Antibiotic and Disinfectant Resistance in *Acinetobacter baumannii* genotyped isolates from the Caracas University Hospital

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The aim of this study was to evaluate the susceptibility to antibiotics and quaternary ammonium disinfectants of *Acinetobacter baumannii* strains isolated from patients with nosocomial infections and the environment of the ICU and Neonatal Service (NS) at the Caracas University Hospital. Resistance to 15 antibiotics was determined. To evaluate the disinfectant resistance phenotypes tests recommended by the AOAC were used. ERIC-PCR and REP-PCR were used for genotyping. We analyzed 74 *A. baumannii*, 14 and 1 from ICU and NS patients, respectively; and 42 and 17 from its corresponding environments. 44.6% of the isolates showed resistance to at least 7 antibiotics. 54% of isolates tested exhibited the resistance phenotypes to the quaternary ammonium hospital disinfectant evaluated. 73% of the isolates from patients were closely related clones, 28.8% of the environmental isolates were grouped in 7 indistinguishable clones groups. The multiple antibiotic and disinfectant resistance profiles and the presence of related bacterial clones demonstrate the need for antibiotic usage surveillance and alternative cleaning methods in this hospital.

Keywords: *Acinetobacter baumannii*; antibiotic; disinfectant; genotyping

1. Background

Acinetobacter baumannii is one of the most important nosocomial pathogens worldwide. High-level antimicrobial resistance is a noteworthy characteristic of this specie. The presence of these bacteria in the hospital environment constitutes a risk factor especially in intensive care units (ICUs) due to the invasive treatments patients are being expose to. Considering bacteria can prevail in inanimate surfaces for long periods of time it is important to maintain the hospital cleaning methods under surveillance [1-3].

Antiseptics and disinfectants are extensively used in hospital infection control. The most widely antiseptics used in hospital infection control include cationic quaternary ammonium compounds (QAC).The daily use of these compounds has led to the selection of resistant bacterial strains that increase the risk of acquiring nosocomial infections. Though it has been reported the cross-resistance between antibiotics and disinfectants [4,5], and considering the multiresistance to antibiotics of microorganisms in hospital settings; to be able to relate bacteria isolated from patients with nosocomial infections and those isolated from their surrounding environment where these disinfectants are being used might bring a different approach to the resistance to antibiotics and disinfectants.

The aim of this study was to determine the susceptibility to antibiotics and QACs of clinically important strains of *A. baumannii* isolated from both patients with nosocomial infections and their surrounding environments, analyzing their genetic relatedness in a period of a year.

2. Materials and Methods

2.1 Bacterial Isolates

Isolates obtained during a one-year study in the ICU and Neonatal Service (NS) at the Caracas University Hospital were used for this study. Environmental isolates were collected both from patients with nosocomial infections (NI) and from the environment of each unit once every two months, performing six samplings during this period. Environmental samples were recovered from sinks, soap dishes, beds, tables, doorknobs, monitors, shelves, cribs, incubators, stethoscopes and personnel hands samples.

Fifteen *Acinetobacter baumannii* (AB) isolated from patients with nosocomial infections in the ICU and NS (one isolate) of the Caracas University Hospital were analyzed. 17 and 42 isolates of *A. baumannii* from the environment of the Neonatal Service and ICU were analyzed (AN and AU, respectively).

2.2 Antimicrobial susceptibility test

To evaluate the antibiotic susceptibility profile of the isolates, the Disc Diffusion method was used following the Clinical and Laboratory Standards Institute (2007) recommendations. Fifteen antibiotics were tested: cefotaxime (30 µg), ceftazidime (30 µg), ceftriazone (30 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), piperacilin-tazobactam (100/10 µg), ampicillin (10 µg), amikacin (30 µg), tobramycin (30 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg) and SXT: trimethoprim-sulphamethoxazole (25 µg). The quality control was carried out by using standard strains of *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27953)

2.3 Disinfectant susceptibility test

To evaluate bacterial resistance to disinfectants we performed the phenotype tests recommended by the Association of Official Analytical Chemistry (AOAC): (i) Quantitative Suspension test (number of viable colonies), and (ii) Cell Death Rate Test (turbidity on liquid culture), both determinations were performed after the exposure of the microorganisms to the disinfectant at its use-concentration (10% dimethyl benzyl lauryl ammonium bromide) according to the protocol of Kawamura-Sato [6].

2.4 Genotyping

Total DNA isolation was performed using the boiling method [7]. For REP-PCR the primer used was 5' GCG CCG ICA TGC GGC ATT 3' [8] and the thermal cycling conditions were as follows: initial denaturation at 94° (5min), 35 cycles of denaturation at 94° (1min), annealing at 50° (1min) extension at 72° (8min), and a final extension at 72° (15min). For the ERIC-PCR the primers used were ERIC1: 5' ATG TAA GCT CCT GGG GAT TCA C 3' and ERIC2: 5' AAG TAA GTG ACT GGG GTG AGC G 3' [9] and the thermal cycling conditions were as follows: initial denaturation at 94° (5min), 35 cycles of denaturation at 94° (1min), annealing at 36° (1min) extension at 68° (10min), and a final extension at 68° (15min). Amplicons were separated by 1.2% agarose gel electrophoresis and digital images analyzed with BioNumerics software package (Applied Maths, Inc., Austin, TX). The Pearson's correlation coefficient and unweighted pair group method with arithmetic means were used to create dendograms. Relatedness was determined by comparing cluster analysis.

3. Results

The resistance to the different classes of antibiotics is presented in Table 1. Around 43% of all isolates were resistant to all antibiotics tested of the different classes, penicillins (46%), cephalosporins (42%), carbapenems (43%), aminoglycosides (42%), and quinolones (39%), with the exception of tetracycline (70%) and the sulfonamide combination SXT (54%). As shown in Figure 1, there were two main groups of *Acinetobacter baumannii* isolates resistant to few (less than 4) antibiotics or those resistant more 11 of the antibiotics tested. 44.6% of the isolates were resistant to at least 7 antibiotics. All *A. baumannii* isolated from patients were resistant to at least 8 antimicrobial agents, 43% (18 out of 42) of isolates from the ICU environment were resistant to at least 7 antibiotics but 97% (16 out of 17) of isolates from NS environment were resistant to 1 to 4 antibiotics.

Table 1 Resistance of *Acinetobacter baumannii* isolates to antibiotic classes

Antibiotic Classes	% resistance (N isolates resistant/N tested)
Penicillins	46 (34/74)
Cephalosporins	42 (31/74)
Carbapenems	43 (32/74)
Aminoglycosides	42 (31/74)
Tetracycline	70 (52/74)
Quinolones	39 (29/74)
Sulfonamide combination	54 (40/74)

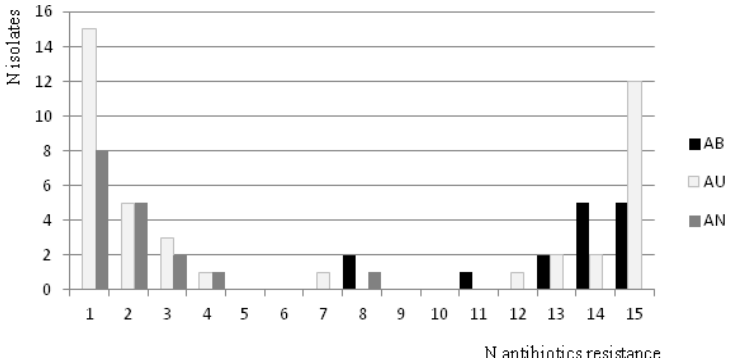

Fig. 1 Number of isolates resistant to a specific number of antibiotics. AB: *A. baumannii* isolated from patients, AU: *A. baumannii* from ICU environment, AN: *A. baumannii* from NS environment.

Our results showed that 27 out of 50 isolates analyzed (54%) exhibited the resistance phenotypes to the quaternary ammonium compound disinfectant agent evaluated, 7 of 14 isolates from patients with nosocomial infections, 6 of 11 isolates from the NS environment, and 11 of 26 isolates from the ICU environment (Table2).

Only 28 isolates of *A. baumannii* were classified in a clonal group which are shown in Figure. 73% (11/15) of the isolates from patient samples, named AB (including the only one from the NS, AB4), were genetically related. AB samples belonging to the clonal group H were classified in five subgroups of indistinguishable clones. Of the environmental samples from the NS, just AN7 & AN8 were indistinguishable clones (group A, Figure 2). 35.7% (15/42) of the ICU environmental isolates AU were grouped in 6 indistinguishable clone groups, noted in Figure 2 from B to G.

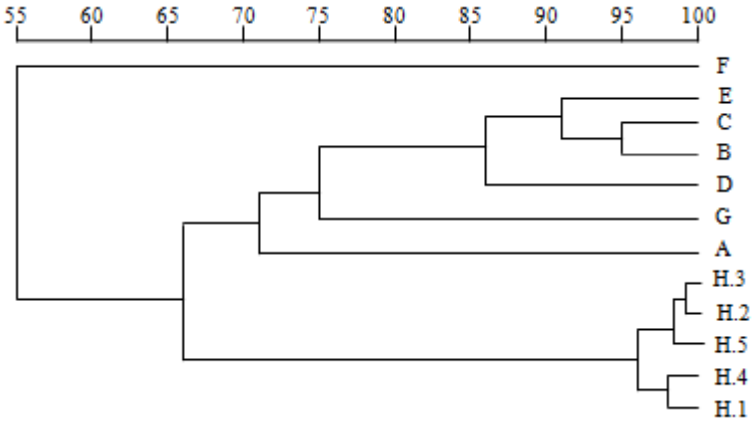

Fig. 2 ERIC-PCR dendrogram of *A. baumannii* related strains isolated from patients with nosocomial infections (AB) and the environment of ICU (AU) and NS (AN), showing the degree of strain similarity. Pearson (Opt 1.5%) (Tol 1.5%). In general, "different" was defined as <95% similarity and three band differences. "Similar" was defined as <97% similarity and up to two band differences. Indistinguishable" was defined as >95% similarity and no banding differences.

There was no correlation neither between multiresistance to antibiotics and resistance phenotype to the quaternary ammonium disinfectant nor between those from the same clonal groups (Table 2). As observed in strains from the H clonal group, isolates as AB9 and AB10, indistinguishable clones (H.3 group) are resistant to 15 and 11 antibiotics and just AB10 was resistant to the disinfectant tested. Strains susceptible to most antibiotics tested as AN7 and AN8 (clonal groups A) were resistant to the QAC disinfectant.

It can be noted that strains AB3, AB9 and AB10 belong to the same clonal group (H.3), showed the persistence and spread of this group from the second to the forth sampling period. Strain AB4 and AB12 from second and fifth sampling period (H.1 clonal group) came from different hospital settings, AB4 being the only strain isolated from a patient with nosocomial infection from the Neonatal Service.

Table 1 Susceptibility of *Acinetobacter baumannii* to 15 antibiotics and the quaternary ammonium disinfectant tested and repetitive sequences PCR clonal groups

Bacterial Isolates	Phenotype to the QAC	N antibiotics resistance	Rep-PCR clonal group
AB1	Resistant	15/15	-
AB2	Resistant	14/15	-
AB3	Sensitive	13/15	H.3
AB4	Sensitive	14/15	H.1
AB7	Resistant	15/15	H.4
AB8	Resistant	12/15	H.4
AB9	Sensitive	15/15	H.3
AB10	Resistant	11/15	H.3
AB11	Sensitive	15/15	H.5
AB12	Resistant	15/15	H.1
AB13	Sensitive	15/15	H.2
AB15	Resistant	15/15	-
AB16	Resistant	15/15	H.5
AB17	Sensitive	15/15	-
AN1	Sensitive	2/15	-
AN2	Resistant	5/15	-
AN4	Resistant	3/15	-
AN5	Sensitive	5/15	-
AN6	Sensitive	4/15	-
AN7	Resistant	3/15	A
AN8	Resistant	2/15	A
AN10	Resistant	3/15	-
AN13	Resistant	1/15	-
AN14	Resistant	2/15	-
AN17	Sensitive	6/15	-
AU1	Resistant	1/15	-
AU2	Resistant	2/15	-
AU3	Resistant	9/15	-
AU4	Resistant	4/15	D
AU7	Sensitive	4/15	D
AU8	Resistant	3/15	-
AU9	Sensitive	4/15	-
AU10	Sensitive	4/15	-
AU11	Sensitive	2/15	-
AU14	Resistant	6/15	-
AU15	Resistant	4/15	-
AU18	Sensitive	5/15	-
AU19	Resistant	4/15	-
AU20	Resistant	5/15	-
AU21	Resistant	15/15	-
AU23	Sensitive	15/15	G
AU29	Sensitive	15/15	-
AU31	Sensitive	15/15	-
AU32	Sensitive	15/15	C
AU33	Sensitive	15/15	B
AU34	Resistant	15/15	C
AU35	Resistant	4/15	-
AU36	Sensitive	15/15	B
AU38	Sensitive	2/15	-
AU41	Sensitive	15/15	C

(-)not in a clonal group

4. Discussion

In the Caracas University Hospital *A. baumannii* is the most important nosocomial pathogen, being found in all areas of critic care in this health care facility, evaluating its antibiotic and disinfectant susceptibility represents an important point to approach this problem in this healthcare facility of Venezuela´s capital city.

The multiple antibiotic resistance profiles demonstrate the need for antibiotic usage surveillance, especially due to the presence of multiresistant bacterial spread in the hospital units tested. The prevalence of resistance to SXT and tetracycline in this study was high. SXT resistance has been associated with the presence of movable elements as integrons and plasmids [10] while tetracycline resistance has been associated with plasmids and conjugative transposones [11]. Given the high incidence of these multiresistant pathogens in the environment of these critic care units and the importance of horizontal gene transfer, hand wash and surface cleaning methodologies have to be evaluated in these services.

Due to the widely distributed QAC resistant strains and the fact that we were not able to link this resistance to any antibiotic resistance mechanism, it is likely that more than one type of mechanism is involve, being a necessity to analyze alternative cleaning methods in the hospital.

The presence of related bacterial clones in different patients in different hospital units and the environment, and given that the problem related to nosocomial infections due to *A. baumannii* did not change in a one year period, the importance of surveillance measures in the hospital have to be a priority in these essential services.

The remarkable environmental adaptability of this microorganism highlights its importance as an opportunistic pathogen. The appearance of isolates resistant to most of the antimicrobial tested need to be considered for further studies because these multiresistant strains are settling in inanimate surfaces in these hospital units, being potential genetic reservoirs for multiple disinfectant and antibiotic resistance genes probably associated with mobile genetic elements.

Though we could not determine the environmental device responsible for any of the nosocomial infections observed, the presence of related bacterial isolates in different patients and the environment, and the high prevalence of resistance to antibiotics and QAC disinfectants have to be considered for further studies.

5. References

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