningitis/Encephalitis *GBS Ultra* CW GBS Ultra CW Group A/C Strep CW HPV (Cervical CA) Breast CA Signature

	17 Tests	24 Tests	32 Tests	35 Tests
International	Xpert® MRSA Xpert® SA Nasal Complete Xpert® MRSA/SA SSTI Xpert® MRSA/SA BC Xpert® *C. difficile* Xpert® *vanA/vanB* Xpert® Norovirus Xpert® Carba-R Xpert® Flu Xpert® EV Xpert® MTB/RIF Xpert® HPV Xpert® CT/NG Xpert® CT Xpert® GBS Xpert® BCR-ABL Monitor	Trichomonas *MRSA Gen 3* Flu/RSV Bladder CA Monitor/Sympt BCR-ABL Ultra HIV-1 Quant HIV-1 Qualitative HCV Quant	*SA Nasal Complete-Next Gen* Group A/C Strep Meningitis/Encephalitis Pertussis GI Panel HSV 1/2 Typing Vaginitis *GBS Ultra* Breast CA Stratifier Breast CA Signature	Pancreatic CA Monitor EBV / NPC Screen EBV / NPC Monitor

Notes: CW – CLIA-Waived; Tests in Italics Represent Product Improvements and are Excluded from Total Test Counts

The product information is intended to outline our general product direction and it should not be relied upon in making a purchasing decision as the development, release, and timing of any of our products remains at our discretion and is also subject to regulatory approvals. Product availability is based on timing of FDA submission and approval in U.S.

For our full test menu currently available, please visit www.cepheid.com/test

CME

Guidelines for Diagnosis, Treatment, and Prevention of *Clostridium difficile* Infections

U nedavno obajvljenim smjernicama, NAATs prepoznat kao superiornija metoda u dijagnosticiranju CDI:

1. Only stools from patients with diarrhea should be tested for *Clostridium difficile* . (Strong recommendation, high-quality evidence)

Because *C. difficile* carriage is increased in patients on antimicrobial therapy, only diarrheal stools warrant testing

2. Nucleic acid amplifi cation tests (NAAT) for *C. difficile* toxin genes such as PCR are superior to toxins A + B EIA testing as a standard diagnostic test for CDI. (Strong recommendation, moderate-quality evidence)
3. Glutamate dehydrogenase (GDH) screening tests for C diffi cile can be used in two- or three-step screening algorithms, but the sensitivity of such strategies is lower than NAATs. (Strong recommendation, moderate-quality evidence)

It should be noted that as many as 10 % of patients with toxigenic organisms can be missed by using GDH screening

4. Repeat testing should be discouraged. (Strong recommendation, moderate-quality evidence)
5. Testing for cure should not be done. (Strong recommendation, moderate-quality evidence)

Surawicz CM, Brandt LJ, Binion DG et al. Guidelines for Diagnosis, Treatment, and Prevention of Clostridium difficile Infections. Am. J. Gastroenterol. 108(4), 478–498 (2013).

Klinički i financijski važne činjenice....

- Spore *C. difficile* mogu perzistirati na površinama godinama i kao takve predstavljaju izvor kontaminacije i infekcije
- Alkohol nije djelotvoran za uklanjanje *C. difficile* spora
- Metronidazol i vankomicin ubijaju vegetativni oblik bakterije,ne spore
- CDAD može produžiti boravak u bolnici za 1 do 2 tjedna
- Dodatni troškovi se povećavaju čak do 7147 eura po pacijentu (Njemačka) £2691 u Irskoj
- U Americi se godišnje potroši 3,2 bilijuna $/god na CDAD

Clinical and economic burden of Clostridium difficile infection in Europe: a systematic review of healthcare-facility-acquired infection. P.N. Wiegand et al. JHI, 81,2012, 1-14

CDAD nadzor

- Neophodno kontinuirano pratiti CDAD kako bi se mogao uočiti porast incidencije ili promjena u težini kliničke slike
- Mikrobiološko testiranje na CD toksin: svi bolesnici koji zadobiju dijareu nakon 3 dana boravka u bolnici
- Odrediti prag incidencije kad će se poduzet posebne mjere kontrole epidemije
  - Ovisi o vrsti institucije, populaciji pacijenata, težini kliničke slike

Prevencija i kontrola CDAD u bolnicama

Prevencija *Clostridium difficile* kolonizacije NOVIH pacijenata

Kontrola bolničkih infekcija

Prevencija pojave CDAD u KOLONIZIRANIH pacijenata

Racionalizacija uporabe antibiotika

Kontrola bolničkih infekcija

1. Kontaktna izolacija

- Jednokrevetne sobe ili kohortiranje
  (Zasebni WC)
- Uporaba zaštitnih sredstava (rukavice, pregače)

- Primjena mjera izolacije do prestanka simptoma / **2d nakon prestanka simptoma** / do otpusta iz bolnice

Kontrola bolničkih infekcija

2. Dezinfekcija ruku

- **Standardna dezinfekcija ruku**: utrljavanje alkohola
  - Alkohol odličan učinak na nesporogene bakterije, ali nije sporicidan

- **Pri njezi CDtox pozitivnog pacijenta dodatno**: pranje ruku sapunom i vodom
  - Mehaničko odstranjivanje spora

Kontrola bolničkih infekcija

3. Dezinfekcija okoline

- Površine značajno kontaminirane
- Dezinfekcija površina sporocidnim dezinficijensom
  - **Hipokloritni dezinficijensi**
  - **Glutaraldehidni**
  - **Dezinficijens na bazi aktivnog kisika**
- Čišćenje i dezinfekcija redovito barem 1x dnevno, a površine koje se često dodiruju i češće (kvake, telefoni, stolovi, stolci)

Kontrola bolničkih infekcija

4. Dezinfekcija instrumenata

- Jednokratna uporaba
- Oprema samo za pacijenta u izolaciji
- Dezinfekcija sporocidnim dezinficijensom (glutaraldehid

Kontrola bolničkih infekcija

5. Edukacija

- Osoblje
- Posjetitelji

  - Potencijalni rezervoari
  - Način prenošenja
  - Uloga okoline / način dezinfekcije
  - Higijena ruku, nošenje rukavica

zaključci...

- Porast incidencije, mortaliteta, težine kliničke slike
- Dokazana pojava i širenje novih hipervirulentnih sojeva

  Novi soj povećane virulencije

  NAP1 / BI / toxinotype III / ribotype 027
- Patogen koji se seli u vanboničku sredinu, zahvaća atipičnu populaciju
- Testiraju se SAMO proljevaste stolice/NEMA aktivnog screeninga na kliconoštvo
- Brza i točna dijagnostika
- Kontrola hospitalnih infekcija!

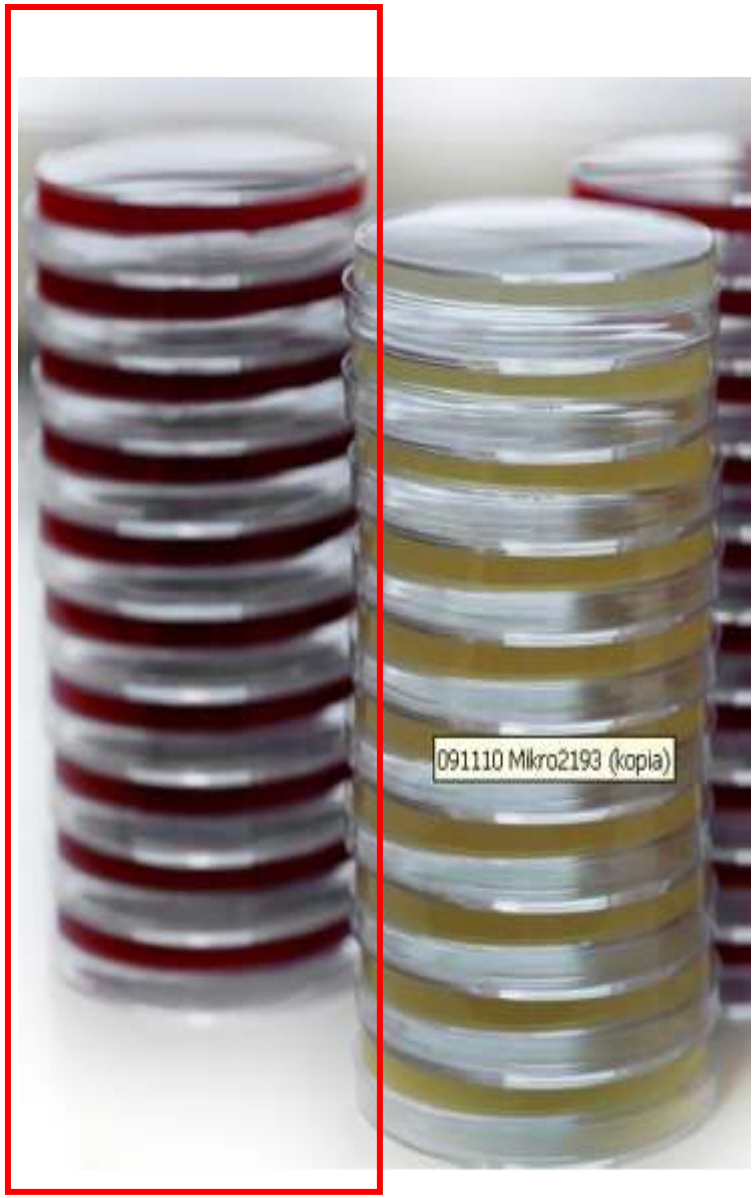
HVALA NA PAŽNJI!!

Testiranje osjetljivosti na antibiotike kod izbirljivih bakterija

Silvija Šoprek
Klinika za infektivne bolesti „Dr. Fran Mihaljević”,
Odjel za kliničku mikrobiologiju,
Zagreb

Vrste medija

Organisms	Medium
Enterobacteriaceae *Pseudomonas* spp. *Stenotrophomonas maltophilia* *Acinetobacter* spp. *Staphylococcus* spp. *Enterococcus* spp.	Mueller-Hinton agar
Streptococcus pneumoniae Streptococcus groups A, B, C and G Viridans group streptococci *Haemophilus* spp. *Moraxella catarrhalis* *Listeria monocytogenes* *Pasteurella multocida* *Campylobacter jejuni* and *coli* *Corynebacterium* spp.	Mueller-Hinton agar + 5% mechanically defibrinated horse blood + 20 mg/L β-NAD (MH-F)
Other fastidious organisms	Pending



Inkubacija ploča

Organism	Incubation conditions
Enterobacteriaceae	35+/-1 ºC in air for 16-20h
Pseudomonas spp.	35+/-1 ºC in air for 16-20h
Stenotrophomonas maltophilia	35+/-1 ºC in air for 16-20h
Acinetobacter spp.	35+/-1 ºC in air for 16-20h
Staphylococcus spp.	35+/-1 ºC in air for 16-20h
Enterococcus spp.	35+/-1 ºC in air for 16-20h (24 h for glycopeptides)
Streptococcus groups A, B, C and G	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Viridans group streptococci	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Streptococcus pneumoniae	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Haemophilus spp.	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Moraxella catarrhalis	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Listeria monocytogenes	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Pasteurella multocida	35+/-1 ºC in air with 4-6% CO_2 for 16-20h
Campylobacter jejuni and *coli*	41+/-1 ºC in microaerobic environment for 24h (40-48h)
Corynebacterium spp.	35+/-1 ºC in air with 4-6% CO_2 for 16-20h (40-48h)
Other fastidious organisms	Pending

EUCAST 2015 Version 5.0

REVIEW 10.1111/j.1469-0691.2011.03703.x

EUCAST expert rules in antimicrobial susceptibility testing

R. Leclercq[1,2], R. Cantón[2,3,4], D. F. J. Brown[4], C. G. Giske[2,4,5], P. Heisig[2,6], A. P. MacGowan[4,7], J. W. Mouton[4,8], P. Nordmann[2,9], A. C. Rodloff[4,10], G. M. Rossolini[2,11], C.-J. Soussy[4,12], M. Steinbakk[4,13], T. G. Winstanley[2,14] and G. Kahlmeter[4,15]

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance

Version 1.0
December 2013

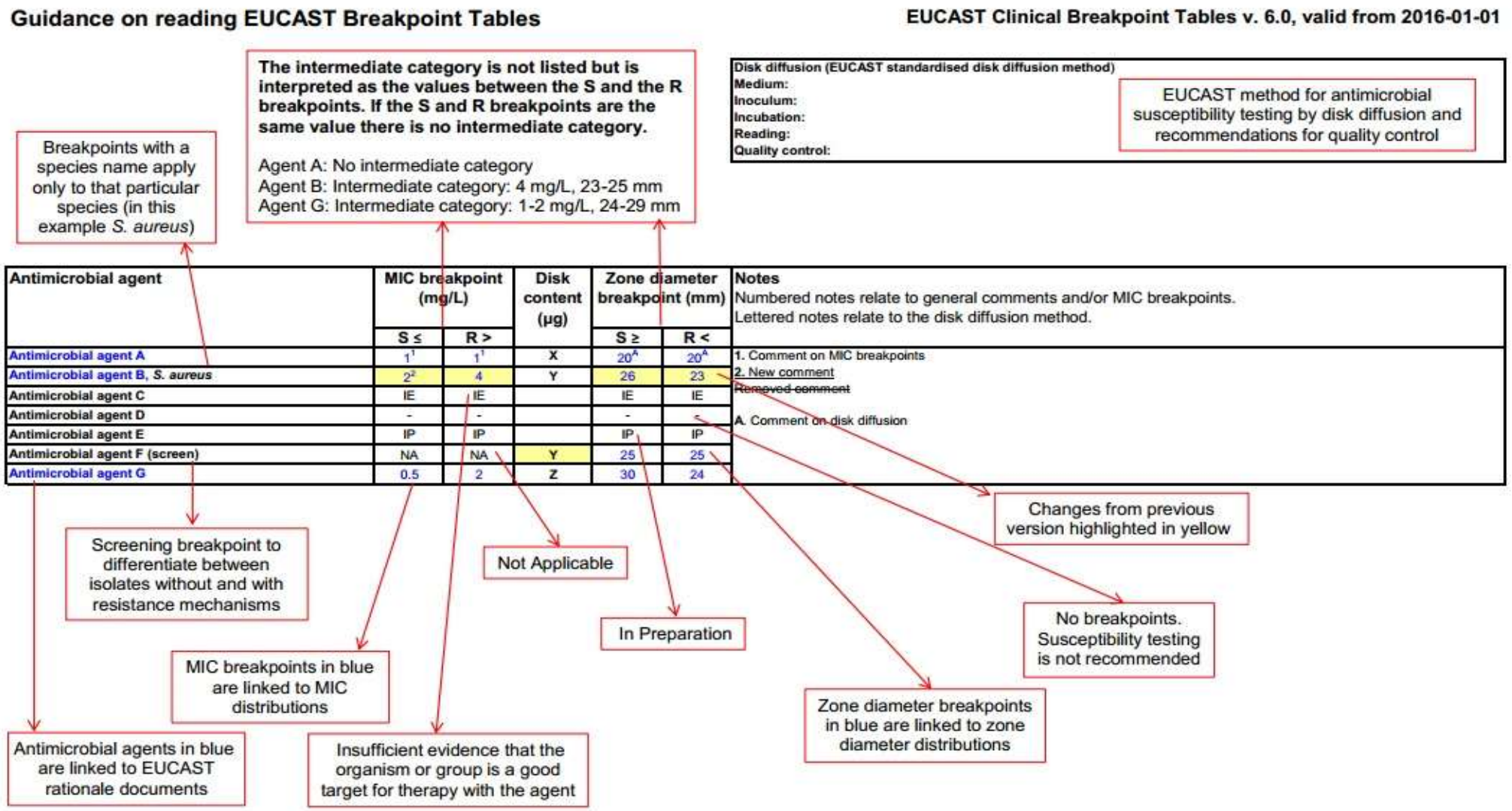
EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

European Committee on Antimicrobial Susceptibility Testing

Routine and extended internal quality control
for MIC determination and disk diffusion
as recommended by EUCAST

Version 6.0, valid from 2016-01-01

EUCAST clinical breakpoint tables



EUCAST expert rules – interpretacijska pravila

Rule no.	Organisms	Agents tested	Agents affected	Rule	Exceptions, scientific basis and comments	Evidence grade	References
8.1	*Staphylococcus* spp.	Oxacillin, cefoxitin (disk diffusion) or detection of *mecA* gene or PBP2a	All beta-lactams	IF resistant to isoxazolyl-penicillins (as determined with oxacillin, cefoxitin, or by detection of *mecA*-gene or of PBP2a) THEN report as resistant to all β-lactams.	Production of PBP2a (encoded by *mecA*) leads to cross resistance to β-lactams except ceftobiprole and ceftaroline.	A	Chambers HF *et al*, 1990 Page MG *et al*, 2006

Agents tested

Agents affected

IF … THEN…

Exceptions, scientific basis and comments

EUCAST expert rules – intrinzična rezistencija

TABLE 3. Intrinsic resistance in Gram-negative bacteria other than *Enterobacteriaceae* and non-fermentative Gram-negative bacteria; Gram-negative bacteria other than *Enterobacteriaceae* and non-fermentative Gram-negative bacteria listed are also intrinsically resistant to glycopeptides, lincosamides, daptomycin, and linezolid

Rule no.	Organisms	Macrolides	Fusidic acid	Streptogramins	Trimethoprim	Nalidixic acid
3.1	*Haemophilus influenzae*	I	R	–	–	–
3.2	*Moraxella catarrhalis*	–	–	–	R	–
3.3	*Neisseria* spp.	–	–	–	R	–
3.4	*Campylobacter fetus*	–	R	R	R	R
3.5	*Campylobacter jejuni, Campylobacter coli*	–	R	R	R	–

R, resistant; I, intermediate.

EUCAST expert rules i interpretacija rezultata

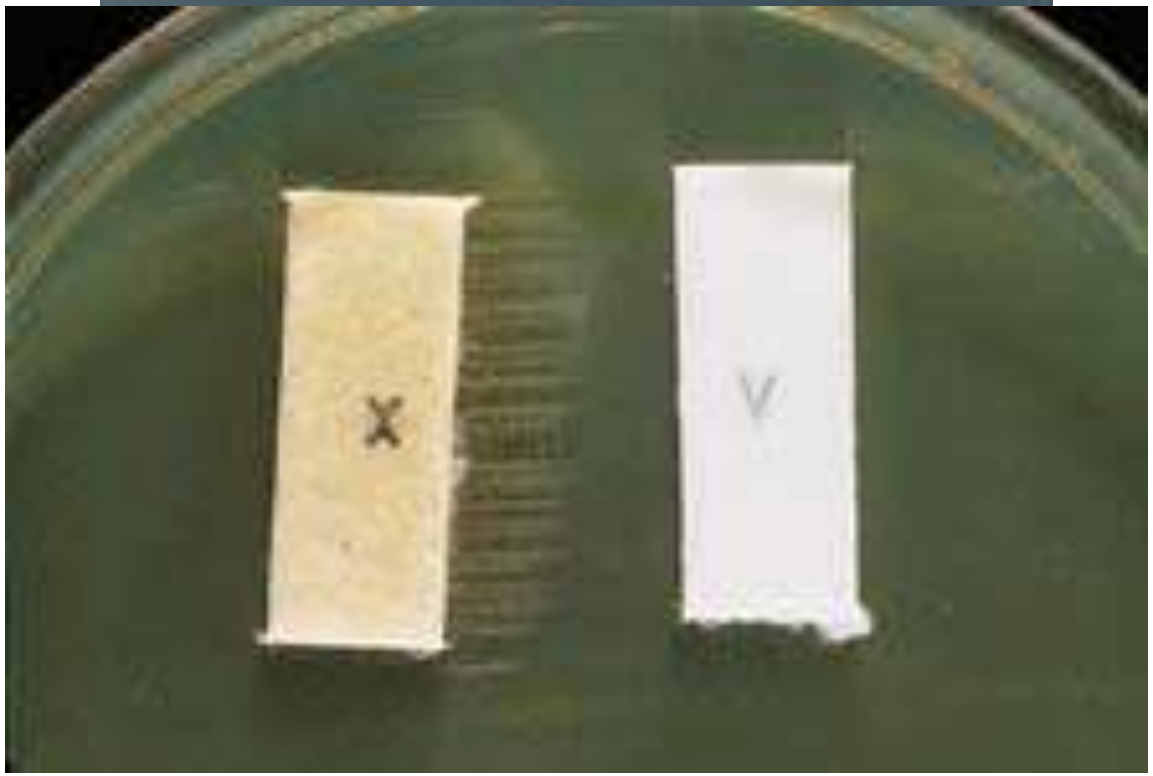
TABLE 10. Interpretive rules for β-lactam agents and other Gram-negative bacteria

Rule no.	Organisms	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
10.1	*Haemophilus influenzae*	Ampicillin or amoxycillin (and β-lactamase detection)	Ampicillin, amoxycillin, and piperacillin	IF β-lactamase-positive, THEN report as resistant to ampicillin, amoxycillin, and piperacillin	Ampicillin is the class representative for amoxycillin. Resistance to ampicillin by production of β-lactamase may be misidentified by the disk diffusion technique. Production of β-lactamase should be examined with a chromogenic test	A	[106,107]
10.2	*Haemophilus influenzae*	Ampicillin or amoxycillin (and β-lactamase detection)	Ampicillin, amoxycillin, amoxycillin–clavulanate, ampicillin–sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin, and piperacillin–tazobactam	IF β-lactamase-negative but ampicillin-resistant (BLNAR), THEN report as resistant to ampicillin, amoxycillin, amoxycillin–clavulanate, ampicillin–sulbactam, piperacillin, piperacillin–tazobactam, cefaclor, cefuroxime, and cefuroxime axetil	BLNAR isolates have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin–tazobactam appear to be less affected by the PBP-mediated resistance mechanisms, evidence regarding clinical efficacy is lacking	C	[48,49,108]
10.3	*Haemophilus influenzae*	Amoxycillin–clavulanate (and β-lactamase detection)	Ampicillin–sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin, and piperacillin–tazobactam	IF β-lactamase-positive and amoxycillin–clavulanate-resistant (BLPACR), THEN report as resistant to ampicillin, amoxycillin, amoxycillin–clavulanate, ampicillin–sulbactam, cefaclor, piperacillin, piperacillin–tazobactam, cefuroxime, and cefuroxime axetil	BLPACR isolates produce β-lactamase and have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin–tazobactam appear to be less affected by the PBP-mediated resistance mechanisms, evidence regarding clinical efficacy is lacking	C	[48,108]

HAEMOPHILUS spp.

Gram negativni sitni kokobacili
OXIDAZA+, H2O2+
”satelitski fenomen”
identifikacija pomoću faktora rasta:
sektor na CA (columbia agar) s faktorima rasta

FAKTOR RASTA / UZROČNIK	X FAKTOR	V FAKTOR	X+V FAKTOR
H.INFLUENZAE	-	-	+
H.PARAINFLUENZAE	-	+	+



Inokulum: McFarland 0.5
Inkubacija: 5% CO2, 35+/-1°C, 18+/-2h

EUCAST zone inhibicije su definirane samo za *H. Influenzae*!!
MIC distribucija *H. influenzae* i *parainfluenzae* je slična
Za interpretaciju osjetljivosti *H. parainfluenzae* koristiti vrijednosti MIC –ova *H. influenzae*

Haemophilus influenzae

SOJEVI ZA KONTROLU KVALITETE

Haemophilus influenzae ATCC 49766

(NCTC 12975, CIP 103570, DSM 11970, CCUG 29539)

Disk diffusion methodology: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD, McFarland 0.5, 5% CO_2, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target[1]	Range[2]		Target	Range
Amoxicillin-clavulanic acid[3,4]	***0.25***	***0.125-0.5***	2-1	***20***	***17-23***
Amoxicillin	***0.25***	***0.125-0.5***	-	-	-
Ampicillin	***0.125***	***0.06-0.25***	2	***22***	***19-25***
Ampicillin-sulbactam[5]	***0.125***	***0.06-0.25***	-	-	-
Azithromycin	***1***	***0.5-2***	-	-	-
Benzylpenicillin	-	-	1 unit	***18***	***15-21***
Cefepime	***0.06***	***0.03-0.125***	30	***33***	***30-36***
Cefixime	***0.03***	***0.016-0.06***	5	***32***	***29-35***
Cefotaxime	***0.008***	***0.004-0.016***	5	***33***	***29-37***
Cefpodoxime	***0.06***	***0.03-0.125***	10	***33***	***30-36***
Ceftaroline	***0.008***	***0.004-0.016***	-	-	-
Ceftibuten	***0.03***	***0.016-0.06***	30	***34***	***31-37***
Ceftriaxone	***0.004***	***0.002-0.008***	30	***38***	***34-42***
Cefuroxime	0.5	0.25-1[6]	30	***30***	***26-34***
Chloramphenicol	***0.5***	***0.25-1***	30	***34***	***31-37***
Ciprofloxacin	***0.008***	***0.004-0.016***	5	***36***	***32-40***
Clarithromycin	***8***	***4-16***	-	-	-
Doripenem	0.125	0.06-0.25[6]	10	***29***	***26-32***
Doxycyline	***0.5***	***0.25-1***	-	-	-
Ertapenem	0.03	0.016-0.06[6]	10	***30***	***27-33***
Erythromycin	***4***	***2-8***	15	***13***	***10-16***
Imipenem	0.5	0.25-1[6]	10	***27***	***24-30***
Levofloxacin	***0.016***	***0.008-0.03***	5	***35***	***31-39***
Meropenem	0.06	0.03-0.125[6]	10	***31***	***27-35***
Minocycline	***0.25***	***0.125-0.5***	30	***29***	***26-32***
Moxifloxacin	***0.016***	***0.008-0.03***	5	***33***	***30-36***
Nalidixic acid	-	-	30	***30***	***27-33***
Ofloxacin	***0.03***	***0.016-0.06***	5	***34***	***31-37***
Rifampicin	***0.5***	***0.25-1***	5	***24***	***21-27***
Roxithromycin	***8***	***4-16***	-	-	-
Telithromycin	***2***	***1-4***	15	***17***	***14-20***
Tetracycline	***0.5***	***0.25-1***	30	***31***	***28-34***
Trimethoprim-sulfamethoxazole[7]	***0.03***	***0.016-0.06***	1.25-23.75	***31***	***27-35***

[1] Calculated by EUCAST.
[2] Established and validated by EUCAST.
[3] For MIC testing, the concentration of clavulanic acid is fixed at 2 mg/L.
[4] *S. aureus* ATCC 29213 (β-lactamase-producing strain) is used to check the inhibitor component (see Routine quality control for β-lactam-β-lactamase inhibitor combination disks).
[5] For MIC testing, the concentration of sulbactam is fixed at 4 mg/L.
[6] From Clinical and Laboratory Standards Institute, M100-S26, 2016, and validated by EUCAST.
[7] Trimethoprim:sulfamethoxazole in the ratio 1:19. MIC values are expressed as the trimethoprim concentration.

Haemophilus influenzae

KONTROLNI SOJ ZA DETEKCIJU MEHANIZAMA REZISTENCIJE

EUCAST
Strains for Detection of Specific Resistance Mechanisms

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Extended quality control as recommended by EUCAST[1]
Version 1.0, valid from 2013-01-01

Escherichia coli	**ATCC 35218[2]**
Klebsiella pneumoniae	**ATCC 700603[3]**
Staphylococcus aureus	**NCTC 12493[3]**
Enterococcus faecalis	**ATCC 51299[3]**
Haemophilus influenzae	**ATCC 49247[3]**

Notes

1. EUCAST quality control strains for extended QC are complementary to the EUCAST routine quality control strains.
2. *E. coli* ATCC 35218 (TEM-1 β-lactamase-producing strain) is recommended specifically to control the inhibitor component of inhibitor-combination disks. This QC test should be performed with each new batch of disks. The active component is controlled by routine QC as recommended in the EUCAST Routine QC Tables.
3. Strains recommended for detection of specific resistance mechanisms (ESBL, MRSA, VRE & HLGR, PBP mutations) are used to validate that the routine susceptibility testing methods will result in the correct S, I and R categorisation. This validation should be performed with every change in the susceptibility testing system (with each new batch of disks or medium) and/or monthly.

Haemophilus influenzae

KONTROLNI SOJ ZA DETEKCIJU MEHANIZAMA REZISTENCIJE-BLNAR soj

Quality control strains for detection of resistance mechanisms on MH-F agar

Mueller-Hinton agar + 5% horse blood and 20 mg/L β-NAD, McFarland 0.5, 5% CO_2, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.

Haemophilus influenzae ATCC 49247

(NCTC 12699, CIP 104604, DSM 9999, CCUG 26214)

β-lactamase negative, ampicillin resistant (BLNAR)

Antimicrobial agent	Disk content (µg)	Target susceptibility[1]	Range[2] (mm)	Comments
				Inhibition zone diameters are particularly affected by variation in medium, inoculum and incubation conditions. Inhibition zones with growth of small colonies within the zone are interpreted as 6 mm (no zone).
Ampicillin	2	R	***6-12***	
Benzylpenicillin	1 unit	R	**6-9**	

Haemophilus influenaze-testiranje osjetljivosti

Test cefinaze!!!

Haemophilus influenzae

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

EUCAST breakpoints have been defined for *H. influenzae* only. Clinical data for other *Haemophilus* species are scarce. MIC distributions for *H. parainfluenzae* are similar to those for *H. influenzae*. In the absence of specific breakpoints, the *H. influenzae* MIC breakpoints can be applied to *H. parainfluenzae*.

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1°C, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Haemophilus influenzae* ATCC 49766 ~~or Haemophilus influenzae NCTC 8468.~~ For control of the inhibitor component of beta-lactam inhibitor-combination disks, use *Staphylococcus aureus* ATCC 29213.

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin	IE	IE		IE	IE
Benzylpenicillin (screen)	NA	NA	1 unit	12[A]	Note[A]
Ampicillin[1]	1	1	2	16[A]	16[A]
Ampicillin-sulbactam[1]	1[2,3]	1[2,3]	10-10	Note[A,B]	Note[A,B]
Amoxicillin[1]	2	2		Note[A,C]	Note[A,C]
Amoxicillin-clavulanic acid[1]	2[4]	2[4]	2-1	15[A]	15[A]
Piperacillin[1]	Note[5]	Note[5]		Note[A,D]	Note[A,D]
Piperacillin-tazobactam[1]	Note[3]	Note[3]		Note[A]	Note[A]
Ticarcillin	IE	IE		IE	IE
Ticarcillin-clavulanic acid	IE	IE		IE	IE
Phenoxymethylpenicillin	IE	IE		IE	IE
Oxacillin	-	-		-	-
Cloxacillin	-	-		-	-
Dicloxacillin	-	-		-	-
Flucloxacillin	-	-		-	-
Mecillinam (uncomplicated UTI only)	-	-		-	-

Notes
Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1. Breakpoints are based on intravenous administration. For penicillins without inhibitors, breakpoints apply to beta-lactamase negative isolates only. For penicillins without inhibitors, beta-lactamase positive isolates should be reported resistant.
2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
3/B. Susceptibility can be inferred from amoxicillin-clavulanic acid.
4. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
5/D. Susceptibility inferred from ampicillin or amoxicillin.

A. Benzylpenicillin 1 unit can be used to screen for, but not to distinguish between, beta-lactamase producing isolates and isolates with PBP mutations. **For interpretation of the benzylpenicillin disk screen, see supplementary table below.**
C. Susceptibility can be inferred from ampicillin.

Haemophilus influenaze-testiranje osjetljivosti

1. PENICILINI

DOKAZ MEHANIZAMA REZISTENCIJE : 1. benzilpenicilin disk (1unit)
2. nitrocefinski disk (test cefinaze)

NITROCEFINSKI DISK – (test cefinaze) provjeriti AMP (R) izolate
▪ ako je soj cefinaza **negativan** - (A, AUG, CXM iv,CRO) +++ (β LAKTAMAZA negativna) ▪ ako je soj cefinaza **pozitivan** - A Ø (AUG,CXMiv,CRO) +++ (β LAKTAMAZA pozitivna) ▪ Ako se rezultati testa cefinaze ne podudaraju s rezultatima antibiograma, pokazati liječniku, učiniti MIK, možda se radi o rijetkim BLNAR (B-lactamase negative ampicillin resistant) sojevima koji mogu biti rezistentni na AP, AUG i CXM i u slučaju cefinaza negativnog soja.

CEFINAZA - → izdati: AMX,AMC,CXMiv,CRO **(S)**
CEFINAZA + → izdati: AMX **(R)**, AMC,CXMiv,CRO **(S)**

Haemophilus influenaze-testiranje osjetljivosti

1. PENICILINI

TEST CEFINAZE (interpretacija):

AKO SE REZULTATI TESTA CEFINAZE **NE PODUDARAJU** S ANTIBIOGRAMOM

↓

Test cefinaze NEG. , a DD AMP, AMP i/ili AMC **R**

↓

Napraviti MIK: AMP

AMC

Haemophilus influenaze-testiranje osjetljivosti

1. PENICILINI

	MIK AMP: > 1 (R)	MIK AMP: ≤ 1 (S)	MIK AMC: > 2 (R)		MIK AMC: ≤ 2 (S)
Test cefinaze	-	-	+	-	-
	BLNAR		**BLPACR**	**BLNAR**	
	Izdati: AMX,AMC,CXM **(R)**	Izdati: AMX,AMC (S)	Izdati: AMX,AMC,CXM **(R)** **CRO (S)**		Izdati: AMC (S) **osim ako je MIK AMP: > 1 = BLNAR** Izdati: AMX,AMC,CXM **(R)**

Haemophilus influenaze-testiranje osjetljivosti

1. PENICILINI

„*Expert rules*" i interpretacija

TABLE 10. Interpretive rules for β-lactam agents and other Gram-negative bacteria

Rule no.	Organisms	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
10.1	*Haemophilus influenzae*	Ampicillin or amoxycillin (and β-lactamase detection)	Ampicillin, amoxycillin, and piperacillin	IF β-lactamase-positive, THEN report as resistant to ampicillin, amoxycillin, and piperacillin	Ampicillin is the class representative for amoxycillin. Resistance to ampicillin by production of β-lactamase may be misidentified by the disk diffusion technique. Production of β-lactamase should be examined with a chromogenic test	A	[106,107]
10.2	*Haemophilus influenzae*	Ampicillin or amoxycillin (and β-lactamase detection)	Ampicillin, amoxycillin, amoxycillin–clavulanate, ampicillin–sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin, and piperacillin–tazobactam	IF β-lactamase-negative but ampicillin-resistant (BLNAR), THEN report as resistant to ampicillin, amoxycillin, amoxycillin–clavulanate, ampicillin–sulbactam, piperacillin, piperacillin–tazobactam, cefaclor, cefuroxime, and cefuroxime axetil	BLNAR isolates have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin–tazobactam appear to be less affected by the PBP-mediated resistance mechanisms, evidence regarding clinical efficacy is lacking	C	[48,49,108]
10.3	*Haemophilus influenzae*	Amoxycillin–clavulanate (and β-lactamase detection)	Ampicillin–sulbactam, cefaclor, cefuroxime, cefuroxime axetil, piperacillin, and piperacillin–tazobactam	IF β-lactamase-positive and amoxycillin–clavulanate-resistant (BLPACR), THEN report as resistant to ampicillin, amoxycillin, amoxycillin–clavulanate, ampicillin–sulbactam, cefaclor, piperacillin, piperacillin–tazobactam, cefuroxime, and cefuroxime axetil	BLPACR isolates produce β-lactamase and have reduced affinity of PBPs for β-lactams. Although piperacillin and piperacillin–tazobactam appear to be less affected by the PBP-mediated resistance mechanisms, evidence regarding clinical efficacy is lacking	C	[48,108]

Bezilpenicilinski screen disk:

Interpretacija i izdavanje nalaza

Screening for beta-lactam resistance in *H. influenzae*

Supplementary table

Benzylpenicillin 1 unit Zone diameter	Beta-lactamase	Further testing and/or interpretation
≥ 12 mm	Do not test	Report susceptible to all beta-lactam agents for which clinical breakpoints are listed (including those with "Note").
< 12 mm	Beta-lactamase negative	A resistance mechanism other than beta-lactamase production is present. As the effect on individual beta-lactam agents differs, test susceptibility to the beta-lactam agent intended for clinical use.
	Beta-lactamase positive	For ampicillin, amoxicillin and piperacillin, report resistant.
		For other beta-lactam agents, test susceptibility to the beta-lactam agent intended for clinical use as another resistance mechanism cannot be excluded by the screen test.

Haemophilus influenaze-testiranje osjetljivosti

Haemophilus influenzae

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-		-	-	~~1. Isolates with MIC values above the susceptible breakpoint are very rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC values above the current resistant breakpoint they should be reported resistant.~~ **A.** Benzylpenicillin 1 unit can be used to screen for beta-lactam resistance. **See Note A on penicillins and supplementary table below.**
Cefadroxil	-	-		-	-	
Cefalexin	-	-		-	-	
Cefazolin	-	-		-	-	
Cefepime	0.25	0.25	30	27[A]	27[A]	
Cefixime	0.125	0.125	5	25[A]	25[A]	
Cefotaxime	0.125	0.125	5	26[A]	26[A]	
Cefoxitin	NA	NA		NA	NA	
Cefpodoxime	0.25	0.5	10	26[A]	23[A]	
Ceftaroline	0.03	0.03		IP	IP	
Ceftazidime	-	-		-	-	
Ceftibuten	1	1	30	25[A]	25[A]	
Ceftobiprole	IE	IE		IE	IE	
Ceftolozane-tazobactam	IE	IE		IE	IE	
Ceftriaxone	0.125	0.125	30	30[A]	30[A]	
Cefuroxime iv	1	2	30	26[A]	25[A]	
Cefuroxime oral	0.125	1	30	50	26	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem[1]	1	1	10	20[A]	20[A]	**1.** Not for meningitis (meropenem is the only carbapenem used for meningitis).
Ertapenem[1]	0.5	0.5	10	20[A]	20[A]	~~2. Isolates with MIC values above the susceptible breakpoint are very rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC values above the current resistant breakpoint they should be reported resistant.~~
Imipenem[1]	2	2	10	20[A]	20[A]	
Meropenem[1] (infections other than meningitis)	2	2	10	20[A]	20[A]	
Meropenem[2] (meningitis)	0.25	1		Note[B]	Note[B]	2. Meropenem is the only carbapenem used for meningitis. **A.** Benzylpenicillin 1 unit can be used to screen for beta-lactam resistance. **See Note A on penicillins and supplementary table below.** **B.** For use in meningitis determine the meropenem MIC value.

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	IE	IE		IE	IE	

2. CEFALOSPORINI

Cefuroksim-DVOJNA INTERPRETACIJA

3. KARBAPENEMI

Kod meningitisa- samo MIK meropenema!!!

Haemophilus influenaze-testiranje osjetljivosti
4. FLUOROKINOLONI

TABLE 13. Interpretive rules for quinolones

Rule no.	Organism	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
13.1	*Staphylococcus* spp.	Ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning of risk for development of resistance during therapy with quinolones	Acquisition of at least one target mutation in *grlA*	C	[86,92]
13.2	*Staphylococcus* spp.	Levofloxacin and moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of combined mutations in *grlA* and *gyrA* leads to complete or partial cross-resistance to all fluoroquinolones	C	[92,116,117]
13.3	*Streptococcus pneumoniae*	Ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning that acquisition of a first-step mutation may lead to resistance development under therapy with other quinolones	Acquisition of at least one target mutation in, for example, *parC* (*parE*). First-step mutations can be more reliably detected in tests with norfloxacin	C	[94,118–120]
13.4	*Streptococcus pneumoniae*	Levofloxacin and moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of combined mutations in, for example, *parC* and *gyrA* leads to complete or partial cross-resistance to all fluoroquinolones	B	[121]
13.5	*Enterobacteriaceae*	Ciprofloxacin	All fluoroquinolones	IF resistant to ciprofloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of at least two target mutations in either *gyrA* or *gyrA* plus *parC*. Exceptionally, production of the AAC(6')-Ib-cr enzyme may affect ciprofloxacin but not levofloxacin	B	[93]
13.6	*Salmonella* spp.	Ciprofloxacin	All fluoroquinolones	IF ciprofloxacin MIC is >0.06 mg/L, THEN report as resistant to all fluoroquinolones	Evidence for clinical failure of fluoroquinolones in cases of resistance caued by the acquisition of at least one target mutation in *gyrA*	A (*Salmonella typhi*), B (other *Salmonella* spp.)	[95,97,98]
13.7	*Haemophilus influenzae*	Nalidixic acid	All fluoroquinolones	IF resistant in nalidixic acid disk diffusion screen test, THEN determine MIC of the fluoroquinolone to be used in therapy (ofloxacin, ciprofloxacin, levofloxacin, or moxifloxacin)	Decreased susceptibility to fluoroquinolones in *H. influenzae* caused by target topoisomerase mutations can be more reliably detected in tests with nalidixic acid. High-level fluoroquinolone resistance in this organism has been rarely described. Until there is evidence of clinical significance of these isolates, they should be reported as resistant	C	[96,122]
13.8	*Neisseria gonorrhoeae*	Ciprofloxacin and ofloxacin	All fluoroquinolones	IF resistant to ciprofloxacin or ofloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of at least two target mutations in either *gyrA* or *gyrA* plus *parC*	C	[123]

Haemophilus influenaze- „expert rules”

TABLE 5. Exceptional phenotypes of Gram-negative bacteria

Rule no.	Organisms	Exceptional phenotypes
5.1	Any *Enterobacteriaceae* (except *Proteae*)	Resistant to meropenem and/or imipenem[a]
5.2	*Serratia marcescens* and *Proteae*	Susceptible to colistin
5.3	*Pseudomonas aeruginosa* and *Acinetobacter* spp.	Resistant to colistin
5.4	*Haemophilus influenze*	Resistant to any third-generation cephalosporin, carbapenems, and fluoroquinolones
5.5	*Moraxella catarrhalis*	Resistant to ciprofloxacin and any third-generation cephalosporin
5.6	*Neisseria meningitidis*	Resistant to any third-generation cephalosporin and fluoroquinolones
5.7	*Neisseria gonorrhoeae*	Resistant to third-generation cephalosporin and spectinomycin

[a]Except in countries in which carbapenemase-producing *Enterobacteriaceae* are not rare.

TABLE 3. In … bacteria; Gr … intrinsically …

… -fermentative Gram-negative … gative bacteria listed are also …

Rule no.	…	…	…	…	…	…	Trimethoprim	Nalidixic acid
3.1							-	-
3.2							R	-
3.3							R	-
3.4							R	R
3.5							R	-

R, resistant; I, …

Haemophilus influenaze-testiranje osjetljivosti
6. OSTALI ANTIBIOTICI

Haemophilus influenzae

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Miscellaneous agents	MIC breakpoint (mg/L)			Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Chloramphenicol	2	2	30	28	28	1. Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Colistin	-	-		-	-	
Daptomycin	-	-		-	-	
Fosfomycin iv	IE	IE		IE	IE	
Fosfomycin oral	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
Mupirocin	-	-		-	-	
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Rifampicin (for prophylaxis only)	1	1	5	18	18	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole[1]	0.5	1	1.25-23.75	23	20	

Haemophilus influenaze: antibiogram....

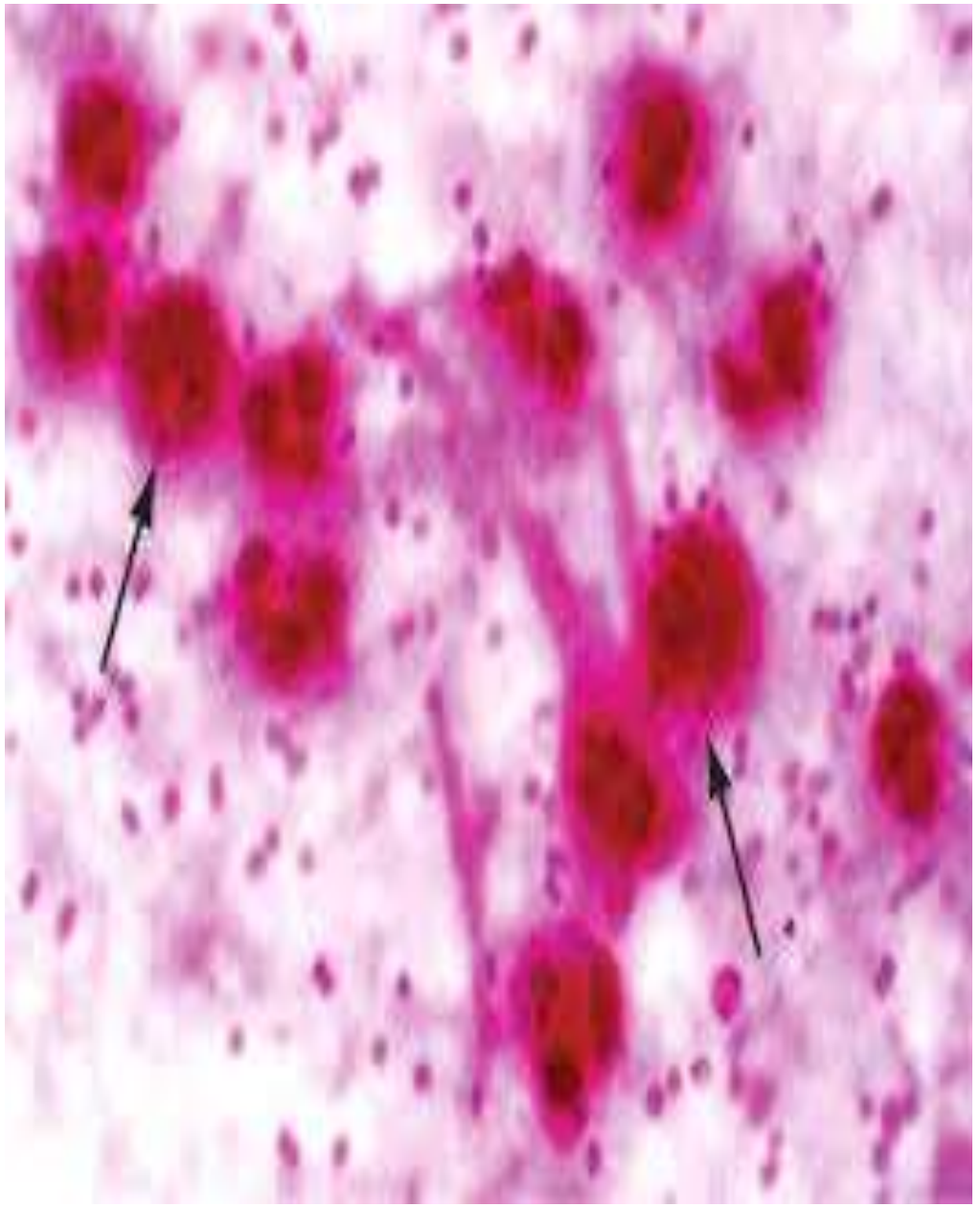
	ANTIBIOTIK	OZNAKA	S	I	R	NAPOMENA
	AMPICILIN 2µg	**AP**	**≥ 16**	**-**	**< 16**	
	rezultate upisati pod **AMOKSICILIN (A)**					
1	**AMOKSICILIN**	**A**				prema AP
2	**AUGMENTIN 3µg** AMOKSICILIN-KLAVULANAT	**AUG**	**≥ 15**	**-**	**< 15**	određuje osjetljivost na SAM i PTZ
3	**CEFUROKSIM iv 30µg**	**CXM**	**≥ 26**	**25**	**< 25**	1 disk = 2 interpretacije
	CEFUROKSIM oralni 30µg		**≥ 50**	**26-49**	**< 26**	divlji tip je umjereno osjetljiv
4	**CEFTRIAKSON 30µg**	**CRO**	**≥ 30**	**-**	**< 30**	
	▪ za sada su svi CRO osjetljivi, A i CXM rijetko rezistentni. Provjeriti ! Učiniti test cefinaze!					
5	**KOTRIMOKSAZOL 25µg** TRIMETOPRIM-SULFAMETOKSAZOL	**TS**	**≥ 23**	**20-22**	**< 20**	

MORAXELLA CATARRHALIS

Gram negativni diplokok
DNAza+
OXIDAZA+
„Gurajuće” suhe kolonije
Većina sojeva producira β laktamazu

Test CEFINAZE + → AMP/AMX **R**

Inokulum: McFarland 0.5
Inkubacija: 5% CO2, 35+/-1°C, 18+/-2h



Moraxella catarrhalis-intrinzična rezistencija („expert rules”)

TABLE 3. Intrinsic resistance in Gram-negative bacteria other than *Enterobacteriaceae* and non-fermentative Gram-negative bacteria; Gram-negative bacteria other than *Enterobacteriaceae* and non-fermentative Gram-negative bacteria listed are also intrinsically resistant to glycopeptides, lincosamides, daptomycin, and linezolid

Rule no.	Organisms	Macrolides	Fusidic acid	Streptogramins	Trimethoprim	Nalidixic acid
3.1	*Haemophilus influenzae*	I	R	–	–	–
3.2	*Moraxella catarrhalis*	–	–	–	R	–
3.3	*Neisseria* spp.	–	–	–	R	–
3.4	*Campylobacter fetus*	–	R	R	R	R
3.5	*Campylobacter jejuni*, *Campylobacter coli*	–	R	R	R	–

R, resistant; I, intermediate.

Moraxella catarrhalis-testiranje osjetljivosti

1. PENICILINI

većina sojeva producira β laktamazu (test cefinaze +) → AMP/AMX R

amoksicilin-klavulanat = (piperacilin-tazobaktam/ampicilin-sulbaktam)

Moraxella catarrhalis

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1°C, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Haemophilus influenzae* ATCC 49766 ~~or Haemophilus influenzae NCTC 8468.~~ For control of the inhibitor component of beta-lactam inhibitor-combination disks, use *Staphylococcus aureus* ATCC 29213.

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)	
	S ≤	R >		S ≥	R <
Benzylpenicillin	-	-		-	-
Ampicillin	-[1]	-[1]		-	-
Ampicillin-sulbactam	1[2,3]	1[2,3]		Note[A]	Note[A]
Amoxicillin	-[1]	-[1]		-	-
Amoxicillin-clavulanic acid	1[4]	1[4]	2-1	19	19
Piperacillin	-[1]	-[1]		-	-
Piperacillin-tazobactam	Note[3]	Note[3]		Note[A]	Note[A]
Ticarcillin	IE	IE		IE	IE
Ticarcillin-clavulanic acid	IE	IE		IE	IE
Phenoxymethylpenicillin	-	-		-	-
Oxacillin	-	-		-	-
Cloxacillin	-	-		-	-
Dicloxacillin	-	-		-	-
Flucloxacillin	-	-		-	-
Mecillinam (uncomplicated UTI only)	-	-		-	-

Notes
Numbered notes relate to general comments and/or MIC breakpoints.
Lettered notes relate to the disk diffusion method.

1. Most *M. catarrhalis* produce beta-lactamase, although beta-lactamase production is slow and may give weak results with *in vitro* tests. Beta-lactamase producers should be reported resistant to penicillins and aminopenicillins without inhibitors.
2. For susceptibility testing purposes, the concentration of sulbactam is fixed at 4 mg/L.
3/A. Susceptibility can be inferred from amoxicillin-clavulanic acid.
4. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.

Moraxella catarrhalis-testiranje osjetljivosti

Moraxella catarrhalis **EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01**

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefaclor	-	-		-	-	
Cefadroxil	-	-		-	-	
Cefalexin	-	-		-	-	
Cefazolin	-	-		-	-	
Cefepime	4	4	30	20	20	
Cefixime	0.5	1	5	21	18	
Cefotaxime	1	2	5	20	17	
Cefoxitin	NA	NA		NA	NA	
Cefpodoxime	IP	IP	10	IP	IP	
Ceftaroline	IE	IE		IE	IE	
Ceftazidime	-	-		-	-	
Ceftibuten	IE	IE		IE	IE	
Ceftobiprole	IE	IE		IE	IE	
Ceftolozane-tazobactam	IE	IE		IE	IE	
Ceftriaxone	1	2	30	24	21	
Cefuroxime iv	4	8	30	21	18	
Cefuroxime oral	0.125	4	30	50	21	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doripenem[1]	1	1	10	30	30	1. Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Ertapenem[1]	0.5	0.5	10	29	29	
Imipenem[1]	2	2	10	29	29	
Meropenem[1]	2	2	10	33	33	

Monobactams	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Aztreonam	IE	IE		IE	IE	

2. CEFALOSPORINI

cefuroksim oralni/iv

ceftriakson

cefepim

cefiksim

3. KARBAPENEMI

neosjetljivih sojeva NEMA

Moraxella catarrhalis-testiranje osjetljivosti

4. FLUOROKINOLONI

Moraxella catarrhalis

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.5	0.5	5	26[A]	26[A]	A. The nalidixic acid disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Levofloxacin	1	1	5	26[A]	26[A]	B. Isolates categorised as susceptible to nalidixic acid can be reported susceptible to ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin. Isolates categorised as non-susceptible may have fluoroquinolone resistance and should be tested against the appropriate agent.
Moxifloxacin	0.5	0.5	5	23[A]	23[A]	
Nalidixic acid (screen)	NA	NA	30	23[B]	Note[B]	
Norfloxacin	-	-		-	-	
Ofloxacin	0.5	0.5	5	25[A]	25[A]	

Disk nalidiksične kiseline (NA)-screen disk za kinolone

Ukoliko je:

NA (S) → izdati sve kinolone (S)

NA (R) → potrebno je testirati svaki od kinolona posebno!

Moraxella catarrhalis-testiranje osjetljivosti

5. MAKROLIDI

ERITROMICIN disk-prema njemu izdati osjetljivost za azitromicin, klaritomicin i roksitromicin

6.TETRACIKLINI

TETRACIKLINSKI disk

Ukoliko je:

TE (S) → izdati doksiciklin i minociklin (S)

TE (R) → potrebno je testirati svaki od tetraciklina zasebno-MIKom!

Moraxella catarrhalis

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Macrolides, lincosamides and streptogramins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Azithromycin	0.25[1]	0.5[1]		Note[A]	Note[A]	1/A. Erythromycin can be used to determine susceptibility to azithromycin, clarithromycin and roxithromycin.
Clarithromycin	0.25[1]	0.5[1]		Note[A]	Note[A]	
Erythromycin	0.25	0.5	15	23[A]	20[A]	
Roxithromycin	0.5[1]	1[1]		Note[A]	Note[A]	
Telithromycin	0.25	0.5	15	23	20	
Clindamycin	-	-		-	-	
Quinupristin-dalfopristin	-	-		-	-	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Doxycycline	1[1]	2[1]		Note[A]	Note[A]	1/A. Isolates susceptible to tetracycline are also susceptible to doxycycline and minocycline, but some resistant to tetracycline may be susceptible to minocycline and/or doxycycline. An MIC method should be used to test doxycycline susceptibility of tetracycline resistant isolates if required.
Minocycline	1[1]	2[1]	30	25[A]	22[A]	
Tetracycline	1[1]	2[1]	30	28[A]	25[A]	
Tigecycline	IE	IE		IE	IE	

Moraxella catarrhalis
6. OSTALI ANTIBIOTICI

Moraxella catarrhalis

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
	S ≤	R >		S ≥	R <	
Chloramphenicol	2[1]	2[1]	30	30[A]	30[A]	**1/A.** Breakpoints relate to topical use only. **2.** Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.
Colistin	-	-		-	-	
Daptomycin	-	-		-	-	
Fosfomycin iv	IE	IE		IE	IE	
Fosfomycin oral	-	-		-	-	
Fusidic acid	-	-		-	-	
Metronidazole	-	-		-	-	
Mupirocin	-	-		-	-	
Nitrofurantoin (uncomplicated UTI only)	-	-		-	-	
Rifampicin	-	-		-	-	
Spectinomycin	-	-		-	-	
Trimethoprim (uncomplicated UTI only)	-	-		-	-	
Trimethoprim-sulfamethoxazole[2]	0.5	1	1.25-23.75	18	15	

Moraxella catarrhalis: antibiogram….

18. ANTIBIOGRAM ZA *Moraxella catarrhalis*						
1.PLOČA MH-F / 0.5 Mc Farland / 16-20 h / 4-6% CO_2						
	Napraviti test cefinaze :	**CEF +**	**AMOKSICILIN**		**AMX**	**R**
		CEF -	**AMOKSICILIN**		**AMX**	**pokazati liječniku**
	DNA +, OX-aza +					
	ANTIBIOTIK	**OZNAKA**	**S**	**I**	**R**	**NAPOMENA**
1	**AMOKSICILIN 30µg**	**A**				**prema CEF**
2	**AUGMENTIN 3µg** AMOKSICILIN-KLAVULANAT	**AUG**	**≥ 19**	**-**	**< 19**	
	ERITROMICIN 15µg	**E**	**≥ 23**	**20-22**	**< 20**	
	• vrijednosti **ERY** upisati za **AZITROMICIN AZM i KLARITROMICIN CLR**					
3	**AZITROMICIN**	**AZM**				prema E
4	**KLARITROMICIN**	**CLA**				prema E
DODATNO TESTIRANJE (DD) :						
	CEFTRIAKSON 30µg	CRO	≥ 24	21-23	< 21	
	NALIDIKSIČNA KIS. 30µg	NA	≥ 23	-	< 23	
	CIPROFLOKSACIN 5µg	CIP	≥ 23	-	< 23	
	MOKSIFLOKSACIN 5µg	MFX	≥ 23	-	< 23	
	KOTRIMOKSAZOL 25µg	TS	≥ 18	15-17	< 15	

NEISSERIA MENINGITIDIS

Gram negativni diplokok
Utilizira maltozu i glukozu
OXIDAZA +

Pri radu s meningokokom preporučene mjere opreza **BSL 2/** postupci kod kojih može doći do stvaranja aerosola izvode se u sigurnosnom kabinetu – BSC

Inokulum: McFarland 0.5
Inkubacija: 5% CO_2, 35+/-1°C, 16-20h

Testiranje osjetljivosti: **SAMO** određivanjem **MIK**-ova jer kriteriji za DD još nisu definirani.

N. meningitidis

TABLE 5. Exceptional phenotypes of Gram-negative bacteria

Rule no.	Organisms	Exceptional phenotypes
5.1	*Any Enterobacteriaceae (except Proteae)*	Resistant to meropenem and/or imipenem[a]
5.2	*Serratia marcescens and Proteae*	Susceptible to colistin
5.3	*Pseudomonas aeruginosa and Acinetobacter spp.*	Resistant to colistin
5.4	*Haemophilus influenze*	Resistant to any third-generation cephalosporin, carbapenems, and fluoroquinolones
5.5	*Moraxella catarrhalis*	Resistant to ciprofloxacin and any third-generation cephalosporin
5.6	*Neisseria meningitidis*	Resistant to any third-generation cephalosporin and fluoroquinolones
5.7	*Neisseria gonorrhoeae*	Resistant to third-generation cephalosporin and spectinomycin

[a]Except in countries in which carbapenemase-producing *Enterobacteriaceae* are not rare.

TABLE 3. Intrinsic resistance in Gram-negative bacteria other than *Enterobacteriaceae* and non-fermentative Gram-negative bacteria; Gram-negative bacteria other than *Enterobacteriaceae* and non-fermentative Gram-negative bacteria listed are also intrinsically resistant to glycopeptides, lincosamides, daptomycin, and linezolid

Rule no.	Organisms	Macrolides	Fusidic acid	Streptogramins	Trimethoprim	Nalidixic acid
3.1	*Haemophilus influenzae*	I	R	–	–	–
3.2	*Moraxella catarrhalis*	–	–	–	R	–
3.3	*Neisseria spp.*	–	–	–	R	–
3.4	*Campylobacter fetus*	–	R	R	R	R
3.5	*Campylobacter jejuni, Campylobacter coli*	–	R	R	R	–

R, resistant; I, intermediate.

N. meningitidis-testiranje osjetljivosti

Neisseria meningitidis

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion criteria for antimicrobial susceptibility testing of *Neisseria meningitidis* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer's instructions.

Penicillins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Benzylpenicillin	0.06	0.25	
Ampicillin	0.125	1	
Ampicillin-sulbactam	IE	IE	
Amoxicillin	0.125	1	
Amoxicillin-clavulanic acid	-	-	
Piperacillin	-	-	
Piperacillin-tazobactam	-	-	
Ticarcillin	-	-	
Ticarcillin-clavulanic acid	-	-	
Phenoxymethylpenicillin	-	-	
Oxacillin	-	-	
Cloxacillin	-	-	
Dicloxacillin	-	-	
Flucloxacillin	-	-	
Mecillinam (uncomplicated UTI only)	-	-	

N. meningitidis-testiranje osjetljivosti

Neisseria meningitidis **EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01**

Cephalosporins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Cefaclor	-	-	1. Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Cefadroxil	-	-	
Cefalexin	-	-	
Cefazolin	-	-	
Cefepime	-	-	
Cefixime	-	-	
Cefotaxime[1]	0.125	0.125	
Cefoxitin	-	-	
Cefpodoxime	-	-	
Ceftaroline	-	-	
Ceftazidime	-	-	
Ceftibuten	-	-	
Ceftobiprole	-	-	
Ceftolozane-tazobactam	-	-	
Ceftriaxone[1]	0.125	0.125	
Cefuroxime iv	-	-	
Cefuroxime oral	-	-	

Carbapenems	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Doripenem	IE	IE	1. Breakpoints relate to meningitis only. 2. Non-susceptible isolates are rare or not yet reported. The identification and antimicrobial susceptibility test result on any such isolate must be confirmed and the isolate sent to a reference laboratory.
Ertapenem	-	-	
Imipenem	-	-	
Meropenem[1,2]	0.25	0.25	

2. CEFALOSPORINI
cefotaksim
ceftriakson

3. KARBAPENEMI
meropenem
(zone vrijede samo za meningitis) neosjetljivih sojeva NEMA

N. meningitidis

Kinoloni

Tetraciklini

Kloramfenikol

Rifampicin

Neisseria meningitidis **EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01**

Miscellaneous agents	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Chloramphenicol	2	4	1. For prophylaxis of meningitis only (refer to national guidelines).
Colistin	-	-	
Daptomycin	-	-	
Fosfomycin iv	-	-	
Fosfomycin oral	-	-	
Fusidic acid	-	-	
Metronidazole	-	-	
Mupirocin	-	-	
Nitrofurantoin (uncomplicated UTI only)	-	-	
Rifampicin[1]	0.25	0.25	
Spectinomycin	-	-	
Trimethoprim (uncomplicated UTI only)	-	-	
Trimethoprim-sulfamethoxazole	-	-	

N. meningitidis: antibiogram….

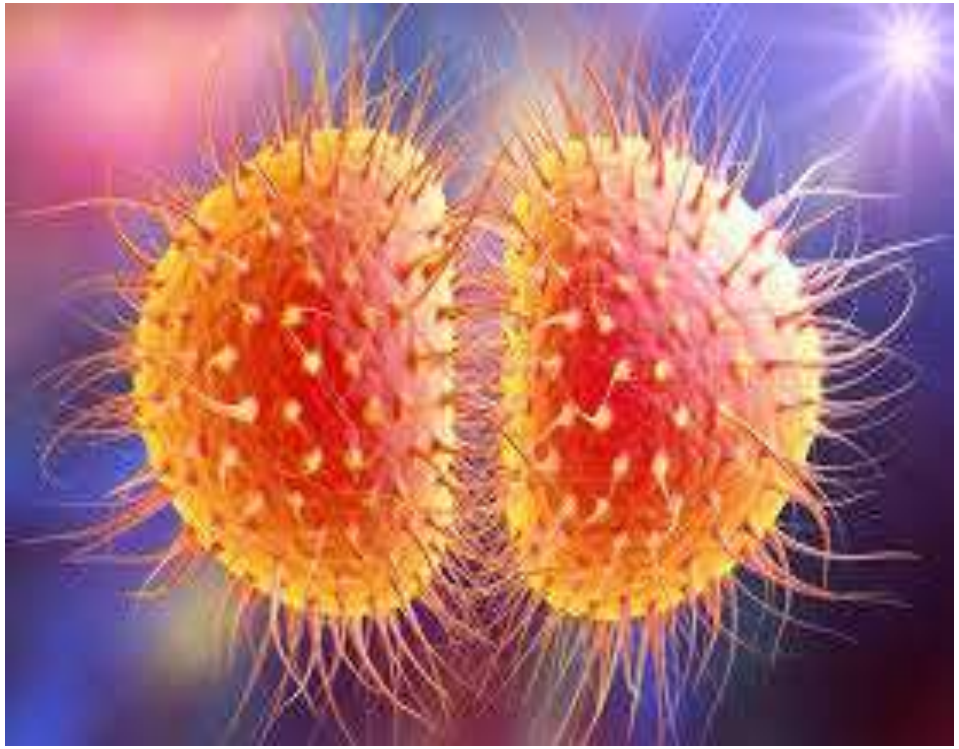
14. ANTIBIOGRAM ZA *N. meningitidis*

Preporučene mjere opreza **BSL 2** :
postupci kod kojih može doći do stvaranja aerosola izvode se u sigurnosnom kabinetu – BSC

kod PRIMARNO STERILNIH UZORAKA osjetljivost se ispituje MIK test trakicom
MH-F / McFarland 0.5 / 16-20 h / 5 % CO2

ANTIBIOTIK	OZNAKA	S	I	R	NAPOMENA
PENICILIN	P	≤ 0.064	0.25-0.125	> 0.25	EUCAST = CLSI 2009
CEFTRIAKSON	CRO	≤ 0.125	-	> 0.125	EUCAST = CLSI 2009
RIFAMPICIN	RD	≤ 0.25	-	> 0.25	EUCAST ≠ CLSI 2009
CIPROFLOKSACIN	CIP	≤ 0.032	-	> 0.032	EUCAST = CLSI 2009

NEISSERIA GONORRHOEAE



Gram negativni diplokok
Morfološki identičan meningokoku
Utilizira **SAMO GLUKOZU**
Posjeduje pile
OXIDAZA +

Inokulum: McFarland 0.5
Inkubacija: 5% CO_2, 35+/-1°C, 16-20h

Testiranje osjetljivosti: **SAMO** određivanjem **MIK**-ova jer kriteriji za DD još nisu definirani

IZOLATE UVIJEK TESTIRATI NA β LAKTAMAZE (penicilinaza)!!!

Neisseria gonorrhoeae-testiranje osjetljivosti

PENICILINI

Ako je izolat βlaktamaza+ izdati → benzilpenicilin, ampicilin i amoksicilin **R**

βlaktamaza- izdati osjetljivost na AMP/AMCX prema penicilinu

Neisseria gonorrhoeae

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion criteria for antimicrobial susceptibility testing of *Neisseria gonorrhoeae* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions.

Penicillins[1]	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Benzylpenicillin	0.06[1]	1	1. Always test for beta-lactamase. If positive, report resistant to benzylpenicillin, ampicillin and amoxicillin. The susceptibility of beta-lactamase negative isolates to ampicillin and amoxicillin can be inferred from benzylpenicillin.
Ampicillin[1]	Note[1]	Note[1]	
Ampicillin-sulbactam	IE	IE	
Amoxicillin[1]	Note[1]	Note[1]	
Amoxicillin-clavulanic acid	Note[1]	Note[1]	
Piperacillin	-	-	
Piperacillin-tazobactam	-	-	
Ticarcillin	-	-	
Ticarcillin-clavulanic acid	-	-	
Phenoxymethylpenicillin	-	-	
Oxacillin	-	-	
Cloxacillin	-	-	
Dicloxacillin	-	-	
Flucloxacillin	-	-	
Mecillinam (uncomplicated UTI only)	-	-	

Neisseria gonorrhoeae-testiranje osjetljivosti

CEFALOSPORINI

cefiksim

cefotaksim

ceftriakson

Neisseria gonorrhoeae

Cephalosporins	MIC breakpoint (mg/L)	
	S ≤	R >
Cefaclor	-	-
Cefadroxil	-	-
Cefalexin	-	-
Cefazolin	-	-
Cefepime	-	-
Cefixime	0.125	0.125
Cefotaxime	0.125	0.125
Cefoxitin	-	-
Cefpodoxime	-	-
Ceftaroline	-	-
Ceftazidime	-	-
Ceftibuten	-	-
Ceftobiprole	-	-
Ceftolozane-tazobactam	-	-
Ceftriaxone	0.125	0.125
Cefuroxime iv	-	-
Cefuroxime oral	-	-

TABLE 5. Exceptional phenotypes of Gram-negative bacteria

Rule no.	Organisms	Exceptional phenotypes
5.1	*Any Enterobacteriaceae* (except *Proteae*)	Resistant to meropenem and/or imipenem[a]
5.2	*Serratia marcescens* and *Proteae*	Susceptible to colistin
5.3	*Pseudomonas aeruginosa* and *Acinetobacter* spp.	Resistant to colistin
5.4	*Haemophilus influenze*	Resistant to any third-generation cephalosporin, carbapenems, and fluoroquinolones
5.5	*Moraxella catarrhalis*	Resistant to ciprofloxacin and any third-generation cephalosporin
5.6	*Neisseria meningitidis*	Resistant to any third-generation cephalosporin and fluoroquinolones
5.7	*Neisseria gonorrhoeae*	Resistant to third-generation cephalosporin and spectinomycin

[a]Except in countries in which carbapenemase-producing *Enterobacteriaceae* are not rare.

Neisseria gonorrhoeae-testiranje osjetljivosti

KINOLONI: ciprofloksacin/ofloksacin (expert rules)

MAKROLIDI: azitromicin

TETRACIKLINI: tetraciklin

SPEKTINOMICIN

TABLE 13. Interpretive rules for quinolones

Rule no.	Organism	Agents tested	Agents affected	Rule	Exceptions, scientific basis, and comments	Evidence grade	References
13.1	*Staphylococcus* spp.	Ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning of risk for development of resistance during therapy with quinolones	Acquisition of at least one target mutation in *grlA*	C	[86,92]
13.2	*Staphylococcus* spp.	Levofloxacin and moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of combined mutations in *grlA* and *gyrA* leads to complete or partial cross-resistance to all fluoroquinolones	C	[92,116,117]
13.3	*Streptococcus pneumoniae*	Ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin	All fluoroquinolones	IF resistant to ofloxacin or ciprofloxacin, but not to levofloxacin or moxifloxacin, THEN report warning that acquisition of a first-step mutation may lead to resistance development under therapy with other quinolones	Acquisition of at least one target mutation in, for example, *parC* (*parE*). First-step mutations can be more reliably detected in tests with norfloxacin	C	[94,118–120]
13.4	*Streptococcus pneumoniae*	Levofloxacin and moxifloxacin	All fluoroquinolones	IF resistant to levofloxacin or moxifloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of combined mutations in, for example, *parC* and *gyrA* leads to complete or partial cross-resistance to all fluoroquinolones	B	[121]
13.5	*Enterobacteriaceae*	Ciprofloxacin	All fluoroquinolones	IF resistant to ciprofloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of at least two target mutations in either *gyrA* or *gyrA* plus *parC*. Exceptionally, production of the AAC(6')-Ib-cr enzyme may affect ciprofloxacin but not levofloxacin	B	[93]
13.6	*Salmonella* spp.	Ciprofloxacin	All fluoroquinolones	IF ciprofloxacin MIC is >0.06 mg/L, THEN report as resistant to all fluoroquinolones	Evidence for clinical failure of fluoroquinolones in cases of resistance caued by the acquisition of at least one target mutation in *gyrA*	A (*Salmonella typhi*), B (other *Salmonella* spp.)	[95,97,98]
13.7	*Haemophilus influenzae*	Nalidixic acid	All fluoroquinolones	IF resistant in nalidixic acid disk diffusion screen test, THEN determine MIC of the fluoroquinolone to be used in therapy (ofloxacin, ciprofloxacin, levofloxacin, or moxifloxacin)	Decreased susceptibility to fluoroquinolones in *H. influenzae* caused by target topoisomerase mutations can be more reliably detected in tests with nalidixic acid. High-level fluoroquinolone resistance in this organism has been rarely described. Until there is evidence of clinical significance of these isolates, they should be reported as resistant	C	[96,122]
13.8	*Neisseria gonorrhoeae*	Ciprofloxacin and ofloxacin	All fluoroquinolones	IF resistant to ciprofloxacin or ofloxacin, THEN report as resistant to all fluoroquinolones	Acquisition of at least two target mutations in either *gyrA* or *gyrA* plus *parC*	C	[123]

Neisseria gonorrhoeae: antibiogram....

isključivo MIK metoda!

15. ANTIBIOGRAM ZA *N. gonorrhoeae* (EUCAST)					
MH-F / 0.5 Mc Farland / 16-20 h / 4-6 % CO_2					
OSJETLJIVOST SE ISPITUJE MIK test trakicama					
ANTIBIOTIK	**OZNAKA**	**S**	**I**	**R**	**NAPOMENA**
PENICILIN	**P**	**≤0.064**	**0.125-1**	**> 1**	
CEFTRIAKSON	**CRO**	**≤0.125**	**-**	**>0.12**	
CEFIKSIM	**CFM**	**≤0.125**	**-**	**>0.12**	
TETRACIKLIN	**TE**	**≤ 0.5**	**1**	**> 1**	
CIPROFLOKSACIN	**CIP**	**≤0.032**	**0.064**	**>0.064**	
AZITROMICIN	**AZM**	**≤ 0.25**	**0.5**	**> 0.5**	

CAMPYLOBACTER spp.

Gram negativni zavijeni štapići „galebova krila”
Kolonije morfološki variraju

Routine QC

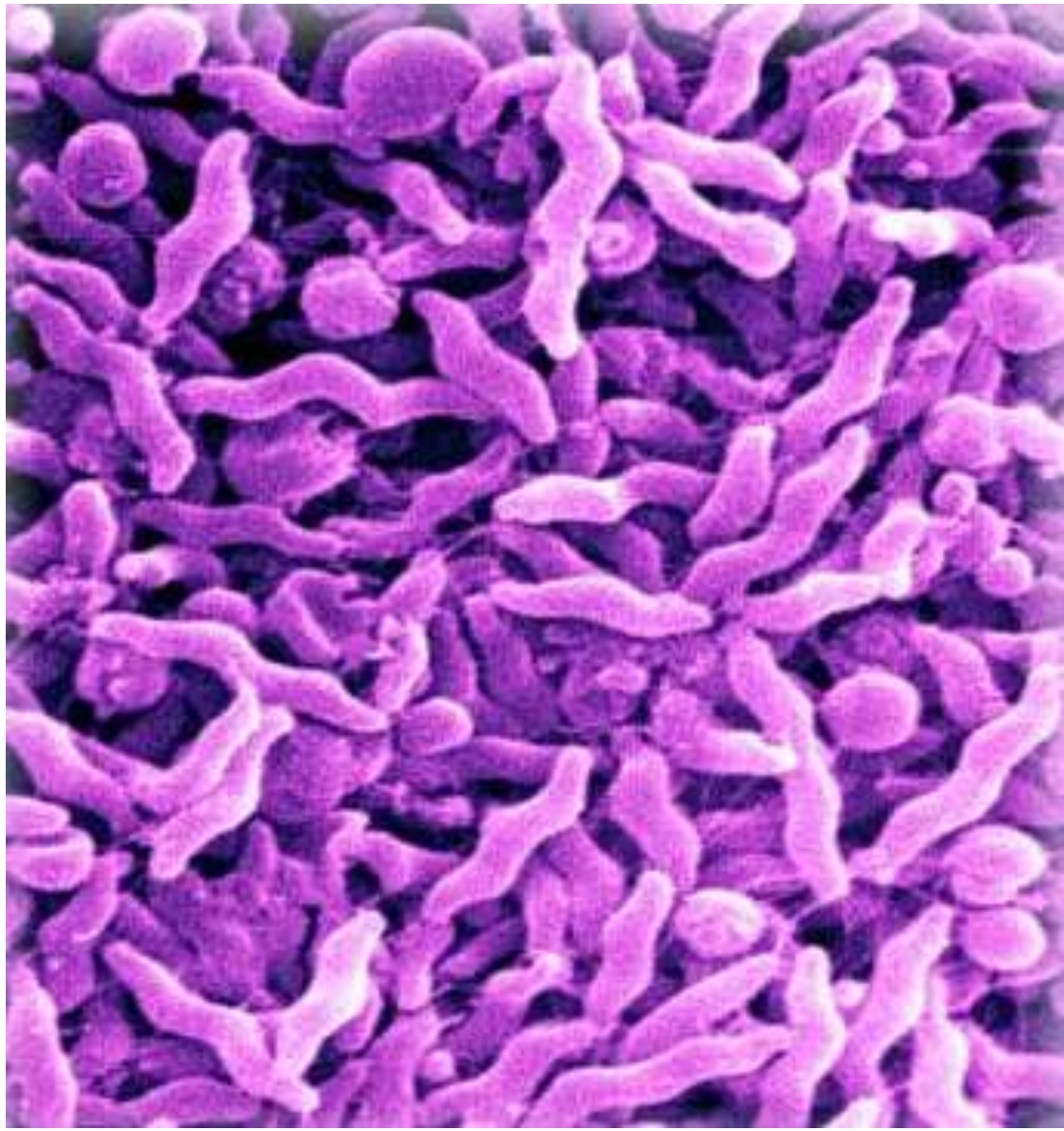
EUCAST QC Tables v. 6.0, valid from 2016-01-01

***Campylobacter jejuni* ATCC 33560**
(NCTC 11351, CIP 702, DSM 4688, CCUG 11284)

Disk diffusion methodology: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD, McFarland 0.5, microaerobic environment, 41±1°C, 24h. Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light. The MH-F plates should be dried prior to inoculation to reduce swarming (at 20-25°C over night or at 35°C, with the lid removed, for 15 min).

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target	Range		Target[1]	Range[2]
Ciprofloxacin	IP	IP	5	*38*	*34-42*
Erythromycin	IP	IP	15	*31*	*27-35*
Tetracycline	IP	IP	30	*34*	*30-38*

[1] Calculated by EUCAST.
[2] Established and validated by EUCAST.
IP = In Preparation



Campylobacter jejuni i coli

MAKROLIDI: testira se **eritromicin**-prema osjetljivosti eritomicina izdaje osjetljivost azitromicina i klaritromicina

različite zone interpretacije osjetljivosti ovisno o kojoj vrsti se radi!!!!

TETRACIKLINI: testira se **tetraciklin-**prema osjetljivosti tetraciklina izdaje se i doksiciklin

Campylobacter jejuni* and *coli

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F). The MH-F plates should be dried prior to inoculation to reduce swarming (at 20-25°C overnight or at 35°C, with the lid removed, for 15 min).
Inoculum: McFarland 0.5
Incubation: Microaerobic environment, 41±1°C, 24h. Isolates with insufficient growth after 24h incubation are reincubated immediately and inhibition zones read after a total of 40-48h incubation.
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Campylobacter jejuni* ATCC 33560

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.5	0.5	5	26	26	

Macrolides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Azithromycin	Note[1]	Note[1]		Note[A]	Note[A]	1/A. Erythromycin can be used to determine susceptibility to azithromycin and clarithromycin.
Clarithromycin	Note[1]	Note[1]		Note[A]	Note[A]	
Erythromycin, *C. jejuni*	4[1]	4[1]	15	20[A]	20[A]	
Erythromycin, *C. coli*	8[1]	8[1]	15	24[A]	24[A]	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	Note[1]	Note[1]		Note[A]	Note[A]	1/A. Tetracycline can be used to determine susceptibility to doxycycline.
Tetracycline	2[1]	2[1]	30	30[A]	30[A]	

Campylobacter jejuni i coli: antibiogram….

22. ANTIBIOGRAM ZA *Campylobacter sp.* iz stolice						
PLOČA MH-F/ Mc Farland 0.5 / 24h (48h) / 42 °C / mikroaerofilni uvjeti						
	ANTIBIOTIK	OZNAKA	S	I	R	NAPOMENA
1	ERITROMICIN 15µg	E	≥ 20	-	< 20	C.jejuni
			≥ 24	-	< 24	C.coli
2	CIPROFLOKSACIN 5µg	CIP	≥ 26	-	< 26	
3	TETRACIKLIN	T	≥ 30	-	< 30	

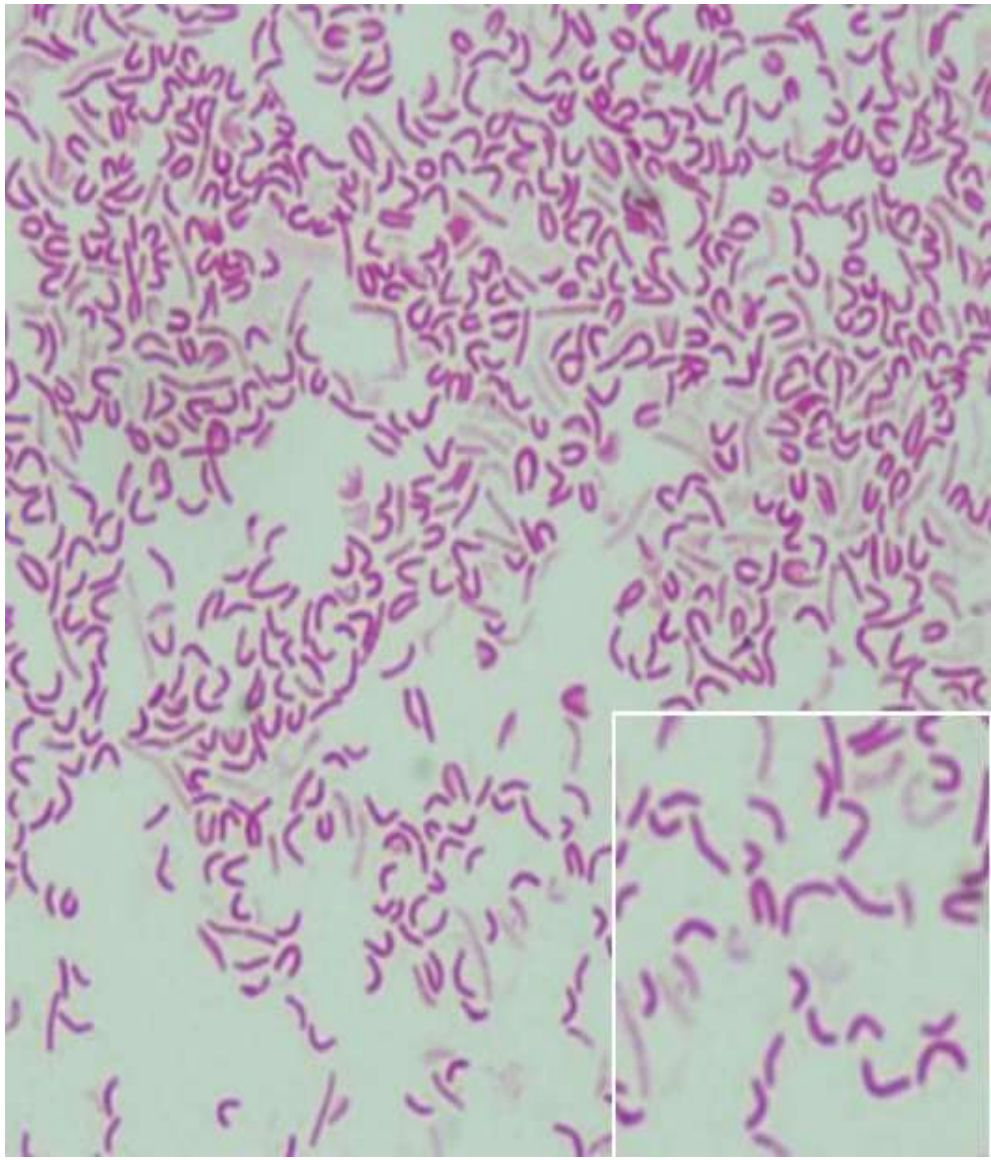
HELICOBACTER PYLORI

Gram negativni zavijeni štapići
Na krvnom agaru: sitne kolonije bez hemolize
OXIDAZA +

Inokulum: McFarland **3.0**
Inkubacija: mirkoaerofilni uvjeti, 35+/-1°C, 72h

Preporuča se **rad u kabinetu**-H.pylori izrazito osjetljiv mikroorganizam (zaštita soja)

Testiranje osjetljivosti: **SAMO** određivanjem **MIK**-ova kriteriji za DD još nisu definirani



H. pylori: antibiogram….

isključivo MIK metoda!

21. ANTIBIOGRAM ZA *Helicobacter pylori*						
OSJETLJIVOST SE ISPITUJE MIK test trakicama: 4 PLOČE MH-F/ Mc Farland 3.0 /72h / 35 °C / mikroaerofilni uvjeti						
	ANTIBIOTIK	**OZNAKA**	**S**	**I**	**R**	**NAPOMENA**
1	**AMOKSICILIN**	**AML**	**≤ 0.125**	**-**	**> 0.125**	
2	**TETRACIKLIN**	**TE**	**≤ 1**	**-**	**> 1**	
3	**METRONIDAZOL**	**MTZ**	**≤ 8**	**-**	**> 8**	
4	**KLARITROMICIN**	**CLR**	**≤ 0.25**	**0.5**	**> 0.5**	

H. pylori-testiranje osjetljivosti

Helicobacter pylori

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion criteria for antimicrobial susceptibility testing of *Helicobacter pylori* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer's instructions.

Penicillins	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Amoxicillin	0.125[1]	0.125[1]	1. The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

Fluoroquinolones	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Levofloxacin	1[1]	1[1]	1. The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

Macrolides	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Clarithromycin	0.25[1]	0.5[1]	1. The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

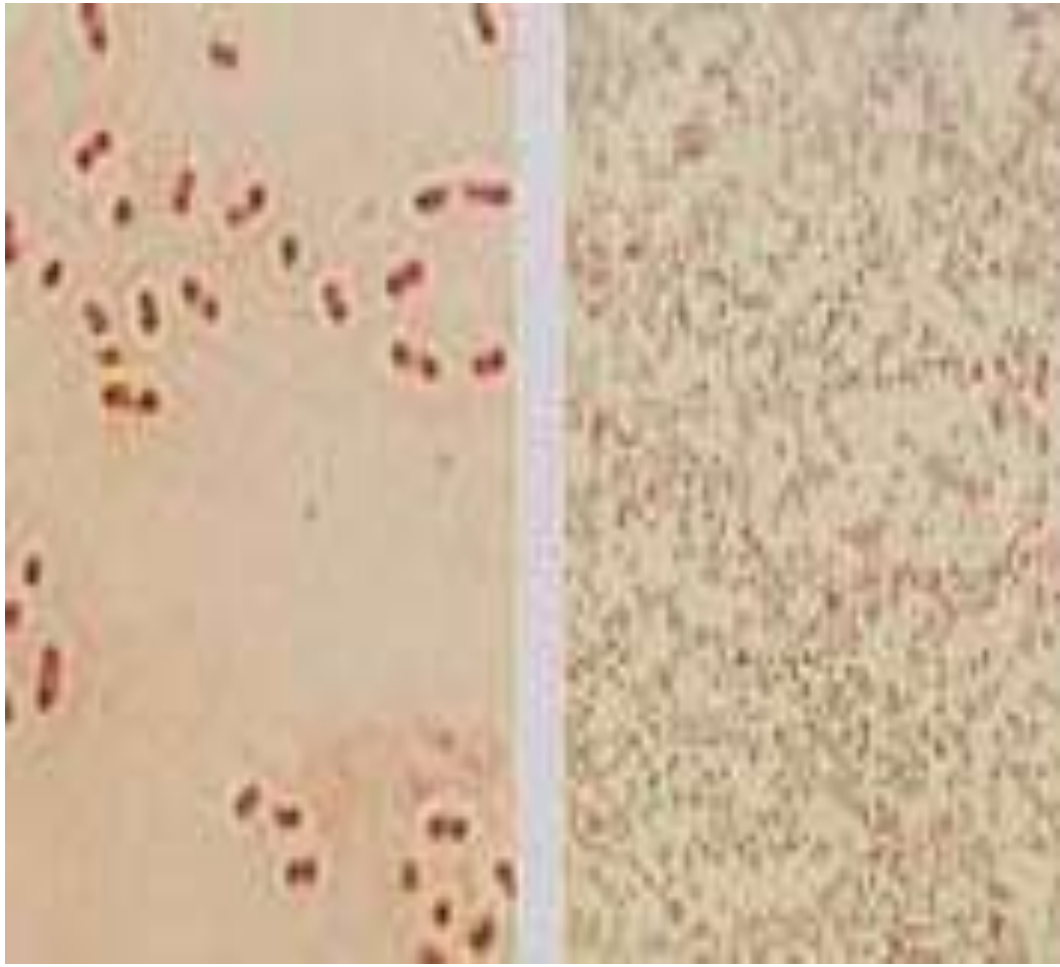
Tetracyclines	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Tetracycline	1[1]	1[1]	1. The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.

Miscellaneous agents	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Metronidazole	8[1]	8[1]	1. The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility.
Rifampicin	1[1]	1[1]	

PASTEURELLA MULTOCIDA

Raste na krvnom i čokoladnom agaru
NE raste na McConkey agaru
Poseban miris
OXIDAZA+
H_2O_2 +
Gram negativan, vrlo sitan mikroorganizam (kokobacil)

Inokulum: McFarland 0.5
Inkubacija: 5% CO_2, 35+/-1°C, 18+/-2h



Pasteurella multocida-testiranje osjetljivosti

NALIDIKSIČNA KISELINA (NA)-screen disk:

ako je NA (S): izdati CIP/LEVO (S)

ako je NA (R): treba testirati svaki od kinolona posebno

Pasteurella multocida

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1°C, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Haemophilus influenzae* ATCC 49766 ~~or *Haemophilus influenzae* NCTC 8468.~~ For control of the inhibitor component of beta-lactam inhibitor-combination disks, use *Staphylococcus aureus* ATCC 29213.

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.5	0.5	1 unit	17	17	1. For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L.
Ampicillin	1	1	2	17	17	A. Susceptibility can be inferred from ampicillin.
Amoxicillin	1	1		Note[A]	Note[A]	
Amoxicillin-clavulanic acid	1[1]	1[1]	2-1	15	15	

Cephalosporins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Cefotaxime	0.03	0.03	5	26	26	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	0.06	0.06	5	27[A]	27[A]	A. The nalidixic acid disk diffusion test can be used to screen for fluoroquinolone resistance. **See Note B.**
Levofloxacin	0.06	0.06	5	27[A]	27[A]	B. Isolates categorised as susceptible to nalidixic acid can be reported susceptible to ciprofloxacin and levofloxacin. Isolates categorised as non-susceptible may have fluoroquinolone resistance and should be tested against the appropriate agent.
Nalidixic acid (screen)	NA	NA	30	23[B]	Note[B]	

Pasteurella multocida-testiranje osjetljivosti

TETRACIKLIN-screen disk: izdati doksiciklin prema osjetljivosti tetraciklina

Pasteurella multocida

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Doxycycline	1	1		Note[A]	Note[A]	A. Susceptibility inferred from tetracycline screen test.
Tetracycline (screen)	NA	NA	30	24[A]	24[A]	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Trimethoprim-sulfamethoxazole[1]	0.25	0.25	1.25-23.75	23	23	1. Trimethoprim-sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.

Pasteurella multocida: antibiogram....

Testira se ampicilin (AP)-vrijednost amoksicilina se izdaje prema AP
Disk tetraciklina-vrijednosti dobivene odnose se i na doksiciklin
Disk nalidiksične kiseline (NA)-screen disk za kinolone
Ukoliko je:
NA (S) → izdati sve kinolone (S)
NA (R) → potrebno je testirati svaki od kinolona posebno!

24. ANTIBIOGRAM ZA *Pasteurella sp.*						
2 PLOČE MH-F / Mc Farland 0.5 / 5% CO_2 / 18 - 20 h						
GN kokobacili, sitni štapići	KA - γ hemoliza	MC - nema porasta	OX-aza +	H_2O_2 +	PG, A, AUG, T, CIP osjetljivi	
	ANTIBIOTIK		OZNAKA	S	I	R	NAPOMENA
1	PENICILIN	1IU	PG	≥ 17	-	< 17	PG mora biti (S)
	AMPICILIN	2µg	AP	≥ 17	-	< 17	
▪ vrijednosti AP upisati pod AMOKSICILIN (A)							
2	AMOKSICILIN		A				prema AP
3	CEFOTAXIME	5µg	CTX	≥ 26	-	< 26	
3	CIPROFLOKSACIN	5µg	CIP	≥ 27	-	< 27	
4	NALIDIKSIČNA KIS.	30µg	NA	≥ 23	-	< 23	skrining test
NA rezistentne izolate pokazati liječniku - izdati uz komentar: Izolat pokazuje početnu rezistenciju na kinolone							
5	TETRACIKLIN	30µg	T	≥ 24	-	< 24	skrining test

LISTERIA MONOCYTOGENES

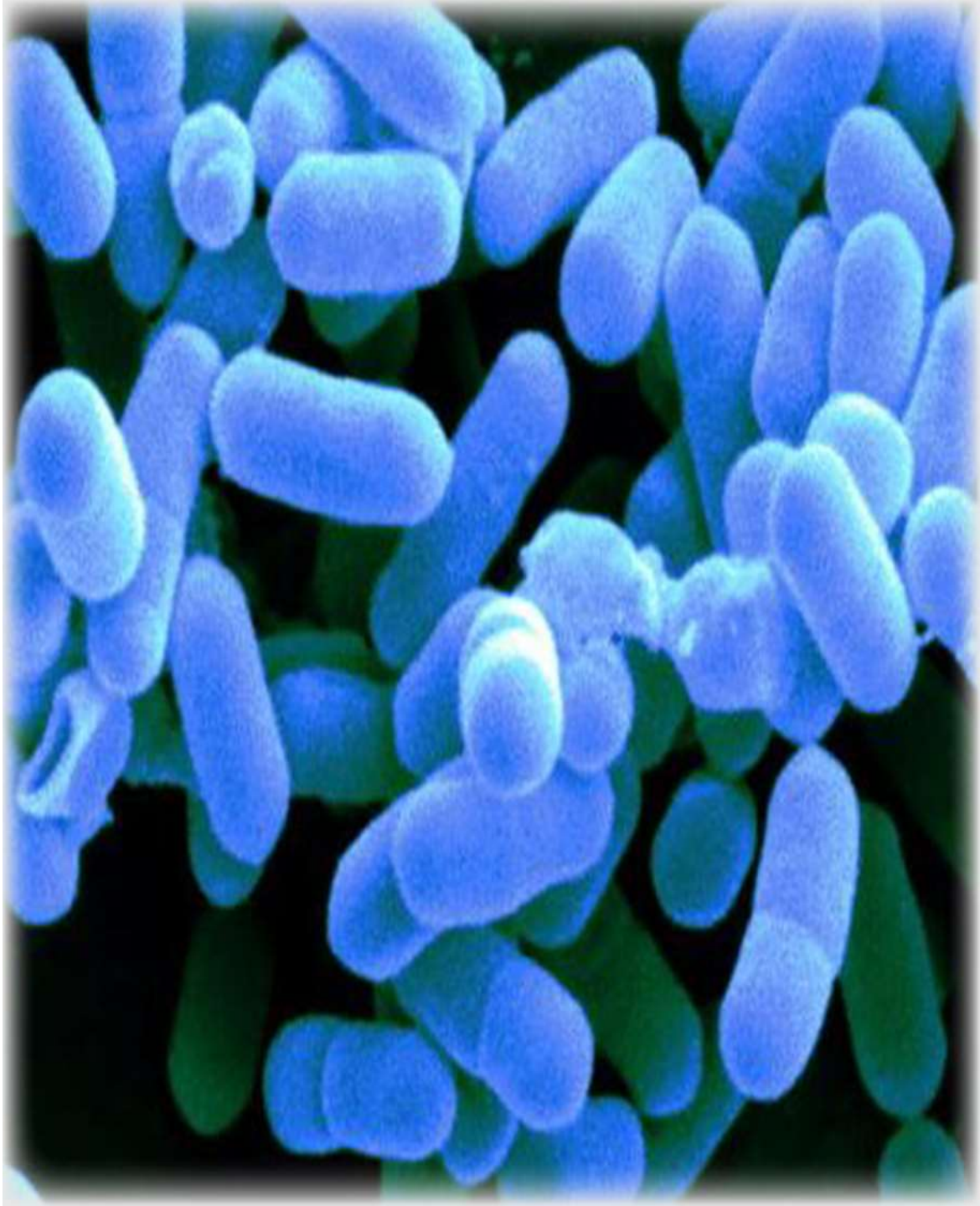
TABLE 4. Intrinsic resistance in Gram-positive bacteria; Gram-positive bacteria are also intrinsically resistant to aztreonam, temocillin, polymyxin B/colistin, and nalidixic acid

Rule no.	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Erythromycin	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulphonamides
4.1	*Staphylococcus saprophyticus*	R	R	-	-	-	-	-	-	-	R	R	-
4.2	*Staphylococcus cohnii, Staphylococcus xylosus*	-	R	-	-	-	-	-	-	-	-	R	-
4.3	*Staphylococcus capitis*	-	R	-	-	-	-	-	-	-	R	-	-
4.4	Other coagulase-negative staphylococci and *Staphylococcus aureus*	-	R	-	-	-	-	-	-	-	-	-	-
4.5	*Streptococcus* spp.	R	-	-	R[a]	-	-	-	-	-	-	-	-
4.6	*Enterococcus faecalis*	R	R	R	R[a]	R	R	R	-	-	-	-	R
4.7	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R[a]	R	R	R	R	-	-	-	R
4.8	*Enterococcus faecium*	R	R	R	R[a,b]	R	-	-	-	-	-	-	R
4.9	*Corynebacterium* spp.	-	-	-	-	-	-	-	-	-	R	-	-
4.10	*Listeria monocytogenes*	-	R	R	-	-	-	-	-	-	-	-	-
4.11	*Leuconostoc* spp., *Pediococcus* spp.	-	-	-	-	-	-	-	R	R	-	-	-
4.12	*Lactobacillus* spp. (some species)	-	-	-	-	-	-	-	R	R	-	-	-
4.13	*Clostridium ramosum, Clostridium innocuum*	-	-	-	-	-	-	-	R	-	-	-	-

R, resistant.

[a]Low-level resistance to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides.

[b]In addition to low-level resistance to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6′) enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin, arbekacin, and streptomycin) and penicillins or glycopeptides.



Listeria monocytogenes-testiranje osjetljivosti

Listeria monocytogenes

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1°C, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	1	1	1 unit	13	13	
Ampicillin	1	1	2	16	16	

Carbapenems	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Meropenem	0.25	0.25	10	26	26	

Macrolides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Erythromycin	1	1	15	25	25	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Trimethoprim-sulfamethoxazole[1]	0.06	0.06	1.25-23.75	29	29	1. Trimethoprim-sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration.

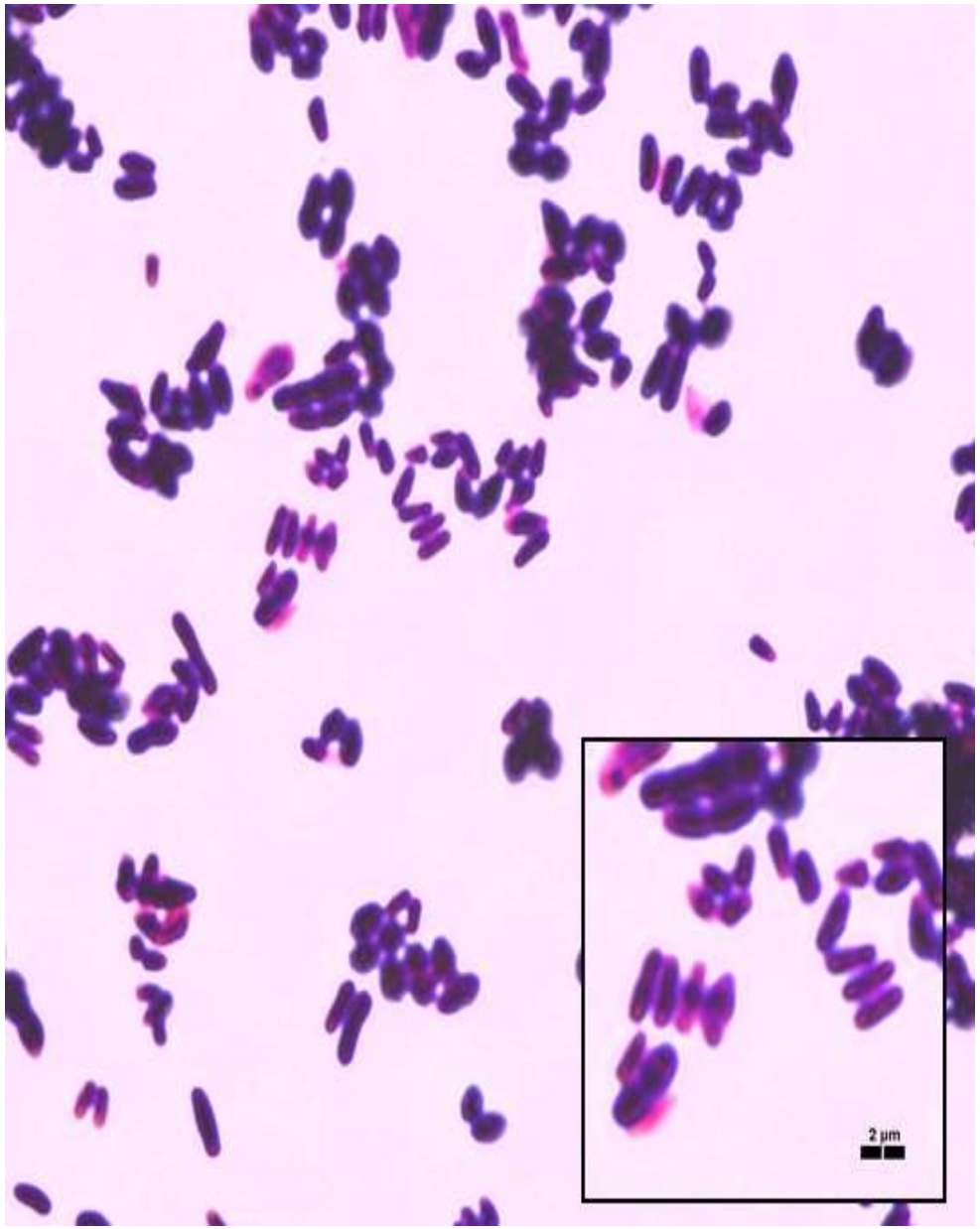
Listeria monocytogenes: antibiogram....

17. ANTIBIOGRAM ZA *Listeria monocytogenes*						
1 PLOČA MH-F / Mc Farland 0.5 / 18-24 h / 5% CO_2						
GP sitni štapići	porast na +4°C	KA - α hemoliza		H_2O_2 +	CAMP test +	
	ANTIBIOTIK	**OZNAKA**	**S**	**I**	**R**	**NAPOMENA**
1	**PENICILIN 1 IU**	**PG**	**≥ 13**	**-**	**< 13**	
2	**AMPICILIN 10µg**	**A**	**≥ 16**	**-**	**< 16**	
3	**ERITROMICIN 15µg**	**E**	**≥ 25**	**-**	**< 25**	
4	**KOTRIMOKSAZOL 25µg** TRIMETOPRIM-SULFAMETOKSAZOL	**TS**	**≥ 29**	**-**	**< 29**	
Napomena: Interpretacija osjetljivosti na gentamicin nije standardizirana za *L. monocytogenes*. Prema literaturi gentamicin je učinkovit u sinergizmu s ampicilinom.						
za izolate iz HEMOKULTURA i LIKVORA učiniti MIK test trakicama: MH-F / Mc Farland 0.5 / 24 – 48 h						
	ANTIBIOTIK	**OZNAKA**	**S**	**I**	**R**	**NAPOMENA**
	PENICILIN	**P**	**≤ 1**		**> 1**	
	AMPICILIN	**AMP**	**≤ 1**		**> 1**	
DODATNO TESTIRANJE (DD):						
	MEROPENEM	**MEM**	**≥ 26**	**25**	**< 25**	

CORYNEBACTERIUM spp.

Gram pozitivni štapići (palisade)
H2O2+

Inokulum: McFarland 0.5
Inkubacija: 5% CO2, 35+/-1°C, 18+/-2h (40-44h)



Corynebacterium spp.-testiranje osjetljivosti

***Corynebacterium* spp.**
~~**except *Corynebacterium diphtheriae***~~

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F)
Inoculum: McFarland 0.5
Incubation: 5% CO_2, 35±1°C, 18±2h. Isolates with insufficient growth after 16-20h incubation are reincubated immediately and inhibition zones read after a total of 40-44h incubation.
Reading: Read zone edges as the point showing no growth viewed from the front of the plate with the lid removed and with reflected light.
Quality control: *Streptococcus pneumoniae* ATCC 49619

Penicillins	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Benzylpenicillin	0.125	0.125	1 unit	29	29	

Fluoroquinolones	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Ciprofloxacin	1	1	5	25	25	
Moxifloxacin	0.5	0.5	5	25	25	

Aminoglycosides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Gentamicin	1	1	10	23	23	

Glycopeptides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Vancomycin	2	2	5	17	17	

Corynebacterium spp.-testiranje osjetljivosti

***Corynebacterium* spp.**
except *Corynebacterium diphtheriae*

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Lincosamides	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Clindamycin	0.5	0.5	2	20	20	

Tetracyclines	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Tetracycline	2	2	30	24	24	

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes
	S ≤	R >		S ≥	R <	Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method.
Linezolid	2	2	10	25	25	
Rifampicin	0.06	0.5	5	30	25	

Corynebacterium spp.: antibiogram...

16. ANTIBIOGRAM ZA *Corynebacterium spp.*						
1 PLOČA MH-F / 0.5 Mc Farland / 16-20 h / 4-6% CO_2						
GPŠ	H2O2 +					
	ANTIBIOTIK	**OZNAKA**	**S**	**I**	**R**	**NAPOMENA**
1	**PENICILIN 1IU**	**PG**	**≥ 29**	**-**	**< 29**	
2	**CIPROFLOKSACIN 5µg**	**CIP**	**≥ 25**	**-**	**< 25**	
3	**KLINDAMICIN 2µg**	**CD**	**≥ 20**	**-**	**< 20**	
4	**VANKOMICIN 5µg**	**VA**	**≥ 17**	**-**	**< 17**	
5	**GENTAMICIN 10µg**	**GM**	**≥ 23**	**-**	**< 23**	
6	**LINEZOLID 10µg**	**LZD**	**≥ 25**	**-**	**< 25**	
7	**RIFAMPICIN 5µg**	**RP**	**≥ 30**	**25-29**	**< 25**	
Za izolate iz HK I LIKVORA učiniti MIK test trakice u dogovoru s kliničarom: MH-F / 0.5 Mc Farland / 16-20 h / 4-6% CO_2						
	PENICILIN	P	≤ 0,125	-	> 0,125	
	CIPROFLOKSACIN	CIP	≤ 1	-	> 1	
	KLINDAMICIN	CD	≤ 0.5	-	> 0.5	
	VANKOMICIN	VA	≤ 2	-	> 2	
	TETRACIKLIN	TE	≤ 2	-	> 2	
	GENTAMICIN	GN	≤ 1	-	> 1	
	LINEZOLID	LNZ	≤ 2	-	> 2	
	RIFAMPICIN	RD	≤ 0.064	0.125-0.25	> 0.25	

ANAEROBNE BAKTERIJE

isključivo MIK metoda!

osjetljivost se ispituje UVIJEK u anaerobnim uvijetima, bez obzira na druge mogućnosti rasta

Gram-negative anaerobes

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion criteria for antimicrobial susceptibility testing of anaerobes have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions.

This group of bacteria includes many genera. The most frequently isolated Gram-negative anaerobes are *Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Bilophila* and *Mobiluncus.* Anaerobes are most frequently defined by no growth on culture plates incubated in a CO_2 enriched atmosphere. For all these species, susceptibility testing should be performed in anaerobic environment.

Gram-positive anaerobes
except *Clostridium difficile*

EUCAST Clinical Breakpoint Tables v. 6.0, valid from 2016-01-01

Disk diffusion criteria for antimicrobial susceptibility testing of anaerobes have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer´s instructions.

This group of bacteria includes many genera. The most frequently isolated Gram-positive anaerobes are: *Clostridium, Actinomyces, Propionibacterium, Bifidobacterium, Eggerthella, Eubacterium, Lactobacillu* s and anaerobic gram-positive cocci.
Anaerobes are most frequently defined by no growth on culture plates incubated in a CO_2 enriched atmosphere, but many Gram-positive, non-spore forming rods such as *Actinomyces* spp., many *P. acnes* and some *Bifidobacterium* spp. can grow on incubation in CO_2 and may be tolerant enough to grow poorly in air, but are still considered as anaerobic bacteria. Several species of *Clostridium,* including *C. carnis, C. histolyticum* and *C. tertium,* can grow but not sporulate in air.
For all these species, susceptibility testing should be performed in anaerobic environment.

Anaerobne bakterije: antibiogram....

23. E - TESTOVI ZA ANAEROBNE BAKTERIJE					
OSJETLJIVOST SE ISPITUJE MIK-om (E-test) 6 PLOČA MH-F / 0.5 Mc Farland / 24 – 48 h / anaerobni uvjeti					
ANTIBIOTIK	**OZNAKA**	**S**	**I**	**R**	**NAPOMENA**
PENICILIN	**P**	**≤ 0.25**	**0,5**	**> 0.5**	
Penicilin (PG) osjetljivi, smatraju se osjetljivima na ampicilin i piperacilin, s ili bez inhibitora					
AMOKSICILIN + KLAVULANAT	**AMC**	**≤ 4**	**8**	**> 8**	
PIPERACILIN + TAZOBAKTAM	**TZP**	**≤ 8**	**16**	**> 16**	
ERTAPENEM	**ETP**	**≤ 1**	**-**	**> 1**	
KLINDAMICIN	**CD**	**≤ 4**	**-**	**> 4**	
METRONIDAZOL	**MTZ**	**≤ 4**	**-**	**> 4**	
TESTIRATI SAMO ZA GRAM POZITIVNE ANAEROBNE BAKTERIJE:					
VANKOMICIN	**VA**	**≤ 2**	**-**	**> 2**	**samo za Gram (+) AN bakterije**

Hvala na pažnji!

Određivanje minimalnih inhibitornih koncentracija

Iva Butić
Klinika za infektivne bolesti "Dr. Fran Mihaljević"

Testiranje osjetljivosti bakterija na antibiotike

1. Kvalitativna metoda:

- disk difuzija
- osjetljiv, umjereno osjetljiv, rezistentan

2. Kvantitativne metode:

- određivanje minimalne inhibitorne koncentracije (MIK)

  a) agar dilucija[1]

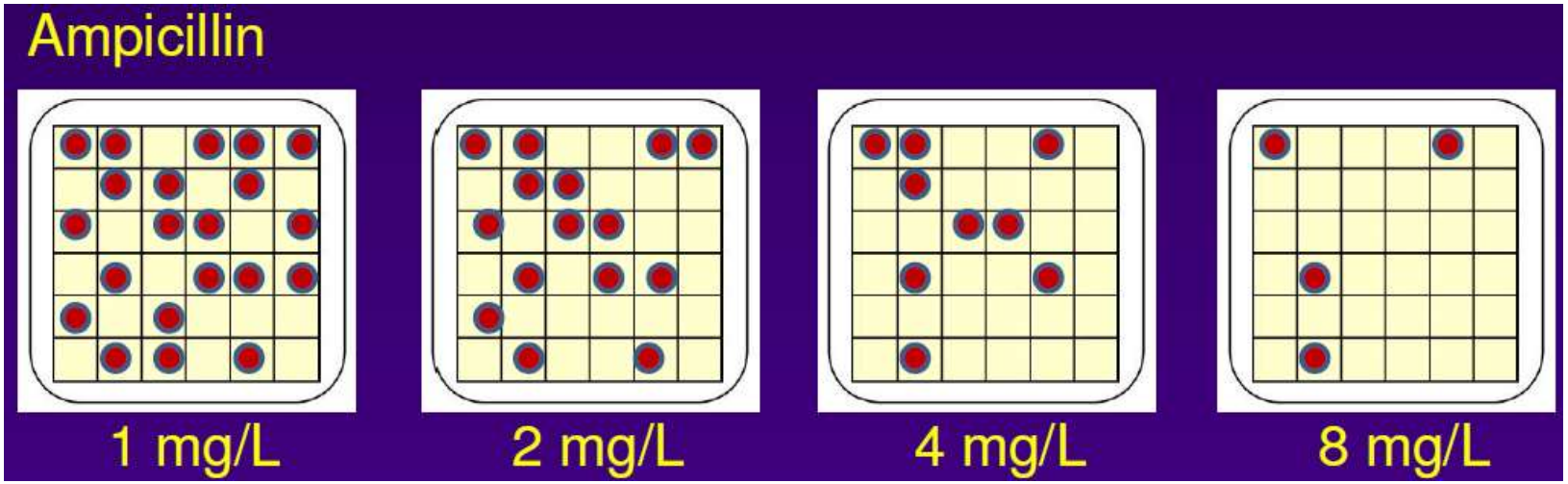
  b) dilucija u bujonu (makrodilucija i mikrodilucija)[2]

  c) metoda gradient testa[3]

  d) automatizirane metode

[1]EUCAST DEFINITIVE DOCUMENT E.Def 3.1, June 2000
[2]EUCAST-CMI, Volume 9 number 8, August 2003
[3]E-test Technical Manual

Agar dilucija

- Različite koncentracije antibiotika otopljene u agaru
- Set ploča s dvostrukim razrijeđenjima antibiotika
- Ispituje se porast bakterija na pločama s različitim konc. antibiotika
- MIK vrijednost -> koncentracija antibiotika prve ploče na kojoj nema vidljivog porasta bakterija
- Pogodna za testiranje osjetljivosti za veliki broj sojeva
- 

Agar dilucija

Materijali:

1. Mueller-Hinton agar (MH)
   (*Hemophilus* spp., streptokoki, *Moraxella catarrhalis* -> MH-F agar)
2. Antibiotik – “stock” otopina
3. ATCC soj (24h kultura)
4. Ispitivani soj/sojevi (24h kultura)

Antibiotik – priprema stock otopine

Table1 Solvents and diluents for antimicrobial agents requiring solvents other than water

Antimicrobial agent	Solvent	Diluent
Amoxycillin	Phosphate buffer 0.1 M, pH 6.0	Phosphate buffer 0.1 M, pH 6.0
Ampicillin	Phosphate buffer 0.1 M, pH 8.0	Phosphate buffer 0.1 M, pH 6.0
Azithromycin	Ethanol 95%	Water
Aztreonam	Saturated sodium bicarbonate solution	Water
Ceftazidime	Saturated sodium bicarbonate solution	Water
Chloramphenicol	Ethanol 95%	Water
Clavulanic acid	Phosphate buffer 0.1 M, pH 6.0	Phosphate buffer 0.1 M, pH 6.0
Erythromycin	Ethanol 95%	Water
Fluoroquinolones	Half volume water, a minimum volume of 0.1 M NaOH to dissolve, then make up to total volume with water	Water
Fusidic acid	Ethanol 95%	Water
Imipenem	Phosphate buffer 0.01 M, pH 7.2	Phosphate buffer 0.01 M, pH 7.2
Mezlocillin	Methanol	Water
Meropenem	Phosphate buffer 0.01 M, pH 7.2	Phosphate buffer 0.01 M, pH 7.2
Nalidixic acid	Half volume water, a minimum volume of 0.1 M NaOH to dissolve, then make up to total volume with water	Water
Nitrofurantoin	Dimethylformamide	Phosphate buffer 0.1 M, pH 8.0
Rifampicin	Methanol	Water
Sulbactam	Phosphate buffer 0.1 M, pH 6.0	Phosphate buffer 0.1 M, pH 6.0
Sulfonamides	Half volume water, a minimum volume of 0.1 M NaOH to dissolve, then make up to total volume with water	Water
Ticarcillin	Phosphate buffer 0.1 M, pH 6.0	Phosphate buffer 0.1 M, pH 6.0
Trimethoprim	Half volume water, a minimum volume of 0.1 M lactic acid or 0.1 M HCl to dissolve, then make up to total volume with water	Water

Priprema ploča s antibiotikom

•Priprema "stock" otopine antibiotika:
10,24 mg antibiotika + 1 mL otapala = 10240mg/L "stock" otopine

Table2 Preparation of dilutions of agents for use in agar dilution susceptibility tests

Antimicrobial concentration (mg/L) in stock solution	Volume stock solution (mL)	Volume distilled water (mL)	Antimicrobial concentration obtained (mg/L)	Final concentration in medium after addition of 19 mL of agar
10 240	1	0	10 240	512
10 240	1	1	5120	256
10 240	1	3	2560	128
2560	1	1	1280	64
2560	1	3	640	32
2560	1	7	320	16
320	1	1	160	8
320	1	3	80	4
320	1	7	40	2
40	1	1	20	1
40	1	3	10	0.5
40	1	7	5	0.25
5	1	1	2.5	0.125
5	1	3	1.25	0.06
5	1	7	0.625	0.03
0.625	1	1	0.3125	0.015
0.625	1	3	0.1562	0.008
0.625	1	7	0.0781	0.004

- "Stock" otopina se može čuvati na -20°C (slijediti upute proizvođača).
- Većina antibiotika je stabilna do 6 mjeseci.
- Klavulanska kiselina i karbapenemi su nestabilni.

ATCC soj (ciljana vrijednost MIK-a)

Table 3 Target MICs (mg/L) for control organisms in cation adjusted Mueller-Hinton broth

Antimicrobial agent	*Escherichia coli* ATCC 25922 NCTC 12241 CIP 76.24 DSM 1103	*Escherichia coli* ATCC 35218 DSM 5564	*Pseudomonas aeruginosa* ATCC 27853 NCTC 12934 CIP 54.127 DSM 1117	*Staphylococcus aureus* ATCC 29213 NCTC 12973 CIP 103429 DSM 2569	*Enterococcus faecalis* ATCC 29212 NCTC 12697 CIP 103214 DSM 2570
Amikacin	1	–	4	2	128
Amoxycillin	8	–	–	0.5	–
Amoxycillin/ clavulanic acid	4/2	8/4	–	–	–
Ampicillin	4	–	–	0.5	1
Ampicillin/sulbactam	4/2	16/8	–	–	–
Azithromycin	–	–	–	1	–
Aztreonam	0.125	–	4	–	–
Carbenicillin	8	–	32	4	32
Cefamandole	0.5	–	–	0.5	–
Cefazolin	2	–	–	0.5	–
Cefepime	0.03	–	2	2	–
Cefixime	0.5	–	–	16	–
Cefonicid	0.5	–	–	2	–
Cefoperazone	0.25	–	4	2	16
Cefotaxime	0.125	–	8	2	–
Cefotetan	0.125	–	–	8	–
Cefoxitin	2	–	–	2	–
Cefpodoxime	0.5	–	–	–	–
Ceftazidime	–	–	–	8	–
Ceftizoxime	0.06	–	32	4	–
Ceftriaxone	0.06	–	16	2	–
Cefuroxime	4	–	–	1	–
Cephalexin	8	–	–	–	–
Cephalothin	8	–	–	0.25	16
Chloramphenicol	4	–	–	4	8
Ciprofloxacin	0.008	–	0.5	0.25	0.5
Clinafloxacin	0.004	–	0.25	0.016	0.06
Clindamycin	–	–	–	0.125	8

- Dozvoljava se jedno razrijeđenje ispod i iznad ciljanog MIK-a (MIC range)

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Agar dilucija

Priprema inokuluma:

1. iz bujona (24h kultura)
   a) 24h bujon = 10^8CFU/mL
   b) razrijediti u 0.85% fiziološke otopine-> 10^7 CFU/mL
   c) inokulacija 1µL suspenzije

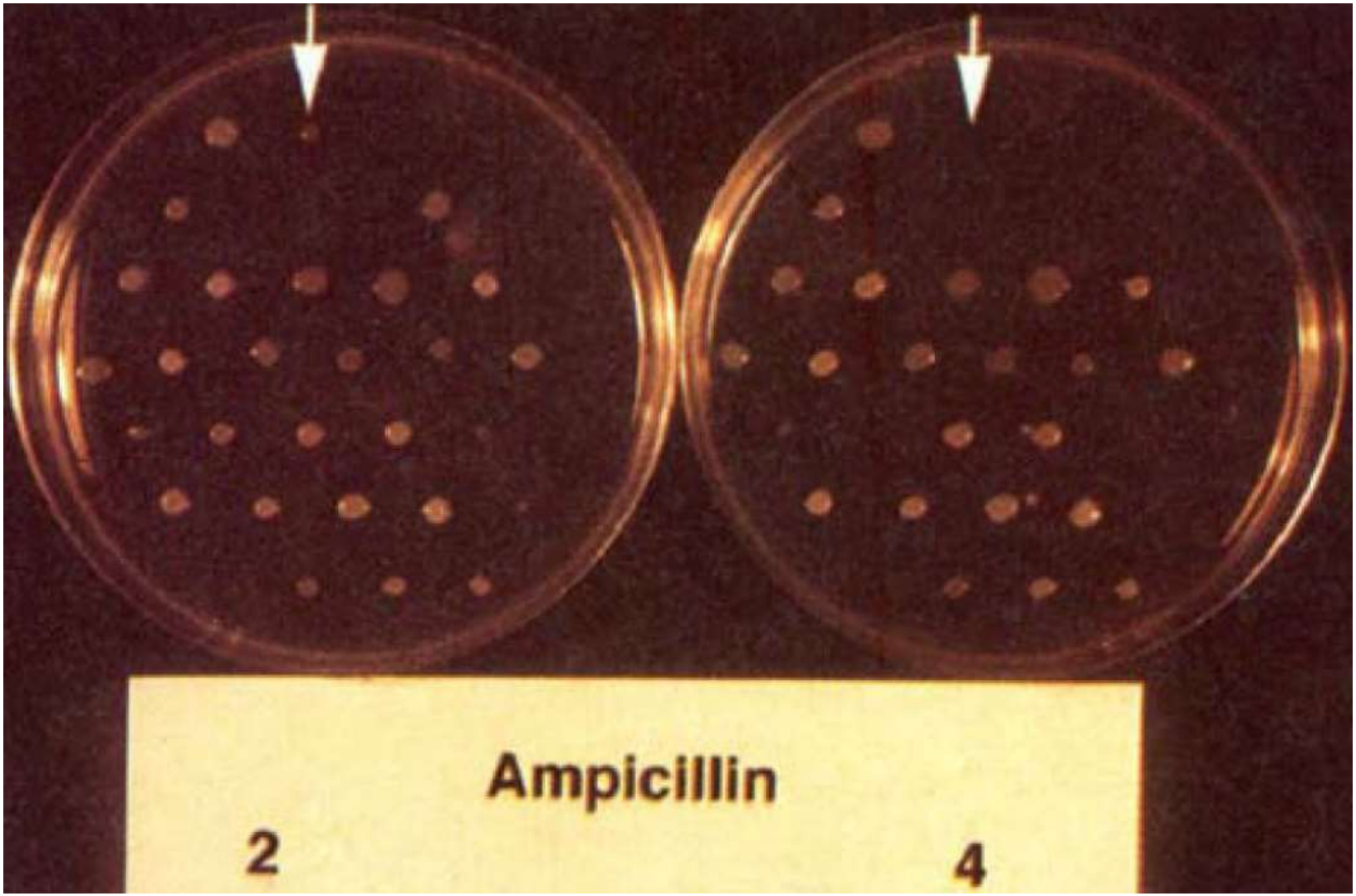
2. s agara (24h kultura)
   a) pikirati 4-5 različitih kolonija i razmutiti u 0.85% fiz.otopine -> 0.5 McFarland
   b) razrijediti u 0.85% fiziološkoj otopini -> 10^7 CFU/mL
   c) inokulacija 1µL suspenzije

Agar dilucija – inokulacija ploča

1. Inokulum- replikator aparat (1µL suspenzije)
2. Mikropipeta (1µL)
3. Eza (1µL)

1µL suspenzije = 10^4 CFU/ubodno mjesto u agaru

Inkubacija: 35-37°C, 18h


Ampicillin
2
4

Dilucija u bujonu

- Makrodilucija u bujonu - volumeni bujona > 1 mL po epruveti
- Mikrodilucija u bujonu - volumeni bujona ≤ 0.5mL po mikrotitarskoj jažici
- Vidljiv bakterijski porast = zamućenje bujona u epruveti/mikrotitarskoj jažici
- MIK -> najniža koncentracija antibiotika u epruveti/mikrotitarskoj jažici gdje nema vidljivog bakterijskog porasta (zamućenje bujona)

Makrodilucija u bujonu

- Referentna metoda ispitivanja osjetljivosti bakterija na antibiotike
- Jedna od najstarijih metoda
- Dvostruka veća/manja razrijeđenja antibiotika -> obavezno uključena konc. 1 mg/L (…0.125, 0.25, 0.5, **1**, 2, 4, 8, 16, 32 mg/L…)
- Testiranje se izvodi u epruvetama
- Bakterijska suspenzija: 0.5 McFarlanda
- Inkubacija 24 sata na temp. 35°C
- Očitavanje vidljivog bakterijskog porasta => zamućenje bujona
- MIK => najniža koncentracija antibiotika gdje nema vidljivog porast bakterija
- Dozvoljeno odstupanje (greška metode): ± 1 razrijeđenje

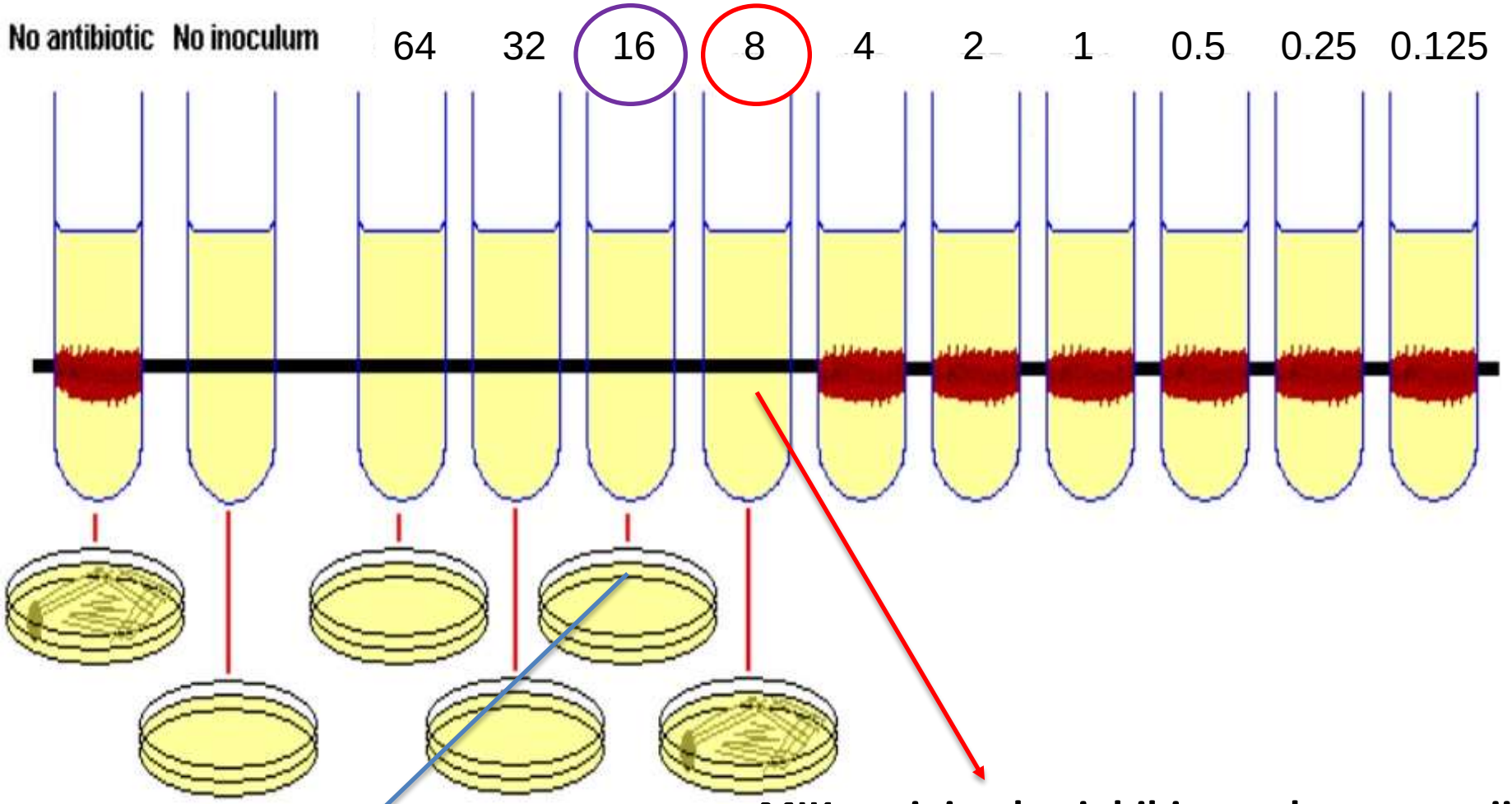
Makrodilucija u bujonu
prednosti i nedostaci metode

Prednosti:

- Kvantitativna metoda osjetljivosti (određujemo MIK vrijednosti)
- Vrijednost MIKa-a je jako važna:
  a) u liječenju teško oboljelih pacijenata
  b) kod oboljenja gdje antibiotik slabo prodire na mjesto infekcije
  c) kod imunokompromitiranih pacijenata

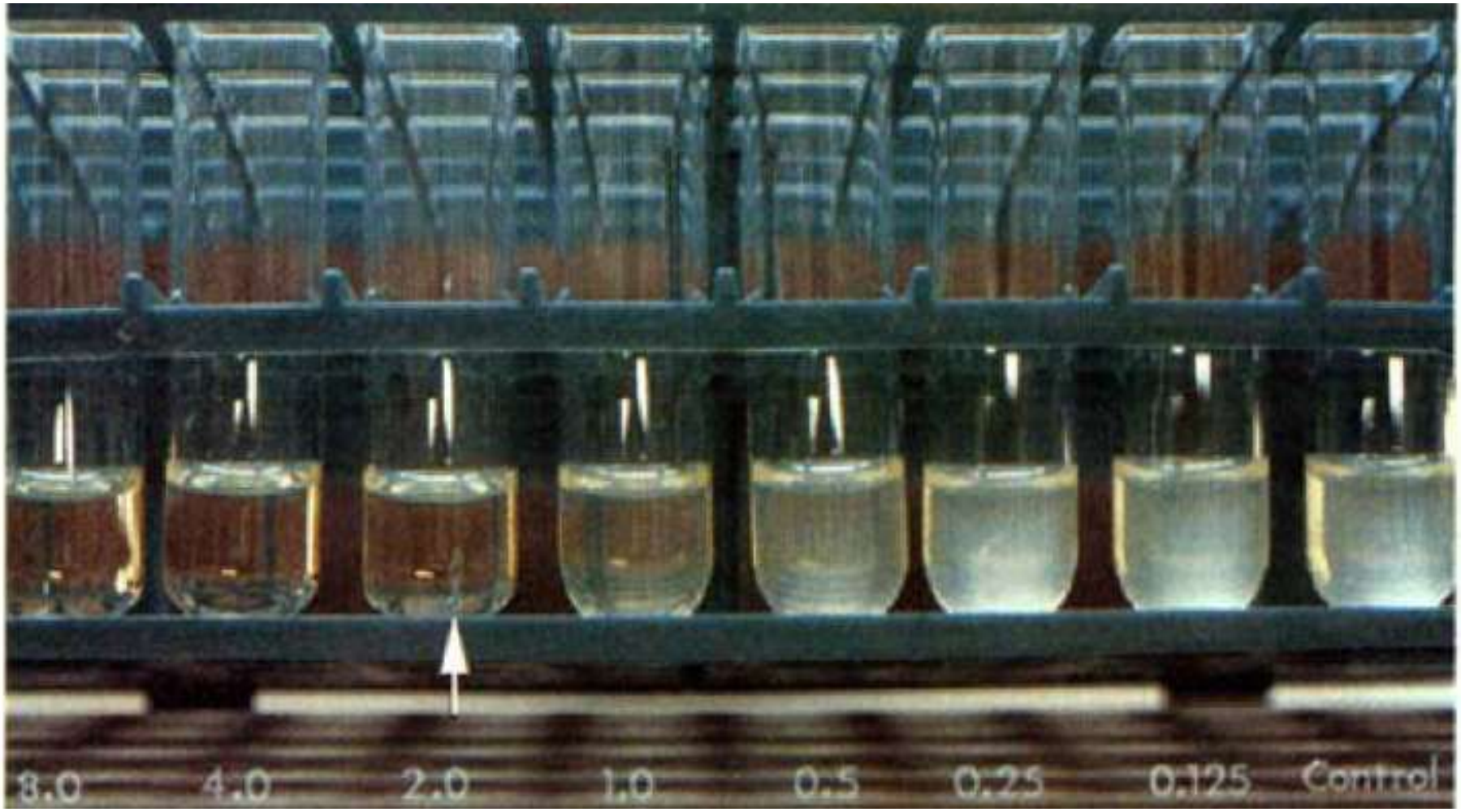
Nedostaci:

- Priprema različitih koncentracija antibiotika je vremenski zahtjevna
- Moguće greške u pripremi različitih koncentracija antibiotika
- Potrebna relativno velika količina reagencija te prostora za izvođenje testa
- Pranje i sterilizacija epruveta



MIK – minimalna inhibitorna koncentracija
najniža koncantracija antibiotika koja zaustavlja vidljivi porast bakterija

MBK – minimalna baktericidna koncentracija –
najniža koncentracija antibitika koja onemogućava porast bakterijskog soja na ploči

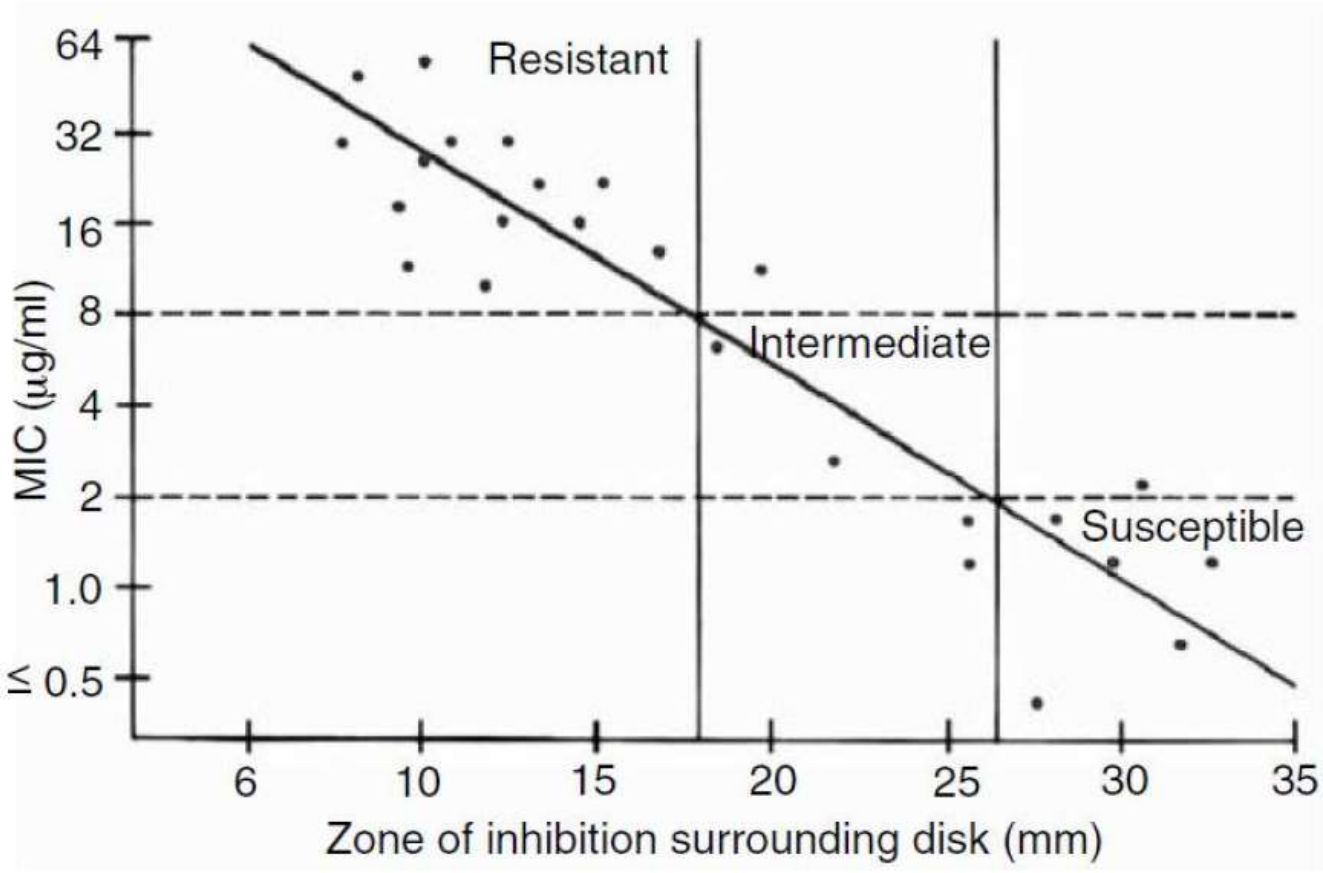


MIK 2.0 mg/L

Mikrodilucija u bujonu

- Praktičnija metoda od makrodilucije u bujonu
- Ekonomična metoda - manji volumeni bujona
- Antibiotik se razrijeđuje u jažicama mikrotitarske pločice
- Mogućnost testiranja nekoliko antibiotika na jednoj mikrotitarskoj pločici
- Priprema panela antibiotika na mikrotitarskoj pločici ovisno o vrsti bakterije (za enterobakterije, *S.aureus*…)
- Pohrana “ready to use” mikrotitarskih pločica do 6 mj na -20°C

Odnos vrijednosti zona inhibicije i MIK vrijednosti



Mikrodilucija u bujonu

izvođenje testa

Materijali:

1. Mueller-Hinton bujon s prilagođenim kationskim vrijednostima (CAMHB)
   - *Hemophilus* spp. i streptokoki: CAMHB + defibirinirana konjska krv

2. Mikrotitarska pločica (jednokratna)

3. ATCC soj (24h kultura)

4. Antibiotik – “stock solution”

5. Ispitivani soj bakterije (24h kultura)

Priprema antibiotika

1. Priprema stock otopine antibiotika (antibiotik u prahu ili otopini)
   (5,12 mg antibiotika u prahu + 1 mL otapala = 5120 mg /L)
2. Priprema razrijeđenja antibiotika iz stock otopine

Antimicrobial concentration (mg/L) in stock solution	Volume stock solution (mL)	Volume broth* (mL)	Antimicrobial concentration obtained (mg/L)
5120	1	9	512
512	1	1	256
512	1	3	128
512	1	7	64
64	1	1	32
64	1	3	16
64	1	7	8
8	1	1	4
8	1	3	2
8	1	7	1
1	1	1	0.5
1	1	3	0.25
1	1	7	0.125

*Broth used for dilution is that used in the susceptibility test. Any supplementation must take place before diluting the antimicrobial agent to maintain the required concentrations.

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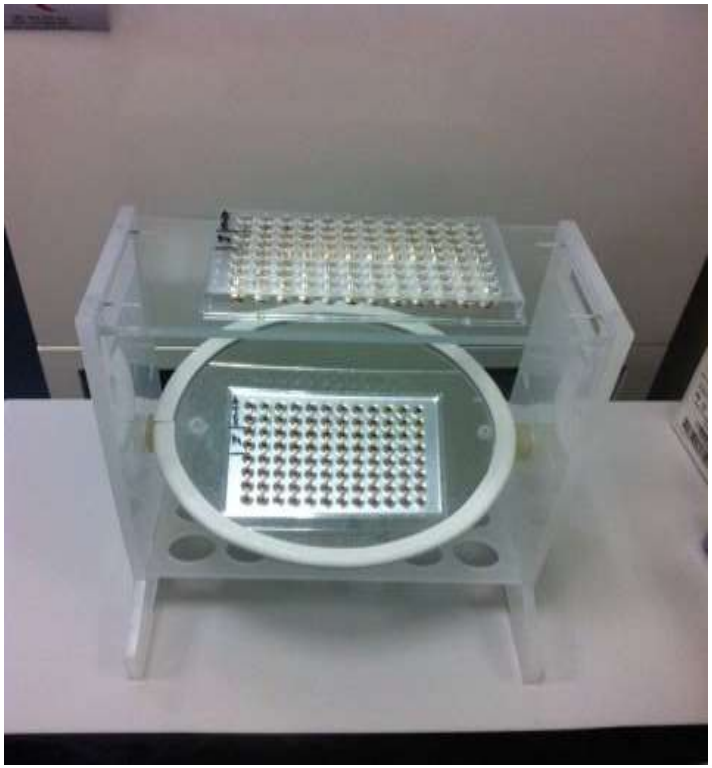
Priprema bakterijske suspenzije

- Inokulum:
  a) bakterijska suspenzija 0.5 McFarlanda
  b) 50µL bakterijske suspenzije + 4950µL CAMHB

- Inkubacija: 35-37°C, 16-20h
  (24h za *Hemophilus* spp, streptokoke i MRSA)

Očitavanje mikrotitarske pločice

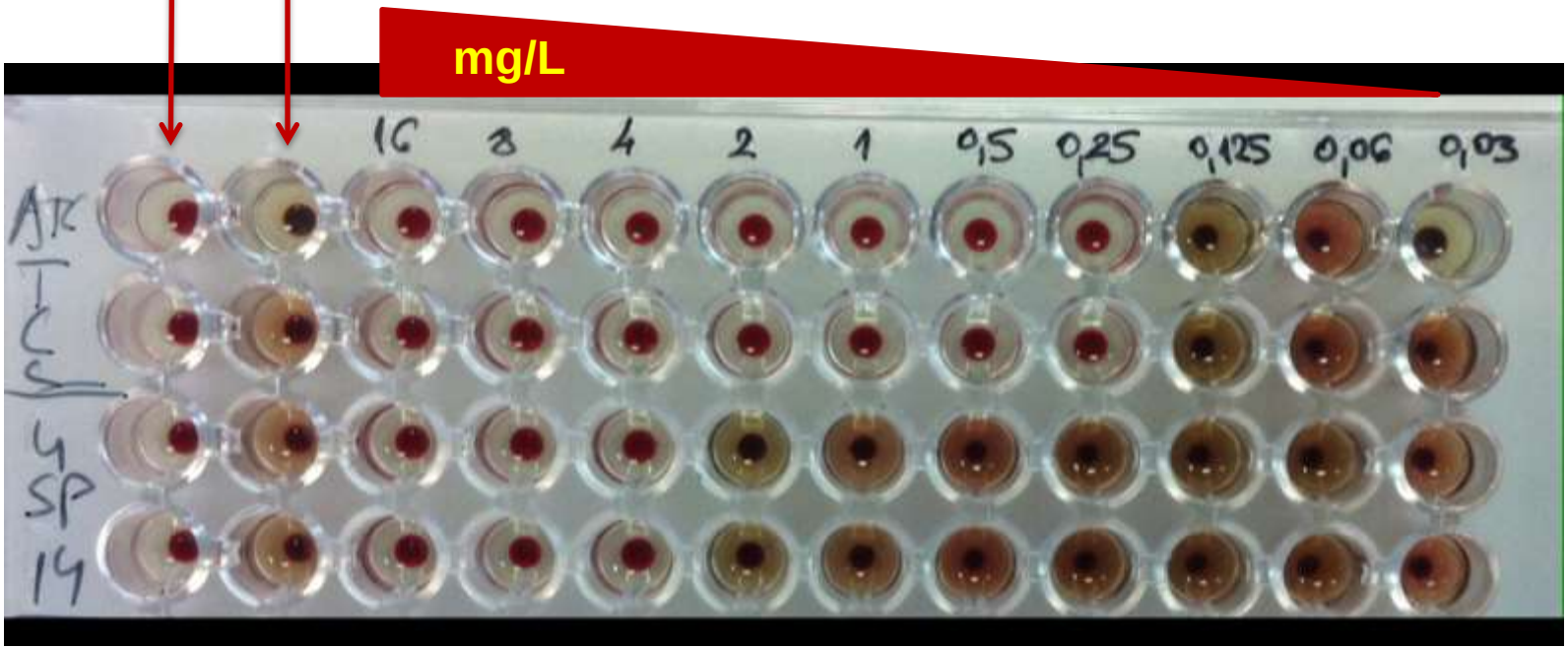
Postolje s ogledalom



Automatizirani čitači

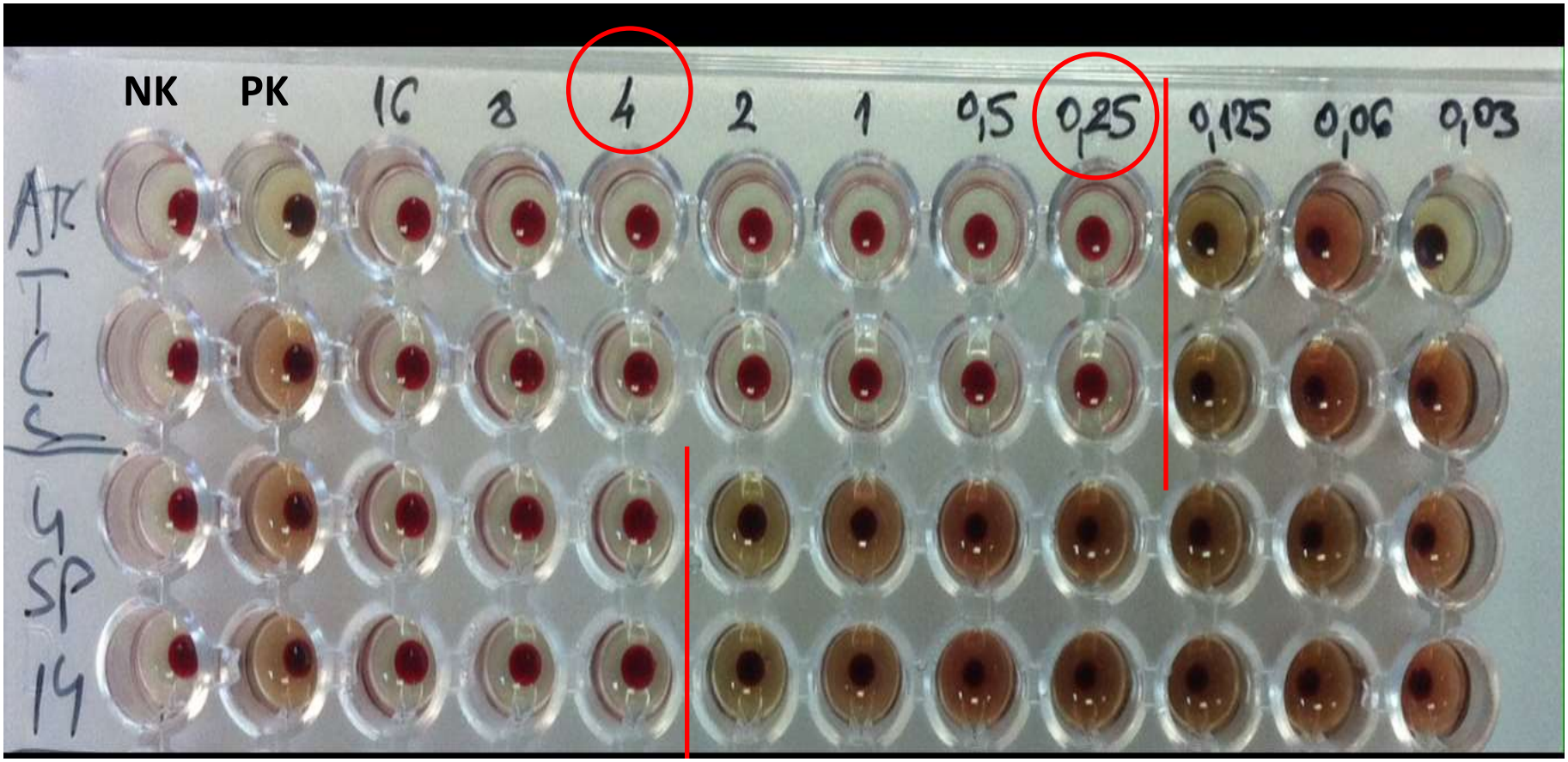
Očitavanje mikrotitarske pločice:

- 1.stupac (**neg.kontrola**)
  - bujon - nema porasta bakterijske kulture
- 2.stupac (**poz.kontrola**)
  - porast bakterijske kulture (zamućenje)
- 3.-12.stupac - bilježimo porast bakterijske kulture



S.pneumoniae ATCC 49619, Target MIC 0.5 mg/L (MIK raspon 0.25-1.0 mg/L)

S.pneumoniae, kliničke vrijednosti penicilina (non meningitis) ≤0.06 (S), >2(R)

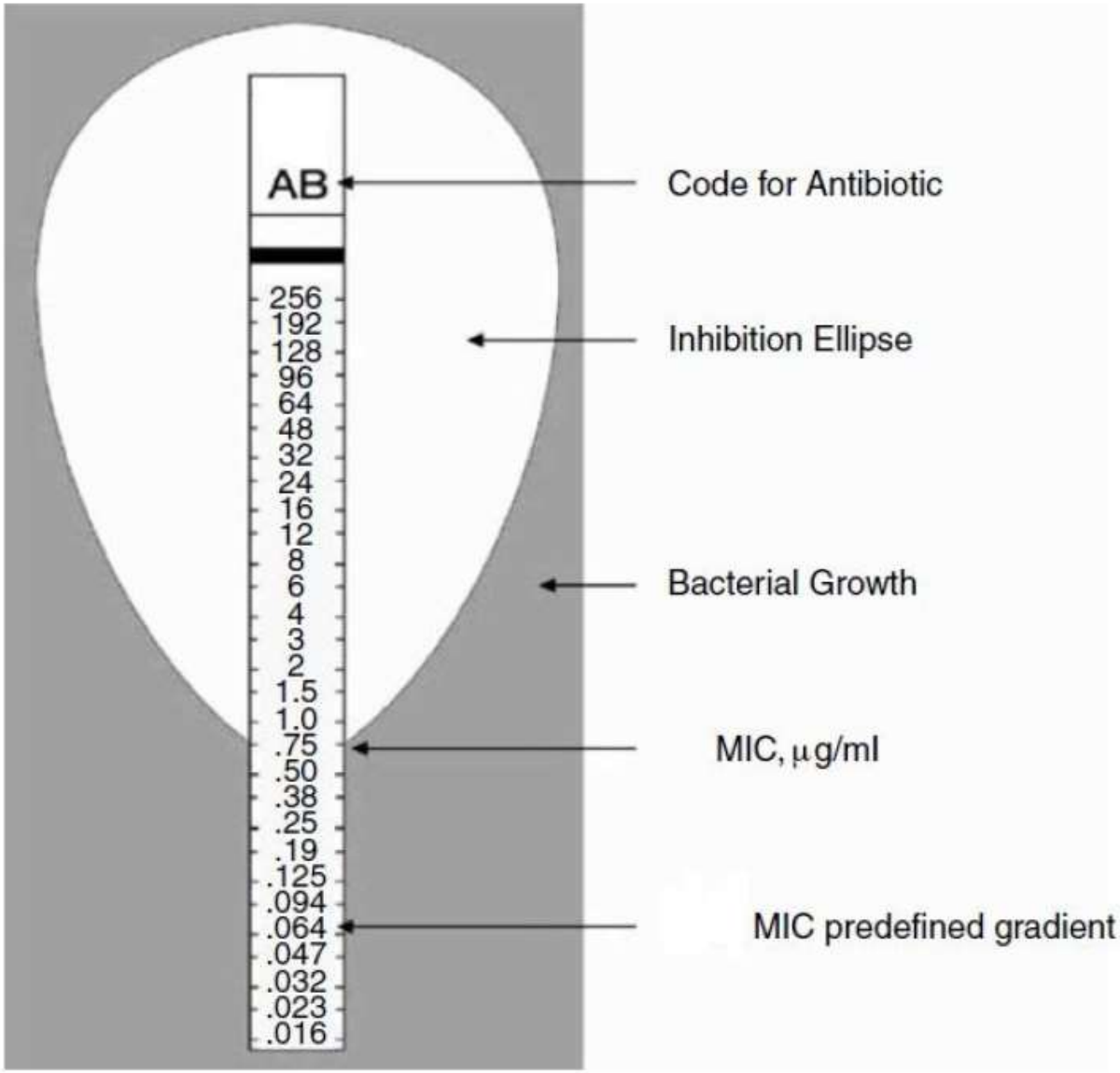


- **Kontrola testa =** MIK vrijednost ATTC soja (unutar dozvoljenog raspona MIK-ova)
- MIK ispitivanog soja = koncentracija antibiotika u prvoj jažici u kojoj nema vidljivog bakterijskog porasta.

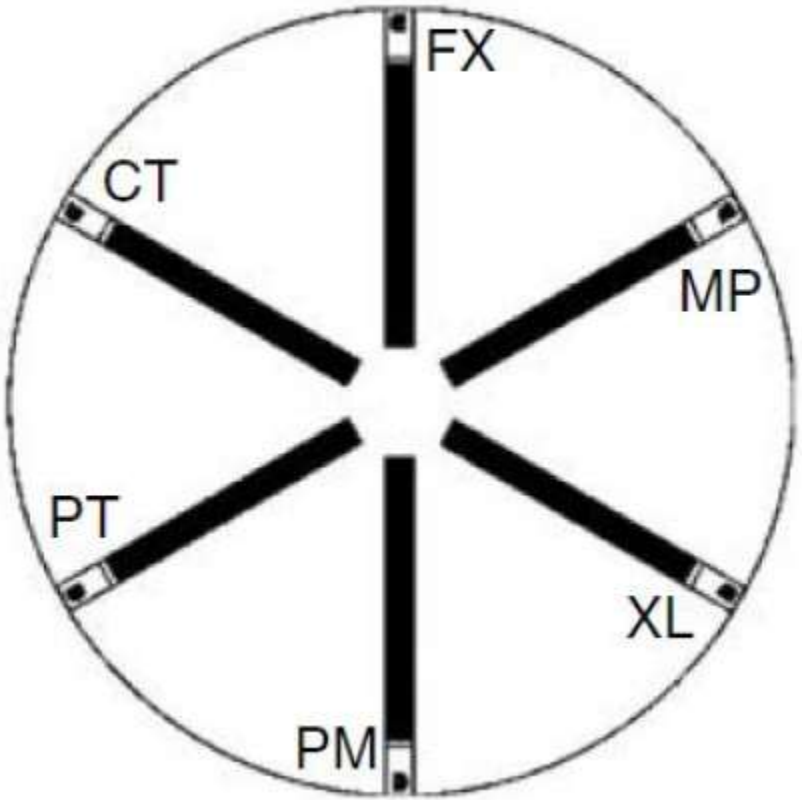
Metoda gradient testa = disk difuzija + agar dilucija

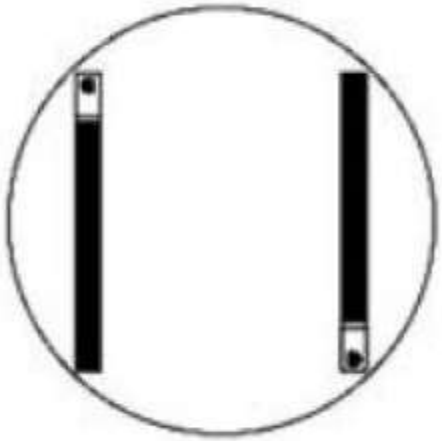
- Koncentracije antibiotika (od najmanje prema najvećoj)impregnirane u suhom obliku na površinu trakice (plastika ili papir)
- Na trakici je 15 dvostruko većih razrijeđenja antibiotika (“prava razrijeđenja)
- MIK vrijednost: očitavamo vrijednost na trakici gdje ju dno elipsoidne zone inhibicije presjeca (E-test = eng. elipsometer test)
- Jednostavna metoda određivanja MIK-a (“user friendly”)
- Prilagođena za rutinski rad u laboratoriju

Metoda gradient testa (shema)



Aplikacija gradient test trakica na ploči
(primjeri)





Očitavanje gradient testa

Očitavanje gradient testa:

•MIK vrijednosti očitavamo na trakici na mjestu inhibicije porasta bakterija

•Baktericidni antibiotici = potpuna inhibicija porasta

•Bakteriostatski antibiotic= 80% inhibicija porasta

•Za pneumokoke, streptokoke enterokoki, fusobakterije dozvolj

•**Izdavanje nalaza:**

u nalazu izdajemo MIK vrijednost koju smo očitali na trakici

•Primjer: MIK vankomicina za *S.aureus*:

S ≤2mg/L R >2 mg/L

Vankomicin osjetljiv

•**Interpretacija MIK vrijednosti između dva "prava" razrijeđenj**

Prije interpretacije rezultata očitanu vrijednost je potrebno

veće razrijeđenje.

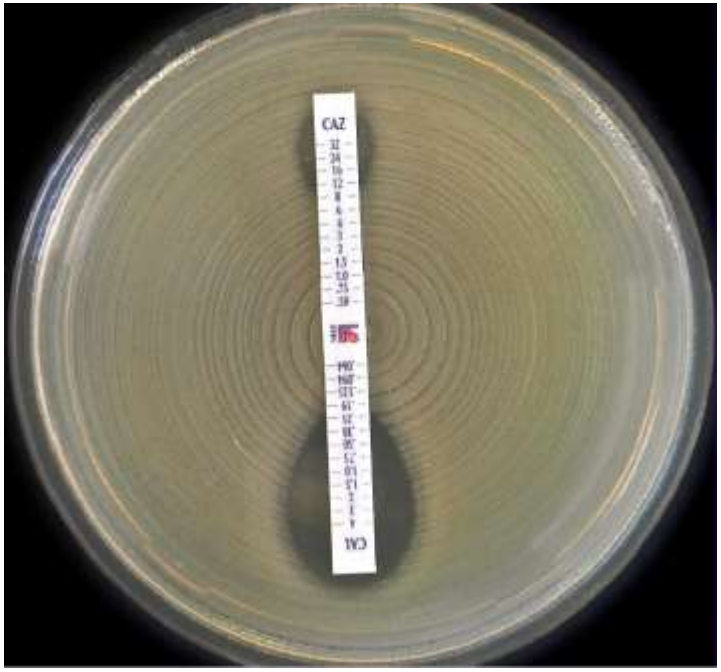
Ostale primjene metode gradient testa

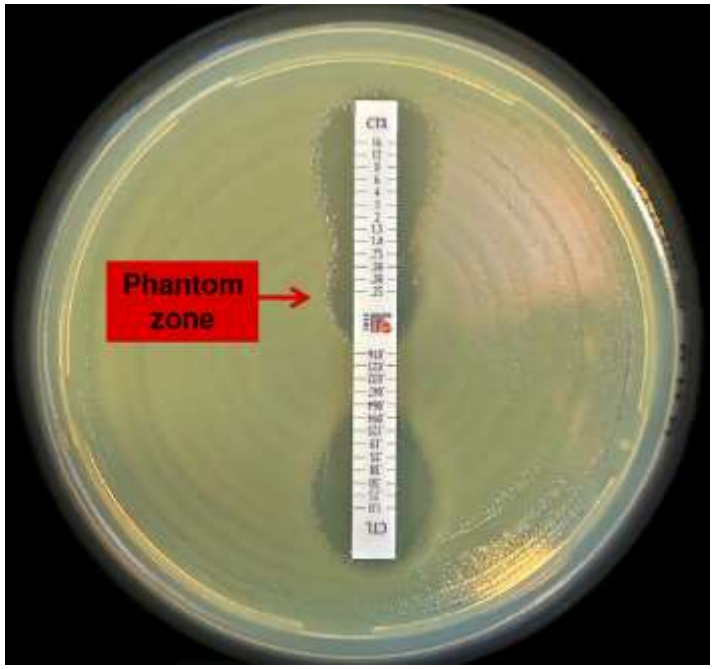
1. **Dokazivanje mehanizama rezistencije:**
   a) ESBL
   b) AmpC β-laktamaze
   c) karbapenemaze

2. **Dokazivanje sinergističkog djelovanja između lijekova**
   a) liječenje infekcija uzrokovanih MDR i PDR bakterijama
   b) liječenje infekcija kod pacijenata s cističnom fibrozom
   c) pacijenti u JIL-u

Dokazivanje produkcije ESBL-a

- Ceftazidim (CAZ): 12 mg/L
- Ceftazidim/klavanska kiselina (CAL): 0.25
- Positivan test = produkcija ESBL eznima:
  vrijednost MIK-a cefalosporina s inhibitorom je 8x manja od vrijednosti MIK-a cefalosporina bez inhibitora ili pojavljivanje tzv. fantomske zone (deformacija elipse)

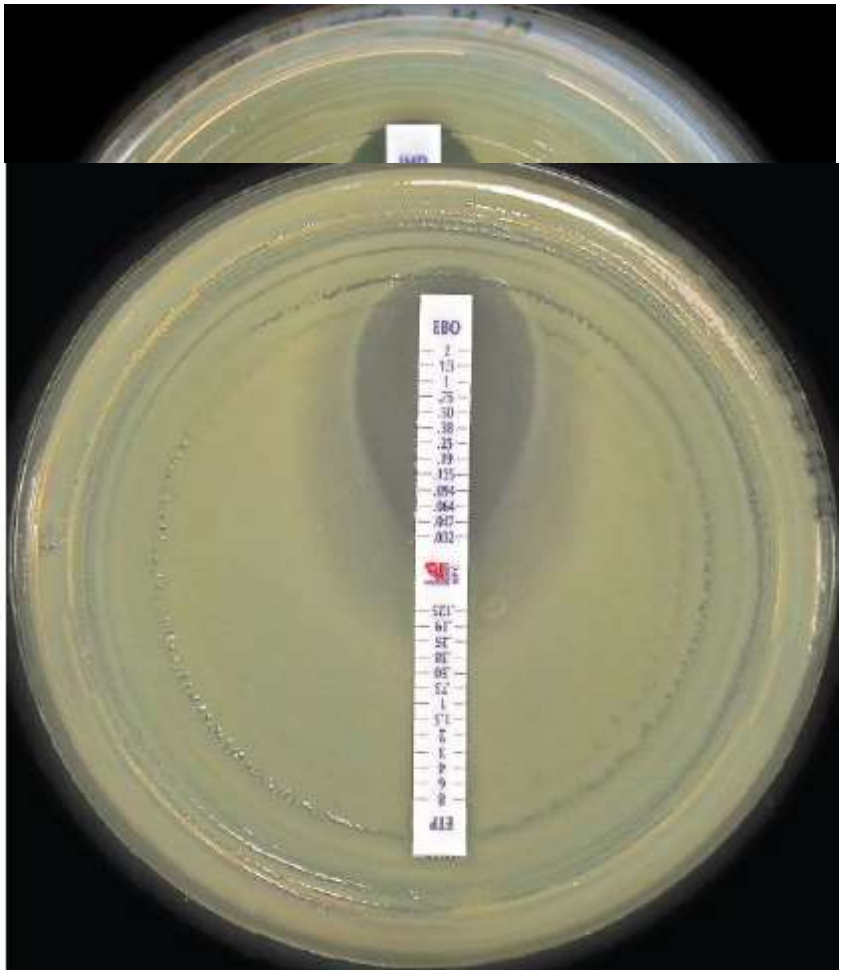




Dokazivanje produkcije AmpC β-laktamaze

- Cefotetan (CTT): > 32 mg/L
- Cefotetan/kloksacilin (CXT): 0.75 mg/L
- Pozitivan test = produkcija AmpC β-laktamaze:
  vrijednost MIK-a cefalosporina s inhibitorom je 8x manja od vrijednosti MIK-a cefalosporina bez inhibitora.

Dokazivanje produkcije karbapenemaza



1. metalo-β-laktamaza

- Imipenem (IMI): > 256 mg/L
- Imipenem+EDTA (IMD): < 1 mg/L

2. KPC

- Ertapenem (ETP): > 8 mg/L
- Imipenem + boronična kis.(EBO):0.032 mg/l

- **Pozitivan test:**

  vrijednost MIK-a karbapenema s inhibitorom je 8x manja od vrijednosti MIK-a karbapenema bez inhibitora.

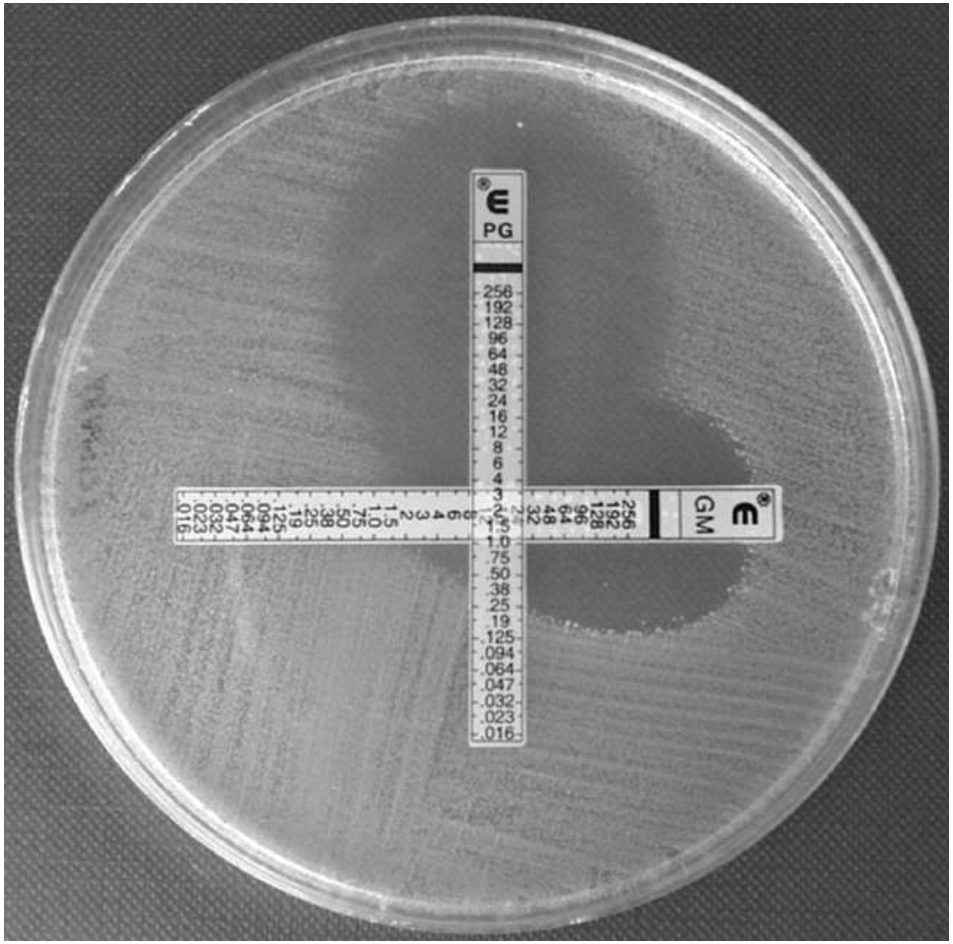
Dokazivanje sinergizma između lijekova

MIK vrijednosti ispitivanih antibiotika:

- Gentamicin (GM):
- Penicillin (PG):

MIK vrijednosti sinergističkog testiranja:

- Gentamicin (GM): 2 mg/L
- Penicillin (PG): 0.38 mg/L



Interpretacija testa detekcije sinergizma

- **Indeks Frakcijske Inhibicijske Koncentracije (FIK Indeks)**

  FIK indeks = (MIK AB / MIK A) + (MIK BA / MIK B)

  MIK A = MIK antibiotika A
  MIK B = MIK antibiotika B
  MIK AB = MIK antibiotika A u kombinaciji s antibiotikom B
  MIK BA = MIK antibiotika B u kombinaciji s antibiotikom A

Mehanizam sinergističkog djelovanja:

- Indiferentan > 1 do 4
- Aditivan > 0.5 do 1
- Sinergistički = 0.5
- Antagonistički > 4

Automatizirane metode određivanja osjetljivosti

- Sadržavaju inkubator i čitač
- Očitava se promjena zamučenja (turbiditeta) u jažicama s poznatim koncentracijama antibiotika
- Program interpretira, pohranjuje i izdaje rezultate (*Data management software*)
- Prepoznaje odstupanja u rezultatima osjetljivosti -> predlaže moguće mehanizame rezistencije (EUCAST Expert rules)
- Mogućnost povezivanja s LIS-om
- **BioMérieux VITEK 2 Compact**
- **Becton Dickinson Phoenix 100**
- **Beckman Coulter MicroScan WalkAway 96 plus**

BioMérieux VITEK 2 Compact

E-test

In summary, with the exception of the Vitek Legacy system, the performance characteristics of all the MIC susceptibility testing methods, when they were measured by essential agreement, were excellent. However, the Phoenix system, Etest, and the Micro-Scan system tended to yield MIC results 1 dilution higher than those obtained by the broth reference method; and agar dilution, the Sensititre system, and the Vitek 2 system yielded results that were 1 dilution lower than those obtained by the broth reference method. The Vitek Legacy system gave no MIC results of 4 or 8 μg/ml, and thus, it is difficult to compare the results obtained with the Vitek Legacy system with those obtained by the reference method. The disk diffusion test did not distinguish vancomycin-intermediate strains from vancomycin-susceptible strains, and the vancomycin agar screen lacked sensitivity for strains with MICs of 4 μg/ml. Clinical laboratories may enhance their ability to detect *S. aureus* isolates with reduced susceptibility to vancomycin by performing further testing (e.g., by the vancomycin Etest) with isolates for which the MICs are 2 μg/ml with one of the commercial systems evaluated in the present study.

VITEK 2

*Swenson J.M. and all. Accuracy of Commercial and Reference Susceptibility Testing Methods for Detecting Vancomycin Intermediate *Staphylococcus aureus*. JCM 2009, 2013–2017

JOURNAL OF CLINICAL MICROBIOLOGY, July 2010, p. 2402–2406 Vol. 48, No. 7
0095-1137/10/$12.00 doi:10.1128/JCM.00267-10

Comparison of Meropenem MICs and Susceptibilities for Carbapenemase-Producing *Klebsiella pneumoniae* Isolates by Various Testing Methods▽

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Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, Connecticut[1]; Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut[2]; New York Presbyterian Hospital, Weill Cornell Medical Center, New York, New York[3]; and Mount Sinai Medical Center, New York, New York[4]

We describe the levels of agreement between broth microdilution, Etest, Vitek 2, Sensititre, and MicroScan methods to accurately define the meropenem MIC and categorical interpretation of susceptibility against carbapenemase-producing *Klebsiella pneumoniae* (KPC). A total of 46 clinical *K. pneumoniae* isolates with KPC genotypes, all modified Hodge test and *bla*KPC positive, collected from two hospitals in NY were included. Results obtained by each method were compared with those from broth microdilution (the reference method), and agreement was assessed based on MICs and Clinical Laboratory Standards Institute (CLSI) interpretative criteria using 2010 susceptibility breakpoints. Based on broth microdilution, 0%, 2.2%, and 97.8% of the KPC isolates were classified as susceptible, intermediate, and resistant to meropenem, respectively. Results from MicroScan demonstrated the most agreement with those from broth microdilution, with 95.6% agreement based on the MIC and 2.2% classified as minor errors, and no major or very major errors. Etest demonstrated 82.6% agreement with broth microdilution MICs, a very major error rate of 2.2%, and a minor error rate of 2.2%. Vitek 2 MIC agreement was 30.4%, with a 23.9% very major error rate and a 39.1% minor error rate. Sensititre demonstrated MIC agreement for 26.1% of isolates, with a 3% very major error rate and a 26.1% minor error rate. Application of FDA breakpoints had little effect on minor error rates but increased very major error rates to 58.7% for Vitek 2 and Sensititre. Meropenem MIC results and categorical interpretations for carbapenemase-producing *K. pneumoniae* differ by methodology. Confirmation of testing results is encouraged when an accurate MIC is required for antibiotic dosing optimization.

Usklađenost s EUCAST-om

http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/2014/Compliance_of_Manufacturers_2014-09-25.pdf

Compliance of manufacturers with EUCAST guidelines, 25 September 2014

VITEK 2 automated system (bioMérieux)

EUCAST terminology implemented	**In computer database**	**S ≤**	Yes
		R >	Yes
		-	Yes
		IE	Yes
	In reports	**S ≤**	Yes
		R >	Yes
		-	Yes
		IE	Yes
EUCAST Expert Rules implemented	All applicable rules dealing with intrinsic resistance, therapeutic interpretation and deduction are implemented beginning with software version V2S 5.03 (2011) . There are additional rules but none should be contrary to EUCAST rules in this software version.		
EUCAST organism groups with no test in the system	*H. influenzae* *M. catarrhalis*	*N. meningitidis* *N. gonorrhoeae*	Gram-negative anaerobes Gram-positive anaerobes
Agents in EUCAST tables but not available in the system	Azithromycin Roxithromycin Ampicillin-sulbactam (fixed 4 mg/L sulbactam) * (*Staphylococcus* spp. and *Enterococcus* spp.) Phenoxymethylpenicillin Cefadroxil		Ceftibuten Cefepime *(S. pneumoniae)* Cefpodoxime (*S. pneumoniae*) Cefuroxime (*S. pneumoniae*) Doxycycline (*S. pneumoniae*) Minocycline (*S. pneumoniae*)
Agents available but EUCAST breakpoints not implemented in the system	Netilmicin (*Staphylococcus* spp.) Trimethoprim (*Enterococcus* spp. and group B streptococci) Amoxicillin-clavulanic acid (fixed 2 mg/l clavulanic acid)* uncomplicated UTI breakpoints (Enterobacteriaceae) Doripenem (Enterobacteriaceae, *Pseudomonas* spp., *Acinetobacter* spp.) Ciprofloxacin (*Salmonella* spp. and *Enterococcus* spp.) Levofloxacin (*Enterococcus* spp.) Norfloxacin (*Enterococcus* spp.)		Trim-sulfa (*Enterococcus* spp.) Ofloxacin (*S. pneumoniae*) Streptomycin (*Enterococcus* spp.) Antifungals All agents with P. *multocida* Benzylpenicillin (CoNS) Cefoxitin screen (*S. saprophyticus* and *S. pseudintermedius*)

*Tests with the agent:inhibitor in a 2:1 ratio are not acceptable using EUCAST methods and breakpoints

Hvala na pažnji!