
CAT

PART I:

EVALUATIE VAN SELECTIEVE ESBL SCREENING AGARS

PART II:

EVALUATIE VAN FENOTYPISCHE ESBL CONFIRMATIE METHODEN IN AMPC- INDUCEERBARE *ENTEROBACTERIACEAE*

Inhoud

Introductie

Part I

-  **Materialen & Methoden**

-  **Resultaten**

-  **Take home message**

Part II

-  **Materialen & Methoden**

-  **Resultaten**

-  **Take home message**



Introductie

Definitie

Extended-spectrum β -lactamase (ESBL)

Hydrolyseren/ veroorzaken resistentie tegen

Penicillines

1^e, 2^e & 3^e generatie cefalosporines

Monobactam (aztreonam)

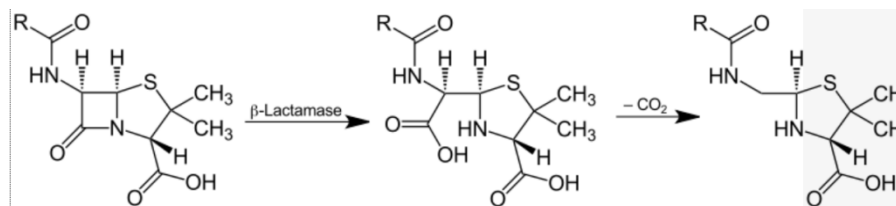
~~Cefamycines (cefoxitine, cefotetan)~~

~~Carbapenem~~

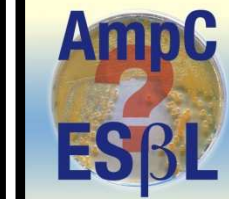
Vnl. plasmidair

Verskillende klassen

TEM, SHV, CTX-M, OXA, VEB, PER,...



Introductie

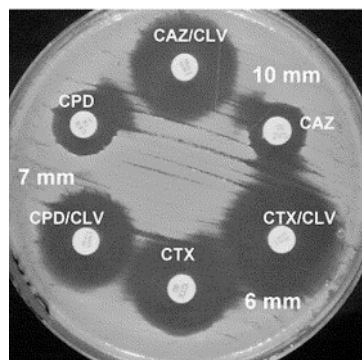


Definitie

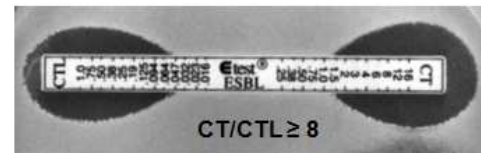
ESBL

Inhibitie door clavulaanzuur

Basis voor fenotypische confirmatiemethoden



CDDST*



Deformatie van cefalosporine inhibitie ellips

Etest® ESBL detection strips

* Combined double-disk synergy test

Introductie

ESBL

-  **Belang van snel opsporen in GNB***
-  ***Optimalisatie AB-therapie***
-  ***Tijdige introductie van maatregelen om verspreiding in ZKH te verhinderen***
-  ***Epidemiologische doeleinden***



* Gram-negatieve bacillen



Introductie

Definitie

AmpC β -lactamase

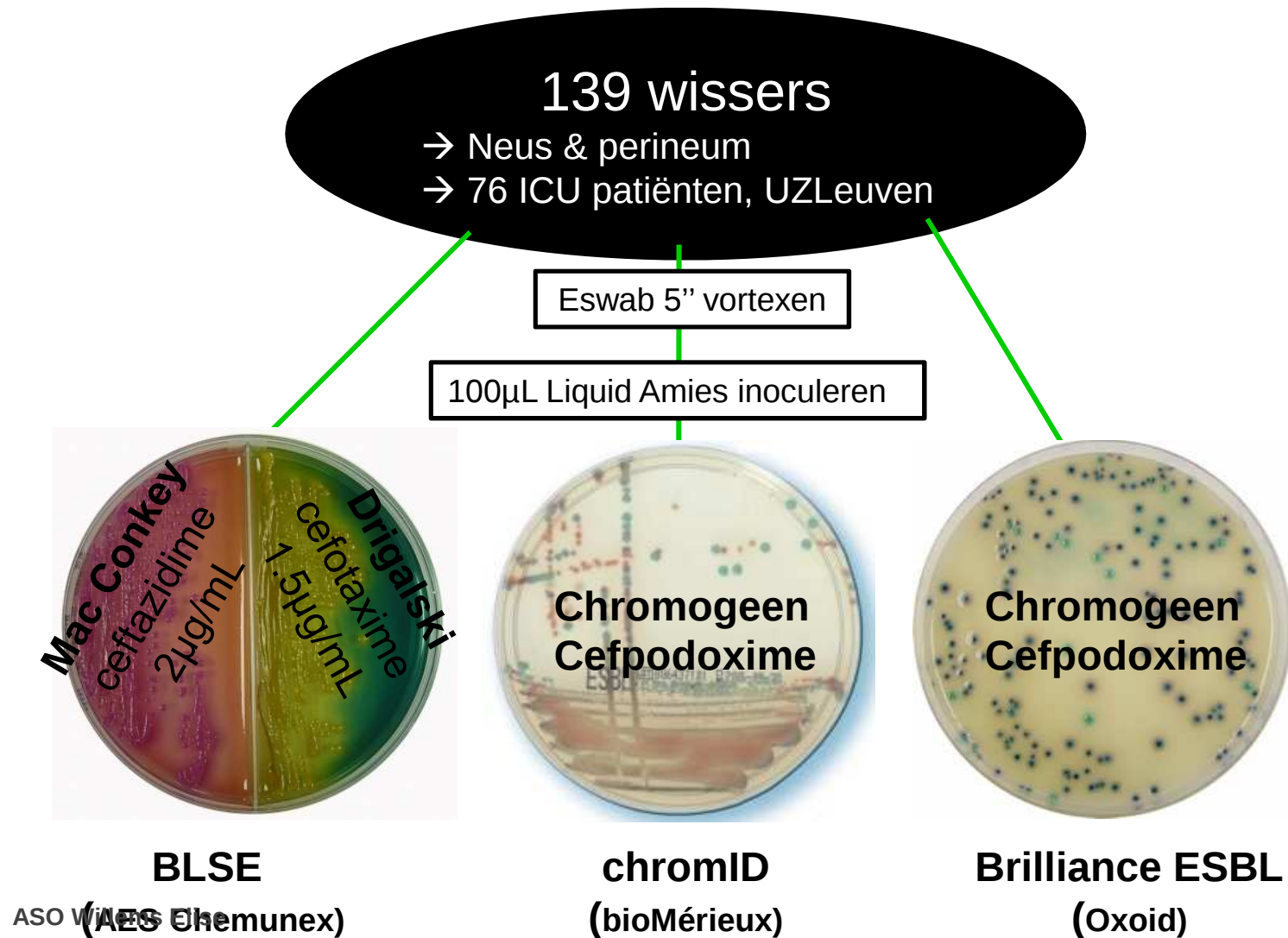
-  Hydrolyseren/ veroorzaken resistentie tegen
 -  Penicillines
 -  1^e, 2^e & 3^e generatie cefalosporines
 -  Monobactam (aztreonam)
 -  Cefamycines (cefoxitine, cefotetan)
 -  ~~Carbapenem~~
-  Chromosomaal of plasmidair
-  Geen inhibitie door clavulaanzuur
 -  Aanwezigheid van ESBL maskeren
-  Inhibitie door cloxacilline

* Combined double-disk synergy test

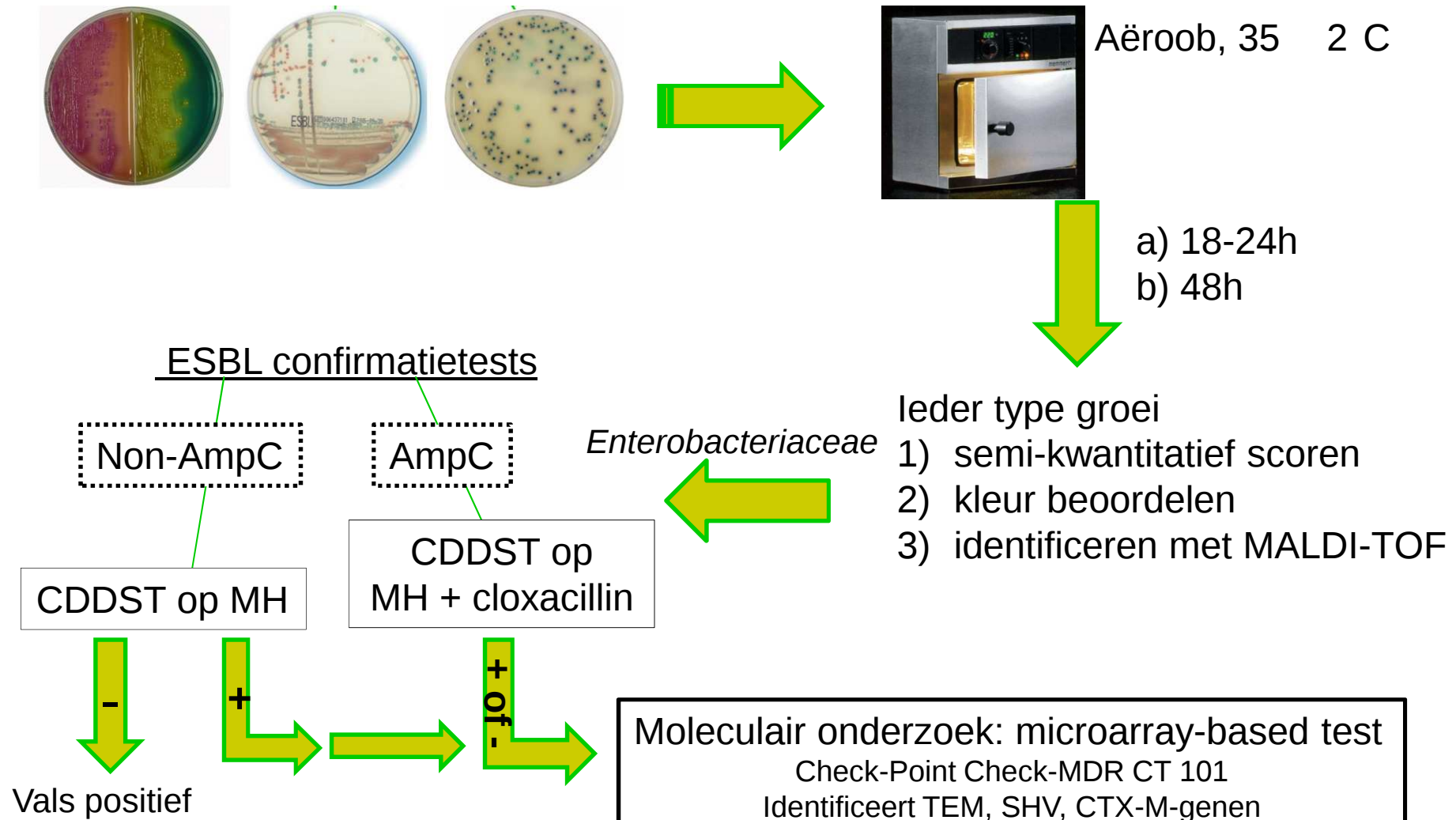
Part I

EVALUATION OF THREE DIFFERENT AGAR MEDIA FOR RAPID DETECTION OF ESBL- PRODUCING *ENTEROBACTERIACEAE* FROM CLINICAL SCREENING SAMPLES.

Part I: Materialen & methoden

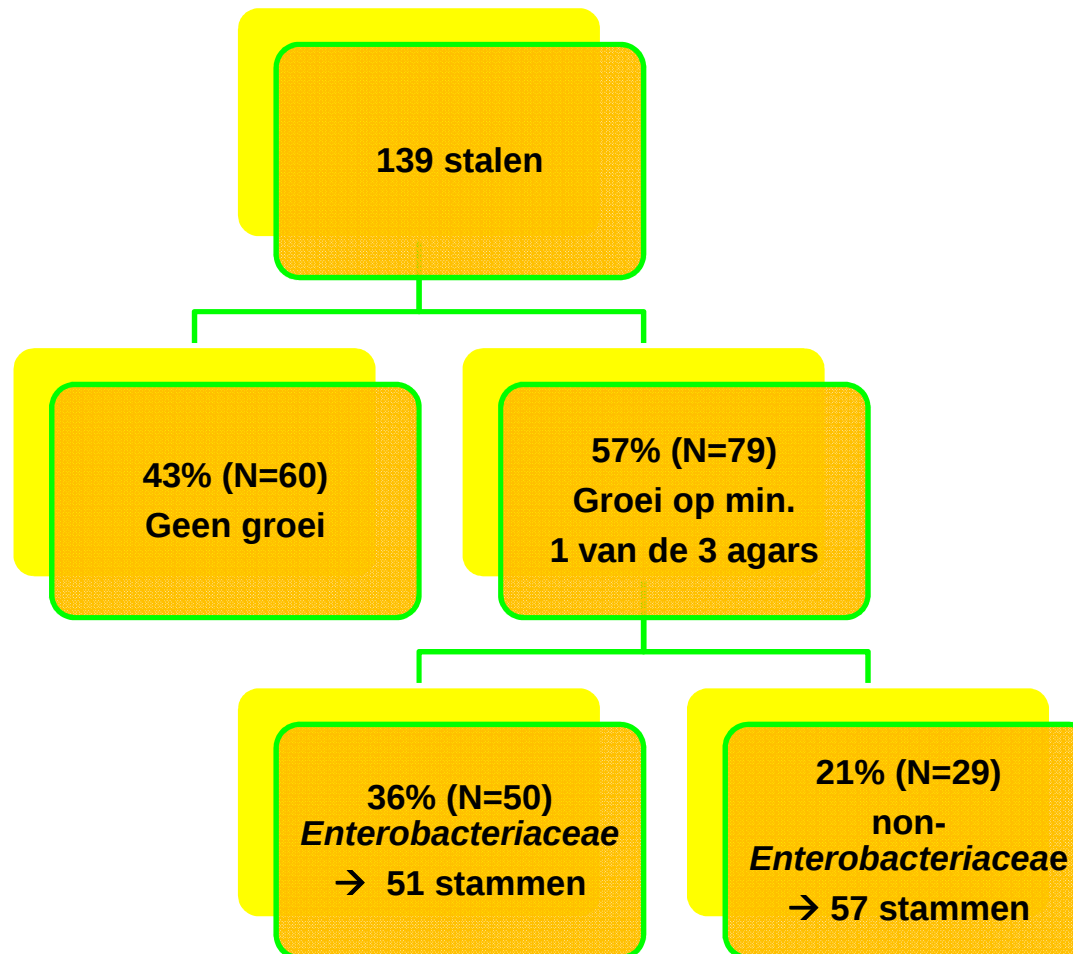


Part I: Materialen & methoden



Part I: Resultaten

 Na 18 – 24h incubatie



Part I: Resultaten

Group/species	No. of isolates recovered after 18 to 24h of incubation on:				
	BLSE	chromID	Brilliance ESBL	All three media	One of three media
<i>Escherichia coli</i>	4	2	3	3	4
KESC-group ^a	38	31	33	27	41
→ <i>Klebsiella pneumoniae</i>	8	9	9	8	10
<i>Klebsiella oxytoca</i>	1	0	1	0	1
→ <i>Enterobacter aerogenes</i>	5	5	4	4	5
<i>Enterobacter cloacae</i>	12	7	10	6	13
<i>Enterobacter ludwigii</i>	2	2	2	2	2
<i>Serratia marcescens</i>	5	3	2	2	5
<i>Citrobacter freundii</i>	5	5	5	5	5
PMP-group ^b	5	3	3	2	6
<i>Proteus mirabilis</i>	0	1	0	0	1
<i>Morganella morganii</i>	5	2	3	2	5
Total no. of Enterobacteriaceae	47	36	39	32	51
Others					
<i>Acinetobacter</i> spp.	2	2	0	0	2
<i>Burkholderia cepacia</i>	0	2	2	0	2
→ <i>Enterococcus</i> spp.	0	2	→ 8	0	9
<i>Pseudomonas aeruginosa</i>	12	8	10	7	14
→ <i>Staphylococcus</i> spp.	0	→ 10	1	0	10
→ <i>Stenotrophomonas maltophilia</i>	→ 6	→ 7	3	3	7
<i>Candida</i> spp.	0	1	12	0	13

^aKESC, *Klebsiella* -*Enterobacter* -*Serratia* -*Citrobacter* group

^bPMP, *Proteus* -*Morganella* -*Providencia* group

Part I: Resultaten



16/51 ESBL + *Enterobacteriaceae* stammen

Species (no. of isolates)	Classes of ESBL enzymes (no. of isolates)		
	TEM	SHV	CTX-M
<i>E. coli</i> (4)	TEM-29 (1)	SHV-5 (1) ^a	CTX-M-1 (3) ^a
<i>K. pneumoniae</i> (10)			CTX-M-1 (10)
<i>C. freundii</i> (2)		SHV-5 (2)	

^a Two ESBLs (CTX-M-1 and SHV-5) were present in one *E. coli* isolate.



Performantie

	BLSE _{18-24h}	BLSE _{48h}		ChromID _{18-24h}	ChromID _{48h}		Brilliance ESBL _{18-24h}	Brilliance ESBL _{48h}	
SN (%)	87,50	87,50	≈	81,25	87,50	≈	87,50	87,50	
SP (%)	60,80	56,00	<	80,65	77,42	≈	82,11	77,42	
PPV (%)	22,22	20,29		35,14	33,33		38,89	33,33	Lage PPV
NPV (%)	97,44	97,22		97,09	97,96		98,06	97,96	Hoge NPV

- Specificiteit: BLSE << chromID & Brilliance ESBL
- Verlengde incubatie (24h → 48h)
 - enkel SN chromID stijgt ($p > 0,05$)
 - SP van de 3 agars daalt ($p > 0,05$ voor BLSE & Brilliance ESBL)

Part I: Resultaten

Specificiteit: BLSE << chromID & Brilliance ESBL?

 **Chromogene media = selectiever voor ESBL-producerende *Enterobacteriaceae***

 Minder VP resultaten t.g.v. non-ESBL producing *Enterobacteriaceae*

 Minder onnodige ESBL confirmatietesten

 Chrom ID: 45% minder t.o.v. BLSE

 Brilliance ESBL: 39% minder t.o.v. BLSE

Chromogeen karakter van chrom ID & Brilliance ESBL

 Minder VP resultaten t.g.v. non-*Enterobacteriaceae*

 Minder onnodige identificaties

 ChromID: 58% minder t.o.v. BLSE

 Brilliance ESBL: 89% minder t.o.v. BLSE

Group/species	No. of isolates recovered after 18 to 24h of incubation on:					
	BLSE		chromID		Brilliance ESBL	
	ESBL	Other	ESBL	Other	ESBL	Other
<i>Escherichia coli</i>	0	0	2	0	3	0
<i>Klebsiella pneumoniae</i>	8	0	9	0	9	0
<i>Moraxella oxyphila</i>	0	1	0	0	0	1
<i>Enterobacter aerogenes</i>	0	5	0	5	0	4

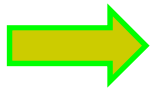
Group/species	No. of isolates recovered after 18 to 24h of incubation on:					
	BLSE		chromID		Brilliance ESBL	
	TN	FP	TN	FP	TN	FP
<i>Acinetobacter</i> spp.	0	2	2	0	0	0
<i>Burkholderia cepacia</i>	0	0	2	0	2	0
<i>Candida</i> spp.	0	0	1	0	12	0
<i>Enterococcus</i> spp.	0	0	2	0	8	0
<i>Pseudomonas aeruginosa</i>	1	11	3	5	8	2
<i>Staphylococcus</i> spp.	0	0	8	2	1	0
<i>Stenotrophomonas maltophilia</i>	0	6	6	1	3	0
Total no.	1	19	24	8	34	2

Part I: Resultaten



Groeidensiteit van 16 ESBL + *Enterobacteriaceae*

-  12 groei op iedere ESBL screen-agar
 -  Vergelijkbare groeidensiteit
-  1/16 groei op BLSE & Brilliance ESBL
 -  BLSE (++++>) > Brilliance (+)
-  3/16 groei op slechts 1 agar (zeldzaam)



Groeidensiteit van ESBL-producerende *Enterobacteriaceae* is vergelijkbaar tussen BLSE, Brilliance ESBL & chrom ID

Part I: Resultaten

 # & types gedetecteerde ESBL enzymes = beperkt in deze studie!

 Performantie data vgl. met voorafgaande studies*

 SN & SP chromogene agars lager in deze studie

 SN_{BLSE} vergelijkbaar

 SP_{BLSE} lager

 Geen significante verschillen in performantie tss. chromogene media

 SP_{chromID} >> SP_{BLSE}

References	BLSE		chromID		Brilliance ESBL	
	SN (%)	SP (%)	SN (%)	SP (%)	SN (%)	SP (%)
Glupczynski et al. [6]			97,7	89,0		
Huang et al. [7]			86,4	95,5	94,9	95,7
Reglier-Poupet et al. [9]	85,0	82,0	88,0	94,4		
Saito et al. [10]			88,2	92,9	100,0	93,3
Willems E. et al.	87,5%	60,8%	81,3%	80,7%	87,5%	82,1%

Glupczynski Y et al., *J Clin Microbiol.* 2007.

Huang TD et al., *J Clin Microbiol.* 2010.

Reglier-Poupet H et al., *J Med Microbiol.* 2008.

Saito R et al., *Lett Appl Microbiol.* 2010.

Part I: Take home message

 **BLSE, Brilliance ESBL & chromID = even betrouwbaar om te screenen naar ESBL- producerende *Enterobacteriaceae* direct vanuit patiëntenstalen.**

-  NPV= 97- 98% (ICU, UZLeuven)
→ *snelle exclusie van patiënten die geen drager zijn van ESBL- producerende Enterobacteriaceae*
-  Specificiteit = laag
→ *nood aan bijkomende ESBL-confirmatietesten*

 **Specificiteit van Brilliance ESBL & chromID >> BLSE**

-  **Minder vals positieve resultaten na 24h**
-  Minder onnodige identificaties ($\pm 70\%$) & ESBL confirmatietesten ($\pm 40\%$)
→ **minder werk met chromogene agars**

Part II

COMPARISON OF DIFFERENT PHENOTYPIC ASSAYS FOR DETECTION OF ESBL PRODUCTION BY INDUCIBLE AMPC-PRODUCING *ENTEROBACTERIACEAE*.

Part II: Materialen & methoden

Bacteriële stammen



73 non-duplicated



AmpC induceerbare *Enterobacteriaceae*....

-  47 *Enterobacter* spp.
-  17 *Morganella morganii*
-  7 *Providencia* spp.
-  8 *Citrobacter freundii*
-  4 *Serratia* spp.



...met vermoeden van ESBL productie...

-  Volgens CLSI criteria (MIC CTX, CTR of CAZ ≥ 1 $\mu\text{g/mL}$, Vitek2) EN/OF
-  Volgens Vitek2's advanced expert system (AES)



...uit klinische patiëntenstalen van UZLeuven (08/2011 - 01/2012)

-  31 urinestalen, 17 respiratoire stalen, 11 wondvochten, 14 andere
-  Vnl. van gehospitaliseerde patiënten (79%)



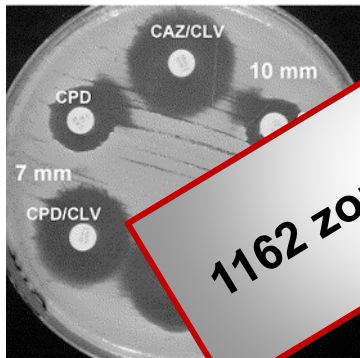
10 extra moleculair getypeerde ESBL-producerende AmpC induceerbare *Enterobacteriaceae* isolaten



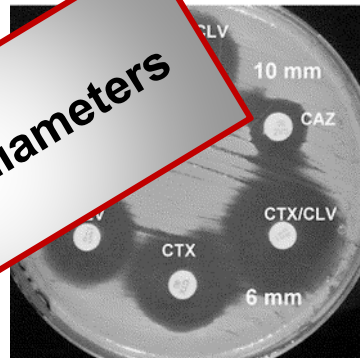
National surveillance laboratorium voor MDR GNB, UCL de Mont-Godinne

Part II: Materialen & methoden

83 AmpC induceerbare
Enterobacteriaceae



1162 zonediameters

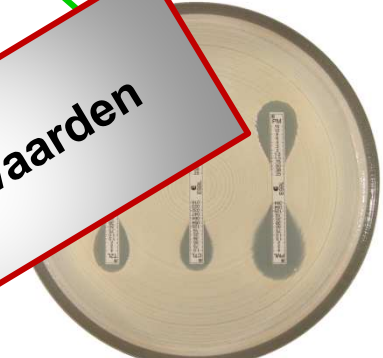


CDDST op MH
CTX, CAZ, FEP
& CTX met cloxacillin

CDDST op MH met
cloxaciline (250 µg/mL)
CTX, CAZ & FEP



ESBL Etest® strips
op MH
CT/CTL, TZ/TZL & PM/PML



ESBL Etest® strips op MH
met cloxaciline (250 µg/mL)
CT/CTL, TZ/TZL & PM/PML

996 MIC-waarden

onderzoek op aanwezigheid van frequent voorkomende
ESBL coderende genen (TEM, SHV, CTX-M, ...)
→ Microarray-based test (Check-Point Check-MDR CT 101)

Gouden standaard

Part II: Resultaten

<i>Enterobacteriaceae</i> isolates	No. of isolates (% of total)	No. of ESBL producers (% of total)	Classes of ESBL enzymes (no. of isolates)		
			TEM	SHV	CTX-M
<i>Enterobacter aerogenes</i>	22 (27)	10 (12)	TEM-15 (1), TEM-24 (1), TEM-26 (6)	SHV-4,-5,-12 (2) ^a	CTX-M 9 (1) ^a
<i>Enterobacter cloacae</i>	25 (30)	8 (10)		SHV-5 (3) ^b , SHV-12 (1) ^b	CTX-M-1 (3), CTX-M 9 (3) ^b
<i>Citrobacter freundii</i>	8 (10)	2 (2)	TEM-24 (1)	SHV-5 (1)	
<i>Morganella morganii</i>	17 (20)	1 (1)			CTX-M-1 (1)
<i>Serratia marcescens</i>	4 (5)	1 (1)		SHV-4,-5,-12 (1)	
<i>Providencia</i> spp.	7 (8)	3 (4)	TEM-17 (1), TEM-26 (2)		
Total	83 (100)	25 (30)	43% of ESBL-encoding genes (12)	28,5% of ESBL-encoding genes (8)	28,5% of ESBL-encoding genes (8)

^a Four ESBL-encoding genes were present in one *E. aerogenes* isolate (CTX-M-9 combined with SHV-4,-5,-12)

^b Two ESBL-encoding genes were present in two *E. cloacae* isolates (CTX-M-9 ones combined with SHV-5 and ones with SHV-12)

1^{ste} studie met verschillende types van ESBL enzymen
in verschillende species van AmpC induceerbare *Enterobacteriaceae*

Part II: Resultaten

$SN_{FEP} > SN_{CAZ}$

↔ Voorgaande studies

↔ FEP stabiel tegen AmpC hydrolase

CAZ + FEP &/of CTX → Geen SN toename

Method	SN (%)	SP (%)	IR (%)
CDDST on MH agar			
CTX	52	91	0
CAZ	92	76	0
FEP	76	93	0
CTX + CAZ	92	76	0
CTX + FEP	76	90	0
CAZ + FEP	92	76	0
CTX + CAZ + FEP	92	76	0
CTX + cloxacillin	↑ 64	100	0
CDDST on MH agar supplemented with cloxacillin			
CTX	↑ 68	98	8
CAZ	92	93	8
FEP	↑ 92	98	8
CTX + CAZ	92	91	8
CTX + FEP	92	97	8
CAZ + FEP	92	93	8
CTX + CAZ + FEP	92	93	8
ESBL Etest on MH agar			
CT/CTL	16	100	61
TZ/TZL	32	98	53
PM/PML	80	100	28
CT/CTL + TZ/TZL	36	98	60
CT/CTL + PM/PML	80	100	60
TZ/TZL + PM/PML	88	98	59
CT/CTL + TZ/TZL + PM/PML	88	98	63
ESBL Etest on MH agar supplemented with cloxacillin			
CT/CTL	68	100	37
TZ/TZL	80	100	34
PM/PML	84	95	52
CT/CTL + TZ/TZL	80	100	39
CT/CTL + PM/PML	88	95	53
TZ/TZL + PM/PML	96	95	52
CT/CTL + TZ/TZL + PM/PML	96	95	52

Contra aanbevelingen fabrikant

Part II: Resultaten



Statistische vergelijking van methoden met 3 hoogste SN%



McNemar



Enkel $SP_{CDDST \text{ op MH, CAZ}}$ is significant lager



Rekening houdend met kostprijs

→ CDDST op MH_{cloxacilline} met CAZ/FEP = meest kostenefficiënt

Method	SN (%)	SP (%)
CDDST on MH agar		
CAZ	92	76
CDDST on MH agar supplemented with cloxacillin		
CAZ	92	93
FEP	92	98
ESBL Etest on MH agar		
TZ/TZL + PM/PML	88	98
ESBL Etest on MH agar supplemented with cloxacillin		
CT/CTL + PM/PML	88	95
TZ/TZL + PM/PML	96	95

Part II: Resultaten



Vitek2 Advanced Expert System (versie 5.03)

-  Resistentiemechanisme voorspellen o.b.v. de MIC-waarde van elk AB
-  Geschikt voor ESBL-screening in AmpC-induceerbare *Enterobacteriaceae* ?
 -  SN= 56%
 -  SP= 50%



Part II: Resultaten



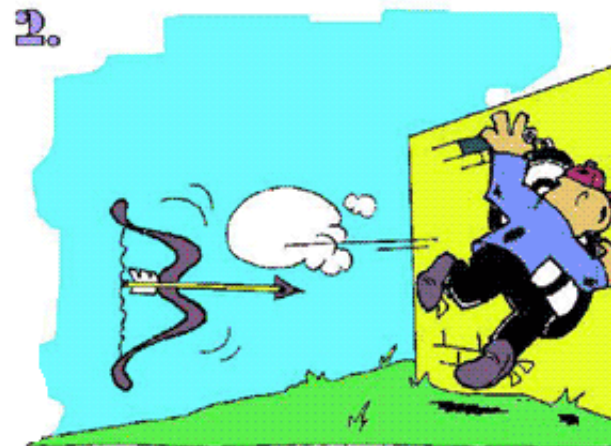
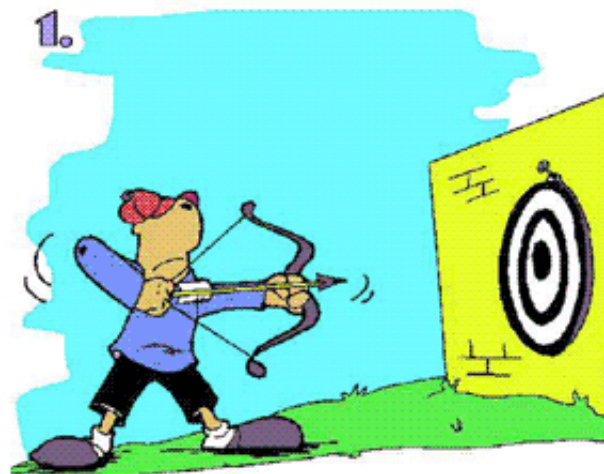
Part II: Take home message

 **Vitek2 AES niet geschikt** voor ESBL-screening in AmpC-induceerbare *Enterobacteriaceae*

 **CDDST op MH_{cloxacilline} met CAZ/FEP = meest kosten-efficiënte** fenotypische ESBL confirmatiemethode in AmpC-induceerbare *Enterobacteriaceae*

VRAGEN?

 Schiet maar af....



Part I: Resultaten



Chromogeen karakter



Non-*Enterobacteriaceae* met kleur van *Enterobacteriaceae* → VP

ChromID (N=8/32, 25%)

Brilliance ESBL (N=2/36, 6%)



Enterobacteriaceae met kleur ≠ instructies van fabrikant

ChromID (N=9/36, 25%)	Brilliance ESBL (N=7/39, 18%)
4 <i>Citrobacter freundii</i>	2 <i>Citrobacter freundii</i>
2 <i>Serratia marcescens</i>	2 <i>Serratia marcescens</i>
2 <i>Enterobacter cloacae</i>	2 <i>Morganella morganii</i>
1 <i>Proteus mirabilis</i>	1 <i>Enterobacter cloacae</i>

2 “verkeerd gekleurde” *C. freundii* stammen op beide media ESBL positief
MAAR kleur van *E. coli* → **geen VN**



1. ID vermoedelijke *Enterobacteriaceae* op chromogene media ≠ betrouwbaar
→ Nood aan confirmatie van identificatie
2. Geen nood tot identificatie van isolaten met non-*Enterobacteriaceae* kleur
→ Geen daling in SN