IN VITRO EVIDENCES OF DISCRET ALTERATIONS IN THE BACTERIAL RESISTANCE/SUSCEPTIBILITY PROFILES INDUCED BY ANTINEOPLASTIC AGENTS

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Summary

This study evaluated the *in vitro* alteration in the resistance/susceptibility profiles of *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* induced by 5-fluorouracil, bleomycin, and *cis*-diammine-dichloroplatinum. Results showed that antineoplastic drugs might induce discrete fluctuations in resistance/susceptibility profiles that are important during the course of a infection in patients undergoing antiproliferative chemotherapy.

Key words: Bacterial resistance, antineoplastic, resistogram.

Introduction

Among the problems concerning to hospital infections, we can point out the rapid arising of multi-resistant strains of many pathogens. Undoubting, this problem may be increased when the patient suffers of malignant diseases and is under a chemotherapy regimen.

In a recent work, it was described that antitumor drugs may induce changes in virulence factors of common oral yeast *Candida albicans* (1). Among the findings, the authors related the increasing of resistance levels against amphotericin B and miconazole when yeast strains were grown in the presence of therapeutic doses of 5-fluoruracil, cis-diammine-dichloroplatinum, and peplomicin. However, there are no references of similar studies with bacteria.

The present study evaluated the eventual possibility of drugs, taken from the antitumor arsenal, to induce any alteration on the susceptibility/resistance balance in bacteria commonly involved in hospital infections.

Material and Methods

For control group, bacterial strains (Table 01) were chosen and processed according to CLSI protocols (2). The bacteria were grown in Muller-Hinton broth (Merck KgaA, Darmstadt, Germany) at 35^0C and normal atmosphere, for 24h. After this, one-milliliter aliquots of each culture suspension were diluted until turbidimetric grade next to 0.5 MacFarland tube. Suspensions were plated on Muller-Hinton Agar plates by swabbing. Antibiogram disks (Newprov Prod. Lab. Ltda, Pinhais, Brazil) (Table 01) were disposed onto culture medium surfaces and the plates were incubated at 35^0C, for 24 hours. The inhibition zones surrounding the paper disks had their diameters measured using a digital caliper Mitutoyo 500-68X (Mitutoyo Co, Kanagawa, Japan). Six repetitions were carried out for each bacterium, in three different occasions.

Table 01. Bacterial strains and antibiotic disks

Bacteria (strain)	Antimicrobial* disks (concentration)
Staphylococcus aureus **ATCC®25923**	**AMI (30μg), CFL (30μg), CLI (2μg), CLO (30μg), ERI (15μg), GEN (10μg), IMI (10μg), OXA (1μg), SXT (20μg), TEC (30μg), TET (30μg), VAN (30μg), NIT (300μg)**
Enterococcus faecalis **ATCC®29212**	**AMP (10μg), CIP (5μg), ERI (15μg), GEN (120μg), PEN (10U), TET (30μg), VAN (30μg), NIT (300μg), NOR (10μg)**
Pseudomonas aeruginosa **ATCC®27853**	**AMI (30μg), CAZ (30μg), CFL (30μg), CFO (30μg), CIP (5μg), CLO (30μg), GEN (10μg), PIP (100μg), SXT (20μg)**
Escherichia coli **ATCC®25922**	**AMI (30μg), CAZ (30μg), CFL (30μg), CFO (30μg), CFT (30μg), CIP (5μg), CLO (30μg), GEN (10μg), PIP (100μg), SXT (20μg)**
Klebsiella pneumoniae **ATCC®700603**	**AMI (30μg), CAZ (30μg), CFL (30μg), CFO (30μg), CIP (5μg), CLO (30μg), GEN (10μg), PIP (100μg), SXT (20μg)**

*AMI (amikacin), AMP (ampicillin), CAZ (cefazolin), CFL (cephalothin), CFO (cefoxitin), CFT (ceftazidime), CIP (ciprofloxacin), CLI (clindamycin), CLO (chloramphenicol), ERI (erythromycin), GEN (gentamicin), IMI (imipenem), NIT (nitrofurantoin), NOR (norfloxacin), OXA (oxacillin), PEN (penicillin G), PIP (pipemidic acid), SXT (sulfazotrim), TEC (teicoplanin), TET (tetracycline), VAN (vancomycin).

In parallel, the strains were grown in Muller-Hinton Broth (Merck KgaA, Darmstadt, Germany) plus 100μM 5-fluorouracil (5-FU), 10μU/mL bleomycin (BLM), or 10μg/mL *cis*-diammine-dichloroplatinum (CDDP). Incubations were carried out at 35^0C and normal atmosphere. After 24h, one-milliliter aliquots of each culture were diluted until turbidimetric grades next to 0.5 MacFarland tube. Suspensions were plated on Muller-Hinton Agar plates plus 100μM 5-fluorouracil (5-FU), 10μU/mL bleomycin (BLM), or 10μg/mL *cis*-diammine-dichloroplatinum (CDDP) by swabbing. Antibiogram disks (Table 01) were disposed onto culture medium surfaces and the plates were incubated at 35^0C, for 18-24 hours. The inhibition disk zones had their diameters measured as above. Six repetitions were carried out for each bacterium, in three different occasions.

To eliminate the null hypothesis that the differences in inhibition halos observed among the experimental groups (Normal, 5-FU, BLM, and CDDP) were due to mere coincidence all the data were checked over in relation to their homogeneity of variances by the Levene index and analyzed by the Games-Howell and Tukey HDS tests with the statistical package SPSS 13.0. (SPSS Inc., Chicago, Il.).

Results

According to figures 1, 2, 3, 4, and 5 the average disk zones for *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* showed no differences ($p > 0.05$) before and after the treatments. However, it is perceptible that in some instances, there are variations in discs' halos due to treatments. In those cases when there were no superpositions of confidence intervals of treatments and controls, we surrounded the peaks with a dotted line.
All the data were analyzed in relation to the resistance degree following the guidelines of CLSI (2005) [CLSI 2005] and in one case the resistance status was altered; only *Escherichia coli* ATCC®25922 decreased its sensibility to cephalotin 30μg from 19.0±0.1mm (susceptible) to 17.2±0.4 (intermediary) when was grown in presence of bleomycin. Also it can be seen in figure 4 that bleomycin reduced the susceptibility to many antimicrobial drugs.

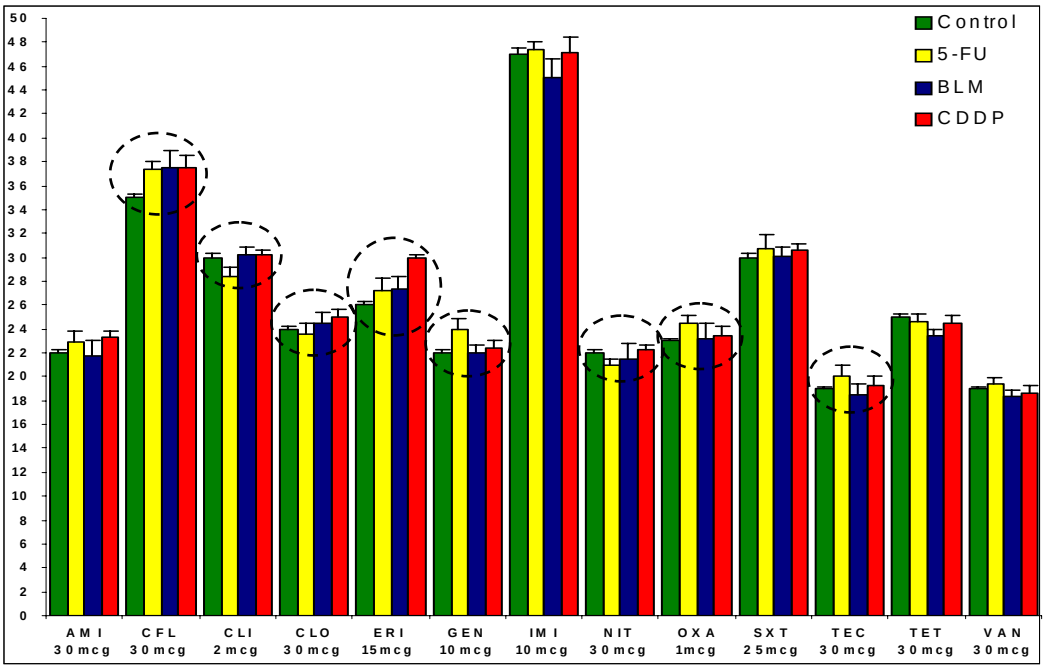

Fig. 1. Comparative resistograms for *Staphylococcus aureus* ATCC®25923 submitted to different antineoplasic drugs regimen. Antibiotics' acronyms listed in table 1. Doted fields indicate cases in which one or more confidence intervals of treatments do not superpose the confidence interval of control. None of the treatments diverged statistically from the control (Games-Howel or Tukey HDS; $p>0.05$). Numbers in ordenate *y*-axis refer to millimeters

Discussion

There is few information concerning to bacterial resistance induced by chemotherapy agents. Based on this, we proposed to evaluate such possibility. For this, the antitumor drugs were diluted until concentrations near to those found after plasmatic distribution (3, 4, 5, 6, 7, 8, 9). The results showed that antineoplastic drugs might induce any degree of fluctuation in disk zones measurements, even though, with no great statistic significance. At least in part, it may be explained due to some bacteria have a considerable number of *ble* gene variants (10) that code products with high affinity by bleomycin, reducing its antibacterial and mutagenic activities. Also, CDDP-resistant cells of *E. coli* show a reduced drug uptake, as well as an increasing in its DNA repairing capacity (11), maybe by RecA protein that is involved in

events of recombination and mutation restoring. An increase in RecA rates in CDDP-resistant *Salmonella typhimurium* strains also was reported (12). Beside the mechanisms described above, many glutathione-synthesizing bacteria are also less prone to the CDDP action (13).

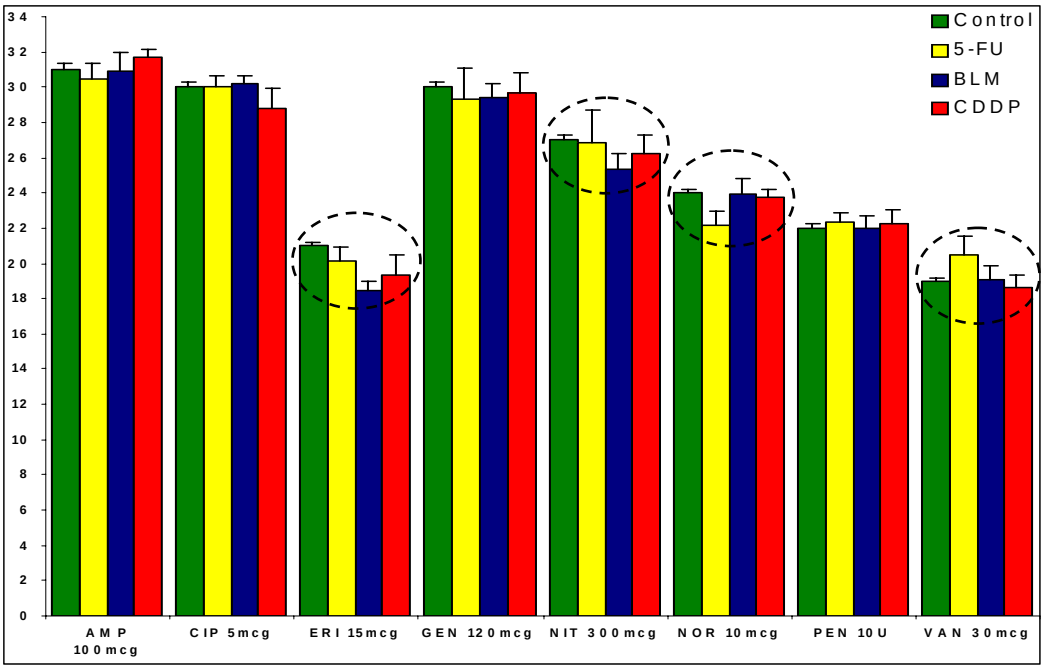

Fig. 2. Comparative resistograms for *Enterococcus faecalis* ATCC®29212 submitted to different antineoplasic drugs regimen. Antibiotics' acronyms listed in table 1. Doted fields indicate cases in which one or more confidence intervals of treatments do not superpose the confidence interval of control. None of the treatments diverged statistically from the control (Games-Howel or Tukey HDS; $p>0.05$). Numbers in ordenate *y*-axis refer to millimeters.

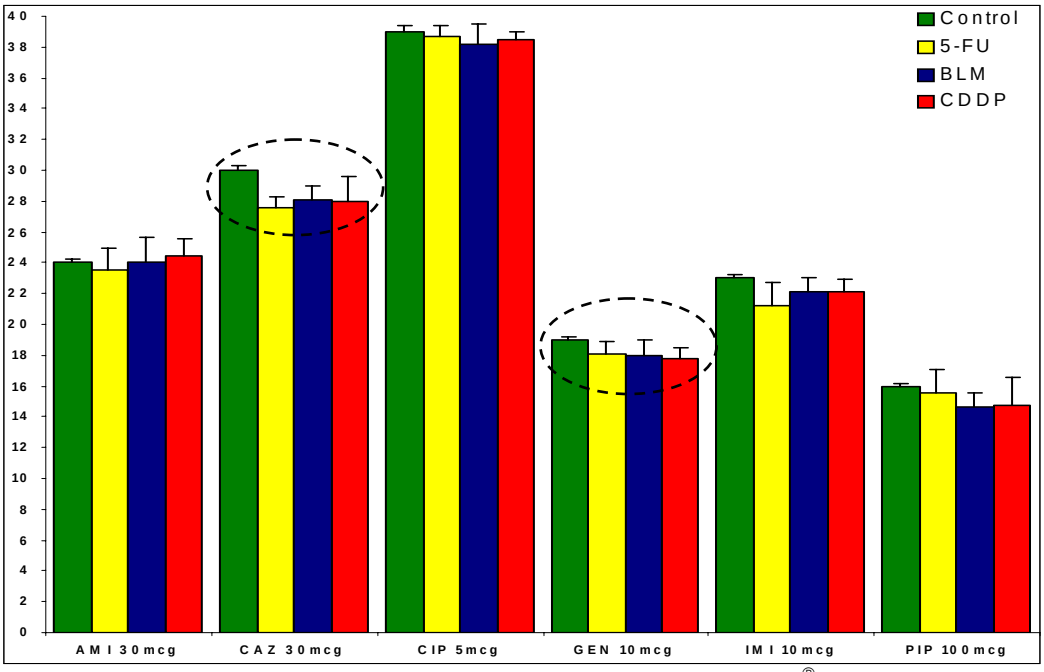

Fig. 3. Comparative resistograms for *Pseudomonas aeruginosa* ATCC®27853 submitted to different antineoplasic drugs regimen. Antibiotics' acronyms listed in table 1. Doted fields indicate cases in which one or more confidence intervals of treatments do not superpose the confidence interval of control. None of the treatments diverged statistically from the control (Games-Howel or Tukey HDS; $p>0.05$). Numbers in ordenate *y*-axis refer to millimeters.

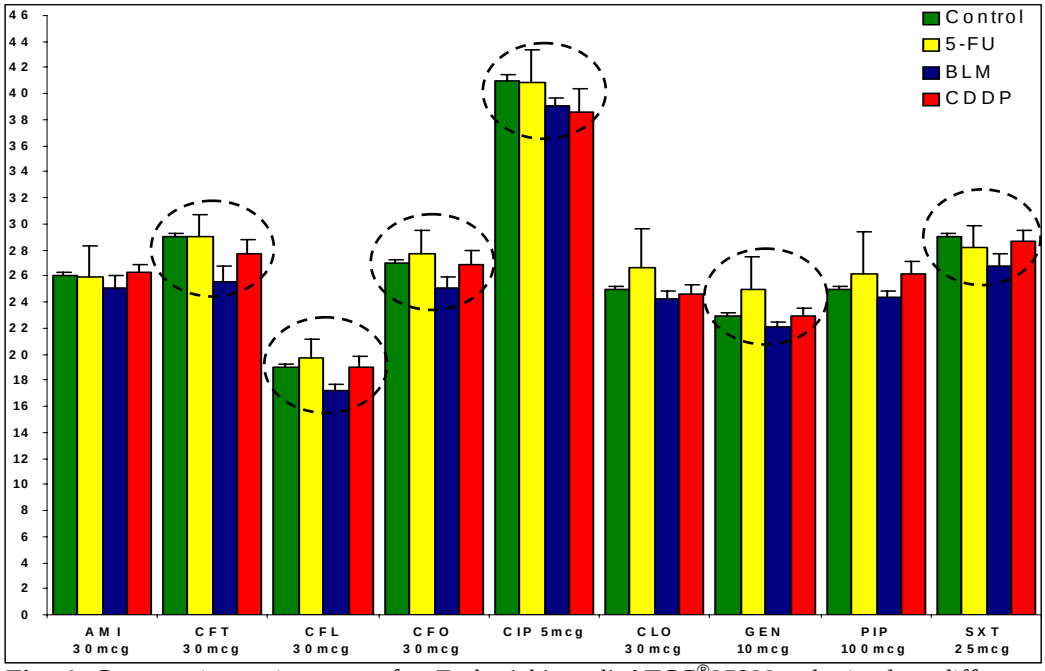

Fig. 4. Comparative resistograms for *Escherichia coli* ATCC®25922 submitted to different antineoplasic drugs regimen. Antibiotics' acronyms listed in table 1. Doted fields indicate cases in which one or more confidence intervals of treatments do not superpose the confidence interval of control. None of the treatments diverged statistically from the control (Games-Howel or Tukey HDS; p>0.05). Numbers in ordenate *y*-axis refer to millimeters.

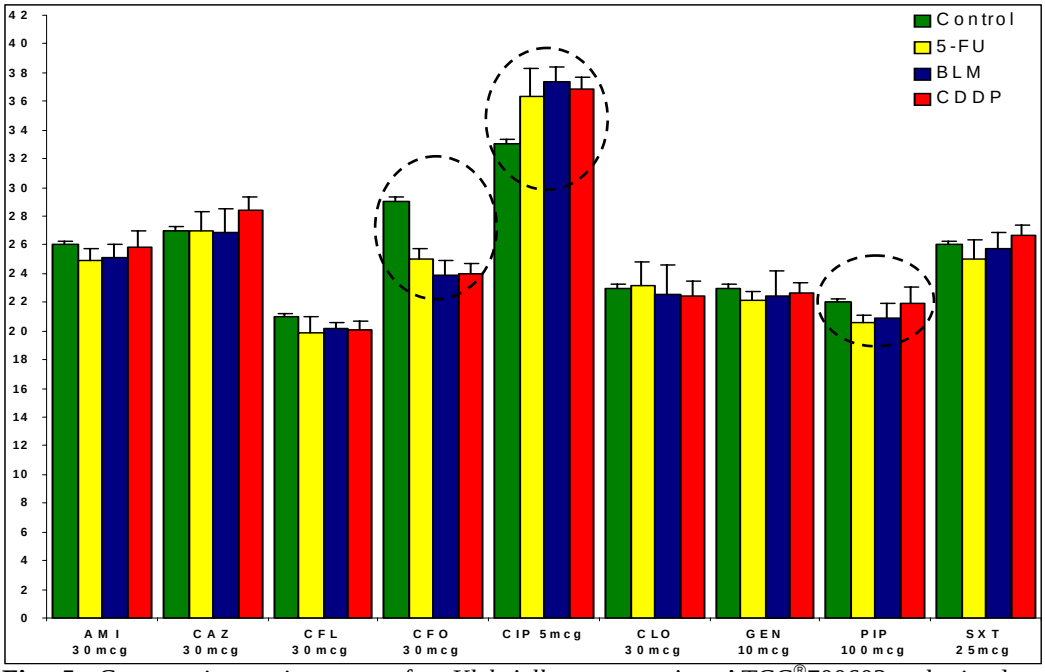

Fig. 5. Comparative resistograms for *Klebsiella pneumoniae* ATCC®700603 submitted to different antineoplasic drugs regimen. Antibiotics' acronyms listed in table 1. Doted fields indicate cases in which one or more confidence intervals of treatments do not superpose the confidence interval of control. None of the treatments diverged statistically from the control (Games-Howel or Tukey HDS; p>0.05). Numbers in ordenate *y*-axis refer to millimeters.

Nyhlen *et al*. (14, 15) evaluated the synergic antimicrobial potential of 5-FU and some antibiotics in *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *E. coli*. They proposed that 5-FU does not interfere in the post-antibiotic effect for the most part of tested bacteria. Moreover, the synergic prolonging of post-antibiotic effect was only induced by 5-FU plus meropenem/ciprofloxacin in *S. epidermidis* and by association with tobramycin in *S. aureus*.
It was previously reported that an interaction between 5-FU and beta-lactams results in a synergistic effect over Gram-negative (16). Such finding was not observed in our study. The disparity found between the studies may be due to the fact that both surveys were conduced following distinct methodologies. Whereas the Gieringer's study (16) involved the antitumor dilution in agar, we opted by to challenge the bacteria with antineoplastic drugs before and during the resistogram. In our conception, the last experimental strategy is clinically more realistic, once presumably, patients begin the antitumor therapeutic regimen before the infection's development.
However, we must assume that these results may not be applied to all clinical cases, once only susceptible bacterial strains were used here (2). Moreover, in many cases we observed non-superposition of confidence intervals. For clinical strains with fewer susceptible phenotypes, as those *borderline* strains, a minimum fluctuation may shift their status from susceptible to resistant or vice-versa as occurred here with *E. coli* ATCC®25922 in the presence of bleomycin.
The main contribution that our data provide is centered on the statement that the clinic and the laboratory personnel must be aware to the fact that infections in patients undergoing antineoplastic treatment may involve bacteria with shifted resistance phenotypes.
Finally, other studies must be conduced prospecting the same resistance behavior *in vivo*, once it was not evaluated important features (e.g., the action of antineoplastic metabolites over the bacteria). Also, studies involving other bacteria as *Haemophyllus* spp., *Neisseria* spp., *Vibrio* spp., *Streptococcus* spp., anaerobes and other fastidious bacteria, as well as clinical specimens, must be carried out.

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