Antimicrobial Susceptibility Patterns of Unusual Nonfermentative Gram-negative Bacilli Isolated from Latin America: Report from the SENTRY Antimicrobial Surveillance Program (1997-2002)

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AMENDED ABSTRACT

Background: Infections due to nonfermentative Gram-negative bacilli (NF-GNB) other than *P. aeruginosa* and *Acinetobacter* spp. are uncommon but their incidence is increasing in the last years.

Methods: The antimicrobial susceptibility (S) of 176 unusual NF-GNB collected from Latin American centers through the SENTRY Program between 1997 and 2002 was evaluated by the NCCLS broth microdilution. The NF-GNB were isolated from blood (118), respiratory tract (44), wound (10) and urine (4).

Results: Nearly 74% of the NF-BGN tested belonged to the following species: *Burkholderia* spp.(83) *Achromobacter* spp. (25), *Ralstonia pickettii* (RP; 16), *Alcaligenes* spp. (12), and *Cryseobacterium* spp. (12). In general, trimethoprim/sulfamethoxazole (MIC_{50}, ≤ 0.5 mg/ml) was the most potent drug followed by levofloxacin (LEV; MIC_{50}, 0.5 μg/ml) and gatifloxacin (GAT, MIC_{50}, 1 μg/ml). The highest S rates were observed for LEV (78.3%), GAT (75.6%) and meropenem (MER; 72.6%). Although IMI (MIC_{50}, 2 μg/ml) and meropenem (MER; MIC_{50}, 2 μg/ml) exhibited similar potency against NF-GNB, a discrepancy on the S rates was observed (69.9% vs. 72.6%, respectively). Against *Achromobacter* spp., MER (MIC_{50}, 0.25 μg/ml) was eight-fold more potent than IMI (MIC_{50}, 2 μg/ml) and exhibited the highest S rate (88.0%). Ceftazidime (CAZ; MIC_{50}, 8 μg/ml) was two-fold more potent than cefepime (CPM; MIC_{50}, 16 μg/ml) and showed higher S rate (64.0% vs. 24.0%). In contrast, against RP, CPM (MIC_{50}, 2 μg/ml; 81.3% S) and IMI (MIC_{50}, 2 μg/ml; 81.3% S) were more active than CAZ (MIC_{50}, >16 μg/ml; 18.8% S) and MER (MIC_{50}, 8 μg/ml; 50.0% S).

Conclusions: Since selection of the most appropriate antimicrobial agents for testing and reporting has not been established by the NCCLS for many of NF-GNB species, results from large multicenter studies may help to guide the best empiric therapy.

INTRODUCTION

Infections due to nonfermentative Gram-negative bacilli (NF-GNB) other than *P. aeruginosa* and *Acinetobacter* spp. are uncommon but their incidence is increasing in the last years. NF-GNB have been implicated as a cause of both infection in immunocompromised hosts and nosocomial outbreaks associated with infusion of contaminated fluids, use of foreign bodies and contaminated tap water.

Identification of some of these unusual NF-GNB is difficult and automated systems may fail in identifying some species. In addition, the taxonomy of many NF-GNB has frequently changed. Decisions about performing susceptibility testing is further complicated by the fact that no interpretative breakpoints have been established for most of the unusual NF-GNB. Furthermore, the results obtained with some organisms by the disk diffusion method do not correlate with those obtained by conventional MIC methods. Thus, clinical microbiology laboratories could face problems in identifying and susceptibility testing these pathogens.

We report the antimicrobial susceptibility profile of unusual NF-GNB isolated from the Latin American medical centers that participate in the SENTRY Antimicrobial Surveillance Program.

MATERIAL & METHODS

Bacterial strains. A total of 176 unusual NF-GNB were collected from the Latin America region through the SENTRY Program between January 1997 and December 2002. The distribution of species is shown in Table 1. All strains were isolated from hospitalized patients. Only a single isolate per patient was evaluated. The isolates were identified to the species level by the participant medical center and sent to the coordinating laboratory for identification confirmation and reference susceptibility testing.

Medical centers. The participant medical centers were distributed throughout twelve cities in seven countries: Brasília (2001-2002), Florianópolis (1997-2002), Rio de Janeiro (1997-1998), São Paulo (1997-2002), and Porto Alegre (1999-2002) in Brazil; Buenos Aires (1997-2002) and San Isidro/Rosario (1997-2002) in Argentina; Santiago in Chile (2 sites, 1997-2000); Medellin in Colombia (1997-2000); Mexico City in Mexico (3 sites, 1997-2002); Montevideo, Uruguay (1997); and Caracas in Venezuela (1998-2002).

Susceptibility testing. Antimicrobial susceptibility testing was performed using the reference broth microdilution method as described by the NCCLS. The susceptibility and resistance rates were calculated according to the NCCLS breakpoints established for testing non-Enterobacteriaceae isolates (M100-S13). Antimicrobial agents were obtained from the respective manufacturers. Quality control was performed by testing *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212.

COMMENTS

- *B. cepacia* (45.5%) was the most frequently isolated NF-GNB from the Latin American medical centers, followed by *Achromobacter xylosidans* (12.5%) and *Ralstonia pickettii* (9.1%).
- The majority (67.0%) of NF-GNB strains were isolated from bloodstream infections. *Cryseobacterium* spp. strains were equally isolated from blood and respiratory tract, and *Alcaligenes* spp. strains were more frequently isolated from skin and soft tissue infections. All other pathogens were more frequently isolated from bloodstream infections.
- Brazil contributed with the largest number of strains (91; 51.7%) followed by Argentina (32; 18.1%) and Colombia (20, 11.4%). A Brazilian medical center (number 048) provided 25.0% of the NF-GNB strains.
- The most active antimicrobial agents overall were levofloxacin (78.3% susceptible) followed by gatifloxacin (75.6%) > meropenem (72.6%) > imipenem (69.9%) > trimethoprim/sulfamethoxazole (68.6%) > piperacillin/tazobactam (67.4%).
- Although levofloxacin (MIC_{50}, 1 μg/ml; 78.3% susceptible) and gatifloxacin (MIC_{50} 1 μg/ml; 75.6% susceptible) were the most active compounds, the other fluoroquinolone evaluated (ciprofloxacin) was active against only 61.4% of strains at the NCCLS susceptible breakpoint established for non-Enterobacteriaceae.
- The aminoglycosides amikacin and gentamicin demonstrated poor in vitro activity against NF-GNB inhibiting less than 30.0% of strains at the NCCLS susceptible breakpoints established for non-Enterobacteriaceae.
- The most active antimicrobial agents against *Burkholderia* spp. were ceftazidime (83.1% susceptible) > levofloxacin (81.9%) > gatifloxacin (79.5%) = meropenem (79.5%).
- The carbapenems meropenem and imipenem were the most active compounds against *Achromobacter* spp. (84.0 - 88.0% susceptible) and *Alcaligenes* spp. (100.0% susceptible), while the fluoroquinolones gatifloxacin and levofloxacin were the most active compounds against *Chryseobacterium* spp. (75.0% susceptible).
- Gatifloxacin (MIC_{50}, 0.25 μg/ml; 87.5% susceptible), ceftriaxone (MIC_{50}, 1 μg/ml; 87.5% susceptible) and piperacillin/tazobactam (MIC_{50}, 8 μg/ml; 87.5% susceptible) were the most active antimicrobial agents against *Ralstonia pickettii*, followed by cefepime, imipenem, levofloxacin and ciprofloxacin (81.3% susceptible; Table 4).

Table 1. Frequency of occurrence of nonfermentative Gram-negative bacilli isolated from Latin American medical centers (SENTRY Program, 1997-2002).

Organism	n (%)	Organism	n (%)
Achromobacter spp.[a]	25 (14.2)	*Ralstonia pickettii*	16 (9.1)
Alcaligenes spp.[b]	12 (6.8)	*Ochrobactrum antropi*	8 (4.5)
Burkholderia spp.[c]	83 (47.2)	*Pseudomonas oryzihabitans*	7 (4.0)
Chryseobacterium spp.[d]	12 (6.8)	Others[e]	9 (5.1)
Comamonas acidovorans	4 (2.3)	Total	176 (100.0)

a. Includes *Achromobacter xylosidans* (22) and *Achromobacter* spp. (3);
b. Includes *Alcaligenes faecalis* (6) and *Alcaligenes* spp. (6);
c. Includes *Burkholderia cepacia* (80) and *Burkholderia gladioli* (3);
d. Includes *Chryseobacterium indologenes* (6) and *Chryseobacterium meningosepticum* (6);
e. Includes CDC Group IVC2 (1), *Empedobacter brevis* (2), *Myroides odoratum* (1), *Sphingomonas paucimobilis* (2), *Sphingobacterium* spp. (1), and other NF-GNB (2).

Table 2. Distribution of the nonfermentative Gram-negative bacilli according to the site of infection (SENTRY Program, Latin America 1997 - 2002).

Organism (n)	Blood n(%)	Respiratory tract n(%)	SST[a] n(%)	Urine n(%)
Achromobacter spp.[b] (25)	16 (64.0)	8 (32.0)	1(4.0)	-
Alcaligenes spp.[c] (12)	3 (25.0)	3 (25.0)	5 (41.7)	1 (8.3)
Burkholderia spp.[d] (83)	52 (62.7)	25 (30.1)	3 (3.6)	3 (3.6)
Chryseobacterium spp.[e] (12)	6 (50.0)	6 (50.0)	0 (0.0)	0 (0.0)
Ralstonia pickettii (16)	16 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others[f] (28)	25 (89.3)	2 (7.1)	1 (3.6)	0 (0.0)
Total	118 (67.0)	44 (25.0)	10 (5.7)	4 (2.3)

a. SST = skin and soft tissue.
b. Includes *Achromobacter xylosidans* (22) and *Achromobacter* spp. (3).
c. Includes *Alcaligenes faecalis* (6) and *Alcaligenes* spp. (6).
d. Includes *Burkholderia cepacia* (80) and *Burkholderia gladioli* (3).
e. Includes *Chryseobacterium indologenes* (6) and *Chryseobacterium meningosepticum* (6).
f. Includes CDC Group IVC2 (1), *Comamonas acidovorans* (4), *Empedobacter brevis* (2), *Pseudomonas oryzihabitans* (7), *Myroides odoratum* (1), *Sphingomonas paucimobilis* (2), *Sphingobacterium* spp. (1), and other NF-GNB (10).

Table 3. In vitro activity of selected antimicrobial agents against the unusual nonfermentative Gram-negative bacilli isolated from the Latin American region (SENTRY Program, 1997 - 2002).

	Cumulative percentage inhibited at MIC (μg/ml):											
Antimicrobial agents	0.12	0.25	0.5	1	2	4	8	16	32	64	$MIC_{50/90}$ (μg/ml)[a]	% Susceptible[b]
β-lactams												
Aztreonam	0.0	0.0	0.0	0.0	2.8	6.9	12.6	23.4	-[c]	-	>16/>16	12.6
Pip/Taz[d]	-	-	13.7	24.0	37.7	52.6	59.4	67.4	78.3	84.0	4/>64	67.4
Ceftriaxone	-	3.4	7.4	11.4	15.3	21.0	30.1	44.3	56.3	-	32/>32	30.1
Ceftazidime	0.0	1.1	8.6	10.2	25.0	52.3	65.3	75.0	-	-	4/>16	65.3
Cefepime	1.1	4.0	5.1	9.7	20.5	28.4	48.3	70.5	-	-	16/>16	48.3
Imipenem	4.0	10.2	21.0	34.1	52.8	69.9	78.4	-	-	-	2/>8	69.9
Meropenem	8.6	20.0	30.9	45.1	60.6	72.6	78.3	-	-	-	2/>8	72.6
Aminoglycosides												
Amikacin	-	-	1.1	5.1	6.3	9.7	14.9	18.3	28.6	44.6	>32/>32	28.6
Gentamicin	-	-	6.8	14.8	19.9	24.4	26.1	-	-	-	>8/>8	24.4
Fluoroquinolones												
Ciprofloxacin	6.3	20.5	47.7	61.4	76.7	-	-	-	-	-	1/>2	61.4
Gatifloxacin	10.2	20.5	40.9	60.2	75.6	88.1	-	-	-	-	1/>4	75.6
Levofloxacin	-	-	44.0	60.6	78.3	90.3	-	-	-	-	1/4	78.3
Others												
Tetracyclines	-	-	-	-	-	29.5	38.6	-	-	-	>8/>8	29.5
Trim/Sulfa[e]	-	-	68.6	76.0	-	-	-	-	-	-	≤0.5/≥2	68.6

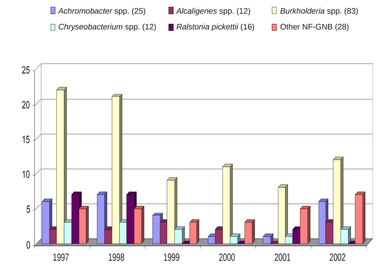
a. Minimal inhibitory concentration (MIC) was determined by broth microdilution method.
b. Susceptibility rates calculated according to the criteria published by the NCCLS for testing non-Enterobacteriaceae except for trimethoprim/sulfamethoxazole. Isolates exhibiting MICs ≥ 1 μg/ml were considered as resistant to trimethoprim/sulfamethoxazole.
c. - Untested concentration.
d. Pip/Taz, piperacillin/tazobactam; Trim/Sulfa, trimethoprim/sulfamethoxazole.

Table 4. Antimicrobial activity of selected antimicrobial agents against the main genera of the nonfermentative Gram-negative bacilli isolated in Latin America (SENTRY Program, 1997 - 2002).

Bacterial species (n)/antimicrobial agent	MIC (μg/ml)[a] MIC_{50}	MIC_{50}	% Susceptible[b]	% Resistant[c]
Achromobacter spp.[d] (25)				
Piperacillin/tazobactam	1	64	76.0	8.0
Ceftazidime	8	>16	64.0	16.0
Cefepime	16	>16	24.0	36.0
Imipenem	2	8	84.0	8.0
Meropenem	0.25	8	88.0	4.0
Ciprofloxacin	2	>2	32.0	48.0
Gatifloxacin	2	>4	60.0	12.0
Levofloxacin	2	>4	68.0	16.0
Amikacin	>32	>32	16.0	72.0
Trimethoprim/sulfamethoxazole	≤0.5	>2	68.0	32.0
Alcaligenes spp.[e] (12)				
Piperacillin/tazobactam	≤0.5	32	83.3	8.3
Ceftazidime	4	>16	75.0	25.0
Cefepime	8	>16	58.3	8.3
Imipenem	1	2	100.0	0.0
Meropenem	0.25	0.5	100.0	0.0
Ciprofloxacin	1	>2	58.3	25.0
Gatifloxacin	1	>4	66.7	25.0
Levofloxacin	1	>4	66.7	25.0
Amikacin	16	>32	50.0	41.7
Trimethoprim/sulfamethoxazole	≤0.5	>2	66.7	33.3
Burkholderia spp.[e] (83)				
Piperacillin/tazobactam	8	64	67.5	9.6
Ceftazidime	4	16	83.1	6.0
Cefepime	8	>16	51.8	30.1
Imipenem	4	>8	60.2	26.5
Meropenem	2	>8	79.5	12.0
Ciprofloxacin	1	>2	61.4	18.1
Gatifloxacin	1	>4	79.5	10.8
Levofloxacin	1	4	81.9	8.4
Amikacin	>32	>32	18.1	60.2
Trimethoprim/sulfamethoxazole	≤0.5	>1	71.1	28.9
Chryseobacterium spp.[f] (12)				
Piperacillin/tazobactam	4	>64	58.3	25.0
Ceftazidime	>16	>16	41.7	58.3
Cefepime	16	>16	41.7	41.7
Imipenem	>8	>8	0.0	100.0
Meropenem	>8	>8	0.0	100.0
Ciprofloxacin	0.5	>2	66.7	25.0
Gatifloxacin	0.5	>4	75.0	25.0
Levofloxacin	0.5	4	75.0	0.0
Amikacin	>32	>32	0.0	75.0
Trimethoprim/sulfamethoxazole	>1	>1	36.4	63.6
Ralstonia pickettii (16)				
Piperacillin/tazobactam	8	32	87.5	6.3
Ceftriaxone	1	>32	87.5	12.5
Ceftazidime	>16	>16	18.8	62.5
Cefepime	2	>16	81.3	18.8
Imipenem	2	8	81.3	6.3
Meropenem	8	>8	43.8	50.0
Ciprofloxacin	0.25	>2	81.3	12.5
Gatifloxacin	0.25	4	87.5	6.3
Levofloxacin	≤0.5	4	81.3	6.3
Amikacin	16	>32	56.3	31.3
Tetracycline	≤4	>8	68.8	18.8
Trimethoprim/sulfamethoxazole	≤0.5	>1	87.5	12.5

a. Minimal inhibitory concentration (MIC) was determined by broth microdilution method.
b. Susceptibility rates calculated according to the criteria published by the NCCLS for testing non-Enterobacteriaceae except for trimethoprim/sulfamethoxazole. Isolates exhibiting MICs ≥ 4 μg/ml were considered as resistant to trimethoprim/sulfamethoxazole.
c. Includes *Achromobacter xylosidans* (22) and *Achromobacter* spp. (3).
d. Includes *Alcaligenes faecalis* (6) and *Alcaligenes* spp. (6).
e. Iudes *Burkholderia cepacia* (80) and *Burkholderia gladioli* (3).
f. Includes *Chryseobacterium indologenes* (6) and *Chryseobacterium meningosepticum* (6).

Figure 1. Distribution of the 176 nonfermentative Gram-negative bacilli strains isolated from Latin American medical centers listed according to the bacterial genera and year of isolation (SENTRY Program, 1997-2002).



CONCLUSIONS

- Nonfermentative Gram-negative bacilli other than *P. aeruginosa* and *Acinetobacter* spp. are uncommon pathogens; however, they represent a real challenge for the routine clinical microbiology laboratories since species identification is complex and antimicrobial susceptibility profiles are unpredictable. In this context, surveillance programs such as the SENTRY Program, are very helpful by providing the most common susceptibility patterns of these infrequent pathogens and guide the best empiric antimicrobial treatment of these infections.

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