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RESEARCH

Minimum Inhibitory Concentrations of Aminoglycoside, β-lactam and Quinolone Antimicrobials for Nosocomial Isolates of *Acinetobacter calcoaceticus-Acinetobacter baumanii* Complex from the Philippine General Hospital

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Acinetobacter calcoaceticus-Acinetobacter baumanii complex isolates from patients of the Philippine General Hospital with hospital-acquired (nosocomial) infections were analyzed for their response to amikacin, gentamicin, netilmicin, tobramycin, ceftazidime, cefotaxime, piperacillin, ciprofloxacin and cefoperazone. The minimum inhibitory concentrations of the antimicrobial agents for the isolates were determined using the agar dilution method. Of the 98 isolates studied, 97 (98.98%) were resistant to at least one of the antimicrobials, with 17 of these 97 isolates (17.53%) being resistant to all of the nine tested. The aminoglycosides netilmicin and amikacin were shown to be the most effective against the isolates, with 61.22% and 56.12% being sensitive to the antimicrobials, respectively. This was followed by the β-lactam ceftazidime, to which 40.82% of the isolates were sensitive. Cefoperazone was the least effective, with 91.83% being resistant to it. The aminoglycosides were shown to be generally more effective than the β-lactam and quinolone antimicrobials.

Key words: antibiotic resistance, hospital-acquired infections, drug resistance

Acinetobacter spp. are gram negative, nonfermentative bacilli that are commonly found in the soil and water. They are also part of the normal flora of the human skin and oropharynx. However, the bacilli are opportunistic pathogens. Their role in nosocomial or hospital-acquired infections has frequently been reported worldwide (Vindenes and Bjerknes 1995; Maruashvili et al. 1995; Kurosu 1995; Bergogne-Berezin and Towner 1996; Du et al. 1996; Seward et al. 1998). Opportunistic infections caused by *Acinetobacter* spp., predominantly *A. baumanii*, include bacteremia, urinary tract infection, meningitis, and nosocomial pneumonia in intensive care units (Seward and Towner 1998). The isolates reported in these and in most other studies have been found to be multiply resistant to a variety of commonly-used antimicrobial agents.

The 1997, 1996 and 1995 antimicrobial resistance surveillance data collected from 11 hospitals in the Philippines by the Committee on Antimicrobial

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Resistance Surveillance of the Department of Health show that 9% (1996), 10% (1995 and 1997) of the pathogens reported from these hospitals were *Acinetobacter* spp. (Carlos 1997b; Carlos 1997a; Carlos 1998). These are in contrast to the data for 1994, where only 5.8% of the pathogens were *Acinetobacter* spp. (Carlos 1996). *Acinetobacter* spp. have also often been associated with nosocomial infections in the Philippine General Hospital although the incidence has not reached a critical level. It was with the objective of determining the levels of resistance (based on breakpoints for sensitivity and resistance) to the USA National Committee for Clinical Laboratory Standards (NCCLS)-listed antimicrobials (Ferraro et al. 1998),and which are also commonly prescribed in the hospital that this study was conducted.

Materials and Methods

Bacterial Isolates

Acinetobacter calcoaceticus-Acinetobacter baumanii complex isolates from patients in the different wards of the Philippine General Hospital (PGH) with nosocomial infections were collected from December 1997 to August 1998. The specimens from which the microorganisms were isolated consisted of endotracheal aspirates, sputum, urine, cerebrospinal fluid, blood and swabs from wounds. A total of 98 isolates were studied.

The isolates were identified as *A. calcoaceticus* var. *anitratus* by the Bacteriology Section, Department of Laboratories of the Philippine General Hospital. The identification was based on the biochemical reactions in triple sugar iron (TSI) agar, lysine iron agar (LIA), sulfide indole motility (SIM), citrate and urea test media, oxidative-fermentative (OF) medium with dextrose, maltose and oxidase test. However, more recent studies have adapted the use of the name *A. calcoaceticus- A. baumanii* complex to include glucose-oxidizing *Acinetobacter* spp. such as *A. calcoaceticus* var. *anitratus* (Bergogne-Berezin and Towner 1996), hence, the use of this name for the isolates in the study. Although *A. baumanii* is the main genomic species associated with nosocomial infections (Berogne-Berezin et al. 1996), and grows at 37°C or higher, as were also shown by the isolates in the study, speciation of the isolates as *baumanii* can not be done in the absence of DNA-DNA relatedness studies.

NCCLS-recommended reference strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 (Ferraro et al. 1998) were tested along with the *Acinetobacter* isolates.

Antimicrobials

The choice of antimicrobials was based on the recommended list of the National Committee on Clinical Laboratory Standards (Ferraro et al. 1998) for *Acinetobacter* spp. Parenteral preparations of the antimicrobials were purchased from a local pharmaceutical supply house.These included the β-lactam antibiotics: ceftazidime (Glaxo), cefoperazone (Pfizer), piperacillin (Lederle), cefotaxime (Hoechst); the aminoglycosides: amikacin (Bristol-Myers Squibb), tobramycin (Elli Lilly), gentamicin (Bristol-Myers Squibb) and netilmicin (Schering-Plough); and the quinolone: ciprofloxacin (Bayer). The concentrations used in the analyses were adjusted according to the % activity of each of the antimicrobial preparations used.

Determination of the Minimum Inhibitory Concentration (MIC)

The MICs of the nine antimicrobials were determined on Mueller Hinton II (MH II) agar using the agar dilution method. Two-fold serial dilutions of the antimicrobials were prepared using sterile distilled water and incorporated into the agar resulting in concentrations ranging from 1 µg/ml to 512 µgs/ml for amikacin, tobramycin, gentamicin, netilmicin, and ciprofloxacin. Concentrations from 1 µg/ml to 1,024 µgs/ml were tested for ceftazidime, cefoperazone, piperacillin and cefotaxime.

Eighteen-hour cultures of the *Acinetobacter* isolates, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 reference strains grown in brain heart infusion broth (BHIB) were subcultured in BHIB and grown to the log phase at 37°C for 4 hours. The turbidity of the inocula was adjusted to equal that of 0.5 MacFarland turbidity standard to approximate 1.5×10^8 cells ml^{-1}. Approximately one microliter (ul) of each inoculum (around 1.5×10^5 CFU) was transferred to the MH II-antibiotic test plates using a Steers-Foltz replicator. Three plates per antibiotic concentration were inoculated for every isolate. Each isolate was also inoculated into MH II agar without antibiotics to check for its growth. The plates were read after incubation at 37°C for 16-18 hours. The minimum concentration of the antimicrobial where no bacterial growth was observed was considered as the MIC of the antimicrobial. Growth of 2 colonies or less or the presence of a slight haze was read as no growth (Baron et al. 1994). The breakpoints for sensitivity and resistance set by the NCCLS for the antimicrobials for *Acinetobacter* spp. were used to interpret the results (Ferraro et al. 1998). The breakpoint of an antimicrobial is the concentration that can be achieved in the serum

or tissue with optimal therapy (Baron et al., 1994). Organisms with MIC at or below the breakpoint are considered susceptible, whereas those with MICs that are above the achievable level or within a range toxic to the host are said to be resistant to the antimicrobial agent.

Results and Discussion

Results of the analyses showed a predominance of the *A. calcoaceticus- A. baumanii* complex isolates which were resistant to the antimicrobials. Of the 98 isolates, only 1% showed complete sensitivity to all of the nine antimicrobials tested. The remaining 99% exhibited either single (13.27%) or multiple (85.71%) resistance (Fig 1). It should be noted that isolates resistant to all the nine antimicrobials comprised the largest group.

The MIC of an antimicrobial agent is the least concentration of the antimicrobial that can inhibit the growth of the microorganism. Based on the breakpoints for sensitivity and resistance, the MIC of an antimicrobial reflects the level of sensitivity or resistance of the microorganism to the agent, hence, the potency of the antimicrobial for the microorganism. The distribution of the percent isolates according to the MIC of the different antimicrobials is shown in Tables 1 and 2. The MICs of all the antimicrobials for a very large percentage of the isolates shown to be resistant were at least twice their MIC breakpoints for resistance. This is exemplified by the MICs of amikacin for 38 of the 43 resistant isolates (88.37%) being at least 64 µgs/ml. The breakpoint for resistance is 32 µgs/ml (Table 1). Another example is that of ceftazidime. The MICs of ceftazidime for 37 of the 50 resistant isolates (74%) were at least 64 µgs/ml. The breakpoint is 32 µgs/ml (Table 2).

The same observation is seen with the rest of the test antimicrobials. The data show a high level of resistance among most of the resistant isolates. The breakpoint for resistance to cefoperazone is 64 µgs/ml. Of the isolates resistant to the antimicrobial, only 7.78% had cefoperazone MIC of 64 µgs/ml. The MIC of cefoperazone for the remaining 92.22% resistant isolates was at least 128 µgs/ml. The MIC of ciprofloxacin was at the sensitive level of ≤1 µg/ml for 20.41% of the isolates. It ranged from 2 to 512 µgs/ml for the remaining 79.59%. Of these, the MIC of ciprofloxacin ranged from 16 to 64 µgs/ml for 14.28% of the isolates; and from 128 µgs/ml to > 512 for the remaining 57.14% isolates. This shows a much higher level of resistance in most resistant isolates than those used in the study of Seward and Towner (1998) where the MIC of ciprofloxacin ranging from 16 µg/ml to 64 µgs/ml were already considered to be of high level of resistance. Ciprofloxacin was also reported to be one

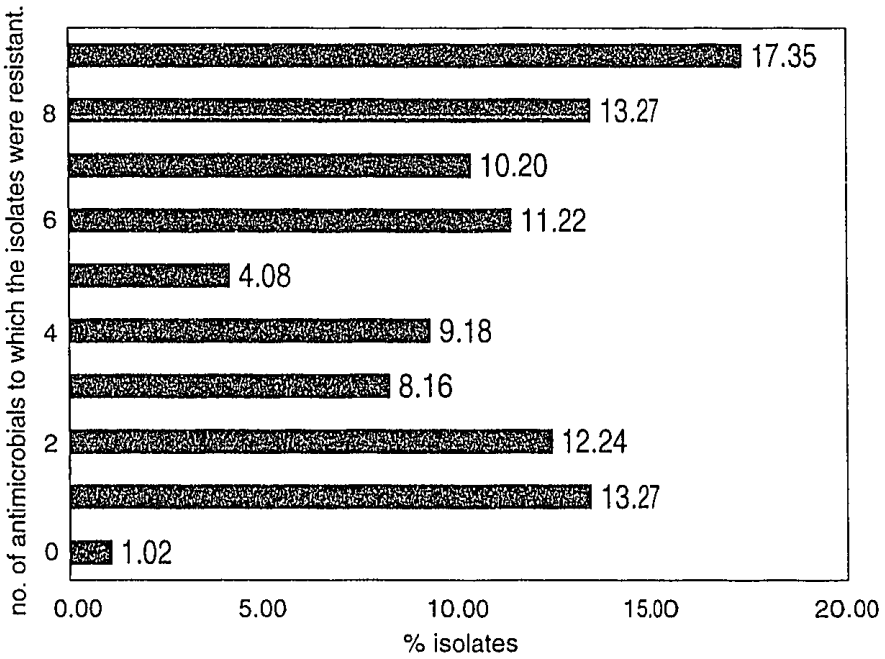

Figure 1. Percent isolates of *Acinebacter calcoaceticus-Acinebacter baumanii* and the corresponding number of antimicrobials to which they were resistant.

Table 1. Distribution of the percentage of *Acinetobacter baumanii- Acinetobacter calcoaceticus* complex isolates[a], *Escherichia coli* (Ec) ATCC 25922 and *Pseudomonas aeruginosa* (Pa) ATCC 27853 according to the MICs of aminoglycoside and quinolone antimicrobials

	aminoglycosides				quinolone
MIC (µgs/ml)	amikacin	gentamicin	netilmicin	tobramycin	ciprofloxacin
≤1	0	16.32, Ec	4.08, Ec	3.06, Ec, Pa	**20.14**, Ec, Pa
2	6.12, Ec[d], Pa[e]	10.20, Pa	18.37, Pa	24.49	3.06
4	11.22	**8.16**	10.20	**9.18**	2.04
8	13.26	5.10	14.29	8.16	3.06
16	[b]**25.51**	4.08	**14.29**	7.14	5.1
32	[c]5.10	6.12	5.10	12.24	1.02
64	5.10	10.20	10.20	3.06	8.16
128	8.16	3.06	2.04	17.35	23.47
256	5.10	11.11	1.02	3.06	26.53
512	7.14	1.02	4.08	9.18	7.14
≤512	13.77	34.69	16.33	3.06	0

[a] total number of isolates tested : 98
[b] bold- faced data are the % isolates with the antimicrobial MICs corresponding to breakpoints for sensitivity
[c] underlined data are the % isolates with the antimicrobial MICs corresponding to breakpoints for resistance
[d] quality control range of MIC for *E. coli* ATCC 25922 (µgs/ml): amikacin: 0.5-4; gentamicin: 0.25-1; netilmicin: ≤0.5- 1; tobramycin: 0.25-1; ciprofloxacin: 0.004-0.0 15(Ferraro et al., 1998)
[e] quality control range of MIC for *P. aeruginosa* ATCC 27853 (µgs/ml): amikacin: 1-4; gentimicin: 0.5-2; netilmicin: 0.5-8; tobramycin: 0.25-1; ciprofloxacin: 0.25-1 (Ferraro et al., 1998)

Table 2. Distribution of the percentage *Acinetobacter baumanii-Acinetobacter calcoaceticus* isolates[a]. *Escherichia coli* (Ec) ATCC 25922, *Pseudomonas aeruginosa* (Pa) ATCC 27853 according to the MICs of β-lactam antimicrobials.

MIC (µgs/ml)	ceftazidime	cefotaxime	cefoperazone	piperacillin
≤1	10.02, Ec[d], Pa[e]	0,Ec	1.02, Ec	0,Ec
2	3.06	1.02	0	0
4	17.35	1.02	0, Pa	1.02, Pa
8	[b]**19.39**	**8.16**, Pa	0	2.04
16	8.16	15.31	0	**12.24**
32	[c]13.27	31.63	7.14	23.47
64	10.20	12.24	7.14	16.33
128	7.14	10.20	26.53	8.16
256	7.14	12.24	13.27	16.33
512	4.08	5.10	23.47	10.20
1024	5.10	3.06	4.08	6.12
≤1024	4.08	0	17.35	4.08

[a] total number of isolates tested : 98
[b] bold-faced data are the % isolates with the antimicrobial MICs corresponding to the breakpoints for sensitivity
[c] underlined data are the % isolates with the antimicrobial MICs corresponding to the breakpoints for resistance
[d] quality control range of MIC for *E. coli* ATCC 25922 (µgs/ml): ceftazidime: 0.06-0.5; cefotaxime: 0.03-0.12; cefoperazone: 0.12-0.5; piperacillin: 1-4 (Ferraro et al., 1998)
[e] quality control range of MIC for *P. aeruginosa* ATCC 27853 (µgs/ml): ceftazidime: 1-4; cefotaxime: 8-32; cefoperazone: 2-8; piperacillin: 1-4 (Ferraro et al., 1998)

of the most active antimicrobials for *A. baumanii* with an MIC of 0.5 µg/ml (Shih et al. 1996).

Among the antimicrobials tested, netilmicin proved to be the most effective, with 61.22% being sensitive to it (Table 3). Still, 38.78% were resistant to the antimicrobial. Amikacin and ceftazidime, with 56.12% and 40.82% of the isolates showing sensitivity to the antimicrobials, respectively, followed this. However, the % isolates resistant to amikacin and to ceftazidime were still a high 43.88% and 51.02%. The study of

Table 3. Response of the *Acinetobacter baumanii-Acinetobacter calcoaceticus* complex isolates[a] to the antimicrobials

Antimicrobials	Resistance % isolates	Intermediate % isolates	Sensitive % isolates
aminoglycosides			
netilmicin	38.78	0[b]	61.22
amikacin	43.88	0	56.12
tobramycin	63.27	0	36.73
gentamicin	65.31	0	34.69
quinolone			
ciprofloxacin	76.53	3.06	20.41
β-lactam			
ceftazidime	51.02	8.16	40.82
piperacillin	44.90	39.80	15.30
cefotaxime	42.86	46.94	10.20
cefoperazone	91.44	7.14	1.02

[a]total number of isolates tested : 98
[b]no intermediate concentration tested

Kiss et al. in 1995 showed that only 2.6% and 4.9% of *A. calcoaceticus* from patients of an intensive care unit were resistant to netilmicin and amikacin respectively, whereas, the result with ceftazidime to which 55.4% were resistant parallels that of the present study.

The least effective was shown to be cefoperazone. Except for 1.02% of the isolates that were susceptible, and 7.14%, that gave intermediate response, the remaining 91.84% were resistant to the antibiotic. The aminoglycosides were found to be generally more effective than the β-lactam and quinolone antimicrobials.

The result of the study which showed that 99% of the isolates tested were resistant to at least one of the antimicrobials is alarming, much more so if we consider the multiplicity and high levels of resistance shown by most isolates. The test antimicrobials are the newer, more promising drugs. These are also commonly-prescribed to treat *Acinetobacter* infections in the hospital. It is worthy to note that, in the Philippine General Hospital, a combination of a β-lactam antibiotic, such as ceftazidime or ceftriaxone, and an aminoglycoside, such as netilmicin, tobramycin or amikacin, is prescribed for *Acinetobacter* infections. However, resistance to multiple antimicrobials renders the use of combination drugs for therapy useless if the organism is resistant to all the drugs used in the combination therapy.

Resistance to aminoglycosides in *Acinetobacter* spp. is frequently plasmid-mediated (Seward et al. 1998). A possible plasmid-encoded extended-spectrum B-lactamase in the microorganism has been suggested (Bergogne-Berezi & Towner 1996). In a hospital setting where rampant use of different antimicrobials serves to positively select for the survival and spread of antimicrobial resistant isolates, the presence of R plasmids in hospital isolates compounds the problem. Aside from usually mediating multiple resistance, some R plasmids can be transferred to other bacteria belonging to the same or to different genera through conjugation. Transfer can occur *in vitro* and *in vivo*, contributing to the spread of multiple antimicrobial resistant strains.

The results of this study serve as a timely reminder for the need to be more discriminating in the prescription and use of antimicrobials. Constant, vigilant monitoring and reporting, critical evaluation of treatment programs and strict implementation of laws regulating dispensing of antimicrobials definitely can not be compromised.

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