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# Antimicrobial Resistance among Gram-Negative Bacilli Isolated from Latin America: Results from SENTRY Antimicrobial Surveillance Program (2008-2010)

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## Abstract

**Objective**: To provide an update on the frequency and antimicrobial resistance (R) of Gram-negative bacilli (GNB) isolated from SENTRY Program Latin American medical centres (LAMC). This program has monitored antimicrobial R in LAMC since 1997.

**Methods**: 12,811 organisms, including 5,704 GNB (44.5%), were consecutively collected (one per patient) between January 2008 and December 2010 from 10 LAMC located in Argentina (ARG; 2), Brazil (BRA; 4), Chile (CHI; 2) and Mexico (MEX; 2). The isolates were susceptibility (S) tested by the CLSI broth microdilution method at a central laboratory. *E. coli* (EC), *Klebsiella* spp. (KSP) and *Enterobacter* spp. (ESP) isolates with MIC, ≥2 mg/L for imipenem (IMI) or meropenem (MER) were screened for carbapenemases by the Modified Hodge test, PCR for $bla_{IMP}$, $bla_{VIM}$, $bla_{KPC}$, $bla_{SME}$, $bla_{GES}$, $bla_{IMI}$, $bla_{NMC-A}$, and $bla_{OXA-48}$, and DNA sequencing. Selected IMI-R *Acinetobacter* spp. (ASP) strains were screened for $bla_{OXA-23,\ 24,\ 51,\ 58}$, $bla_{KPC}$ and MBLs by PCR.

**Results**: The infection sites included bloodstream (BSI; 52.6%), skin and soft tissue (22.9%) and pneumonia (13.1%), among others. The most common causes of BSI were *S. aureus* (SA; 22%), EC (21%) and KSP (13%); while *P. aeruginosa* (PSA; 33%), SA (21%), ASP (18%), KSP (11%) and ESP (5%) represented the top 5 organisms from pneumonia. ESBL rates were 18.0, 12.8, 23.8 and 48.4% among EC and 60.5, 49.9, 59.2 and 33.3% among KSP from ARG, BRA, CHI and MEX, respectively. MER-non-S KSP was highest in BRA (11.1%), followed by ARG (8.2%), CHI (5.0%) and MEX (0.8%). KPC-producing KPN was not detected in 2008, but emerged in 2009 (10 strains) and increased significantly in 2010 (44; p <0.0001). $bla_{KPC-2}$ was detected in 54 of 85 (65.9%) carbapenem-non-S *K. pneumoniae* (KPN). MER-non-S PSA was observed in 53.8, 46.7, 33.3 and 28.8% of strains from ARG, BRA, CHI and MEX, respectively. IMI-S ASP decreased from 38.6, 83.2 and 91.8% in 2003-2005 to 14.5, 28.6 and 47.4% in 2008-2010 in ARG, BRA and CHI, respectively. OXA-producing ASP was documented in ARG (OXA-23), BRA (OXA-23 and -24), CHI (OXA-58) and MEX (OXA-24). Colistin was the only compound with reasonable activity against ASP and PSA.

**Conclusions:** Only COL showed >77% overall coverage against the five most frequently isolated GNB from LAMC participating in the SENTRY Program. Empiric antimicrobial therapy for serious infections caused by GNB requires the combination of two or more agents for adequate coverage.

## Introduction

Infections caused by multidrug-resistant isolates are associated with increased costs, length of hospitalization, and especially, morbidity and mortality rates. Resistance among Gram-negatives is of great concern, since few antimicrobial agents are clinically available to treat infections caused by these pathogens. Surveillance studies like the SENTRY Antimicrobial Surveillance Program are undertaken to help direct antimicrobial use, particularly empiric therapy, based on the local resistance patterns of organisms, and implementation of infection control measures.

Recently, with broader dissemination of KPC-producing Enterobacteriaceae and OXA-producing *Acinetobacter* spp. isolates, a rapid decrease in the susceptibility to carbapenems has been noticed in many parts of the world. The objective of this study was to update the frequency and resistance rates of Gram-negative pathogens isolated from Latin American sites enrolled in the SENTRY Program between 2008 and 2010 and summarize molecular characterization data for subsets of carbapenemase-producing strains.

## Materials and Methods

Bacterial strains. A total of 12,811 bacterial isolates were collected from 10 Latin American sites located in four countries: Brazil (four centres), Argentina (two centres), Chile (two centres), and Mexico (two centres) between January 2008 and December 2010. Among those, 4,964 isolates were analyzed in the present study, including 1,517 *E. coli*, 1,052 *Klebsiella* spp., 451 *Enterobacter* spp., 1,099 *P. aeruginosa* and 845 *Acinetobacter* spp. Furthermore, in order to assess whether there was increase in resistance to carbapenems among *E. coli*, *Klebsiella* spp., *P. aeruginosa* and *Acinetobacter* spp., the rates of resistance to imipenem among isolates collected in three distinct periods of time, 1997-1999 (2,996 isolates), 2003-2005 (3,093 isolates), and 2008-2010 (2,767 isolates) were evaluated. Only isolates collected from medical centres with continuous participation in the SENTRY Program since its beginning (1997) were utilized for these resistance trend analyses. Only a single isolate per patient was included in the study. All isolates were identified in the participant medical centre and forwarded to a central laboratory (JMI laboratories, North Liberty, Iowa, USA) for identification confirmation and susceptibility testing.

Susceptibility testing. Antimicrobial susceptibility testing was performed and interpreted by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method, except for colistin against Enterobacteriaceae, where the EUCAST breakpoint criteria were applied. Quality control (QC) was performed by testing *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, with all QC results within expected ranges. Data analysis was carried out using the SPSS for Windows Release 17.0 – Standard Version (SPSS Inc., Chicago, Illinois, USA). Fisher's exact test and v2 for trend were used to compare differences on antimicrobial susceptibility rates. A *p* value of <0.05 was considered statistically significant.

Screening for carbapenemase encoding genes. Carbapenem-resistant *Acinetobacter* spp. (MIC, ≥8 mg/L for imipenem and/or meropenem) collected in the year 2008 were screened for $bla_{OXA-23,-24,\ -51,-58}$, $bla_{KPC}$, $bla_{IMP}$, $bla_{VIM}$, $bla_{SIM}$, $bla_{GIM}$, and $bla_{SPM}$ by PCR. *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 mg/L) were tested with the Modified Hodge test using imipenem and meropenem disks, and isolates screened for the presence of $bla_{IMP}$, $bla_{VIM}$, $bla_{KPC}$, $bla_{SME}$, $bla_{GES}$ variants as well as for $bla_{NDM-1}$, $bla_{IMI}$, $bla_{NMC-A}$, $bla_{OXA-48}$ by multiplex PCR reactions followed by DNA sequencing.

## Results

- The most common causes of BSI were *S. aureus* (20.7%), *E. coli* (19.0%) and *Klebsiella* spp. (12.3%); while *P. aeruginosa* (31.2%), *S. aureus* (20.0%), *Acinetobacter* spp. (17.7%), *Klebsiella* spp. (10.2%) and *Enterobacter* spp. (5.1%) represented the top five organisms from pneumonia. Gram-negative bacilli accounted for 44.5% of the infections overall.
- Amikacin, carbapenems and colistin showed remarkable activity (≥98.6% overall susceptibility [S]) against the 1,517 *E. coli* isolates. In contrast, high resistance rates to ciprofloxacin (40.2%) and ceftriaxone (23.9%) were observed overall, but especially in isolates collected from Mexican medical centres (Tables 1 and 2).
- Colistin ($MIC_{50}$, ≤0.5 mg/L; 97.0% S) was the most active agent against *Klebsiella* spp., followed by meropenem ($MIC_{50}$, ≤0.12 mg/L; 92.2% S) and imipenem ($MIC_{50}$, 0.25 mg/L; 92.5% S; Tables 1 and 2).
- Overall, 24.7 and 52.7% of the *E. coli* and *Klebsiella* spp. exhibited an ESBL phenotype. The highest ESBL phenotype rates among the *E. coli* strains were observed in Mexico (48.4%), followed by Chile (23.8%), Argentina (18.1%) and Brazil (12.8%). In contrast, among *Klebsiella* spp., ESBL phenotype rates were highest in Argentina (60.4%) > Chile (59.2%) > Brazil (49.9%) > Mexico (33.3%).
- Among *Enterobacter* spp., only 63.6, 82.0 and 92.9% of strains were susceptible to ceftazidime, cefepime and meropenem respectively (Table 1).
- Colistin was the most active antimicrobial agent against *P. aeruginosa* ($MIC_{50,}$ 1 mg/L; 99.7% susceptible) and *Acinetobacter* spp. ($MIC_{50,}$ ≤0.5 mg/L; 98.1% susceptible), followed by amikacin (73.3 and 29.4% S, respectively) and tobramycin 64.7 and 46.6% S, respectively; Tables 1 and 2).
- A significant trend for imipenem resistance was noticed among *Acinetobacter* spp. from Argentina, Brazil and Chile (*p,* ≤0.001); *P. aeruginosa* from Argentina and Chile (*p,* ≤0.001); and *Klebsiella* spp. from Argentina and Brazil (*p,* ≤0.001; Figure 1).
- OXA-carbapenemase encoding genes were detected in 65 of 182 (35.7%) *Acinetobacter* spp. isolates from 2008. $bla_{OXA-23}$ was the most frequent gene being detected in 41 isolates from Argentina (26.8%) and Brazil (73.2%).
- Metallo-β-lactamase encoding genes, $bla_{IMP-18}$ (single isolate) and $bla_{VIM-23}$ (three isolates), were identified in *K. oxytoca* and *E. cloacae* strains from a single Mexican centre. $bla_{OXA-163}$ was identified in one *E. cloacae* and two *K. pneumoniae* strains from Argentina in 2008.
- KPC-2-producing *K. pneumoniae* emerged in 2009 (10 strains) and increased significantly in 2010 in Argentina and Brazil (44 strains; *p,* <0.0001). $bla_{KPC-2}$ was detected in 54 *K. pneumoniae* strains isolated from two Argentinean (15 isolates; 27.8%) and two Brazilian medical centres (39 isolates; 72.2%).

**Table 1.** Antimicrobial activity of antimicrobial agents tested against the five most frequent Gram-negative pathogens collected from Latin American medical centres (SENTRY Program, 2008-2010).

| Organism (No. Tested)/ Antimicrobial Agent | MIC (mg/L) | | % by Category[a] | |
|---|---|---|---|---|
| | 50% | 90% | Susceptible | Resistant |
| *E. coli* (1,517) | | | | |
| Ceftriaxone | ≤0.25 | >8 | 75.3 | 23.9 |
| Ceftazidime | ≤1 | 16 | 82.1 | 9.0 |
| Cefepime | ≤0.12 | >16 | 84.1 | 12.0 |
| Piperacillin/tazobactam | 2 | 16 | 90.5 | 2.8 |
| Imipenem | ≤0.12 | 0.25 | 99.6 | 0.1 |
| Ciprofloxacin | ≤0.5 | >4 | 59.7 | 40.2 |
| Amikacin | 2 | 8 | 98.6 | 0.5 |
| Gentamicin | ≤2 | >8 | 81.2 | 17.5 |
| Colistin | ≤0.5 | ≤0.5 | 99.8 | 0.2 |
| *Klebsiella* spp. (1,052) | | | | |
| Ceftriaxone | >8 | >8 | 47.3 | 52.5 |
| Ceftazidime | 2 | >16 | 54.9 | 40.0 |
| Cefepime | 1 | >16 | 58.8 | 36.3 |
| Piperacillin/tazobactam | 8/4 | >64/4 | 60.9 | 28.8 |
| Imipenem | 0.25 | 1 | 92.5 | 6.2 |
| Ciprofloxacin | 0.5 | >4 | 55.9 | 41.3 |
| Amikacin | 2 | 32 | 86.8 | 7.8 |
| Gentamicin | ≤2 | >8 | 63.1 | 33.0 |
| Colistin | ≤0.5 | ≤0.5 | 97.0 | 3.0 |
| *Enterobacter* spp. (451) | | | | |
| Ceftriaxone | 0.5 | >32 | 54.6 | 44.3 |
| Ceftazidime | ≤1 | >16 | 63.6 | 27.7 |
| Cefepime | ≤0.12 | >16 | 82.0 | 14.0 |
| Imipenem | 0.5 | 1 | 92.9 | 1.3 |
| Piperacillin/tazobactam | 4/4 | >64/4 | 74.1 | 13.3 |
| Ciprofloxacin | ≤0.5 | >4 | 76.3 | 21.1 |
| Amikacin | 1 | 16 | 92.2 | 3.7 |
| Gentamicin | ≤2 | >8 | 78.7 | 18.2 |
| Colistin | ≤0.5 | >4 | 82.7 | 17.3 |
| *P. aeruginosa* (1,099) | | | | |
| Ceftazidime | 4 | >16 | 61.1 | 30.2 |
| Cefepime | 8 | >16 | 61.9 | 21.9 |
| Piperacillin/tazobactam | 16/4 | >64/4 | 55.6 | 25.4 |
| Imipenem | 2 | >8 | 57.6 | 27.6 |
| Ciprofloxacin | 0.5 | >4 | 56.7 | 41.2 |
| Amikacin | 4 | >32 | 73.3 | 23.2 |
| Gentamicin | ≤2 | >8 | 63.2 | 35.2 |
| Tobramycin | 0.5 | >16 | 64.7 | 34.4 |
| Colistin | 1 | 2 | 99.7 | 0.1 |
| *Acinetobacter* spp. (845) | | | | |
| Ceftazidime | >16 | >16 | 12.8 | 81.7 |
| Imipenem | >8 | >8 | 30.5 | 67.8 |
| Piperacillin/tazobactam | >64/4 | >64/4 | 10.2 | 86.3 |
| Ciprofloxacin | >4 | >4 | 12.5 | 87.2 |
| Amikacin | >32 | >32 | 29.4 | 62.6 |
| Gentamicin | >8 | >8 | 37.8 | 53.3 |
| Tobramycin | 8 | >16 | 46.6 | 43.4 |
| Colistin | ≤0.5 | 1 | 98.1 | 1.2 |

a. Susceptibility and resistance rates were calculated according to CLSI guidelines, except for colistin against Enterobacteriaceae, where EUCAST breakpoints were applied.

**Table 2**. Susceptibility rates among Gram-negative bacilli according to the nation of isolation (SENTRY Program, Latin America, 2008-2010).

| Organism/(no. tested)/ Antimicrobial agent | Susceptibility rates by nation (no. tested) | | | |
|---|---|---|---|---|
| *E. coli* | Argentina | Brazil | Chile | Mexico |
| (1,517) | (277) | (429) | (496) | (316) |
| Ceftriaxone | 82.3 | 87.2 | 76.2 | 51.6 |
| Ceftazidime | 87.7 | 83.0 | 84.4 | 52.9 |
| Cefepime | 91.7 | 92.1 | 84.9 | 65.2 |
| Imipenem | 99.6 | 99.3 | 99.8 | 99.7 |
| Meropenem | 100.0 | 99.8 | 100.0 | 100.0 |
| Piperacillin/tazobactam | 94.2 | 91.8 | 92.3 | 82.3 |
| Ciprofloxacin | 58.8 | 72.7 | 64.3 | 35.4 |
| Amikacin | 100.0 | 98.8 | 98.6 | 96.8 |
| Gentamicin | 83.4 | 87.4 | 84.5 | 66.5 |
| Colistin | 100.0 | 99.8 | 99.8 | 99.7 |
| *Klebsiella* spp. | Argentina | Brazil | Chile | Mexico |
| (1,052) | (317) | (405) | (201) | (129) |
| Ceftriaxone | 40.1 | 50.1 | 40.8 | 66.7 |
| Ceftazidime | 46.8 | 52.1 | 44.3 | 66.7 |
| Cefepime | 53.9 | 56.5 | 49.3 | 93.0 |
| Imipenem | 91.7 | 88.7 | 98.0 | 97.7 |
| Meropenem | 91.8 | 88.9 | 95.0 | 99.2 |
| Piperacillin/tazobactam | 49.2 | 62.5 | 63.2 | 81.4 |
| Ciprofloxacin | 48.3 | 55.6 | 52.7 | 80.6 |
| Amikacin | 86.8 | 91.8 | 78.1 | 84.5 |
| Gentamicin | 55.8 | 65.4 | 54.7 | 86.8 |
| Colistin | 95.3 | 96.8 | 98.5 | 99.2 |
| *Enterobacter* spp. | Argentina | Brazil | Chile | Mexico |
| (451) | (74) | (199) | (87) | (91) |
| Ceftriaxone | 58.1 | 55.3 | 50.6 | 53.9 |
| Ceftazidime | 63.5 | 69.4 | 58.6 | 56.0 |
| Cefepime | 78.4 | 78.9 | 83.9 | 90.1 |
| Imipenem | 86.5 | 92.5 | 95.4 | 92.3 |
| Meropenem | 96.0 | 98.5 | 98.9 | 98.9 |
| Piperacillin/tazobactam | 71.6 | 74.4 | 78.2 | 71.4 |
| Ciprofloxacin | 74.3 | 74.4 | 75.9 | 82.4 |
| Amikacin | 94.6 | 92.5 | 89.7 | 92.3 |
| Gentamicin | 64.9 | 78.4 | 75.9 | 93.4 |
| Colistin | 82.4 | 83.4 | 87.1 | 75.8 |
| *P. aeruginosa* | Argentina | Brazil | Chile | Mexico |
| (1,099) | (234) | (490) | (177) | (198) |
| Ceftazidime | 59.4 | 62.0 | 52.5 | 69.0 |
| Cefepime | 64.5 | 58.0 | 58.2 | 71.7 |
| Imipenem | 51.1 | 54.7 | 60.0 | 70.1 |
| Meropenem | 46.2 | 53.3 | 66.7 | 71.2 |
| Piperacillin/tazobactam | 50.1 | 55.7 | 48.0 | 67.7 |
| Ciprofloxacin | 50.4 | 57.1 | 49.7 | 69.7 |
| Colistin | 100.0 | 99.4 | 100.0 | 100.0 |
| Amikacin | 72.2 | 69.4 | 92.1 | 67.7 |
| Gentamicin | 59.0 | 61.0 | 77.4 | 60.6 |
| Tobramycin | 60.3 | 62.2 | 80.2 | 62.1 |
| *Acinetobacter* spp. | Argentina | Brazil | Chile | Mexico |
| (845) | (172) | (355) | (79) | (239) |
| Ceftazidime | 7.6 | 13.0 | 15.2 | 15.5 |
| Imipenem | 14.5 | 27.0 | 47.4 | 41.4 |
| Meropenem | 15.1 | 27.3 | 46.8 | 40.3 |
| Piperacillin/tazobactam | 1.7 | 10.4 | 13.9 | 14.6 |
| Ciprofloxacin | 2.3 | 13.5 | 16.5 | 16.3 |
| Amikacin | 18.6 | 40.9 | 20.3 | 23.0 |
| Gentamicin | 16.9 | 47.6 | 41.8 | 36.8 |
| Tobramycin | 37.8 | 66.2 | 57.0 | 20.5 |
| Colistin | 100.0 | 98.3 | 100.0 | 98.3 |

## Conclusions

- Gram-negative bacilli account for a significant proportion of infections in Latin America, with a high percentage of infections caused by *P. aeruginosa* and *Acinetobacter* spp.
- An important increase in carbapenem resistance was observed among *Acinetobacter* spp. and *K. pneumoniae* isolates mainly due to production of carbapenem-hydrolyzing OXA and KPC-2 enzymes, respectively.
- Only colistin showed reasonable activity (>77% overall coverage) against the five most frequently isolated Gram-negative bacilli from Latin American medical centres participating in the SENTRY Program.
- Empiric antimicrobial therapy for serious infections caused by Gram-negative bacilli requires the combination of two or more agents for adequate coverage in these SENTRY Program participating medical centres.

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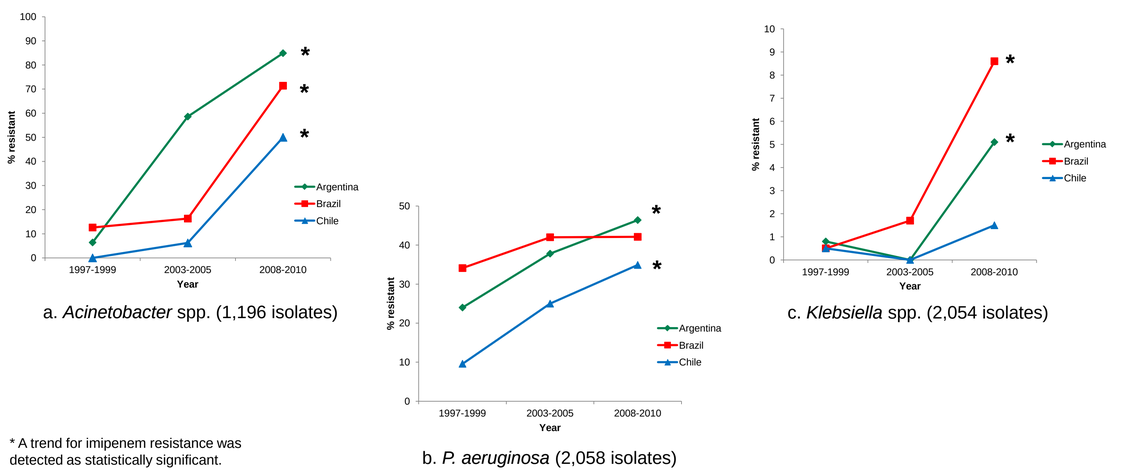
**Figure 1**. Variation in the imipenem resistance rates overtime.




a. *Acinetobacter* spp. (1,196 isolates)

b. *P. aeruginosa* (2,058 isolates)

c. *Klebsiella* spp. (2,054 isolates)

* A trend for imipenem resistance was detected as statistically significant.

**Table 3.** Molecular characterization of selected carbapenem-resistant Gram-negative pathogens.

| Organism (No. tested) | No. of Carbapenem-resistant isolates (%) | No. of isolates with positive Modified Hodge Test (%) | Carbapenemase Encoding Genes (No.) | Nation | Medical Centre No. (No. of isolates) |
|---|---|---|---|---|---|
| *E. coli* (1,517) | 6 (0.4) | 3 (50.0) | $bla_{KPC-2}$ (3) | Brazil | 48 (2), 101 (1) |
| *Klebsiella* spp. (1,052) | 82 (7.8) | 58 (70.7) | $bla_{KPC-2}$ (54) | Argentina | 39 (13), 40 (2) |
| | | | | Brazil | 48 (15), 101 (24) |
| | | | $bla_{OXA-163}$ (2) | Argentina | 39 (1), 40 (1) |
| | | | $bla_{IMP-18}$ (1) | Mexico | 115 (1) |
| | | | $bla_{VIM-23}$ (1) | Mexico | 115 (1) |
| *Enterobacter* spp. (451) | 36 (8.0) | 6 (16.7) | $bla_{KPC-2}$ (3) | Argentina | 39 (3) |
| | | | $bla_{OXA-163}$(1) | Argentina | 39 (1) |
| | | | $bla_{VIM-23}$(2) | Mexico | 115 (2) |
| *Acinetobacter* spp. (303) | 182/303 (60.1) | 65/182 (35.7) | $bla_{OXA-23}$ + $bla_{OXA-51}$ (41) | Argentina | 39 (10), 40 (1) |
| | | | | Brazil | 46 (10), 48 (1), 101 (19) |
| | | | $bla_{OXA-24}$ + $bla_{OXA-51}$ (17) | Argentina | 39 (1) |
| | | | | Mexico | 115 (16) |
| | | | $bla_{OXA-58}$ + $bla_{OXA-51}$ (1) | Chile | 42 (1) |
| | | | $bla_{OXA-51}$ (6) | Argentina | 39 (2) |
| | | | | Brazil | 48 (2) |
| | | | | Chile | 43 (1) |
| | | | | Mexico | 126(1) |