MEDICAL MICROBIOLOGY

A Comparison of the Antimicrobial Resistance Patterns of Gram-Negative Bacilli Isolated from Community-Private and University-Affiliated Hospitals from Puerto Rico

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Few studies have been performed in Puerto Rico concerning the antimicrobial resistance pattern of clinically significant Gram-negative bacilli. The antimicrobial resistance patterns of 5,590 Gram-negative bacteria obtained from three Community-Private Hospitals (CPH) and three University-Affiliated Hospitals (UAH) were evaluated utilizing the institutions' antimicrobial susceptibility reports for the year 2000. The objectives of this study were: to retrospectively evaluate the reported in vitro resistance of clinical isolates of E. coli, K. pneumoniae, E. cloacae, S. marcescens, P. aeruginosa and A. baumannii to selected standard antibiotics and to compare the antimicrobial resistance patterns between Community-Private (CPH) and University Affiliated hospitals (UAH). E. coli was the most common Gram-negative enteric bacilli in both CPH and UAH. In UAH, E. coli demonstrated a statistically significant higher resistance to the selected beta lactams and amikacin antibiotics but not to ciprofloxacin or gentamicin. For K. pneumoniae, the antimicrobial resistant pattern showed that UAH isolates were significantly more resistant to the tested antibiotics with the exception of ceftriaxone. In CPH, E. cloacae isolates

were significantly more resistant to piperacillintazobactam, ciprofloxacin and gentamicin, while in UAH this organism was more resistant to amikacin. In UAH, S. marcescens isolates demonstrated a statistically significant higher resistance to all tested antibiotics with the exception of imipenem, which was similar in both hospitals group. Pseudomonas aeruginosa demonstrated a statistically significant higher resistance in UAH to all selected antibiotics with the exception of ciprofloxacin and gentamicin, which was similar in both hospitals group. Acinetobacter baumannii was the most resistant organisms in both hospitals group. UAH isolates were significantly more resistant than CPH isolates for all tested antibiotics. When compare with other large-scale antimicrobial resistance studies, the present study results suggest an apparent higher resistance in the Puerto Rican isolates. The high numbers of antimicrobial resistant Gram-negative bacilli in our study strongly suggest multiple mechanisms of antimicrobial resistance including the presence of extended spectrum and chromosomally derepressed beta-lactamases.

Key words: Gram-negative bacilli, Antimicrobial resistance, Antimicrobial agents

ntimicrobial resistant bacteria have become an important health issue in the past decades. Infections with these pathogens have been associated with significant increases in health care costs and patient morbidity and mortality. Among the 10 most frequently isolated bacterial pathogens from bloodstream

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Supported by the NIH-RCMI Program (Grant #: G12 RR0 3051), Deanship of Academic Affairs, Medical Science Campus, University of Puerto Rico.

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infections in the United States and Canada are the Gramnegative bacilli Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa, and Enterobacter spp.

The existence of several worldwide antimicrobial surveillance projects reflects the growing concern of emerging antibiotics resistant bacteria. Few studies however, have been performed in Puerto Rico concerning the antimicrobial resistance pattern of clinically significant Gram-negative organisms. In addition, the relation between types of hospital institutions (university affiliated versus community private hospitals) and their antibiotic susceptibility pattern has not been characterized.

The objectives of this study were: 1) to retrospectively evaluate the reported *in vitro* resistance of clinical isolates of *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella*

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pneumoniae, Serratia marcescens, Pseudomonas aeruginosa and Acinetobacter baumannii to selected standard antibiotics and 2) to compare the antimicrobial

resistant patterns between Community-Private (CPH) and University Affiliated hospitals (UAH).

Materials Methods

The antimicrobial susceptibility reports for the year 2000 were obtained from 6 Metropolitan San Juan area hospitals. The hospitals were divided in two groups: Community-Private Hospitals (CPH), which included 3 institutions with a total bed capacity of 772 and the University Affiliated Hospitals (UAH), also including 3 medical school affiliated hospitals with a

total capacity of 701 beds. From each hospital the following data was evaluated, tabulated and subjected to statistical analyses: 1) the total number of Gram-negative bacteria reported per hospital, and 2) the antimicrobial resistance of the selected organisms to standard antibiotics.

The following frequently used antibiotics were selected to evaluate the antimicrobial resistance pattern of the studied Gram-negative bacilli: for the *Enterobacteriacea Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae* and *Serratia marcescens*: piperacillin/tazobactam, ceftriaxone, cefotaxime, ceftazidime, cefepime, imipenem, ciprofloxacin, gentamicin and amikacin, and for the nonfermenter bacilli *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, the same antibiotics were utilized with the exception of ceftriaxone and cefotaxime, which were not evaluated.

Statistical analysis utilizing Chi Square test was performed to determine any significant differences between number of isolates and their resistance patterns obtained from CPH and UAH. A P value ≤0.05 was considered as being statistically significant.

Results

Of a total of 11,574 bacterial isolates reported from 6 hospitals (3 CPH and 3 UAH) for the year 2000, 8,052 institutional antimicrobial susceptibility reports of clinically significant Gram-positive and Gram-negative bacteria were selected for further evaluation. A total of 2,462 selected Gram-positive cocci and 5,590 selected Gram-negative bacilli were identified in the 6 hospitals. Table 1 shows the

total number of unselected and selected Gram-negative bacteria from each participating hospital. The following clinically significant Gram-negative bacilli were further

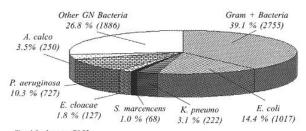
Table 1	1.	Total	Gram-Negative	Bacteria and	Selected	Gram-1	Vegative	Bacilli p	er Hospital
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Hosp	Total GNB Unselected			K. pneumoniae Total %		S. marcescens Total %		E. cloacae Total %		P. aeruginosa Total %		A. baumannii Total %		Total Selected GNB	
CPH															
Α	1286	169	19.7	56	8.7	30	18.2	64	22.0	230	29.3	53	12.0	602	
В	1245	485	56.7	93	14.5	14	8.5	18	6.2	71	9.0	15	3.4	696	
C	1766	363	42.4	73	11.4	24	14.5	45	15.5	426	54.3	182	41.4	1113	
Total	4297	1017	42.2	222	9.2	68	2.8	127	5.3	727	30.2	250	10.4	2411	
UAH															
D	1358	477	55.7	175	27.3	41	24.8	72	24.7	284	36.2	87	19.8	1136	
E	922	195	22.8	187	29.1	71	43.0	64	22.0	248	31.6	81	18.4	846	
F	1220	184	21.5	280	43.6	53	32.1	155	53.3	253	32.2	272	61.8	1197	
Total	3500	856	26.9	642	20.2	165	5.2	291	9.2	785	24.7	440	13.8	3179	
Grand Total	7797	1873	33.5	864	15.5	233	4.2	418	7.478	1512	27.0	690	12.3	5590	

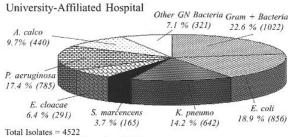
CPH = Community-Private Hospital, UAH = University-Affiliated Hospital, NR = Not Reported, GNB = Gram-negatives Bacteria, Unselected = All isolated Gram-negative bacteria, Selected = E. coli, K. pneumoniae, S. marcescens, E. cloacae, P. aeruginosa, and A. baumannii.

evaluated: Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcescens, Pseudomonas aeruginosa, and Acinetobacter baumannii (Figures 1 and 2). As shown in Figure 1, the Gram-negative

Community-Private Hospitals



Total Isolates = 7052
Total Gram-Negative Bacteria = 60.9 %
Total Gram-Positive Bacteria = 39.1 %



Total Gram-Negative Bacteria = 77.4 %
Total Gram-Positive Bacteria = 22.6 %

Figure 1. Total and percent of Gram-positive and negative bacteria isoleted from Community-Private and University-Affiliated Hospitals

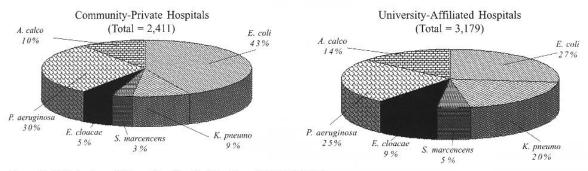


Figure 2. Distribution of Gram-Negative Isolates from CPH and UAH

common in UAH (77.4%) than in according to present and other published studies CPH (60.9%), while conversely Gram-positive isolates were significantly higher in CPH ($P \le 0.05$). The susceptibilities of the Grampositives bacteria were not considered for this report. The distribution of the Gram-negative bacilli isolated from CPH and UAH are shown in Figure 2. A total of 2.411 Gram-negative bacilli were identified in CPH and 3,179 in UAH ($P \le 0.05$). The percent antimicrobial resistance to the beta lactam and non-beta lactam antibiotics, the overall distribution of the studied Gramnegatives and the results from two published studies by Doern et al., (13) and Pfaller et al., (12) for no statistical comparison are shown in Table 2 and 3.

Escherichia coli. As shown in Figure 2, E. coli was the most common Gram-negative bacterium isolated from both hospitals group, 42.2% (1,017) in CPH and 26.9% (856) in UAH, (P ≤0.05). Table 2 and 3 shows the percent of antimicrobial resistance to selected antibiotics according to hospitals groups. University affiliated hospitals demonstrated a statistically significant (P ≤0.05) higher resistance to piperacillin-tazobactam (19.4% vs 1.3%), ceftriaxone (5.8% vs 0%), cefotaxime (19.9% vs 4.2%), $ceftazidime \hspace{0.2cm} \textbf{(10.5\% \ vs \ 0.7\%)}, \hspace{0.2cm} \overline{}_{nd \hspace{0.2cm} = \hspace{0.2cm} not \hspace{0.2cm} done, \hspace{0.2cm} S \hspace{0.2cm} = \hspace{0.2cm} Statistically \hspace{0.2cm} Significant, \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} No \hspace{0.2cm} Statistical \hspace{0.2cm} Significance \hspace{0.2cm} Statistical Significance \hspace{0.2cm} Statistical Significance \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} No \hspace{0.2cm} Statistical Significance \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} Statistical Significance \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} Statistical Significance \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} Statistical Significance \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} Statistical Significance \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIFICANCE \hspace{0.2cm} NS \hspace{0.2cm} = \hspace{0.2cm} NO \hspace{0.2cm} NO \hspace{0.2cm} STATISTICAL SIGNIF$ cefepime (7.3% vs 0%), imipenem Cefepime CPH data from only 2 hospitals except for P. aeruginosa

bacilli were significantly more Table 2. Comparison of percent resistance of Gram-negative bacilli to selected beta lactam antibiotic

Organisms	Antibiotic tested	P	resent Str (2000)	ıdy	Doern et al. 1998	Pfaller et al. 1998 (SENTRY Study)		
Organisms	Andorotte testeu	СРН	UAH	122 12212		USA	Canada	
		%R	%	P < 0.05	% R	%R	%R	
Escherichia coli	Piperacillin/tazobactam	1.3	19.4	S	0	4.5	4.5	
Total isolates CPH = 1017	Ceffriaxone	0	5.8	S	nd	1.7	0.6	
Total Isolates UAH = 856	Cefotaxime	4.2	19.9	S	0	1.7	0.6	
	Ceftazidime	0.7	10.5	S	0	2	1.1	
	Cefepime	0	7.3	S	0	0.7	0	
	Imipenem	0	5.4	S	0	0.5	0.6	
Klebsiella pneumoniae	Piperacillin/tazobactam	21.3	52.3	S	12.5	6	9.8	
Total isolates CPH = 222	Ceffriaxone	24.4	22.3	NS	nd	2.3	3.9	
Total Isolates UAH = 642	Cefotaxime	25.9	53.1	S	1.5	2.3	3.9	
	Ceffazidime	18.2	57.7	S	12.7	3.6	2	
	Cefepime	2.9	20.3	S	0	1.3	2	
	Imipenem	1.3	26.6	S	3.1	0	0	
Enterobacter cloacae	Piperacillin/tazobactam	35.7	11.1	S	19.3	31.2	6.2	
Total isolates CPH = 127	Ceffriaxone	46.6	44.1	NS	nd	30.5	6.2	
Total Isolates UAH = 291	Ceftazidime	49.7	41.1	NS	21.6	30.6	6.2	
	Cefepime	12.7	15.5	NS	0	0.7	0	
	Imipenem	0.5	2.3	NS	0	1.4	0	
Serratia marcescens	Piperacillin/tazobactam	4.2	31.1	S	5.3	nd	nd	
Total isolates CPH = 68	Ceftriaxone	6.9	35.0	S	nd	nd	nd	
Total Isolates UAH = 165	Ceftazidime	24.2	59.7	S	26.4	nd	nd	
	Cefepime	2.4	16.6	S	1.8	nd	nd	
	Imipenem	8.1	5.8	NS	0	nd	nd	
Pseudomonas aeruginosa	Piperacillin/tazobactam	25.0	33.9	S	22.6	7.6	4.5	
Total isolates CPH = 727	Ceftazidime	50.5	44.5	S	16.7	12.9	13.6	
Total Isolates UAH = 785	Cefepime	38.9	46.7	S	20.6	13	15.9	
	Imipenem	30.7	46.9	S	11.3	12	27.3	
	Piperacillin/tazobactam	61.7	80.5	s	52.9	nd	nd	
Acinetobacter baumannii	Ceftazidime	70.6	83.7	S	50	nd	nd	
Total isolates CPH = 250	Cefepime	72.2	83.8	S	50	nd	nd	
Total Isolates UAH = 440	Imipenem	46.8	69.7	S	1.8	nd	nd	

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Table 3. Comparison of percent resistance of Gram-negative bacilli to selected non-beta-lactam antibiotics according to present and other published studies

	Antibiotic	Prese	nt Stud	Pfaller et al 1998			
Organisms	tested	СРН	UAH	D < 0.05	USA	Canad	
		% R	% R	P < 0.05	% R	% R	
Escherichia coli	Ciprofloxacin	13.6	12.4	NS	2.8	3.4	
Total isolates CPH = 1017	Gentamicin	7.2	13.1	NS	4.0	5.7	
Total Isolates UAH = 856	Amikacin	0	6.7	S	1.5	1.1	
Klebsiella pneumoniae	Ciprofloxacin	8.4	22.3	S	3.7	2.0	
Total isolates CPH = 222	Gentamicin	6.2	44.8	S	4.7	5.9	
Total Isolates UAH = 642	Amikacin	4.0	18.0	S	1.0	0	
Enterobacter cloacae	Ciprofloxacin	46.6	21.8	s	7.8	0	
Total isolates CPH = 127	Gentamicin	35.6	24.2	NS	5.7	0	
Total Isolates UAH = 291	Amikacin	3.7	22.0	S	0	3.1	
Serratia marcescens	Ciprofloxacin	9.6	34.8	S	nd	nd	
Total isolates CPH = 68	Gentamicin	18.0	52.6	S	nd	nd	
Total Isolates UAH = 165	Amikacin	19.2	53.2	S	nd	nd	
Pseudomonas aeruginosa	Ciprofloxacin	45.5	44.6	NS	10.9	20.5	
Total isolates CPH = 727	Gentamicin	41.0	45.1	NS	10.3	15.9	
Total Isolates UAH = 785	Amikacin	23.2	35.5	S	1.6	4.5	
Acinetobacter baumannii	Ciprofloxacin	71.3	79.4	S	nd	nd	
Total isolates CPH = 250	Gentamicin	71.3	78.6	S	nd	nd	
Total Isolates UAH = 440	Amikacin	47.6	72.3	S	nd	nd	

nd = not done, S = Statistically Significant, NS = No Statistical Significance

(5.4% vs 0%), gentamicin (13.1% vs 7.2%), and amikacin (6.7% vs 0%). No significant difference in antimicrobial resistance to ciprofloxacin was observed between the two hospitals group (12.4% UAH vs 13.6% CPH).

Klebsiella pneumoniae. As shown in Figure 2, K. pneumoniae represented 20.2% (642) and 9.2% (222) of the total Gram-negative bacteria isolates in UAH and CPH, respectively (P ≤0.05). The percent resistance of K. pneumoniae to selected antibiotics according to hospitals groups is shown in Table 2 and 3. UAH demonstrated a statistically significant (P ≤0.05) higher resistance to piperacillin-tazobactam (52.3% vs 21.3%), cefotaxime (53.1% vs 25.9%), ceftazidime (57.7% vs 18.2%), cefepime (20.3% vs 2.9%), imipenem (26.6% vs 1.3%), ciprofloxacin (22.3% vs 8.4%), gentamicin (44.8% vs 6.2%) and amikacin (18.0% vs 4.0%). No significant difference in antimicrobial resistance to ceftriaxone was observed between the two hospitals group.

Enterobacter cloacae. Figure 2 show that *E. cloacae* represented 5.3% (127) and 9.2% (291) of the total Gram-

negative bacteria isolated in CPH and UAH,
respectively. The antimicrobial resistance
percentage of *E. cloacae* to selected antibiotics
according to hospitals group is shown in Table 2
and 3. UAH isolates demonstrated a statistically
significant higher resistance only to amikacin
(22.0% UAH vs 3.7% CPH, P ≤05), while isolates
from CPH demonstrated a significantly higher
percent resistance (P ≤0.05) to piperacillintazobactam (35.7% CPH vs 11.1% UAH) and
ciprofloxacin (46.6% CPH vs 21.8% UAH). No
significant differences between the two hospitals
group were observed in *E. cloacae* antimicrobial
resistance to ceftriaxone, ceftazidime, cefepime,
imipenem and gentamicin.

Serratia marcescens. This organism represented 2.8% (68) of the total Gram-negative bacteria isolates from CPH and 5.2% (165) of the UAH isolates (Figure 2). Table 2 and 3 shows the resistance of *S. marcescens* to selected antibiotics according to hospitals group. In UAH, there was a significantly higher ($P \le 0.05$) resistance to piperacillin-tazobactam (31.1% vs 4.2%), ceftriaxone (35.0% vs 6.9%), ceftazidime (59.7% vs 24.2%), cefepime (16.6% vs 2.4%), ciprofloxacin (34.8% vs 9.6%), gentamicin (52.6% vs 18.0%) and amikacin (53.2% vs 19.2%). No significant difference in antimicrobial resistance to imipenem (8.1% CPH vs 5.8% UAH) was noted.

Pseudomonas aeruginosa. As shown in Figure 2, P. aeruginosa represented 30.2% (727) and 24.7% (785) of the total Gram-negative bacteria isolates in CPH and UAH, respectively. Table 2 and 3 shows the % antimicrobial resistance of P. aeruginosa to selected antibiotics according to hospitals group. In UAH isolates, there was a significantly higher resistance to piperacillintazobactam (33.9% vs 25.0%), cefepime (46.7% vs 38.9%), imipenem (46.9% vs 30.7%) and amikacin (35.5% vs 23.2%). For ceftazidime, CPH P. aeruginosa isolates had a statistically significant higher resistance than UAH isolates (50.5% vs 44.5%, P ≤0.05). No significant differences in the antimicrobial resistance of P. aeruginosa isolates to gentamicin and ciprofloxacin were observed between the two hospitals group. Both hospitals group demonstrated high numbers of multidrug resistance isolates.

Acinetobacter baumannii. Figure 2 show that A. baumannii represented 10.3% (250) of the total Gramnegative bacterial isolates in CPH and 13.8% (440) of the UAH isolates. The % antimicrobial resistance of A. baumannii to selected antibiotics according to hospitals group is shown in Table 2 and 3. In UAH there was a

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significantly higher (P \leq 0.05) % resistance to all tested antibiotics. piperacillin-tazobactam (80.5% vs 61.7%), ceftazidime (83.7% vs 70.6%), cefepime (83.8% vs 72.2%), imipenem (69.7% vs 46.8%), ciprofloxacin (79.4% vs 71.3%), gentamicin (78.6% vs 71.3%) and amikacin (72.3 vs 47.6%). Both hospitals group antimicrobial reports demonstrated very poor antimicrobial activity against this organism.

Discussion

Antimicrobial resistant bacteria have become a major health issue for the 21st century. The emergence of these microorganisms has been associated with several risk factors, such as: length of hospital stay, proximity to a hospital, severity of illness, recent surgery, immunosuppression, hospital and community antimicrobial utilization, large inner city or teaching hospital, and special areas within hospitals. There are a variety of genetic mechanisms by which bacteria can acquire resistance to antimicrobial agents. This may result in a significant number of antibiotic resistant bacterial pools within a given area.

In a previous study by Doern et al. (13), performed in seven Puerto Rican hospitals, the minimum inhibitory concentration of six broad-spectrum beta-lactam antibiotics against 110 Gram-positive and 459 Gramnegative organisms was evaluated utilizing the E test methodology. This study, together with the data reported by Pfaller et al., (12) utilizing the SENTRY Antimicrobial Surveillance Program for the United States and Canada were utilized to compare our data with their results, as shown in Table 2 and 3. These tables show the antimicrobial resistance patterns of selected Gram-negative bacteria to six beta-lactam antibiotics (Table 2) and to the non-beta-lactam antibiotics ciprofloxacin, gentamicin and amikacin (Table 3). A comparison between the antimicrobial resistance pattern in our two hospitals group and the published reports of Doern et al., (13) and Pfaller et al., (12), suggest that: both, CPH and UAH, showed high numbers of resistant Gram-negative bacilli, with UAH demonstrating a statistically significant higher number of resistant bacteria than the community-private hospitals. When this data is compared with the published antimicrobial study performed in P.R. by Doern et al., (13) an apparent increase in the percent resistance to most of the tested beta-lactam antibiotics was noted in both, UAH and CPH. University affiliated hospitals bacteria showed a consistently higher antibiotic resistance percentage than CPH or Doern et al. (13) isolates. Comparison with Pfaller et al., (12) study demonstrated consistently higher resistance to the beta-lactam and non-beta lactams antibiotics in our isolates when compared with USA or Canadian isolates (Tables 2 and 3).

There may be several explanations for our observed results. Our study included a significant number of isolates from both community and teaching hospitals, however, the yearly susceptibility report lacks important information such as, (1) the anatomical site of infection, (2) community or nosocomial acquired isolate, and/or (3) if several samples with identical organism were obtained from the same patient, which could have shifted our isolates towards a relatively more antimicrobial resistant bacterial population. During the last several years, however, significant increases in the number of antimicrobial resistant bacteria have been observed worldwide. The acquisition of chromosomally or plasmid mediated resistance to antibiotics should be considered in our studied Gramnegative bacilli.

For the family Enterobacteriacea, antimicrobial resistance mechanisms includes among others, the hiperproduction of stably derepressed chromosomal cephalosporinase (AmpC) observed in Enterobacter, Citrobacter, Serratia, and indole positive Proteus species, and the acquisition, usually by E. coli and K. pneumoniae, of a plasmid capable of hydrolyzing the oxymino cephalosporins commonly known as Extended Spectrum Beta Lactamases (ESBLs). In our study, the high percentage of Enterobacter cloacae and Serratia marcescens resistant to the third generation cephalosporins, suggest the presence of stably derepressed chromosomal beta lactamase. The resistance of E. coli to ceftriaxone. cefotaxime and ceftazidime in UAH, and the resistance of K. pneumoniae to the same antibiotics in UAH and CPH. suggest the acquisition of the ESBLs phenotype by these organisms. As for the non-beta lactam antibiotics, ciprofloxacin, gentamicin and amikacin, the same patterns of antimicrobial resistance is observed with, CPH and UAH reports, demonstrating higher resistance to these antibiotics when compare to the SENTRY study (12).

For the non-fermenter Gram-negative bacilli, *P. aeruginosa* and *A. baumannii*, two organisms frequently encountered in immunocompromised or seriously ill patients, there was a consistently high percentage of resistance to both, beta lactam and non-beta lactam antibiotics. *Acinetobacter baumannii* followed by *P. aeruginosa* from both, UAH and CPH, demonstrated the highest resistant to antibiotics of all the studied Gramnegative bacilli.

Conclusions

Significant numbers of antimicrobial resistant Gramnegative bacteria were found in our studied sample, similar or higher than those observed at other geographical areas. University affiliated hospitals had a significantly higher number of resistant organisms than community-private hospitals. The number of Gram-negative bacilli resistant to 3rd generation cephalosporins suggests the presence of ESBLs and chromosomally derepressed beta lactamases in our bacterial isolates.

The significant increases in antibiotic resistance bacteria have become a worldwide health issue as evidenced by several large-scale studies. It is for this reason that antimicrobial resistance surveillance studies coupled with the judicious use of antimicrobial agents should be a priority in the practice of medicine. It is imperative that an island wide antimicrobial susceptibility program be established to monitor the susceptibility trends of the most common bacterial pathogens.

Resumen

Pocos estudios se han llevado a cabo en Puerto Rico concerniente a los patrones de resistencia antimicrobiana de bacilos Gram-negativos clínicamente significativos. Los patrones de resistencia de 5,590 bacterias Gram-negativas obtenidas de tres hospitales privados de la comunidad (CPH) y tres hospitales afiliados a la universidad (UAH) fueron evaluados utilizando los reportes de susceptibilidad antimicrobiana para el año 2000. Los objetivos de este estudio fueron: (1) la evaluación retrospectiva de los reportes in vitro de las resistencias de los aislados clínicos de E. coli, K. pneumoniae, E. cloacae, S. marcescens, P. aeruginosa y A. baumannii a antibióticos selectos y (2) comparar los patrones de resistencia antimicrobiana entre CPH y UAH. E. coli fue el bacilo Gram-negativo entérico más común en CPH y UAH. En UAH, E. coli demostró resistencia estadísticamente significativa a los antibióticos beta lactámicos y amikacina pero no a la ciprofloxacina o gentamicina. Para K. pneumoniae el patrón de resistencia antimicrobiana demostró que los aislados de UAH eran significativamente más resistentes a los antibióticos probados con la excepción de ceftriaxona. En CPH, los aislados de E. cloacae fueron significativamente más resistente a piperacilina/tazobactam, ciprofloxacina y gentamicina, mientras que en UAH este organismo fue más resistente a la amikacina. En UAH, aislado de S. marcescens demostraron una resistencia estadísticamente significativa a todos los antibióticos probados con la excepción del imipenem, el cual fue similar en ambos grupos de hospitales. Pseudomonas aeruginosa demostró una resistencia en UAH significativamente mayor a todos los antibióticos seleccionados con la excepción de ciprofloxacina y gentamicina, que fueron similares en ambos grupos de hospitales. Acinetobacter baumannii fue el organismo más resistente en ambos grupos de hospitales. Aislado del UAH fueron significativamente más resistentes que los de CPH para todos los antibióticos probados. Cuando comparamos este estudio con otros estudios de resistencia, nuestros resultados sugieren una aparente mayor resistencia en nuestras cepas aisladas. El número elevado de bacilos Gram-negativos resistente a antibióticos en nuestro estudio sugiere la presencia de múltiples mecanismos de resistencia a antibióticos incluyendo la presencia de beta lactamasas de espectro extendido y derepresión cromosómica.

References

- Eveillard M, Biendo M, Canarelli B, Daoudi F, Laurans G, Rousseau F, Thomas D. Spread of Enterobacteriaceae producing broad-spectrum beta-lactamase and the development of their incidence over a 16-month period in a university hospital center. Pathol Biol (Paris). 2001;49:515-521.
- Flaws M, Robin T, Punam V. Update on antimicrobial resistance. www.mlo-online.com. 2001;20-29.
- Fluit AC, Verhoef J, and Schmitz FJ. Frequency of isolation and antimicrobial resistance of Gram-negative and Gram-positive bacteria from patients in intensive care units of 25 European university hospitals participating in the European arm of the SENTRY Antimicrobial Program 1997-1998. Eur J Clin Microbiol Infect Dis 2001;20:617-625.
- Fluit AC, Verhoef J, Schmitz FJ. Antimicrobial resistance in European isolates of *Pseudomonas aeruginosa*. European SENTRY Participants. Eur J Clin Microbiol Infect Dis 2000; 19:370-4.
- Gales AC, Jones RN, Turnidge J, Rennie R, Ramphal R. Characterization of Pseudomonas aeruginosa Isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the Global SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis 2001; 32:S146-155.
- Gales AC, Jones RN, Forward KR, Linares J, Sader HS, and Verhoef, J. Emerging importance of multidrug-resistant Acinetobacter species Strenophomonas maltophilia as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance Program (1997-1999). Clin Infect Dis 2001;32:S104-113.
- Jones, RN 1999. ESBLs- the latest threat to therapy. Etest News 1993;23.
- Jorgensen JH, Ferraro MJ, McElmeel ML, Spargo J, Swenson JM, Tenover FC. Etest for ART of pneumococci. J Clin Microbiol 1994;32:159-163.
- Kolar M, Urbanek K, Latal T. Antibiotic selective pressure and development of bacterial resistance. Int J Antimicrob Agents 2001;17:357-363.
- Kumarasinghe G, Chow C, Tambyah PA. Widespread resistance to new antimicrobials in a university hospital before clinical use. Int J Antimicrob Agents 2001;18:391-393.
- 11. Leverstein-van Hall MA, Fluit AC, Blok HE, Box AT, Peters ED, Weersink AJ, Verhoef J. Control of nosocomial multiresistant Enterobacteriaceae using a temporary restrictive antibiotic agent policy. Eur J Clin Microbiol Infect Dis 2001; 20:785-791.
- Pfaller MA, Jones RN, Doern GV, Kugler K, the SENTRY Participants Group. Bacterial pathogens isolated from patients

- with bloodstream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY Antimicrobial Surveillance Program (United States and Canada, 1997). Antimicrob Agents Chemother 1998;42:1762-1770.
- Doern GV, Jones RN, Pfaller MA, Erwin M, Ramirez-Ronda C. Multicenter Evaluation of the *in vitro* activity of six broadspectrum beta lactam antimicrobial agents in Puerto Rico. The
- Puerto Rico Antimicrobial Resistance Study Group. Diag Microbiol Infect Dis 1998;30:113-119.
- 14. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum βlactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific Region. Clin Infect Dis 2001;32:S94-103.

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