SPITALUL CLINIC JUDEȚEAN DE URGENȚĂ
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METODE FENOTIPICE DE IDENTIFICARE A MECANISMELOR DE REZISTENTA LA ANTIBIOTICE

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Testarea sensibilității la antibiotice

ANTIBIOGRAMA

- **Evaluarea răspunsului in *vitro* la acțiunea antibioticelor**

➢ **SCOPURI**

- **Previzionarea succesului sau eșecului terapeutic –relevant pentru pacient**
- **Alertă epidemiologică pentru stabilirea măsurilor de control și prevenție a infecției**
- **Cunoașterea epidemiologiei rezistenței la antibiotice – relevant pentru sănătatea publică**

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METODE DE TESTARE A SENSIBILITĂȚII LA ANTIBIOTICE

- ***FENOTIPICE***

- **Determinarea CMI**
- **Difuzimetric (EUCAST, BSAC, CLSI)**
- **Sisteme automate (Vitek, Phoenix, MicroScan)**

- ***GENETICE si PROTEOMICE***

-detectarea genelor de rezistență *mec* A, *van*A, *van*B

-detectarea PBP2a

-MALDI TOF, PCR și WGS

- ***INTUITIVE-mec*A pozitiv- rezistente la beta lactamine**

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- Metodele fenotipice de detectare a rezistenței la antibiotice sunt încadrate în paradigma "*interpretive reading*" –citirea interpretativă.

- Termenul a fost introdus în 1971 de către Patrice Courvalin, rezident în boli infecțioase la Spitalul Pasteur de Boli Infecțioase din Paris, unde se tratau cazuri rare și dificile.

- După-amiezile petrecute în laboratorul prof. Chabbert l-au comutat definitiv spre microbiologie, domeniu în care lucrează de peste 45 de ani.

- Acesta este un exemplu grăitor privind asocierea și întrepătrunderea între clinică și laborator sau, ”*from bench to bedside*”.
- Pentru anumite combinații între clasele de antibiotice și speciile bacteriene, este de dorit și chiar indicat să se raporteze un rezultat pentru o întreagă clasă de antibiotice, chiar dacă *in vitro* s-a testat un singur membru.

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Patrice COURVALIN
International Senior Award

Sanofi – Institut Pasteur Awards 2016

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JAC

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

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If isolates are speciated and if a sufficient range of antibiotics is tested, underlying resistance mechanisms can often be inferred from the antibiogram data. This allows: (i) anomalous combinations of phenotype and organism to be reconsidered; (ii) prediction of further antibiotics that deserve testing; and (iii) the suppression of susceptibilities that are anomalous in the light of the inferred mechanism. This 'interpretative reading' is widely undertaken in France but is largely precluded in the UK by limited speciation and the testing of narrow ranges of antibiotics. Nevertheless, UK laboratories should be aware of: (i) grossly anomalous combinations of species and phenotype, demanding reference laboratory confirmation; (ii) useful indicator drugs, where resistance implies a mechanism conferring other resistances that may be less obvious in direct tests; and (iii) antibiotics that are prone to select resistant mutants of particular species during therapy. Details of these combinations of organism and resistance are presented. Relationships between antibiogram and mechanism are also presented to allow full interpretative reading for those testing wide panels of drugs versus speciated isolates.

Interpretive reading of in vitro antibiotic susceptibility tests (the antibiogramme)

Patrice Courvalin

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INTRODUCTION

In recent years, there has been important progress in our knowledge of bacterial resistance to antibiotics. The availability of an increasing number of antibiotics has allowed more precise individualization of resistance phenotypes, and enzyme inhibitors have provided clues concerning certain mechanisms of resistance. Study of the biochemical mechanisms of resistance of strains representative of the various phenotypes has led to elucidation of cross-resistance, and examination of large numbers of clinical isolates has provided information on co-resistance to antibiotics. Detailed analysis of the bacteriostatic and bactericidal activity of antibiotics, alone or in combination, has indicated the limits of in vitro antimicrobial susceptibility tests in the detection

production of a single enzyme. It also applies to combined phenotypes resulting from the coexistence in the same host of several mechanisms conferring resistance to antibiotics belonging to the same group. However, the study of strains harboring two cross-resistance determinants indicated that in general the genes contribute, in an additive fashion, to the degree of antibiotic resistance, resulting in unambiguous and easily detectable phenotypes.

RATIONALE

Antibiotics are not lonely individuals but members of tight families

The vast majority of antibiotics used in human therapy

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- Since 1970, he has held many positions at the Institut Pasteur where he was head of the Antibacterial Agents Unit (1983-2015) and director of the National Reference Center for Resistance to Antibiotics (1983-2011) and served as Chairman of the Department of Fundamental and Medical Microbiology (2002-2003).
- He was a Research Associate at the University of Wisconsin–Madison (1974-1977) and was a Visiting Scholar in the Center for Molecular Genetics (1989-1990) and in the Department of Biology (1991-1994), University of California-San Diego (1989-1990).
- Prof. P. Courvalin is Doctor *honoris causa* of the University of El Bosque, Bogota, of the University of Mons, Hainaut, Belgium, of the Technical University of Denmark, and of the University of Zurich, Switzerland.
- Hirsh 97

(https://pcourvalin.wixsite.com/website)

Antibiotic	Microorganism
Amino-, carboxypenicillins	*Citrobacter diversus, Klebsiella*
Aminopenicillins, first-generation cephalosporins	*Citrobacter freundii, Enterobacter, Morganella, Proteus vulgaris, Providencia, Serratia*
Cefoxitin	*C. freundii, Clostridium difficile, Enterobacter*
Cefalotin, Cefotaxime	*Bacillus*
Clavulanic acid	*Campylobacter*
Sulbactam	*Acinetobacter, Pseudomonas cepacia*
Imipenem	*Xanthomonas maltophilia*
Aminoglycosides	*P. cepacia, P. maltophilia*, streptococci, anaerobes
Tetracycline	*Proteus minabilis*
Lincomycin	*Eikenella corrodens, Enterococcus faecalis, Haemophilus, Staphylococcus xylosus, Listeria, Neisseria*, Gram-negative bacteria
Bacitracin	*Streptococcus pyogenes*
Colistin	Sensitivity: *P. aeruginosa* Resistance: *Proteus, Providencia, Serratia, P. cepacia*, Gram-positive bacteria
Trimethoprim	*Acinetobacter, Brucella* spp., *Campylobacter, Neisseria, Nocardia, Pseudomonas* spp.
Nitrofurans	*Proteus, Providencia, Acinetobacter*, micrococci
Novobiocin	*Staphylococcus saprophyticus*
Fosfomycin	*Acinetobacter, S. saprophyticus*
Vancomycin	*Erysipelothrix rhusopathiae, Lactobacillus, Leuconostoc, Nocardia, Pediococcus*, Gram-negative bacteria
Metronidazole	*Gardnerella vaginalis, Propionibacterium*, aerobes
Optochin[a]	Pneumococci
0/129[a]	Differentiation *Micrococcus, Staphylococcus* Differentiation enterobacteria, *Pasteurella, Vibrio*

[a]Not used in therapy.

Antibiotics useful for bacterial identification

Sursa: Patrice Courvalin, "Interpretive reading of in vitro antibiotic susceptibility tests (the antibiogramme)", Clinical Microbiology and Infection, Volume 2 Supplement 1, December 1996

- Clasificarea clinică este deja **INTERPRETARE**

- Scopul este îmbunătățirea detectării mecanismelor de rezistență la antibiotice, prin compararea fenotipului tulpinii sensibile cu fenotipul izolatului clinic.
- Se bazează pe valoarea CMI. Nu există consens internațional asupra punctelor de ruptura
- În practica clinică există două categorii:

succes* sau *eșec terapeutic

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FACILITAREA REZISTENȚEI

- posibilitatea de a anticipa emergența rezistenței;
- Mutațiile ex:*gyrA* care determină rezistența de nivel înalt la enterobacterii - conferă rezistență și la alte chinolone;
- gradul de *cross-rezistență* depinde de activitatea intrinsecă a antibioticului: fluorochinolonele cu CMI usor ridicat rămân active clinic.
- Aceste tulpini poseda un mecanism de rezistență și au potențial de a deveni rezistente la ciprofloxacină sub monoterapie, prin eveniment mutațional secundar.
- Rezistență inductibilă la MLS.

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Antibiotics and combinations that detect best certain resistance mechanisms

Antibiotic	Phenotype	Mechanism[a]	Host
Penicillin+pH indicator	Penicillin resistance	Penicillinase	Gram-positive cocci, *Haemophilus, Neisseria*
Oxacillin	β-Lactam resistance	Additional PBP	*Staphylococcus*
Oxacillin	Penicillin resistance	PBP alteration	*Streptococcus pneumoniae*
Aminopenicillin, aminopenicillin + penicillinase inhibitor	Penicillin resistance	Penicillinase	Enterobacteria
Cefotaxime or ceftazidime +penicillinase inhibitor[c]	β-Lactam (except cephamycins and carbapenems) resistance	Extended spectrum β-lactamase[b] (plasmid-mediated)	
Cefoxitin+cephalosporin[d]			
Clavulanic acid+cephalosporin[d]	Cephalosporin resistance (chromosome-mediated)	Cephalosporinase	
Imipenem+cephalosporin[d]			
Cephamycins, moxalactam+cephalosporin[d]	Antibiotic resistance	Porin alteration	
Cefoxitin	Antibiotic resistance	Porin alteration	*Escherichia coli, Klebsiella*
Kanamycin	Amikacin resistance	APH(3'), ANT(4')	Gram-positive cocci
Gentamicin	Aminoglycoside resistance	APH(2")-AAC(6')	
2'-*N*-Ethylnetilmicin+6'-*N*-ethylnetilmicin[e]	Aminoglycoside resistance	AAC(2'), AAC(6')	Gram-negative bacteria
Apramycin[e]	Aminoglycoside resistance	AAC(3)-IV	
Fortimicin[e]	Aminoglycoside resistance	AAC(3)-I	
Amikacin+tobramycin	Aminoglycoside resistance	APH(3')-VI	*Acinetobacter*
Netilmicin+tobramycin	Aminoglycoside resistance	AAC(3)	*Pseudomonas*
Erythromycin + lincomycin	Inducible MLS resistance	Ribosomal methylation	Gram-positive cocci
Nalidixic acid	Quinolone resistance	DNA gyrase or porin alteration	Gram-negative bacteria
Tinidazole	Imidazole resistance	Reductase	Anaerobes

[a]AAC, aminoglycoside acetyltransferase; ANT, aminoglycoside nucleotidyltransferase; APH, aminoglycoside phosphotransferase; PBP, penicillin binding protein.
[b]Except *Proteus penneri* and *P. vulgaris*, which resist through production of a cephalosporinase susceptible to penicillinase inhibitors.
[c]In case of synergism.
[d]In case of indifference
[e]Not used in therapy.

Sursa: Patrice Courvalin, "Interpretive reading of in vitro antibiotic susceptibility tests (the antibiogramme)", Clinical Microbiology and Infection, Volume 2 Supplement 1, December 1996

Antibiotic	Microorganism
Penicillins (except benzylpenicillin) Cloxacillin, dicloxacillin, flucloxacillin, nafcillin Cephalosporins Clindamycin	*Staphylococcus*
Amikacin Netilmicin	Gram-positive cocci
Penicillins susceptible to penicillinases (except benzylpenicillin)	*Haemophilus*[a], *Neisseria*
Topical drugs Prodrugs	All bacteria

[a]Except against strains that are intermediate or resistant to penicillins and do not produce a penicillinase.

Antibiotics used in therapy that should not be tested

Sursa: Patrice Courvalin, "Interpretive reading of in vitro antibiotic susceptibility tests (the antibiogramme)", Clinical Microbiology and Infection, Volume 2 Supplement 1, December 1996

Examples of impossible antibiotic resistance phenotypes

Resistance phenotype	Microorganism
Gentamicin^{R}, other aminoglycosides^{S} Minocycline^{R}, tetracycline^{S}	Gram-positive cocci
Methicillin or oxacillin^{R}, cephalosporin^{S} or carbapenem^{S} Methicillin or oxacillin^{R}, penicillin^{S}	*Staphylococcus aureus*
Penicillin^{R} Teicoplanin^{R}, vancomycin^{S}	Group A, C, G streptococci Enterococci
Amino-, carboxypenicillins^{S}	*Citrobacter diversus*
Aminopenicillins^{S}, first-generation cephalosporins^{S}, aminopenicillin+clavulanic acid^{S} (except *Proteus penneri* and *P. vulgaris*)	*Citrobacter freundii, Enterobacter cloacae, Morganella morganii, Proteus vulgaris, Providencia, Serratia, Yersinia*
Cefamandole or cefuroxime^{S}	*P. vulgaris, Serratia*
Cefoxitin^{S}	*Citrobacter freundii, Enterobacter*
Third-generation cephalosporins^{R}, amino-, carboxypenicillins,^{S} and/or first-generation cephalosporins^{S}	Enterobacteriaceae
Colistin^{S}	*Proteus, Providencia, Serratia*

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IMPLICAȚII

- *A la carte* sau meniu?
- Nu se testează toate antibioticele existente;
- Alegerea bateriei de testare - minim de antibiotice din aceeași familie; prezența *cross - rezistenței;*
- Se testează cei mai folosiți agenți;
- Testarea pentru comparare și a altor antibiotice în funcție de indicații;
- Evaluarea noilor agenți antimicrobieni.

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REZISTENȚE NEOBIȘNUITE

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

Note to all tables: β-lactam groups
First generation cephalosporins: cephalexin, cephalothin, cephazolin and cephradine.
Second generation cephalosporins: cefamandole, cefaclor and cefuroxime.
Third generation cephalosporins: cefotaxime, cefpodoxime, ceftazidime and ceftriaxone.
Fourth generation cephalosporins: cefepime and cefpirome.
Oxyimino cephalosporins: cefepime, cefotaxime, cefpirome, cefpodoxime, ceftazidime, ceftriaxone and cefuroxime.
Cephamycins: cefoxitin, cefotetan.
Aminopenicillins: amoxycillin, ampicillin, mezlocillin and piperacillin.
Carboxypenicillins: carbenicillin and ticarcillin.

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CITIREA INTERPRETATIVĂ

- **Mecanismul de rezistență din rezultatul testării sensibilității;**
- **Permite estimarea răspândirii rezistenței;**
- **Identifică pattern-urile anormale de rezistență;**
- **Introduce în bateria de testare a unor antibiotice mai puțin folosite.**

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Phenotypes: interpretation of mechanisms and editing of antiobiograms: beta-lactams vs. Enterobateriaceae (I)

AMP	AMX/ CLAV	TIC	TIC/ CLAV	PIP	PIP/ TAZ	CEF	FOX	CXM	CAZ	CTX CRO	CPR FEP	ATM	IMP MEM	Interpretation	Frequency	Edit/action
E. coli, *P. mirabilis*, *Salmonella*, *Shigella* spp.																
S	S	S	S	S	S	S	S	S	S	S	S	S	S[a]	classical	common	
R	S	R	S	r	S	r	S	S	S	S	S	S	S	penicillinase-low	common	Edit 1st gen cephs to R except in UTI.
R	r/R	R	r/R	R	r/R	R	S	S	S	S	S	S	S	penicillinase-high	common	Edit 1st gen cephs to R except in UTI.
R	R	r	R	R	R	R	R	R	R	r/R	S	r/R	S	AmpC high	rare	Consider TEMO as therapy alternative.
R	any[b]	R	any[b]	R	any[b]	R	S	R	R	R	R	R	S	ESBL-broad	rare	ESBL test; if +ve, edit 2/3/4 gen cephs to R.[c]
R	any[b]	R	any[b]	R	any[b]	R	S	r	R	r	r	r	S	ESBL-ceftazidimase	rare	ESBL test; if +ve, edit 2/3/4 gen cephs to R.[c]
R	R	R	R	R	r/R	S	S	S	S	S	S	S	S	IRT[d]	???	
any	any	any	any	any	any	any	any	any	any	any	any	any	R[1]	!!!	!!!	Refer.
Klebsiella spp., *C. diversus*																
R	S	R	S	r	S	S	S	S	S	S	S	S	S	classical-low SHV-1 or K1	common	Edit all penicillins (except TEMO) to R.
R	r/R	R	any[b]	R	r/R	R	S	S	S	S	S	S	S	penicillinase-high	common	
R	any[b]	R	any[b]	R	any[b]	R	S	R	R	R	R	R	S	ESBL-broad	scattered	ESBL test; if +ve, edit 2/3/4 gen cephs to R.[c]
R	any[b]	R	any[b]	R	any[b]	R	S	r	R	r	r	r	S	ESBL-ceftazidimase	scattered	ESBL test; if +ve, edit 2/3/4 gen cephs to R.[c]
R	R	R	R	R	r/R	S	S	S	S	S	S	S	S	IRT,[d]	???	
R	R	R	R	R	R	R	S	R	S	S	S	R	S	K1 high, *K. oxytoca* only	scattered	Edit CTX to R; ??? CAZ.
R	R	R	R	R	R	R	R	R	R	R	S	r/R	S	AmpC acquired	rare	
any	any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.
Enterobacter, *C. freundii*																
R	R	S	S/r	S	S	R	R	S/r	S	S	S	S	S	classical AmpC inducible	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	any[b]	R	S/r	R	R	S	S	S	S	S	S	penicillinase	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	any[b]	R	S/r	R	R	R	R	R	R	R	S	ESBL-broad	rare	Edit 2/3/4 gen cephs to R.[f]
R	R	R	any[b]	R	S/r	R	R	r	R	r	R	r	S	ESBL-ceftazidimase	rare	Edit 2/3/4 gen cephs to R.[f]

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Phenotypes: interpretation of mechanisms and editing of antiobiograms: beta-lactams vs. Enterobateriaceae (II)

R	R	R	R	R	R	R	R	R	R	R	S	R	S	AmpC derepressed	common	Consider TEMO as therapy alternative.
any	any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.
M. morganii/Providencia spp.																
R	R	S	S/r	S	S	R	r	R	S	S	S	S	S	classical	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	S	R	S/r	R	r	R	S	S	S	S	S	penicillinase	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	R	R	S	R	r	R	R	R	S	R	S	AmpC derepressed	scattered	Consider TEMO as therapy alternative.
any	any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.
P. vulgaris																
R	S	R	S	S	S	R	S	R	S	S	S	S	S	classical	common	
R	S	R	S	R	S	R	S	R	S	S	S	S	S	penicillinase	common	
R	S	R	S	R	S	R	S	R	S	R	S	S	S	Chr β-lactamase derepressed	rare	
any	any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.
Serratia spp.																
R	R	S	S	S	S	R	r	R	S	S	S	S	S	classical	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	any[b]	R	any	R	r	R	S	S	S	S	S	penicillinase	common	Advise against use of 2/3 gen cephs.[e]
R	R	R	R	R	r/R	R	r	R	S	R	S	R	S	AmpC derepressed	rare	Consider TEMO as therapy alternative.
any	any	any	any	any	any	any	any	any	any	any	any	any	R	!!!	!!!	Refer.

See note to all tables in Table I.
General notes for Tables V–XI:
Classical means the historic phenotype of the species, without acquired resistance; **common** means seen in >10% of isolates; **scattered** means seen in 5–10% of isolates; **uncommon** means seen in 1–5% of isolates; and **rare** means seen in <1% of isolates. Local frequencies may be very different, especially during outbreaks and in specialist units. **Refer** and **!!!** mean send to an appropriate reference or academic laboratory for confirmation (see text); **???** means uncertain as insufficient data.
Abbreviations: AMK, amikacin; AMX, amoxycillin; AMP, ampicillin; AMX/CLAV, co-amoxiclav; ATM, aztreonam; CAZ, ceftazidime; Ceph, cephalosporin; FEP, cefepime; CLI, clindamycin; CEF, cephalothin; CPR, cefpirome; CRO, ceftriaxone; CTX, cefotaxime; CXM, cefuroxime; ERY, erythromycin; FOX, cefoxitin; GEN, gentamicin; IMP, imipenem; KAN, kanamycin; MEM, meropenem; NEO, neomycin; NET, netilmicin; OXA, oxacillin/cloxacillin; PCG, penicillin G; PIP, piperacillin; TZP, piperacillin/tazobactam; Q-D, quinupristin/dalfopristin; TEMO, temocillin; TIC, ticarcillin; TIM, ticarcillin/clavulanate; TOB, tobramycin; 1st gen ceph, first generation cephalosporin; 2/3/4 gen ceph, second/third/fourth generation cephalosporin; R, resistant; r, reduced zones but likely to remain susceptible at BSAC breakpoints; B, borderline (MICs for typical strains of the species without acquired resistance, fall around the zone/MIC breakpoints); S, susceptible.
[a]Discount low-level imipenem resistance in *P. mirabilis*.
[b]Varies with amount of β-lactamase produced.
[c]See Livermore and Brown[6] for ESBL tests for these species.
[d]IRT: inhibitor-resistant TEM mutant.
[e]If second- or third-generation cephalosporins are used, there is substantial risk of selection of derepressed, mutants during therapy. See Table III.
[f]ESBL tests[6] are of dubious value for AmpC-inducible species since clavulanate-induced AmpC enzymes (which evade the action of clavulanate) are prone to attack the indicator cephalosporin. The pattern of cefotetan-susceptible, ceftazidime-resistant would imply ESBL production, but this principle has not been evaluated critically.[6]

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Phenotypes: interpretation of mechanisms and editing of antiobiograms: beta-lactams vs. non-fermenters

TIC	TIC/ CLAV	PIP	PIP/ TAZ	CAZ	CPR/ FEP	ATM	IMP	MEM	Interpretation	Frequency	Edit/action
P. aeruginosa[a]											
S	S/r	S	S	S	S	S	S	S	classical	common	beware mutational resistance; see Table III
R	any	R	any	S	S	S	S	S	penicillinase	rare	
r	R	r	r	r	r	R	S	S	AmpC part derepressed	common	
r/R	r/R	R	R	R	S/r	R	S	S	AmpC fully derepressed	rare	
R	R	r/R	r/R	r/R	R	r/R	S	r	increased efflux[b]	common	
S	S/r	S	S	S	S	S	R	r	loss of OprD porin	scattered	
Acinetobacter spp.[c]											
S. maltophilia[d]											

See note to all tables in Table I and general notes for Tables V–XI in Table V.

[a]Isolates may have multiple mechanisms, with profiles superimposed on each other.

[b]Isolates typically also have r/R to quinolones.

[c]Relationships between antibiogram and mechanisms poorly defined. Carbapenems have the most consistent activity against the genus: refer carbapenem-resistant *Acinetobacter* isolates.

[d]May appear susceptible to penicillins and cephalosporins on IsoSensitest agar, but is generally resistant on Mueller–Hinton agar. Among β-lactams, ticarcillin/clavulanate has best provenance, although co-trimoxazole (not trimethoprim alone) is the usual drug of choice.

Sursa: David M. Livermore,Trevor G. Winstanley, Kevin P. Shannon, "Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes".. Journal of Antimicrobial Chemotherapy (2001) 48, Suppl. S1, 87–102.

Phenotypes: interpretation of mechanisms and editing of antiobiograms: beta-lactams vs. fastidious Gram-negative bacteria and M. catarrhalis

PCG	AMP/ AMX	AMX/ CLAV	CCL	CTX/CRO/ CFIX	MEM	IMP	Interpretation	Frequency	Edit/action
H. influenzae									
R	S	S	S	S	S	S	classical	common	
R	R	S	S	S	S	S	β-lactamase +ve	common	Confirm with β-lactamase test.[a]
R	r/R	r/R	R	r	S	S/R[b]	intrinsic resistance-altered PBPs; impermeability or efflux	rare	
any	any	any	any	any	R	any	!!!	!!!	Refer.
any	any	any	any	R	any	any	!!!	!!!	Refer.
N. gonorrhoeae									
S	S	S	–	S	–	–	classical	common	
R	R	S	–	S	–	–	β-lactamase +ve	common	Confirm with β-lactamase test.[a]
r/R	r/R	r/R	–	S	–	–	intrinsic impermeability or efflux	common	
any	any	any	any	R	–	any	!!!	!!!	Refer.
N. meningitidis									
S	S	S	–	S	–	–	classical	common	
r	R	r	–	S	–	–	impermeability or efflux	common	
Substantial R to any β-lactam							!!!	!!!	Refer.
M. catarrhalis									
R	S	S	S	S	S	S	classical	common	Confirm β-lactamase negative by direct test; if +ve, report as ampicillin/amoxycillin-resistant.
R	R	S	S	S	S	S	BRO-1/2 β-lactamase +ve	common	

See note to all tables in Table I and general notes for Tables V–XI in Table V.

[a]See Livermore & Brown[6] for β-lactamase tests.

[b]*H. influenzae* with intrinsic resistance to penicillins and cephalosporins are either fully susceptible to imipenem, or show a high level of resistance, implying that the group encompasses at least two different genotypes.

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Phenotypes: interpretation of mechanisms and editing of antiobiograms: beta-lactams vs. s Gram-positive cocci

PCG	AMP/ AMX	AMX/ CLAV	OXA	Any cephalosporin	IMP/ MEM	Interpretation	Frequency	Edit/action
Staphylococci								
S	S	S	S	S	S	classical, now uncommon	scattered	
R	R	S	S	S	S	β-lactamase +ve	common	Edit all penicillins except oxacillin and methicillin to R.
any	any	any	R	any	any	methicillin/oxacillin resistant	common	Edit all β-lactams to R.
S. pyogenes								
S	S	S	S	S	S	classical	common	
R	any	any	any	any	any	!!!	!!!	Refer.
S. pneumoniae								
S	S	S	S	S	S	classical		
any	any	any	R	any	any	PenR pneumococcus	common	Determine MICs of drugs intended for use. Cefotaxime and ceftriaxone, also meropenem, often remain active, with oral cephalosporins mostly less active than amoxycillin.
E. faecalis								
r	S	S	R	R	S	classical	common	
R	R	S	R	R	S	β-lactamase +ve	!!!	Refer.
R	R	R	R	R	R	probably *E. faecium*	error	Check speciation.
E. faecium								
R	S	S	R	R	S	classical, now rare	scattered	
R	R	R	R	R	R	uses PBP-5 to cross-link peptidoglycan	common	

Sursa: David M. Livermore,Trevor G. Winstanley, Kevin P. Shannon, "Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes".. Journal of Antimicrobial Chemotherapy (2001) 48, Suppl. S1, 87–102.

GEN	NET	TOB	AMK	KAN	NEO	Interpretation	Frequency	Edit/action and comments
E. coli and other Enterobacteriaceae not shown separately								
S	S	S	S	S	S	classical	common	
R	S	S	S	S	S	AAC(3)I	rare	Also R to fortimicin.
R	R	R	S	R	S	AAC(3)II	rare	Greater R to GEN than to TOB or NET.
R	R	R	S	r	R	AAC(3)IV	rare	Also R to apramycin (used in veterinary practice). Mostly in *E. coli*.
S/r	R	R	R	R	R	AAC(6′)	rare	One component of GEN remains active but *in vivo* use best avoided.
R	S	R	S	R	S	ANT(2′)	rare	Equal R to GEN and TOB.
S	S	S	S	R	R	APH(3′)	common	Usually more R to KAN than NEO. Was common, now rarely seen.
r/R	r/R	r/R	r/R	r/R	r/R	'impermeability'	rare	Low level R to all aminoglycosides.
Klebsiella spp.								
S	S	S	S	S	S	classical	common	
R	S	S	S	S	S	AAC(3)I	rare	Also R to fortimicin.
R	R	R	S	r	S	AAC(3)II	scattered/rare	Greater R to GEN than to TOB or NET.
S/r	R	R	R	R	R	AAC(6′)	rare	One component of GEN remains active, but *in vivo* best avoided.
R	S	S	S	R	S	ANT(2′)	scattered/rare	Equal R to GEN and TOB.
S	S	S	S	R	R	APH(3′)	common?	Usually more R to KAN than NEO. Was common, probably remains so.
r/R	r/R	r/R	r/R	r/R	r/R	'impermeability'	rare	Low-level R to all aminoglycosides.
Serratia spp.								
S	S	S	S	S	S	classical	common	Chromosomal AAC(6′) expressed weakly: risk of selection of over-producers in therapy with AMK, TOB, NET.
R	S	S	S	S	S	AAC(3)I	rare	Also R to fortimicin.
R	R	R	S	r	S	AAC(3)III	rare	Greater R to GEN than TOB or NET.
S/r	R	R	R	R	R	AAC(6′)	common	Mutation causes over-production of chromosomal AAC(6′).
R	S	R	S	R	S	ANT(2′)	rare	Equal R to GEN and TOB.
S	S	S	S	R	R	APH(3′)	rare	Usually greater R to KAN than to NEO.
r/R	r/R	r/R	r/R	r/R	r/R	'impermeability'	rare	Low-level resistance to all aminoglycosides.
Providencia stuartii								
R	r	R	S	S	R	AAC(2′)	classical	Chromosomal. AAC(2′); poorly expressed.
R	R	R	S	S	R	AAC(2′)	common	Mutation causes overproduction of AAC(2′).
P. aeruginosa								
S	S	S	S	R	R	classical	common	
R	S	S	S	R	R	AAC(3)I	rare	Also R to fortimicin.
R	S	R	S	R	R	AAC(3)III	rare	
S/r	R	R	R	R	R	AAC(6′)	rare	One component of GEN remains active, but *in vivo* use best avoided.
R	R	R	S	R	R	AAC(6′)II	rare	R pattern not obviously predictable from enzyme activity.
R	S	R	S	R	R	ANT(2′)	rare	Equal levels of R to GEN and TOB.
S	S	S	S	R	R	APH(3′)	common	Usually more R to KAN than to NEO.
r/R	r/R	R/R	r/R	r/R	r/R	'impermeability'	rare	Low-level R to all aminoglycosides.

Phenotypes: interpretation of mechanisms and editing of antiobiograms: amynoglicosides vs. Gram-negative bacteria

Sursa: David M. Livermore,Trevor G. Winstanley, Kevin P. Shannon, "Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes".. Journal of Antimicrobial Chemotherapy (2001) 48, Suppl. S1, 87–102.

Phenotypes: interpretation of mechanisms and editing of antiobiograms: amynoglicosides vs. Gram-positive bacteria

GEN	NET	TOB	AMK	KAN	NEO	Interpretation	Frequency	Edit/action and comments
Staphylococci								
S	S	S	S	S	S	classical	common	
S	S	R	R	R	S	ANT(4′) (4″)I	rare	Unlike 'Gram-negative' ANT(4′), also modifies dibekacin at 4″.
R	r	R	r	R	r	APH(2′)	rare	Greater R to TOB.
						AAC(6′)	scattered	
S	S	S	S	R	R	APH(3′)	common	Usually more R to KAN than NEO.
S	S	S	R	R	R	APH(3′)III	rare	Rare.
r/R	r/R	R/R	r/R	r/R	r/R	'impermeability'	rare	Low-level R to all aminoglycosides.
E. faecalis								
R	R	R	R	R	R	classical	common	Intrinsic low-level resistance.
R	R	HLR	HLR	HLR	R	ANT(4′) (4″)I	rare	
HLR	R	HLR	R	HLR	R	APH(2″)/	scattered	Greater R to GEN than TOB.
						AAC(6′)		
R	R	R	R	HLR	HLR	APH(3′)	common	Usually more R to KAN than NEO.
R	R	R	HLR	HLR	HLR	APH(3′)III	rare	Rare.
E. faecium								
R	R	R	R	R	R	AAC(6′)I	classical	Chromosomal AAC(6′), intrinsic to *E. faecium*.
R	R	HLR	HLR	HLR	R	ANT(4′) (4′)	rare	
R	R	R	R	HLR	HLR	APH(3′)	common	Usually greater R to KAN than NEO.
R	R	R	HLR	HLR	HLR	APH(3′)III	rare	

Sursa: David M. Livermore,Trevor G. Winstanley, Kevin P. Shannon, "Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes".. Journal of Antimicrobial Chemotherapy (2001) 48, Suppl. S1, 87–102.

ERY[a]	CLI	Q-D	Interpretation	Frequency	Edit/action
Staphylococci					
S	S	S	classical	common	
R	S	S	may be MLS_B inducible may have macrolide efflux	common	Check if erythromycin antagonizes clindamycin; if antagonism seen, isolate has MLS_B, and clindamycin should be used with caution (if at all).
R	R	S	MLS_B constitutive	common	Note Specification of Product Characteristics recommendation that Q-D should be given thrice daily even in skin and soft tissue infection.
any	any	R	!!!	refer	
Streptococci, including *S. pneumoniae*					
S	S	S	classical	common	
R	R	S	MLS_B constitutive/inducible	common	NB-inducible resistance usually affects clindamycin as well as erythromycin in streptococci.
R	S	S	efflux; MLS_B inducible	common	
any	any	R	!!!	refer	
E. faecalis					
S	S	R	classical	common	
R	S	R	may be MLS_B inducible may have macrolide efflux	common	Check if erythromycin antagonizes clindamycin, e.g. with a double disc test. If antagonism is seen, the isolate has MLS_B, and clindamycin should be used with caution, if at all.
R	R	R	MLS_B constitutive	common	
any	any	S	probable mis-speciation		If also AMP resistant, almost certainly *E. faecium*, not *E. faecalis*. Refer if confirmed as *E. faecalis*.
E. faecium					
S	S	S	classical	common	
R	S	S	may be MLS_B inducible may have macrolide efflux	common	Check if erythromycin antagonizes clindamycin; if antagonism seen, isolate has MLS_B, and clindamycin should be used with caution (if at all).
R	R	S	MLS_B constitutive	common	
any	any	R	probable mis-speciation; possible quinupristin efflux or modification		If also AMP susceptible, almost certainly *E. faecalis*, not *E. faecium*. Refer if confirmed as *E. faecium*.

Phenotypes: interpretation of mechanisms and editing of antiobiograms: MLS drugs vs. Gram-positive cocci

Sursa: David M. Livermore,Trevor G. Winstanley, Kevin P. Shannon, "Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes".. Journal of Antimicrobial Chemotherapy (2001) 48, Suppl. S1, 87–102.

LIMITE

- **Nu înlocuiește identificarea mecanismului de rezistență determinat biochimic și genetic;**
- **Asocierea mai multor mecanisme de rezistență dau limitele metodei;**
- **Nu toți microbiologii sunt experți în antibiotice - rolul computerului în deducerea mecanismului de rezistență;**
- **Nu toate mecanismele de rezistență sunt cunoscute.**

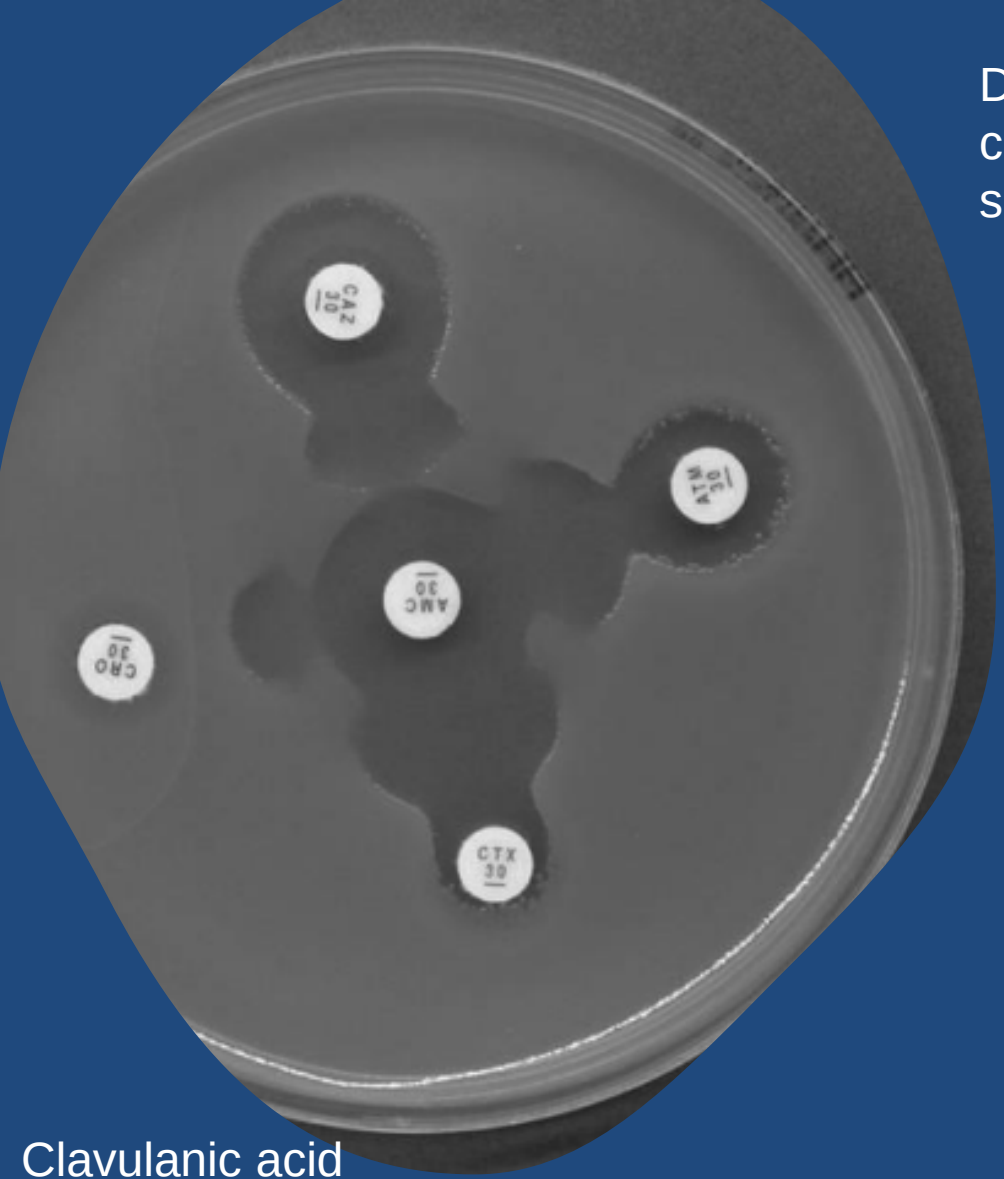
Roxana Filip: roxana_filip@yahoo.com

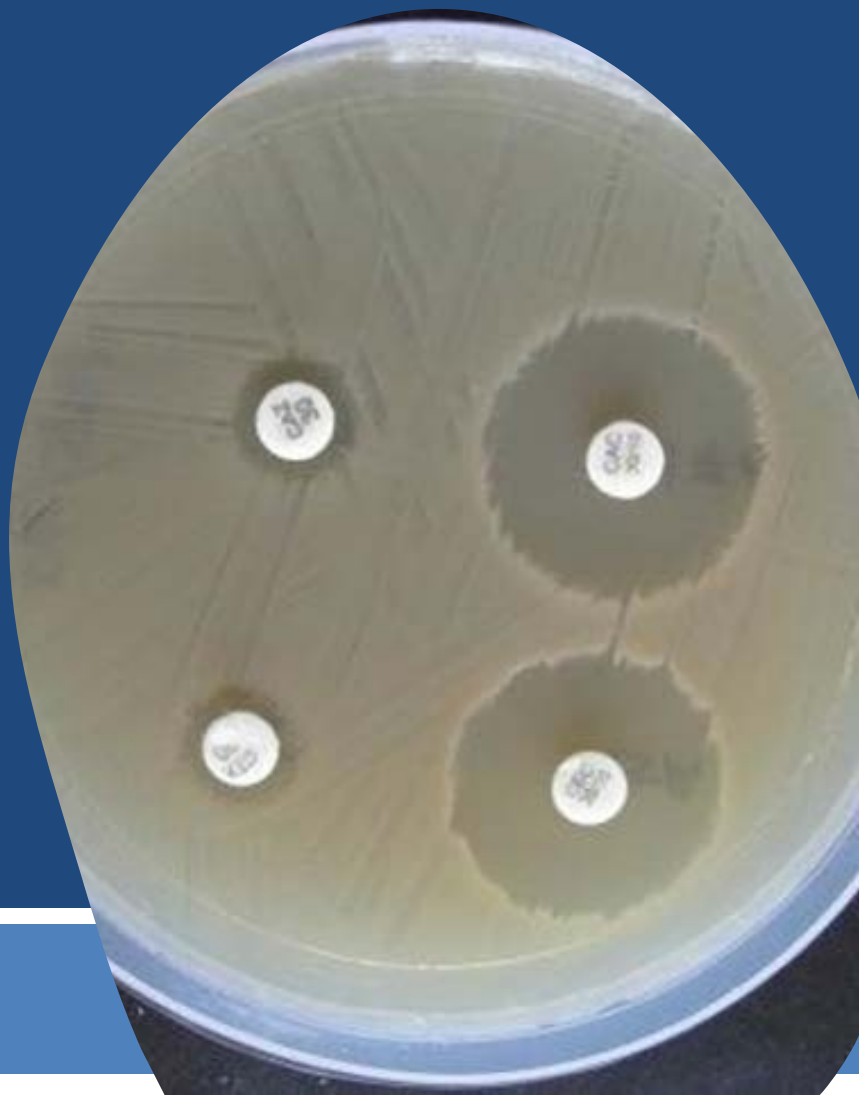
Paulina Siniatkina Don,t speak supravietuitoare de tuberculoza- Moscova 2015

- A fost sfatuita sa nu vorbeasca despre tuberculoza, dar si-a folosit talentul artistic pentru a-si exprima emotiile si frustrarile
- In prezent este o luptatoare in domeniul tuberculozei
- Figura centrala acoperita de o masca- arat stigma asociata cu acest diagnostic, teama si depresia-calmata doar de smulgerea petalelor din floarea alba pe care o tine in mana
- 40-70% dintre pacienti sufera de anxietate sau depresie
- Anual 10 milioane de cazuri- 1.5 milioane de decese
- Eliminarea globala a tuberculozei include si terapia psihiatrica
- 24 martie ziua internationala a tuberculozei- ziua in care Robert Koch a anuntat la Societatea de Fiziologie din Berlin descoperirea agentului etiologic

Roxana Filip: roxana_filip@yahoo.com

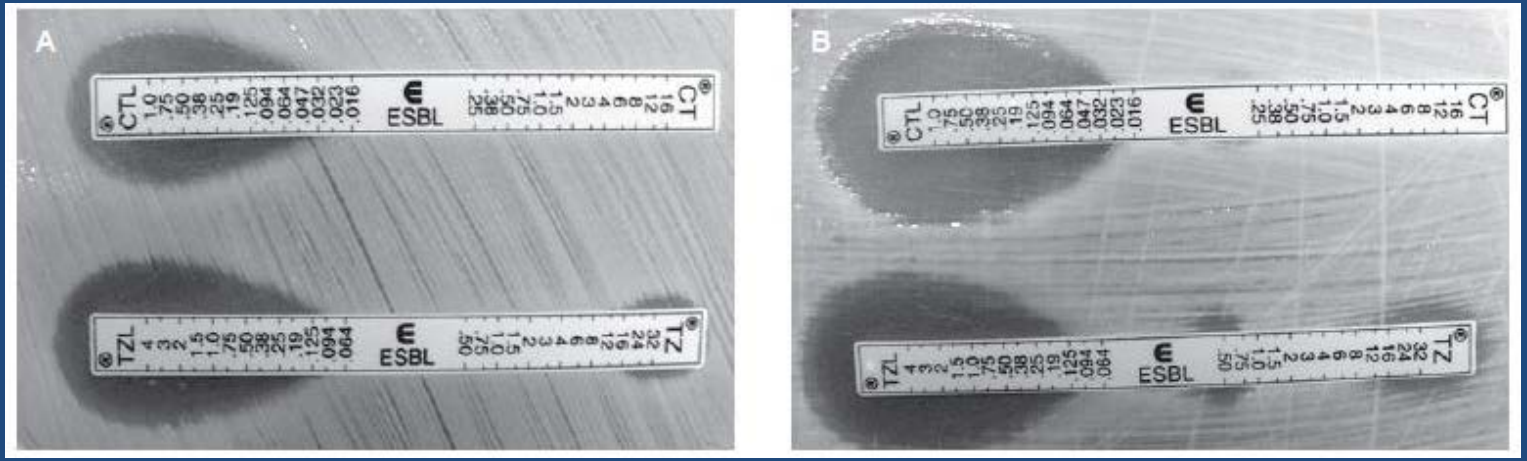
Double disk (> 5mm) between 3rd gen cephalosporin and the disk supplemented with clavulanic acid





Clavulanic acid synergism test

MIC assay (automatic, E-test etc.)

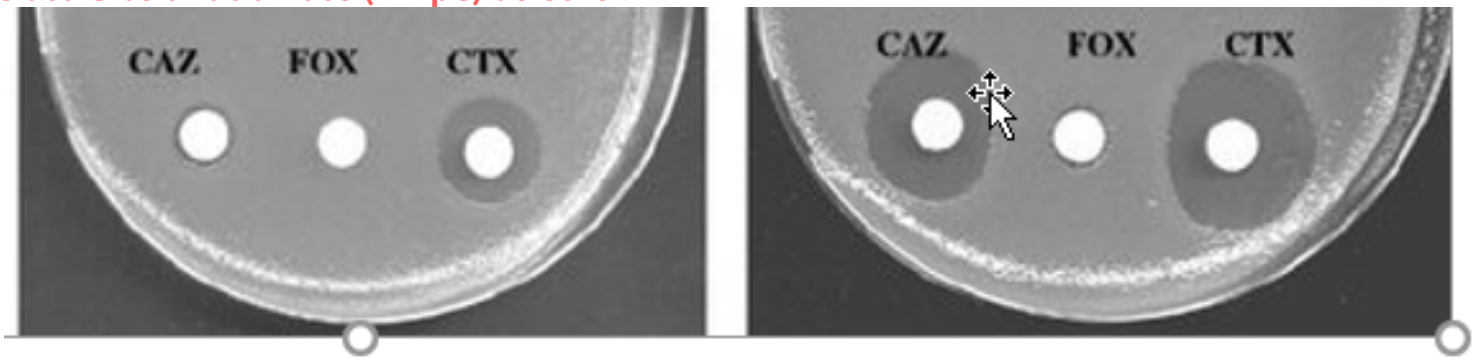


CromID ESBL

AmpC Cephalosporinase-Producing *Enterobacterales*

- **Incidence** in children 14.2% -29%

- Transmissible AmpC, less frequent, most commonly CMY-2, but also ACT/MIR-type AmpC genes.
- **AMPCES** (*Acinetobacter, Morganella, Proteus/ Providencia/Pseudomonas, Citrobacter, Enterobacter, Serratia*)
- Inducible/derepressed
- R to Clavulanic Acid (CLA) and Cefoxitin (FOX)
- Therapeutic options:
  - 4th gen cephalosporins
  - Carbapenemes
- **Class C beta-lactamase (AmpC) detection**

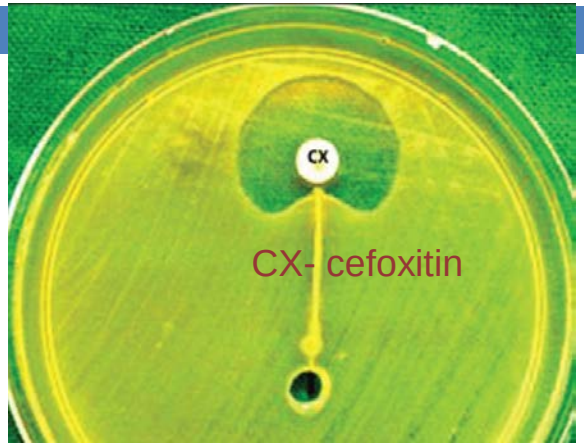


Derepressed AmpC

inducible AmpC

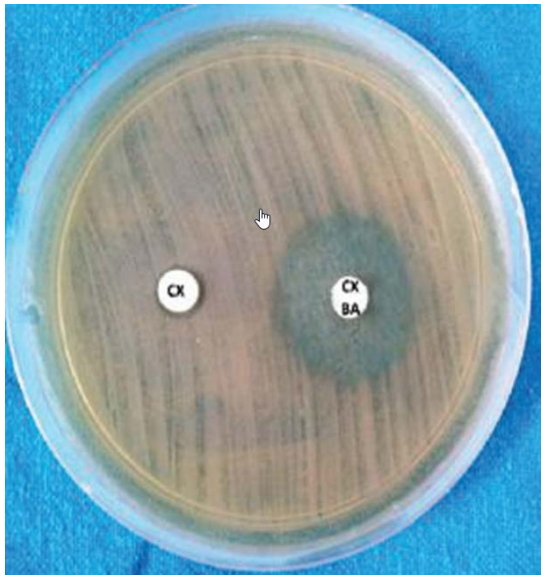
Class C beta-lactamase (AmpC) detection

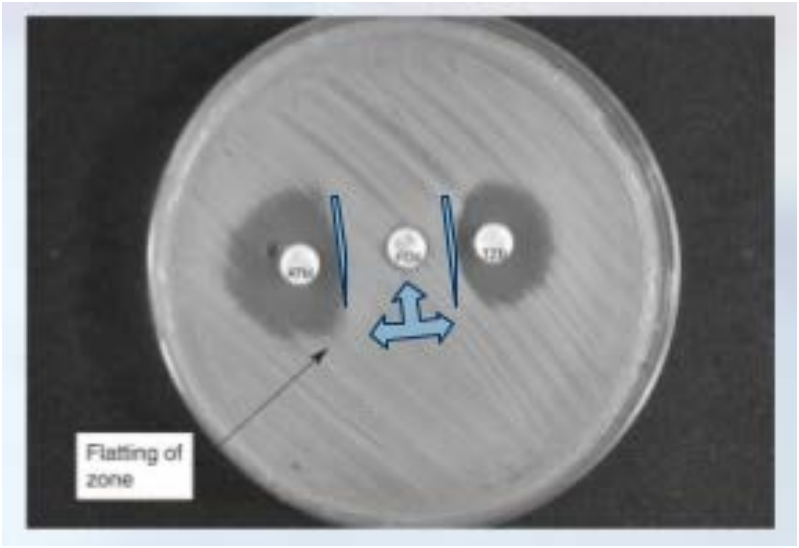
1. Modified Hodge test



3. Antagonism

2. DD test





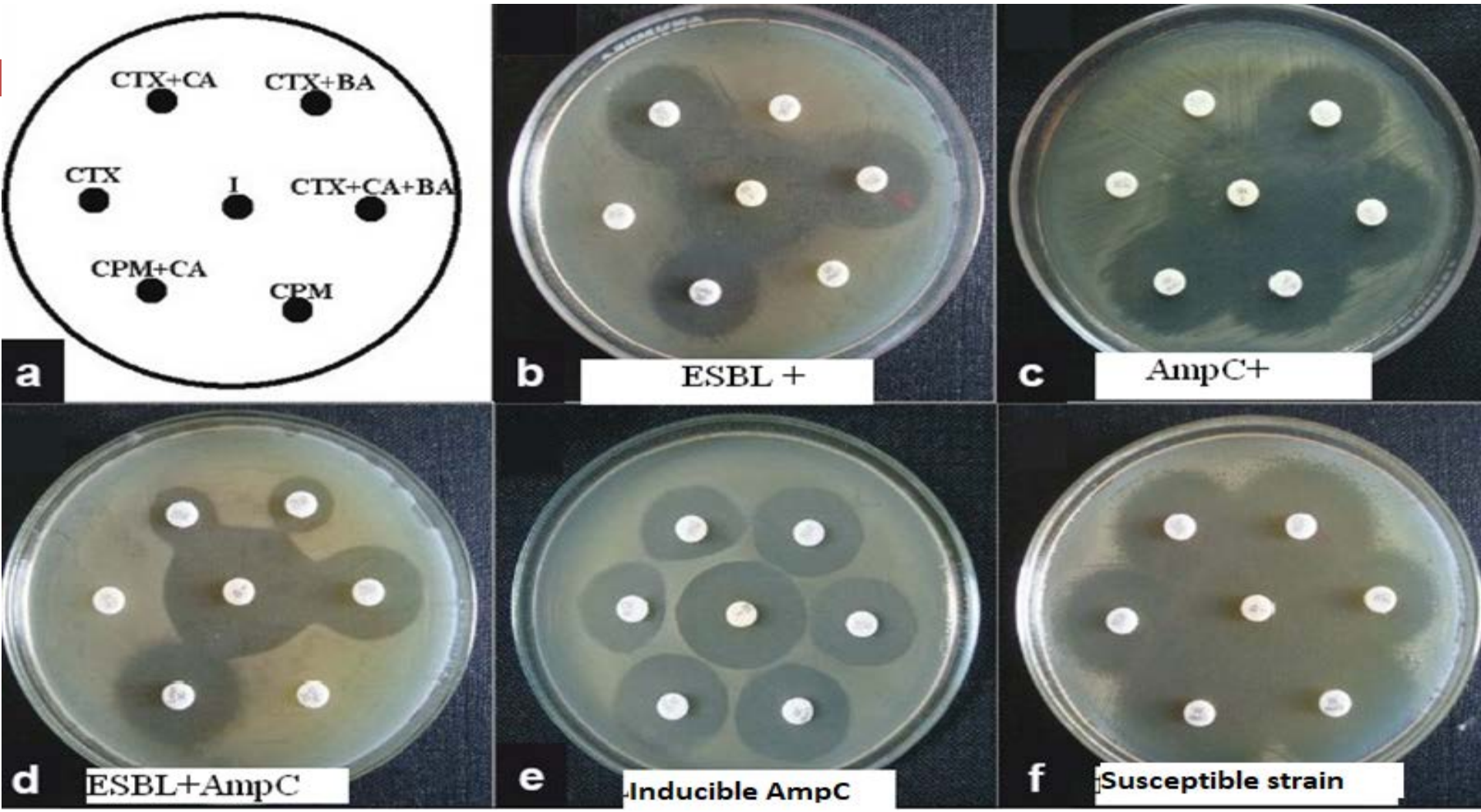
Test strain *E. cloacae* - AmpC

Indicator strain *E. coli* NCTC10418

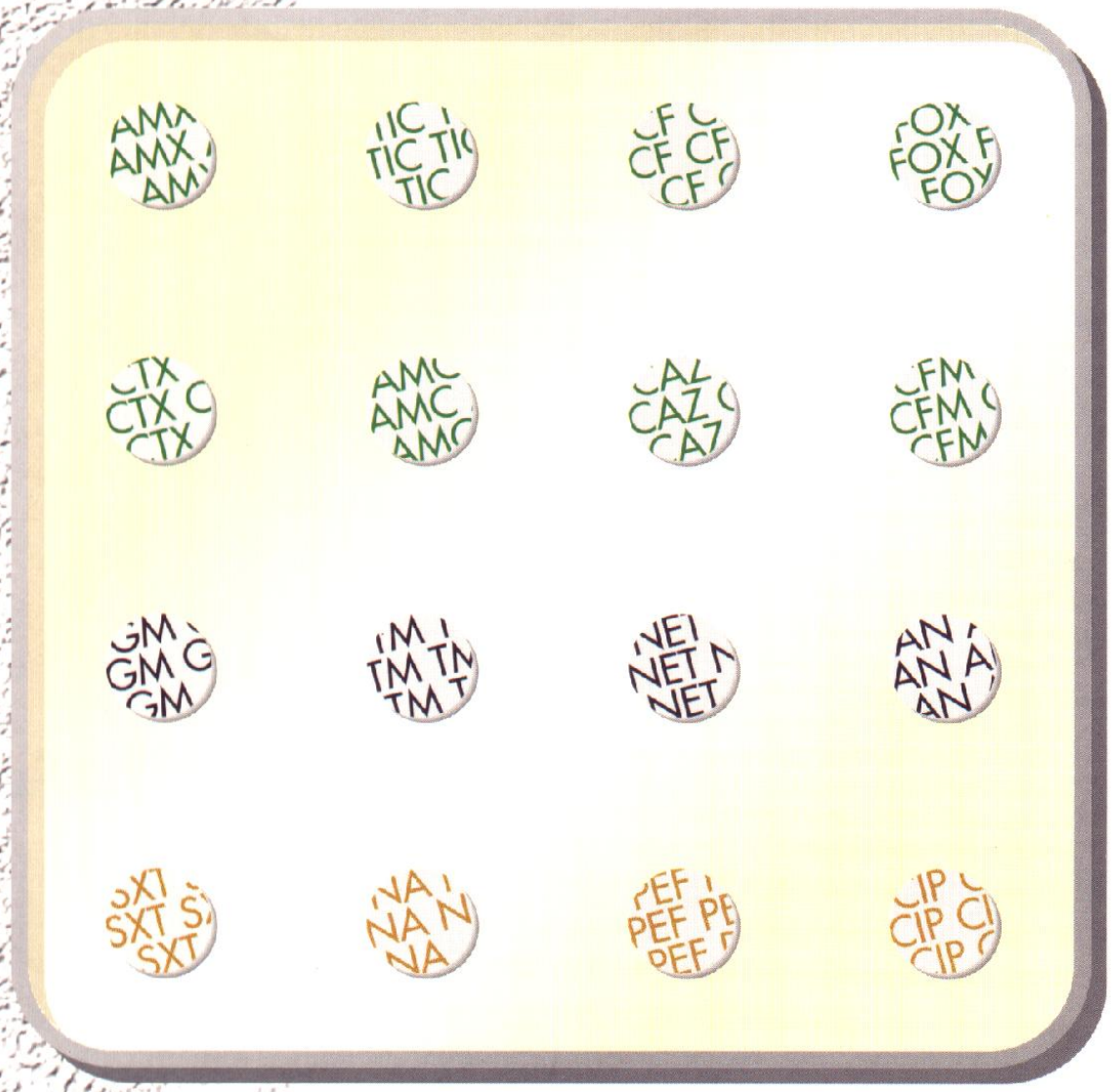
Disk Cefotaxim 30 μg

4. Commercial kits - Rosco, Mast, AB Biodisk –E-Test etc.

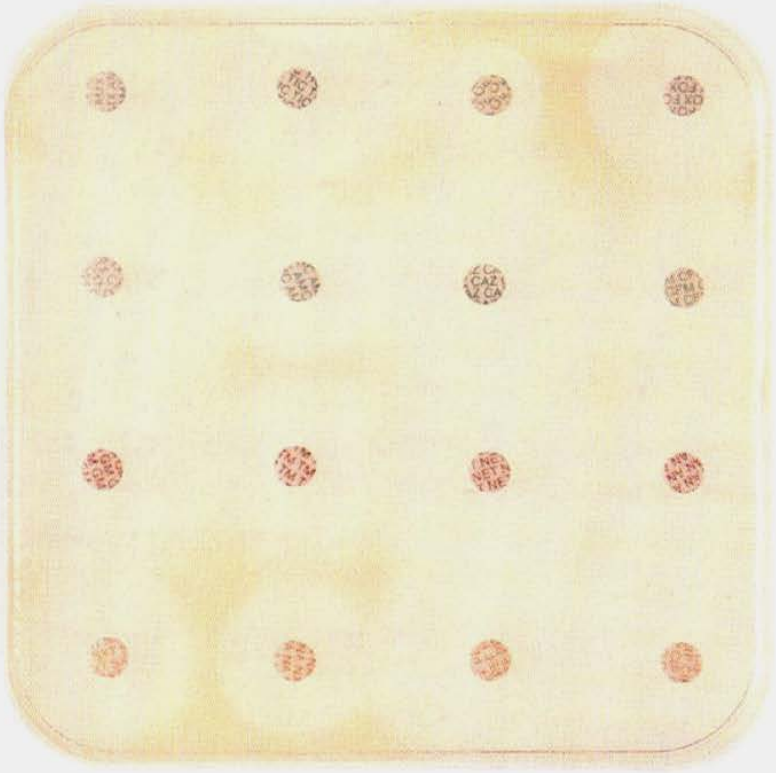
Differentiation of ESBL, AmpC, ESBL+AmpC phenotypes using specific inhibitors



I-imipenem;
CA/CLA- clavulanic acid;
BA – boronic acid;
CPM-cefpirome (4th gen cephalosporin);
CTX-cefotaxime (3rd gen cephalosporin).

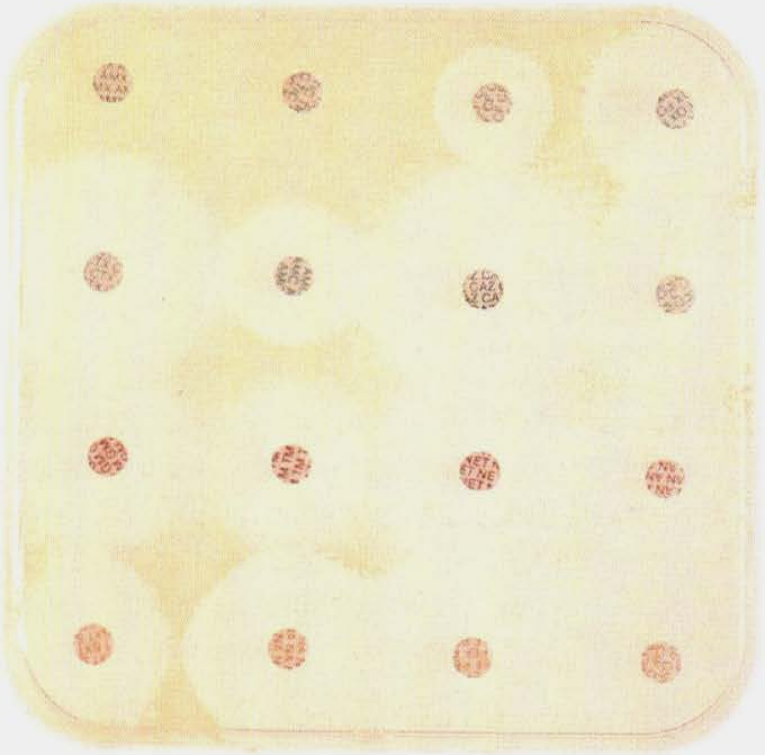


AMX	Amoxicillin	**GM**	Gentamicin
TIC	Ticarcillin	**TM**	Tobramycin
CF	Cephalotin	**NET**	Netilmicin
FOX	Cefoxitin	**AN**	Amikacin
CTX	Cefotaxime	**SXT**	Trimethoprim/ sulfamethoxazole
AMC	Amoxicillin/ clavulanic acid	**NA**	Nalidixic acid
CAZ	Ceftazidime	**PEF**	Pefloxacin
CFM	Cefixime	**CIP**	Ciprofloxacin



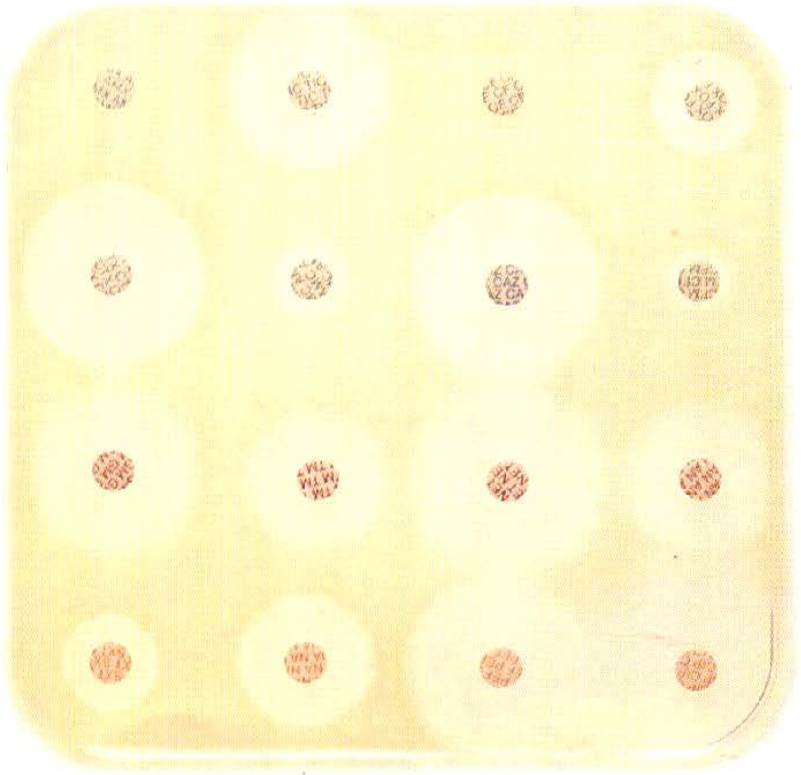
E. coli

Phenotype 1: this strain is **susceptible** to all the antimicrobials tested.



E. coli

Phenotype 2: this **penicillinase**-producing strain is resistant to amoxicillin and ticarcillin.



E. coli

Phenotype 3: this **cephalosporinase**-producing strain is resistant to amoxicillin, cephalotin, and amoxicillin/clavulanic acid.

The strain is susceptible to the other antimicrobials tested.

Rembrandt van Rijn (1606–1669), *The Night Watch* (1642). Oil on canvas, 149 in x 171 in /379.5 cm × 453.5 cm. Digital image courtesy of Rijksmuseum, Amsterdam, the Netherlands.

- ***Rembrandt Rondul de noapte- 1642,*** **anul în care soția sa, Saskia a murit după o suferință îndelungată;**
- **Printre personajele principale, active este o fetiță- care seamană cu Saskia;**
- **Noțiunea de rond de noapte - presupune inclusiv agențiile de sănătate publică care trebuie să fie pregătite pentru o gamă largă de evenimente infecțioase;**

- **Un sistem eficient, modern, de monitorizare și prelucrare a informației este crucial;**
- **Alexander Langmuir, epidemiologul care a pus bazele CDC Atlanta nota în 1962: *Supravegherea bună nu asigură decizia corectă, dar măcar scade șansa unei decizii greșite*.**

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