Antimicrobial Resistance Patterns of *Acinetobacter antitratus* from Cases of the Philippine General Hospital and the Conjugative Transferability of the Resistance

M. C. A. MADSON, E. R. SARNE[1] and E. C. CABRERA
Department of Biology, De La Salle University
Taft Avenue, Manila

ABSTRACT

Acinetobacter anitratus isolates from patients of the Philippine General Hospital were studied for their antimicrobial or drug resistance patterns and the transferability of the resistance to Escherichia coli SF-800 by conjugation. Twenty four of the 25 isolates were found to be resistant to at least one of the following test drugs, namely: ampicillin with sulbactam, amikacin, gentamicin, tobramycin, trimethoprim-sulfamethoxazole and nalidixic acid. Eighteen of the 25 isolates showed multiple resistance, some to as many as five drugs. Results of the conjugation studies showed that all isolates tested were able to transfer their resistance to E. coli SF-800. Three isolates transferred all of the resistance tested while the other ten isolates transferred only some of their resistance.

INTRODUCTION

Nosocomial or hospital-acquired infections are transmitted to patients either by hospital personnel or by other patients. They can be acquired through surgical procedures, bladder catheters, endotracheal tubes, intravenous fluids and other equipment (Joklik et al., 1984). Infection and colonization with *Acinetobacter sp.* occur when the organism is introduced into traumatic wounds particularly if there has been soil or water contamination. *Acinetobacter sp.* is also found in the normal flora of the skin and mucous membranes of humans. It has also been isolated from hospital walls and fixtures (Zembrzuska-Sadkowska, 1995). Although it possesses very little invasive activity, it had been found to be an etiologic agent of pneumonia and urinary tract infections (Finegold and Baron, 1986). Likewise, it has recently emerged as an important nosocomial pathogen because of its resistance to the majority of commonly-used antimicrobial agents (Triantafilo et al., 1997; Sing and Yeo, 1996; Roussel-Delvallez et al., 1996).

[1]Health And Sciences Campus, College of Medicine, De La Salle University
Dasmarinas, Cavite

One of the serious problems faced today in the treatment of diseases is the development of bacterial resistance to drugs used. Drug resistance can be inherent in the bacteria or caused by chromosomal mutation to resistance. More importantly, it can also be caused by extrachromosomal genetic elements called R plasmids or resistance plasmids. R plasmids usually code for resistance to multiple drugs. Some plasmids are transferable to other bacteria belonging to the same or to different genera and species through conjugation. This results in the conversion of the drug sensitive recipients to resistant strains, usually with multiple resistance.

This study aimed to determine the response of nosocomial *A. anitratus* to commonly-used antimicrobial agents and to determine the conjugative transferability of the resistance characteristics in these isolates to *E. coli*.

MATERIALS AND METHODS

A. anitratus isolated from patients of the Philippine General Hospital from September to November, 1996 were studied. The isolation and identification were done by the staff of the Bacteriology Section of the Department of Laboratories of the hospital. Twenty five isolates were tested for their susceptibility to the following drugs, namely: ampicillin with sulbactam [10 micrograms (µgs)], amikacin (30 µgs), gentamicin (10 µgs), tobramycin (10 µgs), trimethoprim-sulfamethoxazole (1.25 µgs, 23.75 µgs) and nalidixic acid (30 µgs). *E. coli* SF800 which is $Lac^{+}Res^{-}$ was used as the recipient for the conjugative transferability.

The drug susceptibility patterns of the isolates were determined using the disc-agar diffusion method modified from that of Bauer *et al.* (1966). The inocula used were four-hour cultures in tryptic soy broth (TSB) prepared from 18-hour TSB cultures. Turbidity of the isolates was adjusted to equal that of 0.5 MacFarland standard to approximate an inoculum concentration of 1.5×10^{8} cells ml^{-1}. The isolates were plated on Mueller-Hinton II agar. The diameters of the zones of inhibition were measured to the nearest millimeter and interpreted with the use of the zone diameter interpretative chart of the National Committee on Clinical Laboratory Standards (N.C.C.L.S.).

Conjugation was carried out using a modification of the method of Sinclair *et al.*,(1981). Of the 25 isolates, thirteen isolates which were multiply resistant to the test drugs were used as donors in the conjugation studies. Fifty microliters (uls) and 200 uls of donor and recipient cells, respectively, were inoculated in tryptic soy agar plates using the spread plate method. The plates were incubated at 37°C for four to six hours. Sterile saline solution with a volume of 1.5 ml was added to each plate after which the growth was harvested. One hundred uls of the growth mixture were inoculated into Mac Conkey agar plates containing each of the following: ampicillin with sulbactam (10 µgs/ml), amikacin (30 µgs/ml), gentamicin (10 µgs/ml), tobramycin (10 µgs/ml), and trimethoprim sulfamethoxazole (1.25 µgs, 23.75 µgs/ml). The resulting *E. coli* transconjugants were lactose fermenters showing resistance to the test drug(s). These were differentiated from the donor, *A. anitratus* which were non-lactose fermenters and from *E. coli* SF 800 which did not receive the resistance genes. The latter did not grow on the selective Mac Conkey agar plates with the drugs. Using the Modified Kirby-Bauer diffusion method, the responses of the transconjugant suspects to the test drugs were confirmed.

The 18-hour cultures of *A. anitratus* donors, and *E. coli* SF 800 were also inoculated in selective plates with the individual antibiotics to serve as controls. This was done to check for the emergence of spontaneous mutants to resistance and also to check the efficacy of the drugs in the plates used.

RESULTS AND DISCUSSION

The resistance phenotypes of the *A. anitratus* isolates are shown in Table 1. The results show that out of the 25 isolates, 24 (96%) were resistant to at least one drug, with 18 out of the 24 (75%) being multiply-resistant. Four (16 %) of these were resistant to two drugs and five (20 %) isolates were resistant to three drugs. Six (24 %) isolates were resistant to four drugs and three (12 %) were resistant to five drugs. Results correlate well with those of studies done in other countries which also show multiple drug resistance in most if not all of the isolates tested (Triantafilo et al., 1997; Shi-Zy et al., 1996; Roussel-Delvallez et al., 1996; Ling et al., 1996; Sing and Yeo, 1996; De and Deodhar, 1995). Literature search done by the authors did not yield published data on the drug resistance profiles of local *Acinetobacter* isolates.

Table 1 Resistance phenotypes of the *Acinetobacter anitratus* isolates from patients of the Philippine General Hospital.

Isolate Number	Resistance Phenotype
1	NA, GM, TS, TN, AN
2	SAM, NA, GM, TS
3	NA, GM, TS, TN
4	NA, GM, TS, TN, AN
5	SAM, NA, TS
6	NA, TS
7	NA, GM, TS
8	NA, GM, TS
9	NA
10	NA, GM, TS
11	NA, TS
12	NA
13	NA
14	NA, TS, TN
15	NA, GM, TS, TN
16	NA, GM, TS, TN
17	NA, GM, TS, TN, AN
18	NA
19	NA, TS
20	NA
21	SAM, NA, TS
22	NA
23	NA, GM, TS, TN
24	sensitive
25	NA, TS

SAM - ampicillin with sulbactam
AN - amikacin
GM - gentamicin
NA - nalidixic acid
TN - tobramycin
TS - trimethroprim-sulfamethoxazole

Table 2 shows that 24 (96%) of the 25 isolates tested were resistant to nalidixic acid. The study of Ferreira et al. (1984) shows that 48% of the *Acinetobacter* isolates tested were still sensitive to the drug in contrast to the results of the present study where only 4% of the isolates (one isolate) was sensitive to it. Amikacin was shown to be the antibiotic to which most isolates (84%) were sensitive. This was followed by sensitivity to ampicillin/sulbactam (80%).

Table 2: Response of the 25 isolates of *Acinetobacter anitratus* from patients of the Philippine General Hospital to the different test antibiotics.

Antibiotics	Susceptible Isolates		Resistance Isolates	
	Number	%	Number	%
SAM (10 ugs)	20	80	4	16
NA (30 ugs)	1	4	24	96
GM (10 ugs)	14	56	11	44
TS (25 ugs)	7	28	18	72
TN (10 ugs)	17	68	8	32
AN (30 ugs)	21	84	3	12

ugs - micrograms
SAM - ampicillin with sulbactam
AN - amikacin
GM - gentamicin
TN - tobramycin
TS - trimethroprim-sulfamethoxazole
NA - nalidixic acid

Ferreira et al. (1984) also showed that the *Acinetobacter* isolates tested were most sensitive to amikacin. Ling et al. (1996) found that amikacin was "reliably active" against the organism. In the study of Gales et al. (1996) however, only 16% of the isolates tested was sensitive to amikacin. Results of Urban et al. (1993) and Gales et al. (1996) among others, also show that *A. anitratus* was susceptible to ampicillin with sulbactam. Sulbactam, a beta-lactamase inhibitor is directed against plasmid-mediated enzymes and various extended-spectrum enzymes. Sulbactam is also active against some of the chromosomally-mediated enzymes and is active against *Acinetobacter* and *Bacteroides* (Williams, 1997). Resistance to trimethoprim-sulfamethoxazole, gentamicin and tobramycin were shown by 72%, 44% and 32% of the isolates, respectively.

Data on the transferability of the drug resistance to *E. coli* SF800 through conjugation show that all of the thirteen isolates transferred their resistance to the recipient (Table 3). Of these thirteen isolates, three effected complete transfer (i.e., all resistance tested were transferred). The other isolates transferred only some of their

Table 3 Resistance transferred and not transferred to *Escherichia coli* SF 800 from *Acinetobacter anitratus* isolates from patients of the Philippine General Hospital after conjugation.

Isolate Number	Resistance transferred	Resistance not transferred	Interpretation
SF-800 + isolate number 1	GM, TN, TS	SAM, AN	Partial transfer
SF-800 + isolate number 2	GM, TN, TS	SAM, AN	Partial transfer
SF-800 + isolate number 3	GM, TN	TS	Partial transfer
SF-800 + isolate number 4	AN, TN, TS	GM	Partial transfer
SF-800 + isolate number 5	TS	SAM, TN	Partial transfer
SF-800 + isolate number 7	GM, TS	none	Complete transfer
SF-800 + isolate number 8	GM, TS	none	Complete transfer
SF-800 + isolate number 14	TN	TS	Partial transfer
SF-800 + isolate number 15	TS	GM, TN	Partial transfer
SF-800 + isolate number 16	GM, TN, TS	none	Complete transfer
SF-800 + isolate number 17	GM	AN, TN, TS	Partial transfer
SF-800 + isolate number 21	TS	SAM	Partial transfer
SF-800 + isolate number 23	GM, TS	TN	Partial transfer

SAM - ampicillin with sulbactam
AN - amikacin
GM - gentamicin
TN - tobramycin
TS - trimethoprim-sulfamethoxazole

resistance. Resistance to nalidixic acid, on the other hand has been reported to be chromosomally mediated (Joklik et al., 1984), so its transfer was not expected. The same result was obtained in the study.

Local studies also show the conjugative transfer of drug resistance from nonfermentative, gram negative bacilli like *Pseudomonas aeruginosa* (Cabrera et al., 1997), and from fermentative, gram negative bacilli like *Vibrio cholera* (Cabrera, 1994), *Salmonella* spp. (Cabrera, 1987) and *E. coli* (Hernandez and Raymundo, 1989; Cabrera, 1988). The results are of significance to public health. When bacteria

become resistant to drugs by the acquisition of R-plasmids, they acquire a fast recovery rate, are selectively filtered out for survival and are disseminated in the environment, most especially in an environment with heavy usage of antibiotics such as in a hospital setting. Considering that R plasmids are also often transferable even to those belonging to a different family, such as between *Acinetobacter sp.* and *E. coli,* their presence in bacteria increases the chance of nosocomial infections caused by multiple drug resistant strains (Saunders, 1984). This definitely results in difficult therapeutic problems.

RECOMMENDATION

Considering the rapid emergence of *Acinetobacter* sp. as a nosocomial pathogen in other countries, many isolates of which are multiple drug resistant strains, and the finding that its resistance is transferable to other bacteria belonging to a different family, it is recommended that more intensive monitoring of cases and more extensive study of local *Acinetobacter* isolates, in particular, their drug resistance characteristics, be pursued.

REFERENCES

BAUER, A., KIRBY, K., SHERRIS, J., TURCK, M.1966. American Journal of Clinical Pathology as cited by Cabrera, E. 1994. Conjugative transferability of the multiple drug resistance in *Vibrio cholerae* isolates. Acta Manilana 42:15-19.

CABRERA, E. 1987. Plasmid-mediated multiple antibiotic resistance in *Salmonella* isolates from cases of the Philippine General Hospital. The Philippine Journal of Science.116

CABRERA, E. 1988. Antibiograms and conjugative transferability of multiple antibiotic resistance in Escherichia coli of chickens and poultry farm workers. The Philippine Journal of Science. 117: 385-394

CABRERA, E. 1994. Conjugative transferability of the multiple drug resistance in *Vibrio cholera* isolates. Acta Manilana. 42:15-19.

CABRERA, E., HALOS, S. and VELMONTE, M. 1997. Antibiograms, O serotypes and R plasmids of nosocomial *Pseudomonas aeruginosa* isolates from the ICU-CCU of the Philippine General Hospital. The Philippine Journal of Microbiology and Infectious Diseases. 26: 121-128.

DE, A. and DEODHAR, L. 1995. Antibiotic resistance patterns and R plasmids of *Acinetobacter calcoaceticus* subsp. *anitratus*. Indian Journal of Pathology and Microbiology. 38: 185-188.

FERREIRA, M., VIEU, J., KLEIN, B. 1984. Phage-types and susceptibility to 26 antibiotics of nosocomial strains of *Acinetobacter sp.* isolated in Portugal. Journal of International Medical Research. 12 (1):364-367.

FINEGOLD, S. and BARON, E. (ed.). 1986. Bailey and Scott's Diagnostic Microbiology. 7th ed. C.V. Mosby Company. U.S.A.

GALES, A., SADER, H., SINTO, S., SANTOS, O. and MENDES, C. 1996. In-vitro activity of ampicillin-sulbactam against clinical multiple resistant *Acinetobacter baumanii* isolates. Journal of Chemotherapy. 1996. 8 ; 416-419.

HERNANDEZ, M. and RAYMUNDO, A. 1989. Incidence and conjugal transfer of antibiotic resistance in *Escherichia coli* from pig feces. Philippine Agriculture. 71: 403-408.

JOKLIK, W., WILLET, H., and AMOS, B. ed. 1984. Zinsser Microbiology. Appleton Century Crafts. Prentice Hall. Int.

LING, J., NG, T., CHENG, A., NORRBY, S. 1996. Susceptibilities to 23 antimicrobial agents and beta-lactamase production of blood culture isolates of *Acinetobacter* sp. in Hongkong. Scandinavian Journal of Infectious Diseases. Suppl. 1996.101: 21-25.

ROUSSEL-DELVALLEZ, M., WALLET, F., DELPIERRE, F. and COURCOL, R. 1996. In-vitro bactericidal effect of a beta-lactam and aminoglycoside combination against multiple resistant *Pseudomonas aeruginosa* and *Acinetobacter baumanii*.. Journal of Chemotherapy. 8: 365-368

SAUNDERS, J. 1984. Genetics and evaluation of antibiotic resistance. British Medical Bulletin. 408:54-60.

SHI-, Zy, LIU, P., LAU, Y., LIN, Y., HU, B., SHIR, J. 1996. Antimicrobial susceptibility of clinical isolates of *Acinetobacter baumanii*. Diagnostic Microbiology of Infectious Diseases. 24: 81-85.

SINCLAIR, M., ASCHE, V., MORGAN, A., HOLLOWAY, B. 1981. The Medical Journal of Australia as cited by Cabrera, E. 1994. Conjugative transferability of the multiple drug resistance in *Vibrio cholera* isolates. Acta Manilana. 42:15-19.

SING, L. and YEO, M. 1996. Changing trends in the antimicrobial susceptibility of clinical isolates of *Acinetobacter baumanii*. Annals of the Academy of Medicine of Singapore. 25: 179-183.

SYNDMAN, D. 1991. Clinical implications of multi-drug resistance in the intensive care unit. Scandinavian Journal of Infectious Diseases. 5.Suppl. 78:54-63. Verbist, L. 1991. Incidence of multi-resistance in gram-negative bacterial isolates from intensive care units in Belgium: a surveillance study. Scandinavian Journal of Infectious Diseases 4:45-53 as cited by Bergogne Berezin, E., Decre, D., Joly-Guillou, M. 1993. Opportunistic nosocomial multiply resistant bacterial-infections-their treatment and prevention. Journal of Antimicrobial Chemotherapy. 32. Suppl. A:39-47.

SY, E. 1987. Antibiotic resistance pattern of *Escherichia coli* isolated from antibiotic-treated suckling pigs and transferability by conjugation of the antibiotic resistance to antibiotic sensitive *Escherichia coli.* Unpublished Bachelor's Thesis. DLSU.

TRIANTAFILO, V., FICA, A., SILVA, M. and THOMPSON, L. 1997. E-test to study inhibitory concentration, bacterial diversity and to identify presumptively beta-lactamase production in strains of *Pseudomonas aeruginosa* and *Acinetobacter baumanii* associated with nosocomial infections. Rev. of Med. Chile. 125: 149-160.

URBAN, C., GO, E., MARIANO, N., BERGER, B.J., AVRAHAM, I., RUBIN, D., RAHAL, J.J. 1993. Effect of sulbactam on infections caused by imipenem-resistant *Acinetobacter calcoaceticus* biotype *anitratus.* Journal of Infectious Diseases. 167(2): 448-451.

VOLK, W. and WHEELER, M. 1984. Basic Microbiology. Harper and Row Publishers, Inc. pp. 251-256; 463.

WATANABE, T. and FUKASAWA. 1961. Episome-mediated transfer of drug resistance in Enterobacteriaceae : Transfer of resistance by conjugation. Journal of Bacteriology. 81:668-678.

WILLIAMS, J. 1997. Beta-lactamase inhibition and in vivo activity of sulbactam and sulbactam/cefoperazone. Clinical and Infectious Diseases. 24: 494-497.

ZEMBRZUSKA-SADKOWSKA, E. 1995. The danger of infection of the hospital patients with the microorganisms present in preparations and in the hospital environment. Acta Pol. Pharm. 52: 173-178.