Assessment of Pathogens and Resistance Patterns among Bacterial Isolates from Pediatric Patients Hospitalized in Latin American Intensive Care Units: Report of the SENTRY Program (2003)

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AMENDED ABSTRACT

Objective: To assess the frequency of occurrence and the resistance patterns (RP) of pathogens causing infections in pediatric patients hospitalized in ICUs of Latin American medical centers (LAMC) participating in the SENTRY Program, a unique data analysis.
Methods: Following a SENTRY objective, the LAMCs were asked to submit the first 50 consecutive bacterial isolates from any source of infection beginning on JAN/2003. The susceptibility (S) to antimicrobial agents was tested by NCCLS broth microdilution methods to >30 drugs. Imipenem (IMI)-resistant (R) *P. aeruginosa* (PSA) isolates were ribotyped and screened for metallo-ß-lactamase (M9L).
Results: 344 isolates were collected from 9 LAMC (4 countries). Frequency of recovery was: *S. aureus* (16.3%) > PSA (11.6%) > coag. negative staphylococci (CoNS; 11.4%) > *E. coli* (EC; 10.5%) > *Klebsiella* spp. (KSP; 9.9%) > ß-haemolytic streptococci (ßHS; 8.5%). *S. aureus* S to oxacillin, ciprofloxacin (CIP), gatifloxacin and linezolid were 60.7%, 64.3%, 96.4% and 100.0%, respectively. Polymyxin B ($MIC_{90} \leq 1$ µg/ml; 100.0% S) was the most active agent against PSA followed by CIP (MIC_{50}, 0.12 µg/ml; 75.0% S). PSA S to IMI and meropenem were 52.5% and 67.5%, respectively. Only 2 clusters of IMI-R PSA were observed: Brasilia (2 strains) and Buenos Aires (3 strains); and no M9L-producing strain was detected. Overall, 19.4% of *E. coli* and 56.0% of KSP isolates showed an ESBL phenotype with high rates of cross-resistance to other agents. No penicillin-resistant or macrolide-resistant ßHS were detected.
Conclusions: The occurrence of pathogens found in the pediatric population seen in LAMC was similar to that observed for adult patients in ICUs. Higher rates of resistance to oxacillin among CoNS and to carbapenem among PSA isolates were documented. In contrast, the quinolones remained very active against most of the pathogens evaluated, possibly due to limited use in this population.

INTRODUCTION

Nosocomial infections in pediatric patients may differ from those in adult patients in several aspects, including site of infection and etiology. Viral infections of the upper respiratory and gastrointestinal tracts are the most common type of nosocomial infection among patients hospitalized in general pediatric ward. In contrast, urinary tract infection is the most common type of nosocomial infection in adult patients. Among neonatal and pediatric intensive care unit (ICU) patients, the bloodstream is the most common site of nosocomial infection, followed by the lower respiratory tract while among adult ICU patients, the lower respiratory tract is the most common site.

The antimicrobial susceptibility pattern of isolates causing infections in pediatric patients may also vary from those causing infections in adults. The main objective of this study was to assess the frequency of occurrence and the resistance patterns of bacterial pathogens causing infections in pediatric patients hospitalized in ICUs of the Latin American medical centers participating in the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Protocol Design. The SENTRY Antimicrobial Surveillance Program participating medical centers were guided by a common protocol. Each medical center submitted the first 50 consecutive bacterial isolates collected from patients hospitalized in pediatric ICUs beginning on JAN/2003. The isolates were identified to the species level by the participant medical center and shipped to the monitoring laboratory (JMI Laboratories, North Liberty, IA, USA) for identification confirmation and reference antimicrobial susceptibility testing. Only a single isolate per patient could be referred to the monitoring center.

Medical centers. The participant medical centers were located in eight cities (four countries): Brasília, Florianópolis, Porto Alegre, and São Paulo in Brazil; Buenos Aires and San Isidro in Argentina; Santiago in Chile (two sites); and Caracas in Venezuela.

Susceptibility testing. Antimicrobial susceptibility testing was performed and interpreted following the guidelines for reference broth microdilution method as described by the NCCLS. Dry-form microdilution panels and broth for inoculation were purchased from Trek Diagnostics Inc. (Cleveland, OH, USA). Testing of quality control strains *Escherichia coli* ATCC 25922 and 35218, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Haemophilus influenzae* ATCC 49247 and 49766, and *Streptococcus pneumoniae* ATCC 49619 was performed for quality assurance purposes. *E. coli* and *Klebsiella pneumoniae* isolates with increased MIC results (≥ 2 µg/ml) for ceftazidime and/or ceftriaxone and/or aztreonam were considered extended-spectrum ß-lactamase (ESBL)-producing phenotypes according to NCCLS criteria. The production of ESBL was confirmed by disk approximation test.

Metallo-ß-lactamase (M8L) screening. Among other selected pathogens, *P. aeruginosa* strains resistant to imipenem (MIC, ≥ 16 µg/ml), meropenem (MIC, ≥ 16 µg/ml), and ceftazidime (MIC, $\geq$ 32 µg/ml) were routinely screened for production of M6Ls by a disk approximation test. Briefly, a 100mm Mueller-Hinton agar plate was inoculated using a 0.5 McFarland suspension from a fresh culture. Imipenem, meropenem, and ceftazidime disks were strategically aligned around disks contained either EDTA (750 µg) or 2-mercaptoproioinic acid (MPA, 360 µg). The appearance of growth-inhibitory zone between the carbapenems and/or ceftazidime and either one of the disks containing the M8L inhibitor (EDTA or MPA) was considered a positive test.

Molecular typing. Multiple isolates of imipenem-resistant *P. aeruginosa* (MIC, ≥ 16 µg/ml) from the same medical center were molecularly typed by ribotyping. Ribotyping was performed by using automated Riboprinter Microbial characterization system (DuPont Qualicon, Inc.). In short, genomic DNA was isolated and digested using EcoRI. DNA was separated by agarose gel electrophoresis. Southern hybridization using probes derived from *E. coli* rRNA operon created characteristic band patterns. These patterns were matched to pre-existing patterns by computer analysis and those with $\geq$ 93% identity were assigned to the same ribogroup.

RESULTS

- A total of 344 bacterial isolates were collected from pediatric ICU patients hospitalized in the Latin American medical centers. Only 4.8% of the pathogens were isolated from neonatal ICU patients. Most of the isolates were recovered from Brazil (46.5%), followed by Chile (26.2%) > Argentina (22.9%) > Venezuela (4.4%).
- In general, bloodstream (38.1%) was the most frequent body site of infection, followed by the respiratory tract (19.8%) > urine (12.2%) > skin and soft tissue (10.4%).
- Overall, *S. aureus* (16.3%) was the most frequently isolated pathogen followed by *P. aeruginosa* (11.6%) > CoNS (11.4%) > *E. coli* (10.5%) > *Klebsiella* spp. (9.9%; Table 1).
- Variations in the pathogen rank order among the Latin American nations might have been influenced by the frequency of the body site of infection in each center (Table 2). For example, *E. coli* was the most frequently isolated pathogen in the Argentinean medical centers probably because the urinary tract was the most frequent site of infection (34.2%) in these medical centers.
- *S. aureus* was the most frequently isolated pathogen in the age groups of $\leq$ 1 month (43.7%) and 2 months-1y-o (17.8%). Conversely, *P. aeruginosa* (21.7%) and *E. coli* (22.2%) were the most frequently isolated pathogens in the age categories of 2-6 y-o and > 6 y-o, respectively (Table 3).
- Oxacillin-resistance was observed in 84.6% of CoNS and 39.3% of *S. aureus* isolates. In addition, 5.1% of CoNS strains showed reduced susceptibility to teicoplanin. All staphylococci isolates were susceptible to linezolid (MIC_{50}, ≤ 2 µg/ml), vancomycin (MIC_{50}, 1 µg/ml), and quinupristin/dalfopristin ($MIC_{90} \leq 0.5$ µg/ml). Among the fluoroquinolones, gatifloxacin was the most active compound against CoNS (MIC_{50}, 0.12 µg/ml; 97.4% susceptible) and oxacillin-resistant *S. aureus* (MIC_{90}, 2 µg/ml; 90.9% susceptible; Table 4).
- ß-haemolytic streptococci (ßHS) were responsible for 8.5% of the infections in the Latin American pediatric ICU patients in the period evaluated. However, > 80% of strains were sent by three medical centers located in Chile and Venezuela. No resistance to penicillin or macrolides was noticed. On the other hand, 31.0% of strains showed resistance to tetracycline (Table 5).
- Overall, 19.4% of *E. coli* and 56.0% of *Klebsiella* spp. isolates showed an ESBL phenotype with high rates of cross-resistance to other antimicrobial agents. Against *E. coli*, the highest susceptibility rate was achieved by imipenem (100.0%) followed by amikacin (91.7%) > piperacillin/tazobactam (88.9%) > gatifloxacin = levofloxacin (86.1%).

Table 1. Frequency of the bacterial pathogens causing infections in Pediatric ICU patients hospitalized in the Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2003).

Rank Order	Microorganism	No. of isolates (%)
1	*S. aureus*	56 (16.3)
2	*P. aeruginosa*	40 (11.6)
3	CoNS[a]	39 (11.4)
4	*E. coli*	36 (10.5)
5	*Klebsiella* spp.[b]	34 (9.9)
6	Beta-haemolytic streptococci[c]	29 (8.5)
7	*Enterobacter* spp.[d]	19 (6.5)
8	*Haemophilus* spp.[e]	15 (4.4)
9	*Acinetobacter* spp.[f]	14 (4.1)
	Serratia spp.[g]	14 (4.1)
11	*Stenotrophomonas maltophilia*	13 (3.8)

a. Includes: *Staphylococcus capitis* (1 strain), *Staphylococcus epidermidis* (9 strains), *Staphylococcus haemolyticus* (5 strains), *Staphylococcus hominis* (1 strain), *Staphylococcus warneri* (3 strains), and coagulase-negative *staphylococcus* (20 strains).
b. Includes: *Klebsiella pneumoniae* (29 strains), *K. oxytoca* (4 strains), and *Klebsiella* spp. (1 strain).
c. Includes: Group A *Streptococcus* (23 strains), Group B *Streptococcus* (4 strains), Group C *Streptococcus* (1 strain), and beta haemolytic *Streptococcus* (1 strain).
d. Includes: *E. aerogenes* (4 strains), *E. cloacae* (9 strains), and *Enterobacter* spp. (6 strains).
e. Includes: *H. influenzae* (13 strains), and *H. parainfluenzae* (2 strains).
f. Includes: *A. baumannii* (10 strains), *A. lwoffii* (2 strains), and *Acinetobacter* spp. (2 strains).
g. Includes: *S. marcescens* (11 strains), and *Serratia* spp. (3 strains).

Table 2. Occurrence of the most frequently isolated pathogens from pediatric ICU patients hospitalized in Latin American medical centers listed by nation (SENTRY Program, 2003).

Rank Order	Nation			
	Argentina	Brazil	Chile	Venezuela
	N=79 (%)	N=160 (%)	N=90 (%)	N=15 (%)
1	*E. coli* (24.1)	*S. aureus* (18.8)	*S. aureus* (23.3)	ßHS[b] (60.0)
2	*Klebsiella* spp. (17.8)	CoNS[a] (18.1)	ßHS[b] (16.6)	CoNS[a] (20.0)
3	*P. aeruginosa* (16.4)	*Klebsiella* spp. (10.0)	*Haemophilus* spp. (15.5)	*E. coli* (6.7)
				Klebsiella spp. (6.7)
4	*S. aureus* (7.6)	*P. aeruginosa* (8.8)	*P. aeruginosa* (14.4)	-
	Enterobacter spp. (7.6)			
5	CoNS[a] (6.3)	*Enterobacter* spp. (8.1)	*S. pneumoniae* (8.9)	-
	S. maltophilia (6.3)			

a. CoNS, coagulase-negative staphylococci.
b. ßHS, ß-haemolytic streptococci.

Table 3. Occurrence of the top ten pathogens isolated from pediatric ICU patients hospitalized in Latin American medical centers listed by age category (SENTRY Antimicrobial Surveillance Program, 2003).

Rank order	Age Category (% Frequency)			
	≤ 1 month	2 months-1 y-o	2-6 y-o	> 6 y-o
	(N=16)	(N=196)	(N=69)	(N=54)
1	*S. aureus* (43.7)	*S. aureus* (17.8)	*P. aeruginosa* (21.7)	*E. coli* (22.2)
2	*E. coli* (12.5)	CoNS[a] (15.3)	ßHS[b] (14.4)	*S. aureus* (14.8)
3	*K. pneumoniae* (12.5)	*P. aeruginosa* (9.7)	*E. coli* (10.1)	*Klebsiella* spp. (11.1)
4	CoNS[a] (12.5)	*Klebsiella* spp. (9.7)	*S. aureus* (8.7)	*P. aeruginosa* (11.1)
5	*Proteus* spp. (6.2)	*Enterobacter* spp. (8.2)	*Klebsiella* spp. (8.7)	ßHS[b] (9.2)
6	*Enterococcus* spp. (6.2)	*E. coli* (7.1)	*Haemophilus* spp. (5.8)	*Acinetobacter* spp. (7.4)
7	*Haemophilus* spp. (6.2)	ßHS[b] (6.1)	*Enterococcus* spp. (4.3)	*S. maltophilia* (7.4)
8	-	*Serratia* spp. (5.6)	*S. maltophilia* (4.3)	*Haemophilus* spp. (7.4)
9	-	*Acinetobacter* spp. (4.1)	CoNS[a] (4.3)	*Burkholderia* spp. (1.9)
10	-	*S. pneumoniae* (3.6)	*S. pneumoniae* (4.3)	*Citrobacter* spp. (1.9)

a. CoNS, Coagulase-negative staphylococci.
b. ßHS, ß-haemolytic streptococci.

Table 4. Antimicrobial activity of 13 drugs tested against *Staphylococcus* spp. causing infections in pediatric ICU patients hospitalized in the Latin American Medical centers (SENTRY Program, 2003).

	CoNS[a] (39)			Oxacillin-susceptible *S. aureus* (34)			Oxacillin-resistant *S. aureus* (22)		
	MIC (µg/ml)[b]			MIC (µg/ml)[b]			MIC (µg/ml)[b]		
Antimicrobial agent	MIC_{50}	MIC_{90}	%S[c]/%R[c]	MIC_{50}	MIC_{90}	%S[c]/%R[c]	MIC_{50}	MIC_{90}	%S[c]/%R[c]
Oxacillin	>2	>2	15.4/84.6	0.5	1	100.0/0.0	>2	>2	0.0/100.0
Ceftriaxone	16	>32	35.9/10.3	4	4	94.1/0.0	>32	>32	0.0/86.4
Cefepime	4	16	79.5/7.7	2	4	100.0/0.0	>16	>16	13.6/86.4
Clindamycin	0.12	>8	56.4/43.6	0.12	0.12	100.0/0.0	>8	>8	22.7/77.3
Gentamicin	8	>8	38.5/46.2	≤2	≤2	94.1/5.9	>8	>8	13.6/77.3
Vancomycin	1	2	100.0/0.0	1	1	100.0/0.0	1	1	100.0/0.0
Teicoplanin	≤2	8	94.9/0.0	≤2	≤2	100.0/0.0	≤2	≤2	100.0/0.0
Quin/Dal[d]	≤0.25	0.5	100.0/0.0	≤0.25	0.5	100.0/0.0	0.5	0.5	100.0/0.0
Linezolid	1	1	100.0/-[e]	2	2	100.0/-[e]	1	2	100.0/-[e]
Ciprofloxacin	0.25	>4	61.5/38.5	0.25	0.5	97.1/2.9	>4	>4	13.6/86.4
Levofloxacin	0.25	>4	64.1/20.5	0.12	0.25	100.0/0.0	4	>4	13.6/18.2
Gatifloxacin	0.12	2	97.4/0.0	0.06	0.12	100.0/0.0	2	2	90.9/0.0
Trim/Sulfa[f]	2	>2	53.8/46.2	≤0.5	≤0.5	100.0/0.0	≤0.5	>2	68.2/31.8

a. Includes: *Staphylococcus capitis* (1 strain), *Staphylococcus epidermidis* (9 strains), *Staphylococcus haemolyticus* (5 strains), *Staphylococcus hominis* (1 strain), *Staphylococcus warneri* (3 strains), and Coagulase-negative *staphylococcus* (20 strains).
b. Minimal inhibitory concentration (MIC) determined by broth microdilution method [NCCLS, 2003].
c. Percentages of susceptibility (%S) and resistance (%R) calculated by the NCCLS criteria [2004].
d. Breakpoints not established by the NCCLS criteria.
e. Quinupristin/Dalfopristin
f. Trimethoprim/Sulfamethoxazole

- Among *P. aeruginosa*, only 52.5% and 67.5% of the isolates were susceptible to imipenem and meropenem, respectively. Polymyxin B ($MIC_{50} \leq 1$ µg/ml; 100.0% susceptible) was the most active agent, followed by ciprofloxacin (MIC_{50}, 0.12 µg/ml; 75.0% susceptible).
- Imipenem-resistant *P. aeruginosa* (IMI-R PSA) strains were detected in all Latin American medical centers evaluated, except medical center 043 located in Santiago, Chile. No M8L-producing isolate was detected. Two small clusters of IMI-R PSA were identified in Argentinean (three strains, ribogroup 197.8) and Brazilian (two strains, ribogroup 658.2) medical centers (Table 7).

Table 5. Antimicrobial activity of 13 drugs tested against 29 ß-hemolytic streptococci causing infections in pediatric ICU patients hospitalized in the Latin American Medical centers (SENTRY Antimicrobial Surveillance Program, 2003).

	ß-haemolytic streptococci[a] (29)		
	MIC (µg/ml)[b]		
Antimicrobial agent	MIC_{50}	MIC_{90}	%S[c]/%R[c]
Penicillin	≤0.016	0.06	100.0/-[d]
Oxacillin	≤0.25	0.5	-[d]/-[d]
Erythromycin	≤0.06	≤0.06	100.0/0.0
Ceftriaxone	≤0.25	≤0.25	100.0/-[d]
Cefepime	≤0.12	≤0.12	100.0/-[d]
Clindamycin	≤0.06	≤0.06	100.0/0.0
Vancomycin	0.25	0.5	100.0/-[d]
Teicoplanin	≤2	≤2	-[d]/-[d]
Quinupristin/Dalfopristin	≤0.25	≤0.25	-[d]/-[d]
Linezolid	1	1	100.0/-
Levofloxacin	0.5	1	100.0/0.0
Gatifloxacin	0.25	0.25	100.0/0.0
Trimethoprim/Sulfamethoxazole	≤0.5	≤0.5	-[d]/-[d]

a. Includes: group A *Streptococcus* (23 strains), group B *Streptococcus* (4 strains), group C *Streptococcus* (1 strain), and ß-haemolytic *Streptococcus* (1 strain).
b. Minimal inhibitory concentration (MIC) determined by broth microdilution method.
c. Percentages of susceptibility (%S) and resistance (%R) calculated by the NCCLS criteria.
d. Breakpoints have not been established by the NCCLS.

Table 6. Antimicrobial activity of 14 drugs tested against the most prevalent gram-negative bacilli causing infections in pediatric ICU patients hospitalized in the Latin American Medical centers (SENTRY Program, 2003).

	Escherichia coli (36)			*Klebsiella pneumoniae* (29)			*Pseudomonas aeruginosa* (40)		
	MIC (µg/ml)[a]			MIC (µg/ml)[a]			MIC (µg/ml)[a]		
Antimicrobial agent	MIC_{50}	MIC_{90}	%S[b]/%R[b]	MIC_{50}	MIC_{90}	%S[b]/%R[b]	MIC_{50}	MIC_{90}	%S[b]/%R[b]
Aztreonam	≤0.12	>16	83.3/16.7	16	>16	48.3/41.4	8	>16	57.5/22.5
Pip/Taz[c]	2	32	88.9/5.6	4	>64	86.2/10.3	8	256	80.0/20.0
Cefoxitin	4	8	94.4/5.6	4	16	89.7/0.0	>32	>32	-/-[d]
Ceftriaxone	≤0.25	>32	80.6/19.4	>32	>32	37.9/58.6	>32	>32	2.5/80.0
Ceftazidime	≤1	4	97.2/2.8	4	>16	65.5/27.6	4	>16	62.5/32.5
Cefepime	≤0.12	>16	83.3/16.7	8	>16	62.1/27.6	4	16	67.5/10.0
Imipenem	≤0.5	≤0.5	100.0/0.0	≤0.5	≤0.5	100.0/0.0	2	>8	52.5/32.5
Meropenem	0.03	≤0.06	100.0/0.0	0.03	0.06	100.0/0.0	2	>8	67.5/22.5
Ciprofloxacin	≤0.03	>4	83.3/16.7	≤0.03	0.5	93.1/3.4	0.12	>4	75.0/22.5
Levofloxacin	≤0.03	4	86.1/8.3	0.06	0.5	96.6/3.4	0.5	>4	75.0/25.0
Gatifloxacin	≤0.03	4	86.1/8.3	0.06	1	96.6/3.4	1	>4	67.5/20.0
Amikacin	2	16	91.7/2.8	8	>32	86.2/10.3	4	32	82.5/10.0
Gentamicin	≤2	>8	75.0/25.0	8	>8	48.3/48.3	≤2	>8	67.5/27.5
Polymyxin B	≤1	≤1	97.2/2.8[e]	≤1	≤1	94.2/5.8[e]	≤1	≤1	100.0/0.0[e]

a. Minimal inhibitory concentration (MIC) determined by broth microdilution method.
b. Percentages of susceptibility (%S) and resistance (%R) calculated by the NCCLS criteria.
c. Breakpoints have not been established by the NCCLS.
d. Percentages of susceptibility and resistance calculated by the NCCLS criteria for *Acinetobacter* spp. [2005, in press].
e. Piperacillin/Tazobactam

Table 7. Molecular typing results of imipenem resistant *P. aeruginosa* (MIC, ≥ 16 µg/ml) strains.

Isolate #	Medical center	Patient age (years)	Culture date	Specimen type	Ribotyping
359	039	1	12/3/2003	Catheter	ND
9	040	2	7/14/2003	BAL[a]	ND
10	040	3	7/14/2003	Other	197.8
62	040	2	8/12/2003	Blood	197.8
183	040	<1	10/3/2003	Catheter	197.8
78	040	9	9/5/2003	Other	1033.3
1	042	5	6/23/2003	Sputum	241.1
34	042	<1	7/31/2003	Bronchial wash	241.4
19	042	11	7/14/2003	Catheter	1421.2
154	048	7	6/17/2003	Tracheal aspirate	ND
174	057	7	10/19/2003	-	ND
253	101	<1	3/30/2003	CSF[b]	658.2
268	101	<1	5/22/2003	Eye secretions	658.2

a. Bronchoalveolar lavage.
b. Cerebrospinal fluid.

CONCLUSIONS

- The rank order of pathogens causing infections in pediatric ICU patients hospitalized in Latin American medical centers was similar to those published in literature, and slightly different from those found in adult patients. A higher proportion of ß-haemolytic streptococci and *H. influenzae* was observed in pediatric ICU patients when compared to the general adult population.
- High rates of antimicrobial resistance were encountered among bacterial isolates collected from this pediatric population.
- The results provided by comprehensive surveillance programs, such as the SENTRY Program, allows for the characterization of local patterns of resistance and represent an important tool for guiding empiric antimicrobial therapy.

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