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Healthcare Associated Infection

Latin American Quality Control Program in Bacteriology and Antimicrobial drug Resistance (LA-EQAS): Is Latin American prepared to detect emerging resistance mechanism?

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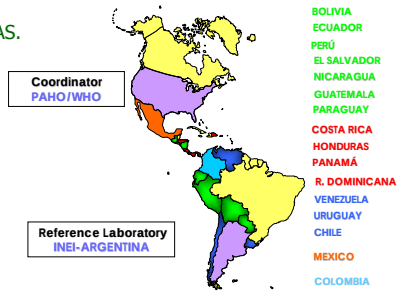
INTRODUCTION

In 1996, the Pan American Health Organization (PAHO) sponsored the creation of a "Latin American Network for Surveillance of Antimicrobial Drug Resistance" (RELAVRA). Its objective was to obtain reliable microbiological data and to strengthen surveillance through the establishment of quality assurance programs. In 2000, a "Latin American Quality Control Program in Bacteriology and Antimicrobial Resistance" (LA-EQAS) was created in the context of the "Prevention and Control of antimicrobial resistance in the Americas Project " and, the INEI "Dr. Carlos Malbrán" from Argentina was designed as the Reference Laboratory. Currently, 17 laboratories (16 countries) participate of LA-EQAS.

Table 1 : Participating Laboratories in LA-EQAS.

LABORATORY	COUNTRY	MICROBIOLOGY
Inst. Nac. de Laboratorios de Salud. INLASA	Bolivia	E.Damiani, G. García
Hospital Vozandes	Ecuador	Jeannette Zurita
Inst. Nac. de Higiene y Med. Trop. "L. Izquieta Perez"	Ecuador	Carmen Pesantes
Laboratorio Central Dr. Max Bloch.	El Salvador	Zandra Fuentes
Centro Nacional de Diagnóstico y Referencia.CNDR	Nicaragua	Javiera Mejía
Laboratorio Central de Salud Pública.	Paraguay	Mario Martínez Mora
Instituto Nacional de Salud.	Perú	Rosa Sacsaquispe
Laboratorio Nacional de Salud.	Guatemala	Mercy L. Cabrera Morales
Inst. Costarricense de Inv. y Enseñanza en Nut. y Salud.	Costa Rica	H. Bolaños, A. Jimenez
Departamento de Laboratorios.	Honduras	Carmen Morales
Laboratorio Central de referencia en Salud Pública.	Panamá	Raquel Bolaños
Laboratorio Nacional de Salud Pública. "Dr. Defilló"	R. Dominicana	Reyna Ovalles
Instituto Nacional de Higiene "Rafael Rangel". INHRR	Venezuela	Daniel Marcano
Instituto de Salud Pública.	Chile	A. Maldonado, M.S. Pratt
Laboratorio Nacional de Higiene Pública.	Uruguay	Teresa Camou
Instituto de Diagnóstico y Referencia Epidemiológicos	México	Irma Hernandez Monroy
Instituto Nacional de Salud	Colombia	María Elena Realpe



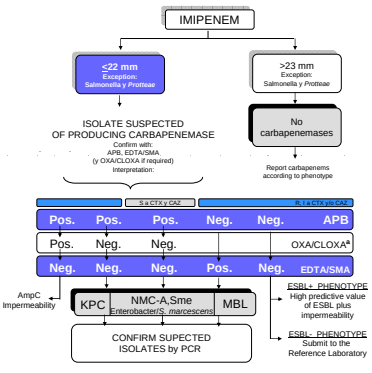
AIM

I) To evaluate laboratory assurance in detecting **emergent resistance mechanisms** as: a) **carbapenem resistance by KPC** (*Klebsiella pneumoniae* carbapenemase) in *Enterobacteriaceae*, b) **carbapenem resistance mediated by metallo-β-lactamases** (MBL-IMP type) in *Enterobacteriaceae* and c) vancomycin intermediate resistance in *Staphylococcus aureus* (**VISA**).

II) To evaluate the agreement among the inhibition zones reported by participating laboratories and the reference range.

III) To evaluate the performance of the proposed flow chart for screening of suspected carbapenemase-producing strains of *Enterobacteriaceae.* (Fig 1)

Figure 1 **:** Proposed flow chart for screening of suspected class A carbapenemase and MBL-producing strains of *Enterobacteriaceae.*



[a] "Double modified" Hodge test for confirmation of suspected class A carbapenemases in AMP-C producers (*Citrobacter freundii, Enterobacter cloacae, Serratia* spp, *Providencia* spp. and *Morganella* spp).

APB: 3-aminophenylboronic acid, EDTA: ethylenediaminetetraacetic acid, SMA: sodium mercaptoacetic acid, OXA: oxacillin, CLOXA: cloxacillin, CTX: cefotaxime, CAZ: ceftazidime, KPC: *Klebsiella pneumoniae* carbapenemase, MBL: metallo-β-lactamase, ESBL: extended-spectrum β-lactamase.

MATERIALS AND METHODS

Strains, susceptibility tests and molecular characterization of resistance mechanisms: *K. pneumoniae* OPS-161(KPC-2), *E. cloacae* OPS-166 (PER-2 and IMP-8) and *S. aureus* OPS-165 (VISA VAN MIC 4ug/ml) were submitted to the participating laboratories for susceptibility analysis. Strains were evaluated by disk diffusion and MICs according to M100-S20 CLSI guidelines. The resistance mechanisms of the strains were assessed by PCR/DNA sequencing for: *bla*KPC-2, *bla*PER-2 and *bla*IMP-8 genes.

RESULTS

I. Agreement in the detection of the resistance mechanisms

Table 2 : Agreement in the detection of the resistance mechanisms

Strains	Agreement in the detection of the resistance mechanisms (%)
***Klebsiella pneumoniae* OPS-161**	**76**
***Enterobacter cloacae* OPS-166**	**73**
***S. aureus* OPS-165**	**67**

Global agreement for the three mechanisms was 72%.

Table 3A : Resistance mechanisms reported in KPC- producing *K. pneumoniae* OPS-161.

Resistance mechanisms reported by laboratories	No. of laboratories	Comments
KPC[a]	**4 (23%)**	Positive sinergy between the discs of carbapenems and APB[d]
Carbapenemase (MBL[b] or KPC)	**4 (23%)**	Imipenem inhibition zones ≤ 22mm. Hodge test: positive for carbapenems
ESBL[c] and KPC	**2 (12%)**	Positive sinergy between the discs of carbapenems and APB[d]
ESBL and Carbapenemase	**3 (18%)**	Imipenem inhibition zones ≤ 22mm. Hodge test: positive for carbapenems
ESBL	1 (6%)	
ESBL and Impermeability	1 (6%)	
No interpretation reported	2 (12%)	

76%

❖ **KPC and carbapenemases, with or without ESBL, were considered correct.**

[a] **KPC**: *Klebsiella pneumoniae* carbapenemase, [b] **MBL**: metallo-β-lactamase, [c] **ESBL**: extended-spectrum β-lactamase, [d] **APB**: 3-aminophenylboronic acid (300µg/disc)

Table 3B : Resistance mechanisms reported in *E. cloacae* OPS-166 MBL producing, IMP type

Resistance mechanisms reported by laboratories	No. of laboratories [a]	Comments
MBL[b]	**6 (40%)**	Positive sinergy between the discs of carbapenems and EDTA/SMA[d]
Carbapenemase	**5 (33%)**	Imipenem inhibition zones ≤ 22mm. Hodge test: positive for carbapenems
ESBL[c]	3 (20%)	
No interpretation reported	1 (7%)	

73%

❖ **MBL or carbapenemases were considered as correct.**

[a] two laboratories were not included in the analysis of the carbapemem resistance mechanism, [b] **MBL**: metallo-β-lactamases, [c] **ESBL**: extended-spectrum β-lactamase. [d] **EDTA/SMA**: ethylenediaminetetraacetic acid/sodium mercaptoacetic acid (SMA)

Table 3C : Vancomycin MIC values reported in *S. aureus* OPS-165

	MIC (µg/ml)					Essential Agreement (EA)[b]	Mechanism detection (%)	Error		
	≤1	2	4	8	16			VMa[c]	Ma[d]	Mi[e]
No. of laboratories with VAN MIC [a]	1	3	7	3	1	13	67%	---	---	5
	7%	20%	47%	20%	7%	87%				

❖ **Reference VAN MIC: 4 ug/ml (agar dilution, broth macrodilution, Etest, MICE, Vitek2)**
❖ **VISA (VAN MIC 4 and 8 ug/ml) interpretation was considered correct.**

[a] two laboratories didn´t report vancomycin MIC, [b] **EA**: reference MIC +/- 1 dilution. [c] **VMa**: Very Major error, [d] **Ma**: Major error, [e] **Mi**: Minor error

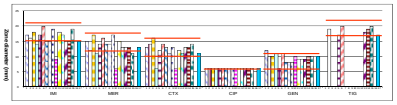
II. Agreement between inhibition zones obtained by Participating Laboratories and the reference range

Table 4 : Agreement among the inhibition zones and the reference range.

	Agreement among the inhibition areas (%)
***Klebsiella pneumoniae* OPS-161**	**91**
***Enterobacter cloacae* OPS-166**	**93**
***S. aureus* OPS-165**	**89**

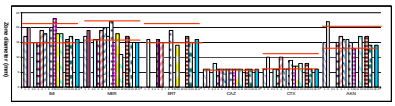
Global agreement for the inhibition zones was 91%.

Figure 2A : Distribution of inhibition zones in KPC- producing *K. pneumoniae* OPS-161.



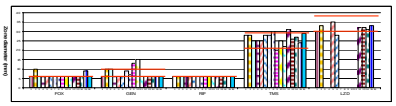
Reference range(mm): Imipenem (IMI): 15-21, Meropenem (MER): 12-18, Cefotaxime (CTX): 10-16, Ciprofloxacin (CIP): 6, Gentamicin (GEN): 6-11, Tigecyclina (TIG): 17-23

Figure 2B : Distribution of inhibition zones in *E. cloacae* OPS-166 MBL, IMP type, producing.



Reference range Imipenem (IMI): 15-21, Meropenem (MER): 16-23, Ertapenem (ERT): 15-21, Ceftazidime (CAZ): 6, Cefotaxime (CTX): 6-11, Amikacin (AKN): 13-19

Figure 2C : Distribution of inhibition zones in *S. aureus* OPS-165 (VISA).



Reference range (mm): Cefoxitin (FOX): 6, Gentamicin (GEN): 6-10, Rifampicin (RIF): 6, Trimethoprim/Sulfamethoxasole (TMS): 21-29, Linezolid (LZD): 30-37

CONCLUSION

❖ Although the global agreement in the detection of the resistance mechanisms was not ideal (72%), we can conclude that Reference Latin American laboratories are prepared to recognize emerging resistance mechanisms as KPC, MBL and VISA.
❖ The proposed flow chart resulted satisfactory for detecting carbapenemase-producing strains of *Enterobacteriaceae* (76 % and 73% of agreement for KPC and MBL carbapenemases, respectively).
❖ Vancomycin intermediate resistance in *S. aureus* was the mechanism which presented major difficulty (67% of agreement).
❖LA-EQAS became an excellent tool to highlight the detection and reporting difficulties, to improve training in the recognition of resistance mechanisms and to design strategies for the enhancement of the quality of the diagnosis of infectious diseases in the Region.
❖ KPC, MBL and VISA are becoming more prevalent in the Region. Proficient methods are needed for their early detection in clinical microbiology laboratories for targeting optimal antimicrobial therapy and controlling the spread of health care infections.