



XIV Congresso Brasileiro de
Controle de Infecção e
Epidemiologia Hospitalar
19 A 22 DE NOVEMBRO DE 2014 | EXPO UNIVED CURTIBA | CURTIBA | PR



DISSEMINAÇÃO DE ENTEROBACTÉRIAS RESISTENTES A CARBAPENÊMICOS E O IMPACTO NAS IRAS: "EXISTE ALGUMA LUZ NO FIM DO TÚNEL?"

Impacto no Paraná

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Conflitos de Interesse

- Coordenador da Microbiologia
DB Medicina Diagnóstica
- Bacteriologista
LACEN/PR
- Professor de Microbiologia
PUCPR



ANTIBIOTIC RESISTANCE THREATS **in the United States, 2013**



**U.S. Department of
Health and Human Services**
Centers for Disease
Control and Prevention

NATIONAL SUMMARY DATA



Estimated minimum number of illnesses and deaths caused by antibiotic resistance*:

At least  **2,049,442** illnesses,
 **23,000** deaths

**bacteria and fungus included in this report*



U.S. Department of
Health and Human Services
Centers for Disease
Control and Prevention

Nível de Ameaça – URGENTE

- Bactérias que são ameaças imediatas à Saúde Pública
- Requerem ação agressiva e urgente



- *Clostridium difficile*
- **Enterobactérias Resistentes a Carbapenêmicos (CRE)**
- *Neisseria gonorrhoeae*

THREAT LEVEL
URGENT



U.S. Department of
Health and Human Services
Centers for Disease
Control and Prevention

Quem são as MDR mais preocupantes atualmente?


- Enterobactérias : KPC e NDM
- *Pseudomonas* MBL (SPM)
- *Acinetobacter* Oxa-23 e NDM-1
- *Klebsiella* R à tigeciclina
- *Klebsiella*, *Acinetobacter* e *Pseudomonas*
 - Resistentes à Polimixina/Colistina



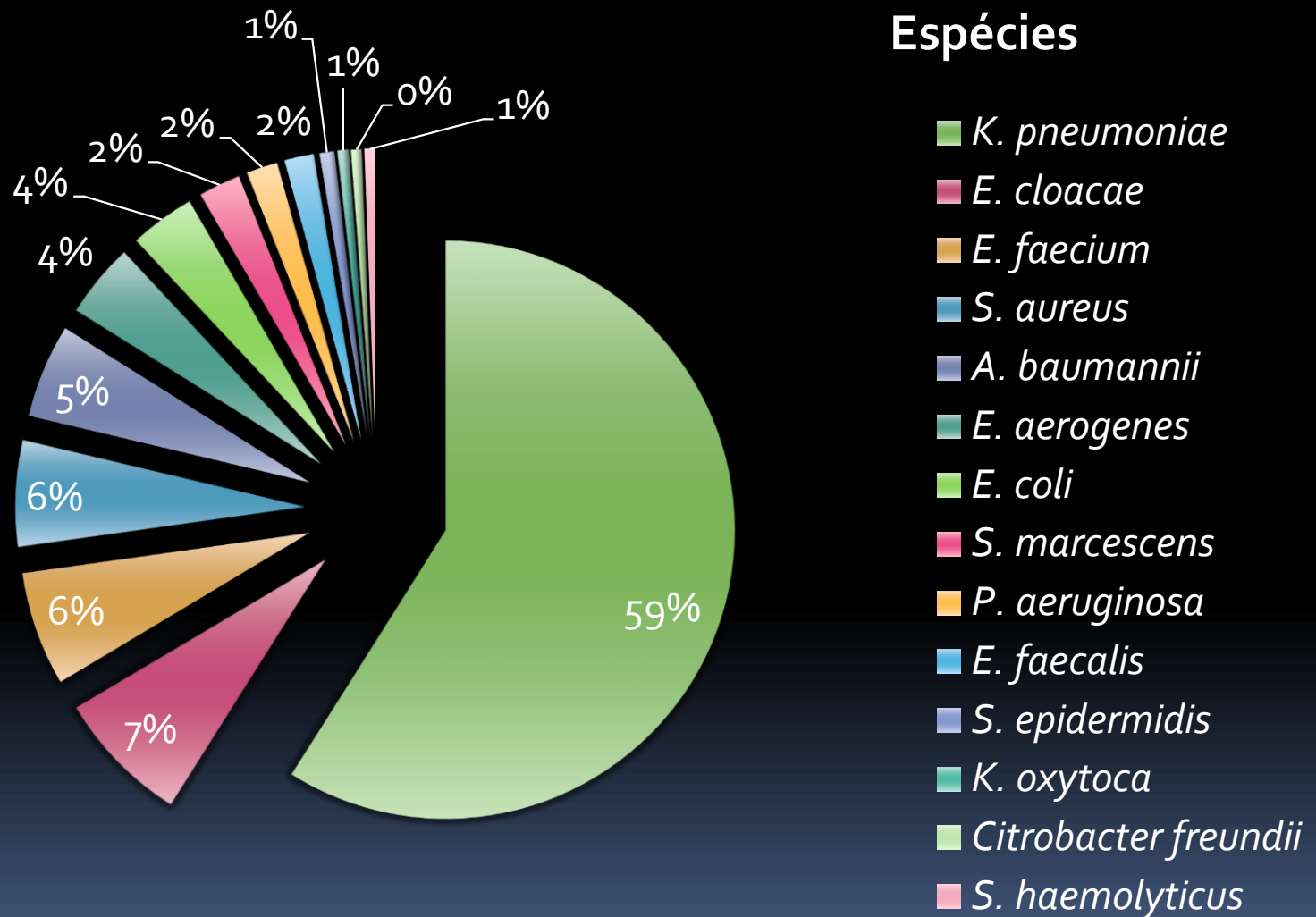
IMPACTO NO PARANÁ



Dados do LACEN-PR

- Período
 - 2012 a 2014
 - Amostragem
 - 3.942 isolados enviados de 52 hospitais – PR, SC E RS
 - Viés
 - cepas resistentes –confirmação fenótipo ou genótipo
- 

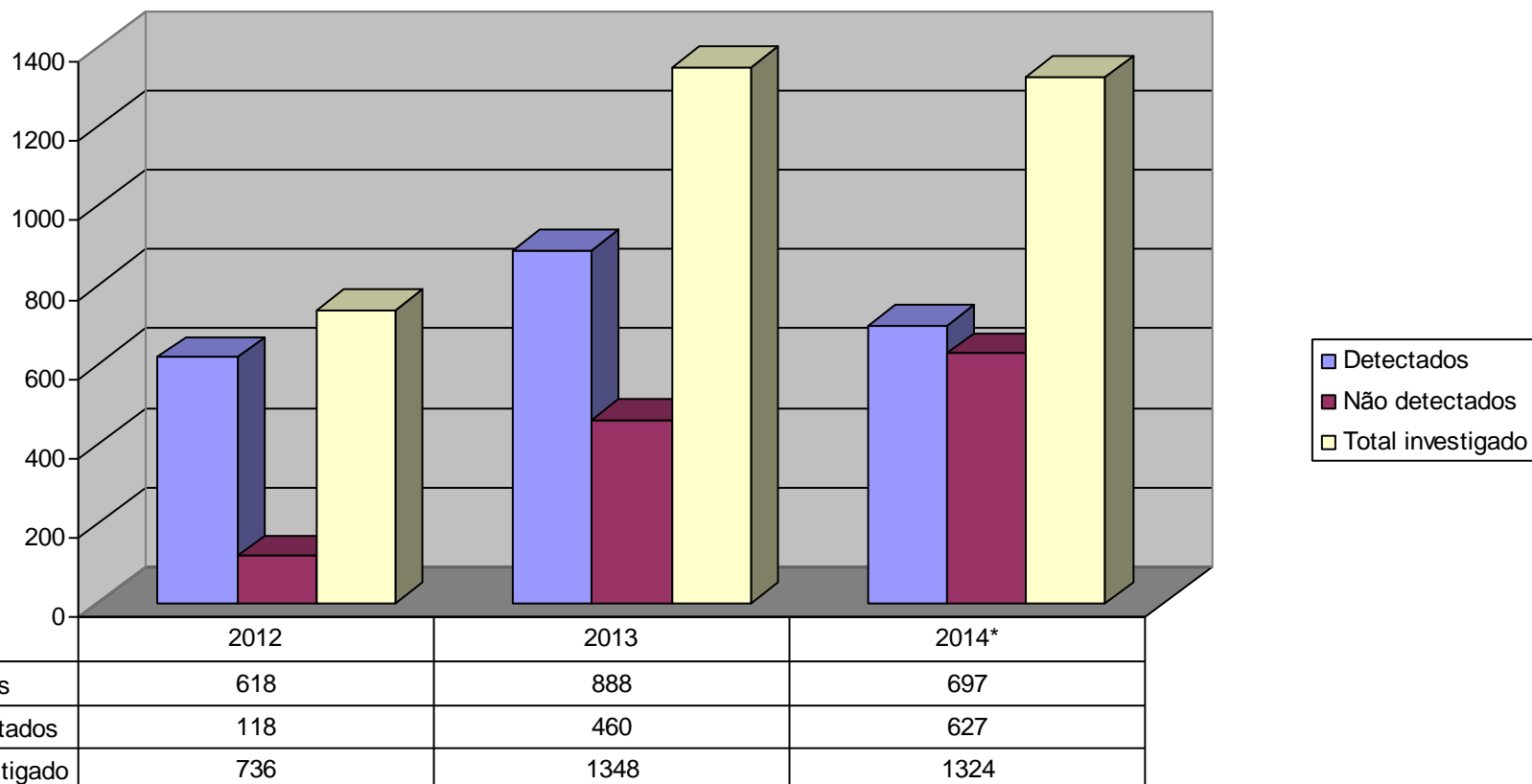
MDR – LACEN/PR – 2012-2014



Enterobacteriaceae: 72%; 61% são CRE

PESQUISA DO GENE *blaKPC* EM ENTEROBACTÉRIAS

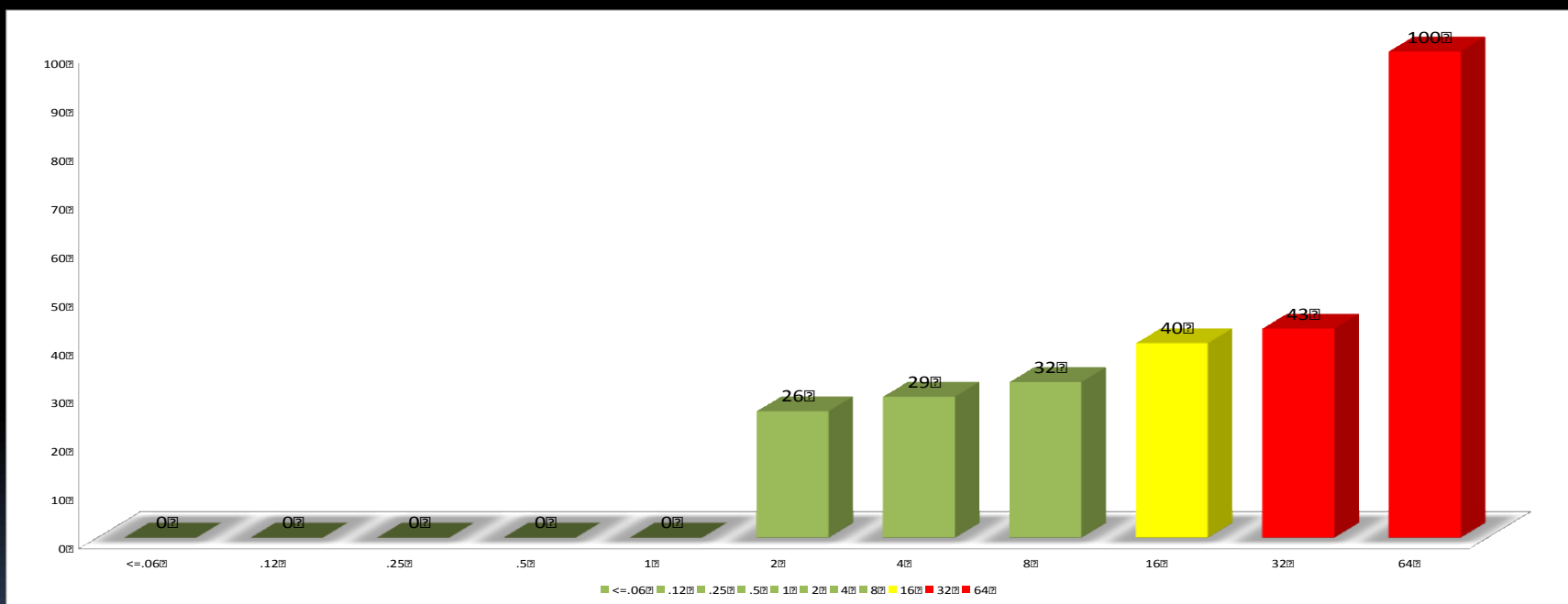
CEPAS INVESTIGADAS NO LACEN/PR 2012-2014



* Dados preliminares (jan-jun/2014)

MIC 90 - *K.pneumoniae* x Amicacina

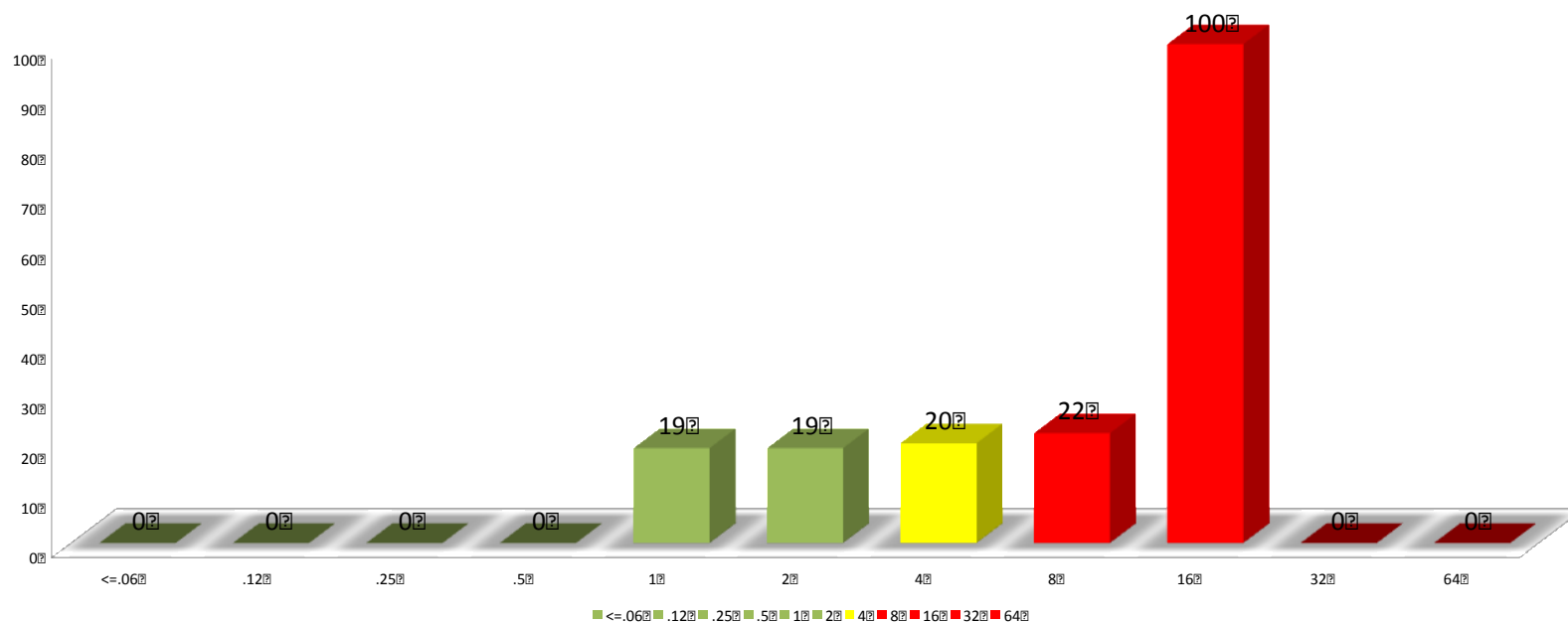
S I R



n=1127; 2012 a 2014;

MIC 90 - *K.pneumoniae* x Gentamicina

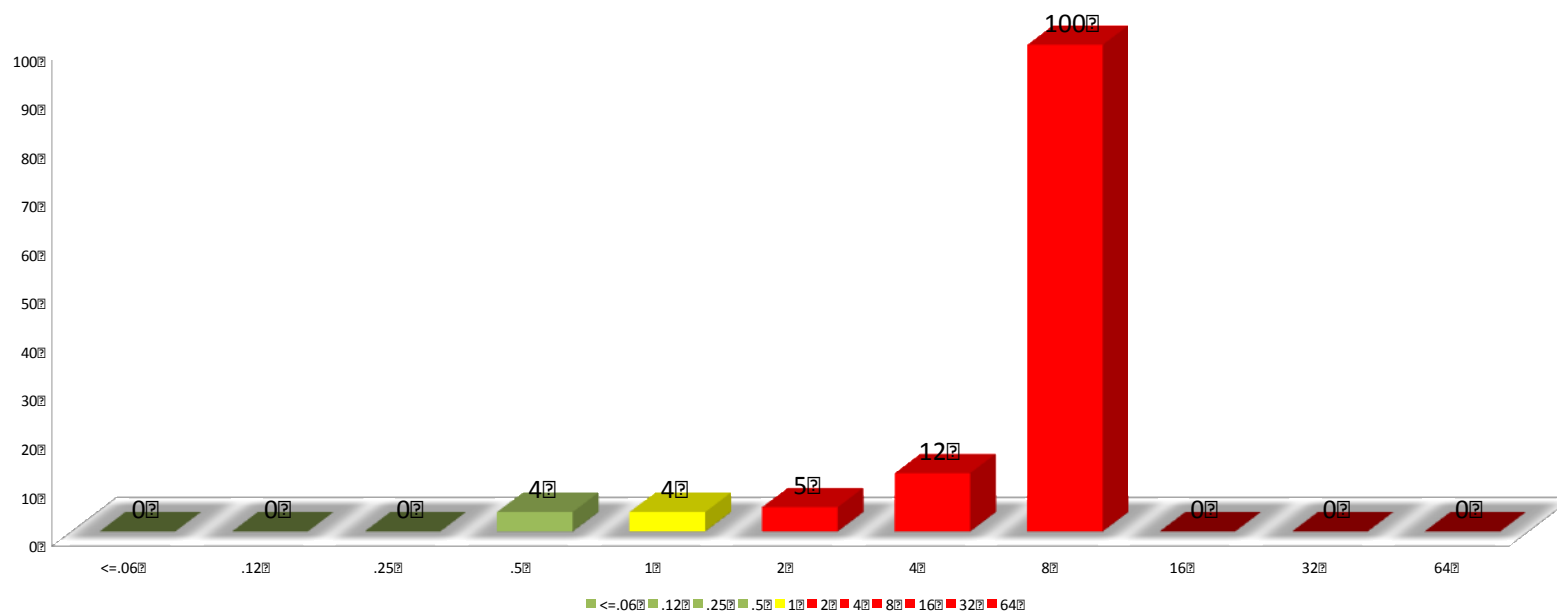
S I R



n=1127; 2012 a 2014

MIC 90 - *K.pneumoniae* x Ertapenem

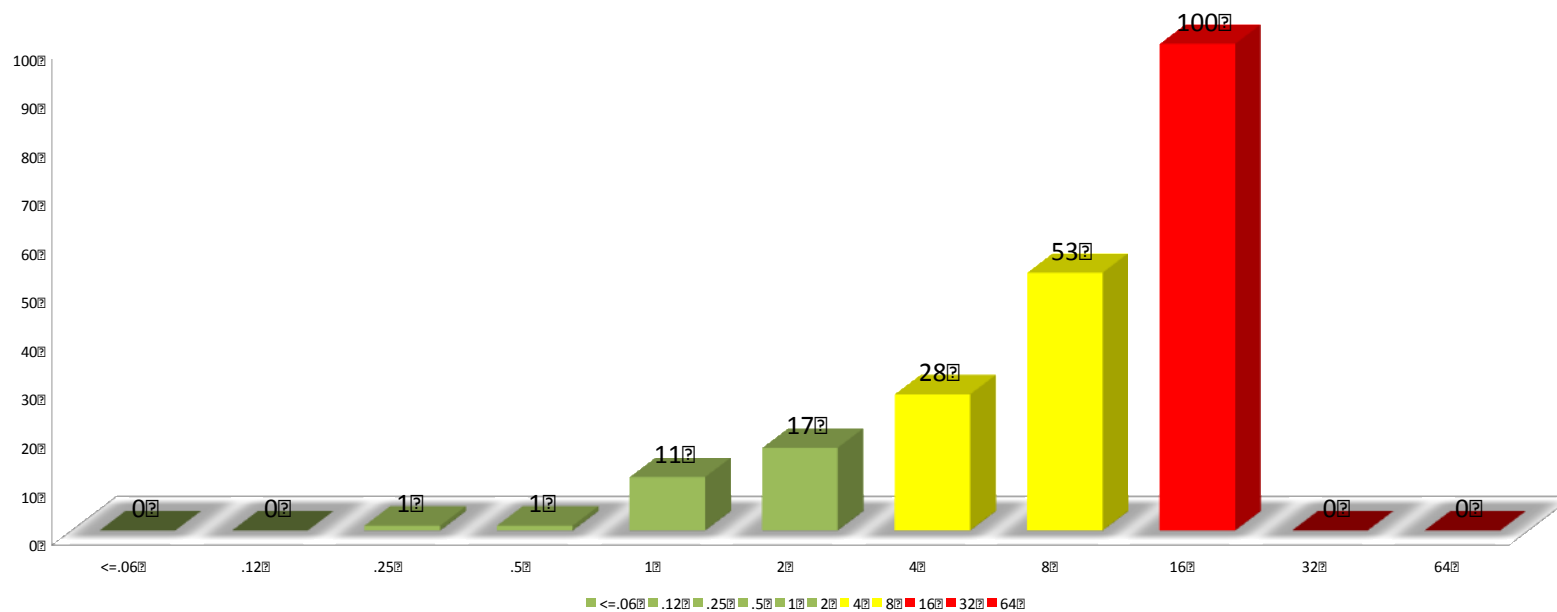
S I R



n=1127; 2012 a 2014

MIC 90 - *K.pneumoniae* x Imipenem

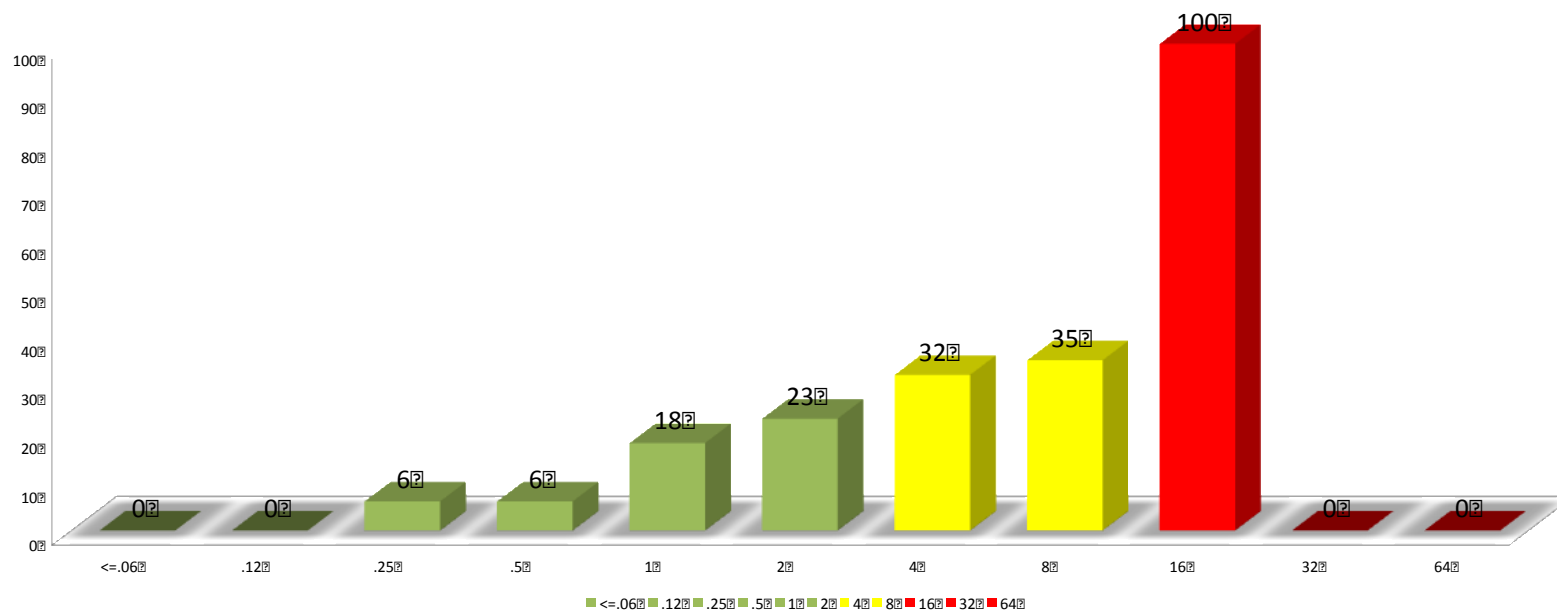
S I R



n=1127; 2012 a 2014

MIC 90 - *K.pneumoniae* x Meropenem

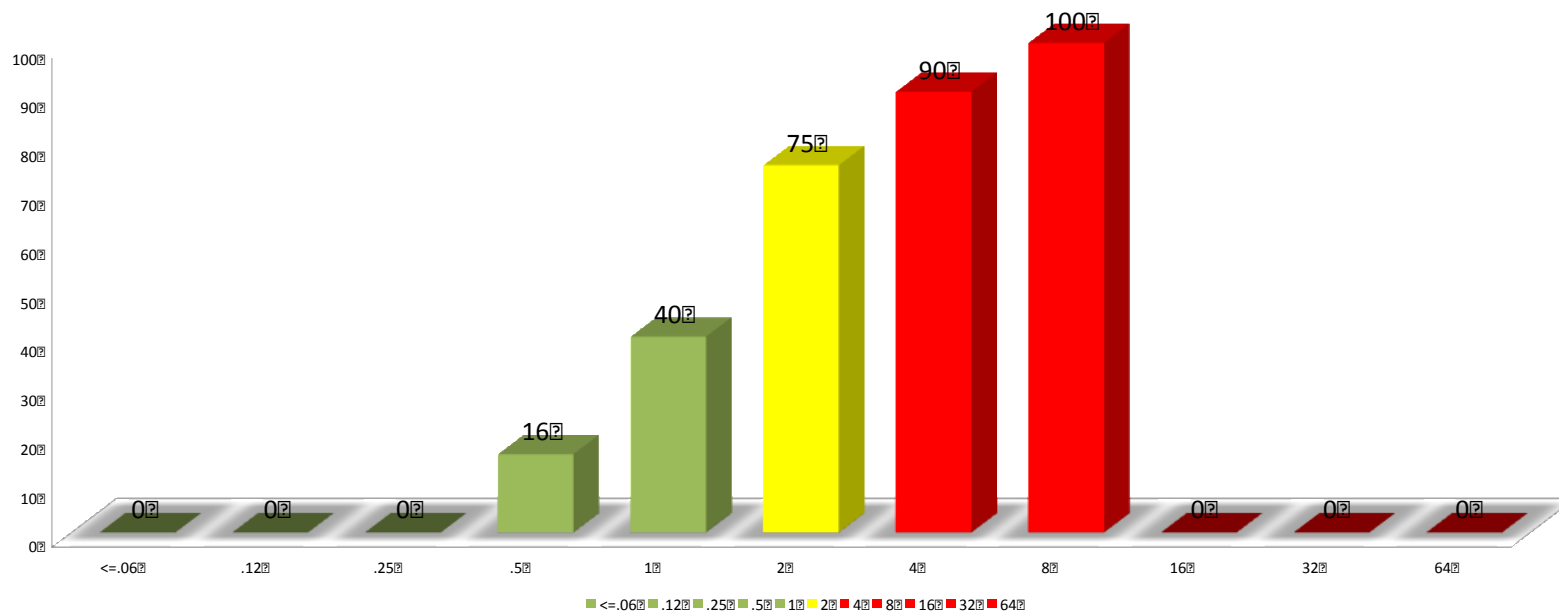
S I R



n=1127; 2012 a 2014

MIC 90 - *K.pneumoniae* x Tigeciclina

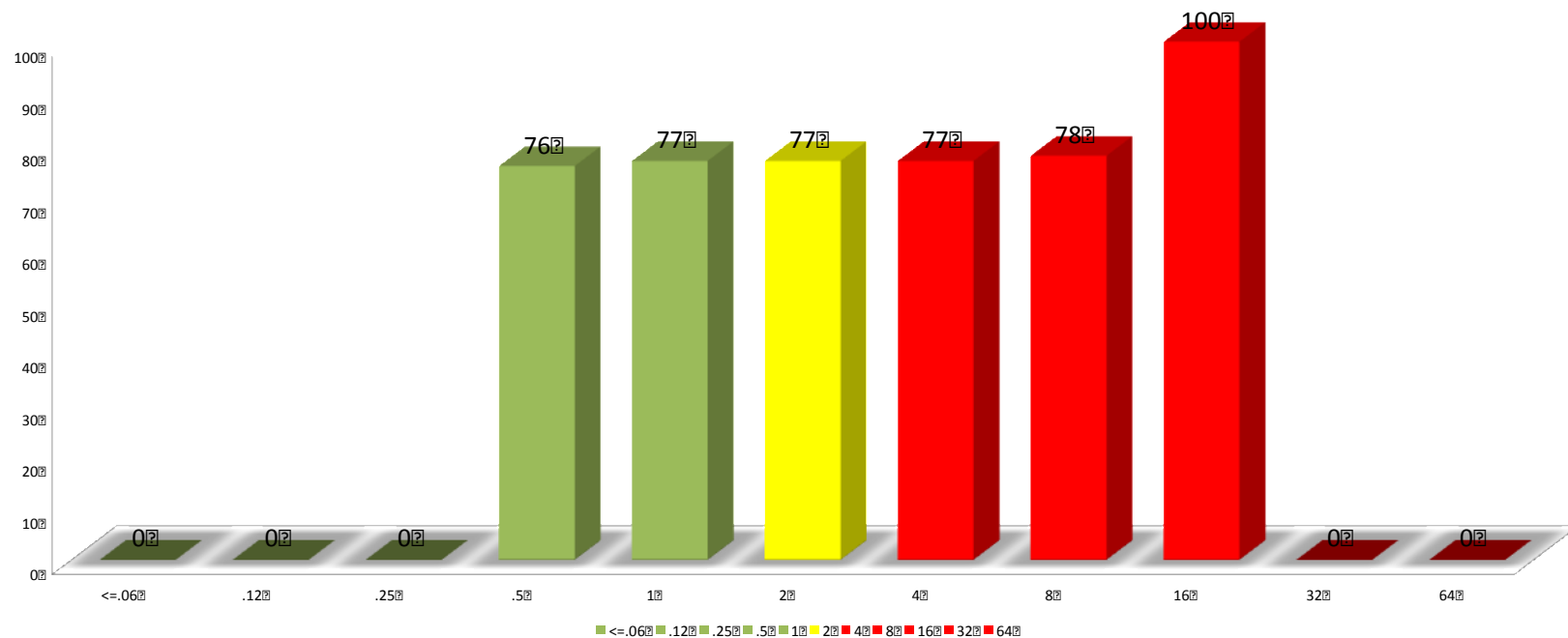
S I R



n=1127; 2012 a 2014

MIC 90 - *K.pneumoniae* x Polimixina

S I R




n=1127; 2012 a 2014



Qual a conduta?

Mitigar o problema

- Prevenção: detecção rápida + controle
 - Usar tecnologia a nosso favor
 - Terapias multi-drogas – Parâmetros (?)
 - Novas drogas (?)
- 



DETECÇÃO RÁPIDA DE GENES DE RESISTÊNCIA

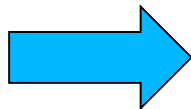


PCR *end-point*

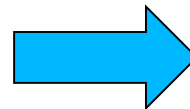
Procedimentos



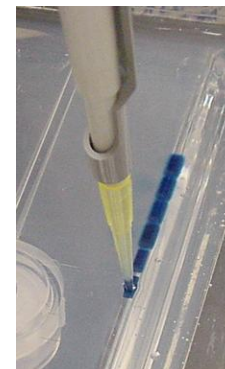
1- Extração



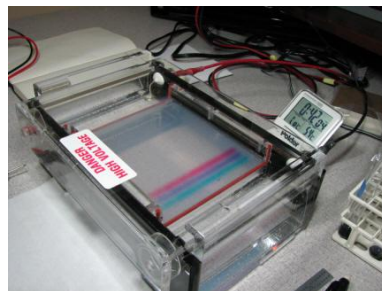
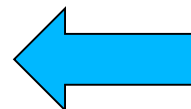
2- Preparo da Mix



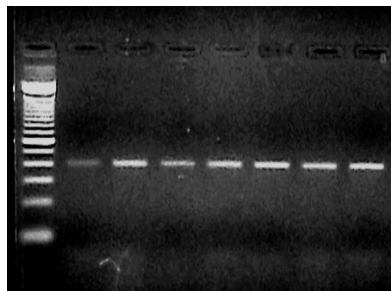
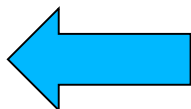
3- Amplificação



4- Aplicação



5- Eletroforese



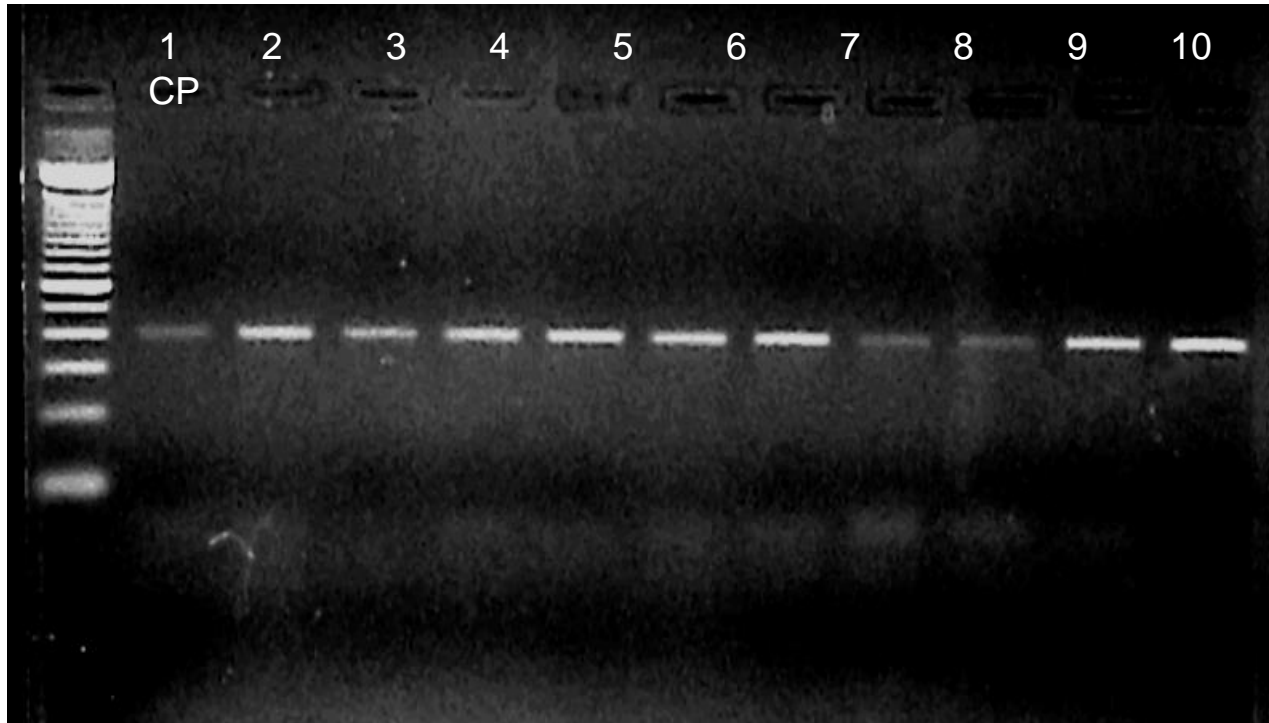
6- Análise



PCR *end point*

Interpretação

Detecção de genes de resistência



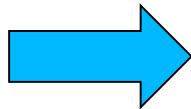


PCR *Real Time SYBR Green*

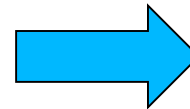
Procedimentos



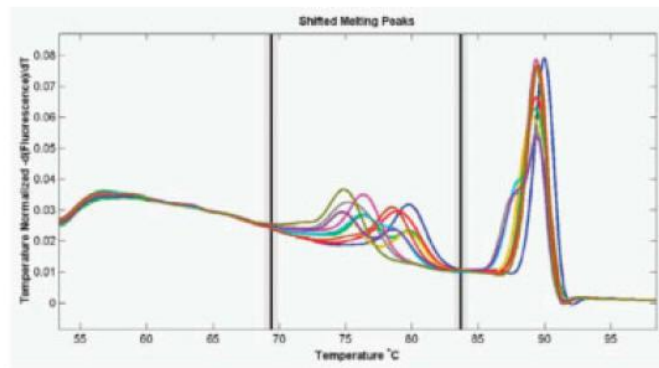
1- Extração



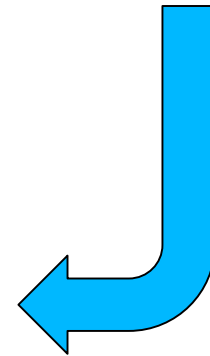
2- Preparo da Mix



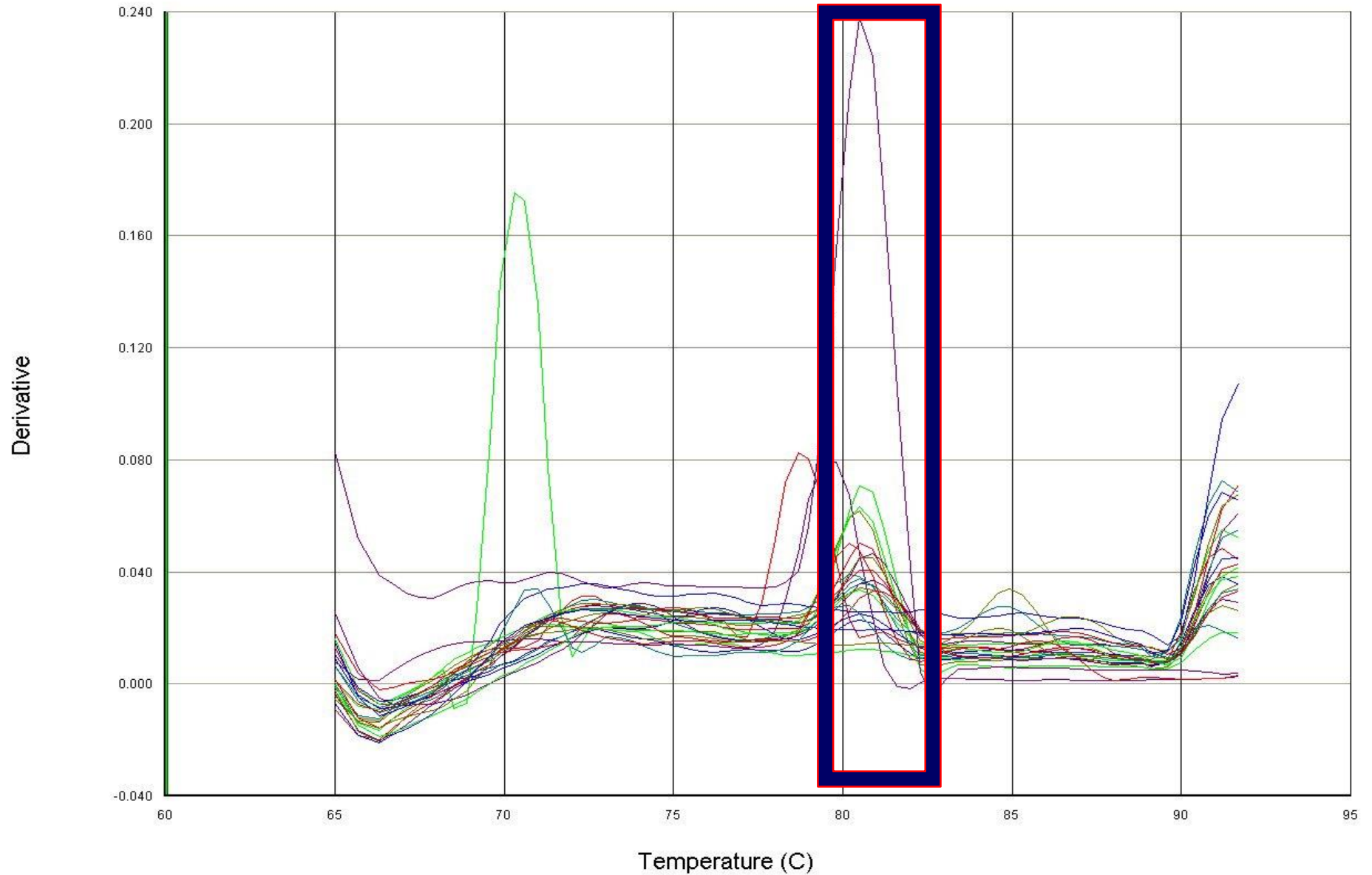
3- Amplificação



4- Leitura e Interpretação



Dissociation Curve



Detector = SYBR SPM, T_m = 60.0 °C
Well(s): A1-H12
Document: SPM- IMP -VIM 16052013 (Standard Curve)

T_m: 80,1 ± 0,2

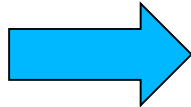


PCR *Real Time* TaqMan

Procedimentos



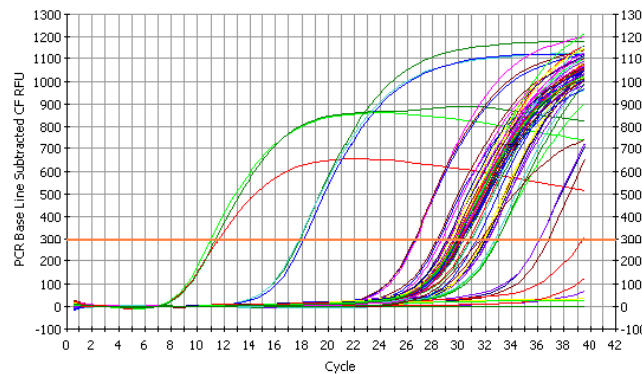
1- Extração



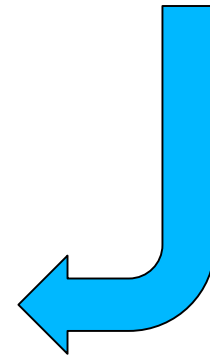
2- Preparo da Mix

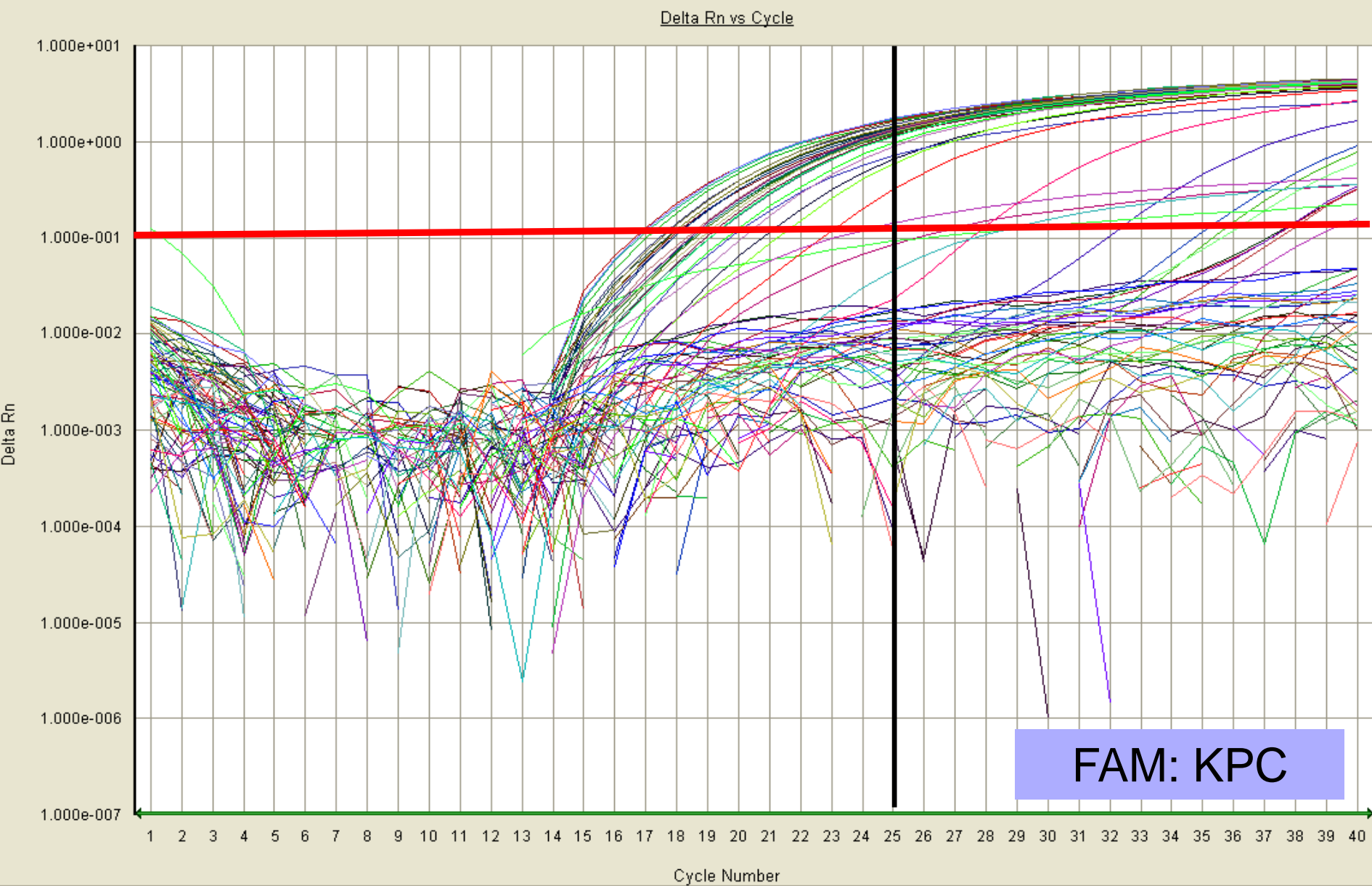


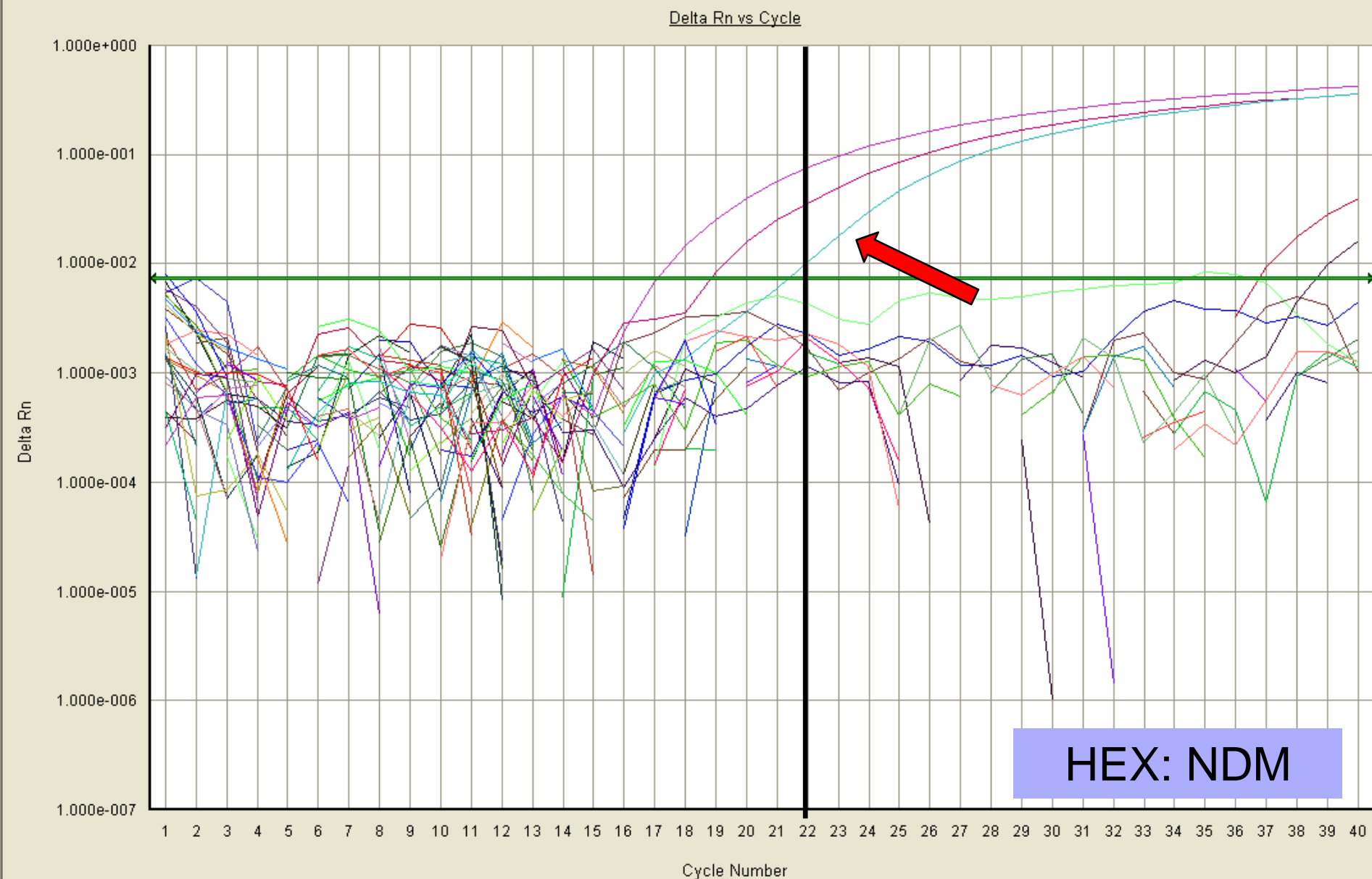
3- Amplificação



4- Leitura e Interpretação









High Resolution Melting

HRM + Automação



Light Cycler 480 - Roche

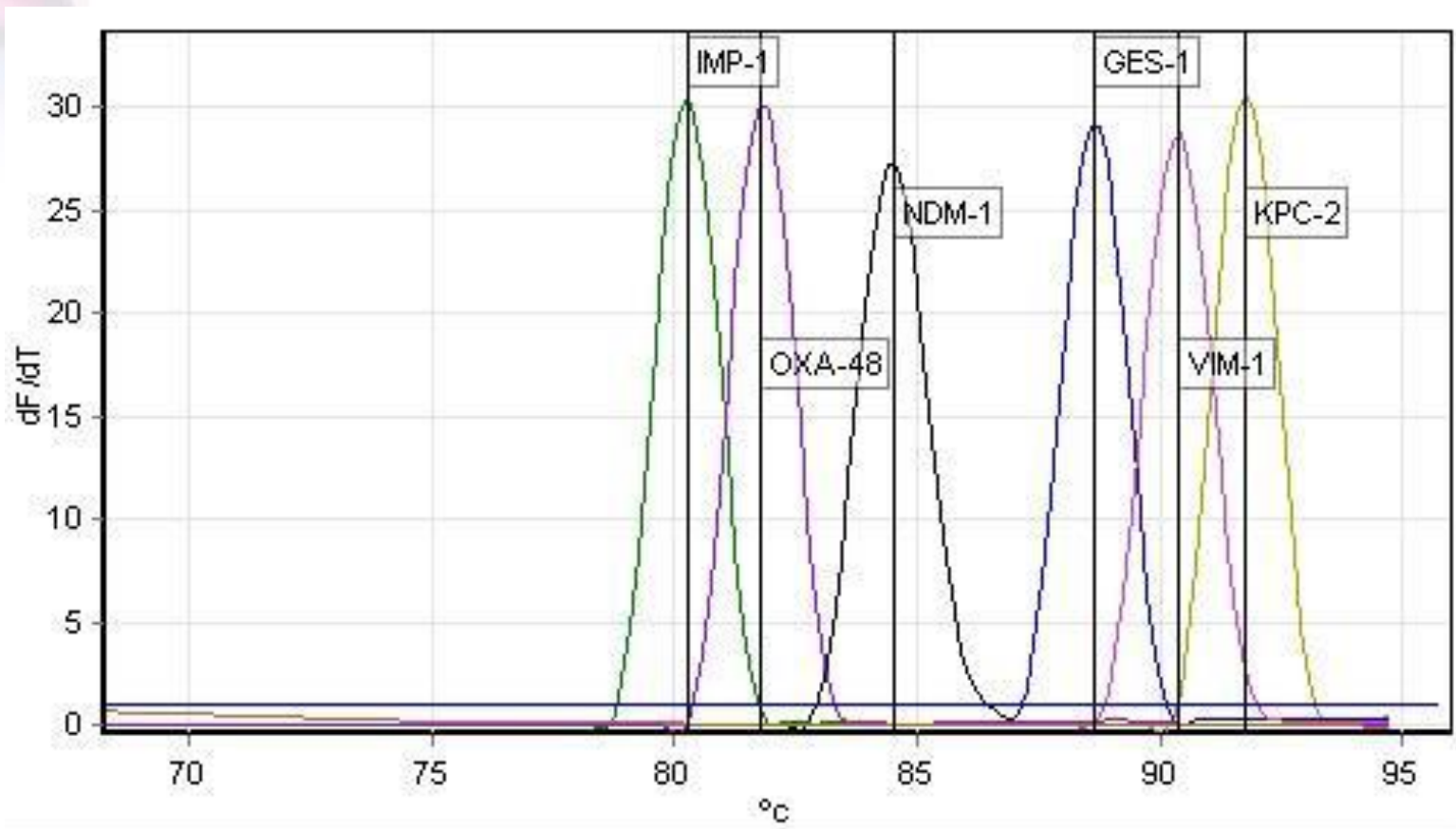


BD- MAX – Becton e Dickinson



High Resolution Melting

HRM + Automação



qPCR Multiplex* automatizado



* KPC + NDM + OXA-48

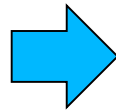


PCR *Real Time* Automatizado

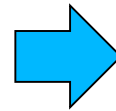
Procedimentos



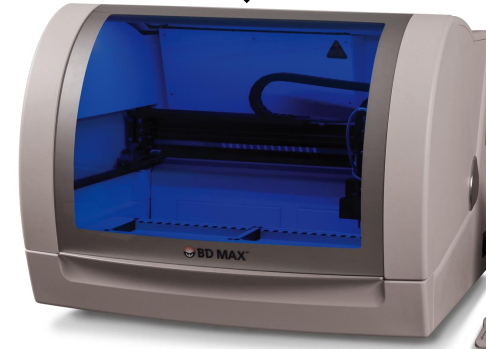
1- Rack



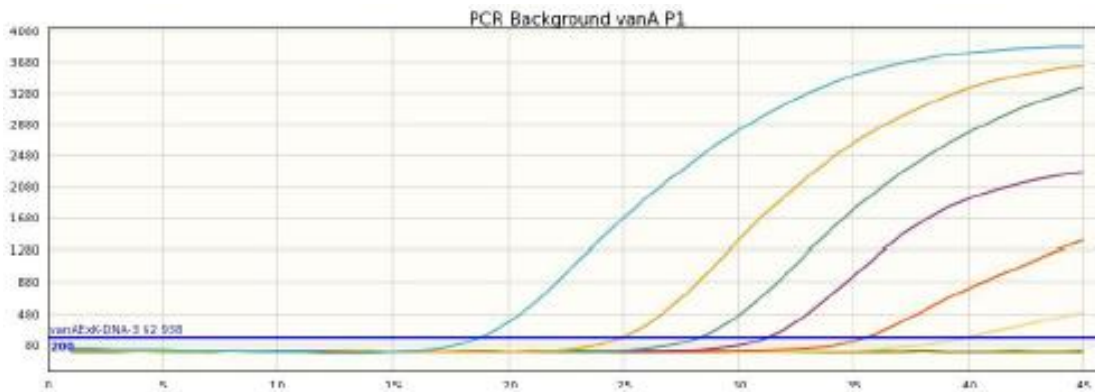
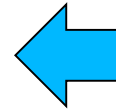
2- Reagentes



3- Extração



4- Amplificação



5- Leitura e Interpretação



Tempo de Execução dos Exames

- PCR Ponto Final

- 18-24h + 4h = 22-28h

- PCR Melting

- 18-24h + 3h = 21-27h

- PCR TaqMan

- 18-24h + 2h = 20-26h

- PCR Automatizado

- ~~■ 18-24h + 2h = 2h~~

- MALDI – TOF

- ~~■ 18-24h + 3h = 3h~~

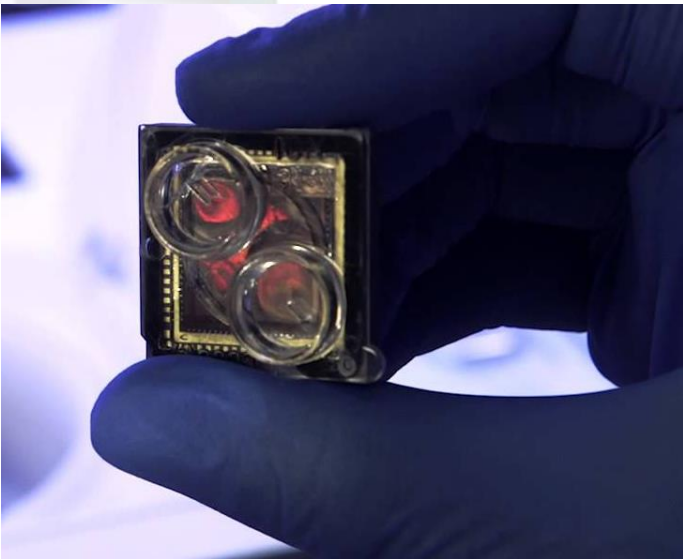
- PCR Automatizado

- Multiplex Plus (> 90 genes)

- ~~■ 18-24h + 2h = 2h~~



Next Generation Sequencing





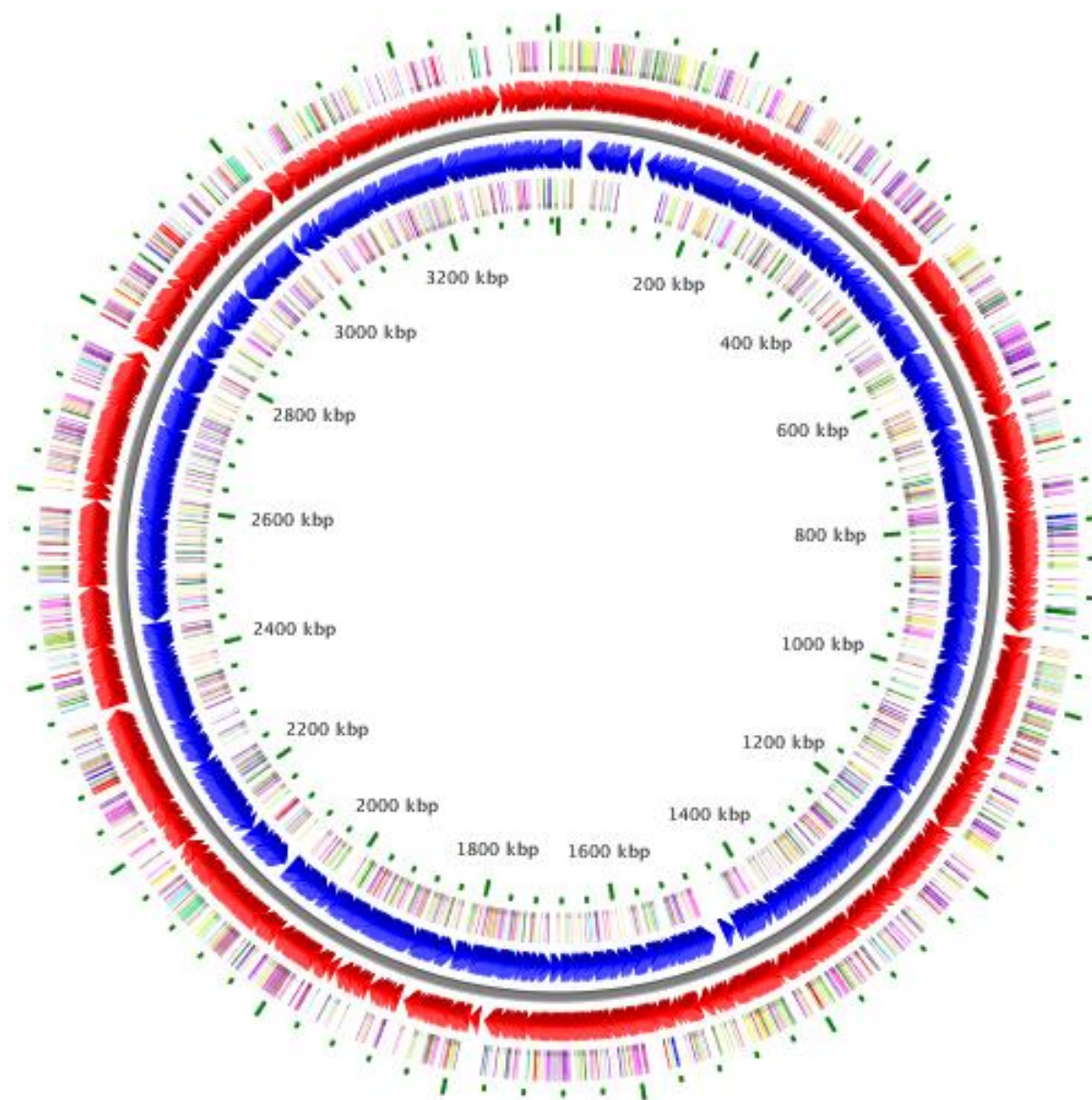
Next Generation Sequencing

- Sequenciamento de Próxima Geração
 - Genoma completo
 - Pesquisa de TODOS os genes simultaneamente
 - Define o **Resistoma!**
 - Detecção de variantes alélicas
 - Custo / Trabalhoso (muitas etapas manuais)



NGS High Throughput

- 3,5 dias para preparo e sequenciamento
- 5 dias incluindo análise
- Custo atual
 - Brasil: R\$ 1.200,00 / isolado
 - UE: EU\$ 150,00 / isolado (\approx R\$ 500,00)
 - Aplicação: alguns isolados (hoje)



Genes encoding proteins

- Forward strand
- Reverse strand

Genes encoding functional RNA

- Forward strand
- Reverse strand

COG functional categories

Information storage and processing

- Translation, ribosomal structure and biogenesis
- Transcription
- DNA replication, recombination and repair

Cellular processes

- Cell division and chromosome partitioning
- Posttranslational modification, protein turnover, and chaperones
- Cell envelope biogenesis, outer membrane
- Cell motility and secretion
- Inorganic ion transport and metabolism
- Signal transduction mechanisms

Metabolism

- Energy production and conversion
- Carbohydrate transport and metabolism
- Amino acid transport and metabolism
- Nucleotide transport and metabolism
- Coenzyme metabolism
- Lipid metabolism
- Secondary metabolites biosynthesis, transport, and catabolism

Poorly characterized


- General function prediction only
- Function unknown



CONTROLE DE INFECÇÕES DE MULTI-RESISTENTES



AÇÕES

- Reativação da Comissão Estadual
 - VISA, VE, LACEN
 - Revisão da Portaria 0674/2010
 - Fluxo de envio de cepas
 - Prioridades
- 

Ações Laboratoriais LACEN/PR

- Participação ativa na Rede RM – ANVISA
 - Pesquisa de BMR
 - Referência para PR, SC, RS, MT e MS
- Métodos Multiplex de Rotina
 - CRE: KPC, NDM, OXA-48
- Métodos Multiplex em Implantação
 - SPM + IMP + VIM + NDM + KPC + OXA-23 + OXA-48




Proposta Integradora

- HUB de Resistência
 - Curitiba – Londrina – Maringá – Foz – Cascavel
 - Uso do MALDI-TOF para *screening*




Necessidades

- Fortalecer as equipes de Controle de Infecção
 - Apropriar-se das novas tecnologias
 - Laboratório COM Microbiologista
 - Entender e utilizar regras de PK/PD
- 



Perspectivas “Futuras”

- Pesquisa de múltiplos genes por qPCR
 - Resistoma por NGS <Us\$100,00
 - Metagenômica ambiental
- 

1000000



1000000





TERAPIA COM MULTI-DROGAS

Combination therapy for carbapenem-resistant Gram-negative bacteria

**Mical Paul^{1*}, Yehuda Carmeli², Emanuele Durante-Mangoni³, Johan W. Mouton⁴, Evelina Tacconelli⁵,
Ursula Theuretzbacher⁶, Cristina Mussini⁷ and Leonard Leibovici^{8,9}**

¹*Division of Infectious Diseases, Rambam Health Care Campus and The Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel;* ²*Division of Epidemiology, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel;* ³*Internal Medicine, University of Naples S.U.N., Monaldi Hospital, Napoli, Italy;* ⁴*Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Centre Rotterdam, Rotterdam, The Netherlands;* ⁵*Infectious Diseases and Internal Medicine I, Tuebingen University Hospital, Tuebingen, Germany;* ⁶*Center for Anti-Infective Agents, Vienna, Austria;* ⁷*Clinic of Infectious Diseases, University of Modena and Reggio Emilia, Modena, Italy;* ⁸*Medicine E, Rabin Medical Center, Beilinson Hospital, Petah-Tikva, Israel;* ⁹*Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel*

Currently, however, there is no evidence-based support for most combination therapies against CR-GNB, including colistin/carbapenem combination therapy. Maio, 2014



NOVAS DROGAS



TO BE CONTINUED...





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