

*Full Length Research Paper*

# Antimicrobial resistance and disinfectants susceptibility of persistent bacteria in a tertiary care hospital

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It is well known that putative pathogenic bacteria are ubiquitous and widely distributed in the hospital environment. This study aimed to detect bacterial persistence in the nosocomial environment (different critical areas of the hospital) after mopping by the cleaning staff. Susceptibility patterns to antimicrobial drugs and disinfectants commonly used in health services were also investigated by disk diffusion and agar dilution tests. Rinse water from mops was processed for isolation of *Enterobacteriaceae* (GNR), non-fermenting Gram-negative rods (NFGNR), coagulase-negative staphylococci (CNS) and enterococci (ENT). Microorganisms were biochemically characterized and 547 strains were recovered. Only CNS and NFGNR were isolated in all critical areas. Overall 67% of the isolated bacteria were resistant to more than three drugs, being considered as multiresistant. Disinfectants were effective in concentrations ranging from 0.125 to 1%. Hospitals provide reservoirs of multiresistant microorganisms borne by patients and staff, but the hospital environment may be an important repository. Preventing the spread of relevant bacteria depends on the quality of hospital routine cleaning services. Monitoring bacteria susceptibility to antimicrobials and disinfectants may help the management of nosocomial infections.

**Key words:** Nosocomial environment, hospital cleaning, antimicrobial drugs, disinfectants.

## INTRODUCTION

Hospital infections, a severe public health issue, are widespread and have high economic and social impact (Blanc, 2004). Most infections may be related to unbalanced microbiota and host defense mechanisms, but undoubtedly hospital environments are a great source of

potentially pathogenic microorganisms (Bryce et al., 2007). Several bacteria are associated to nosocomial infections, mainly representatives of Gram negative rods from the *Enterobacteriaceae* family (GNR), non-fermenting Gram-negative rods (NFGNR), Gram positive cocci *Staphylococcus*, especially coagulase-negative species (CNS) and *Enterococcus* (ENT) (Sader et al., 2001). Antimicrobial resistance turns into a complex both ecological and clinical problems when considering the genetic variability in microorganisms. Its contention is one of the greatest challenges of the 21st century, and originates appeals from several international Health Organizations asking for regional data in bacterial susceptibility patterns, especially for strains of nosocomial circulation (ASM, 2009). Microorganisms may

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**Abbreviations:** GNR, Gram negative rods from the *Enterobacteriaceae* family; NFGNR, non-fermenting gram negative rods; CNS, coagulase-negative staphylococci; ENT, enterococci.

be associated to several biological materials in the hospital environment such as floors, walls, ceiling, doors, windows, electro-electronic equipment and specific hospital articles in use for assistance to patients (Rossi et al., 2008). Thus, the quality of cleaning services is an important condition in the prevention and control of microbial spread, as well as the type of disinfectants used to diminish risks of cross infections during healthcare assistance (Kramer et al., 2006).

The most commonly used chemical agents in the nosocomial environment for high level of disinfection are glutaraldehyde, the association of peracetic acid/hydrogen peroxide (0.5 to 2%) and sodium hypochlorite (1%). For medium level of disinfection the products generally used are sodium hypochlorite (0.3 to 0.5%), iodofors, phenol derivatives, 70% ethyl alcohol and 92% isopropyl alcohol. Quaternary ammonium compounds and low concentration sodium hypochlorite (0.2%) are used for low level cleaning and disinfection (Rutala and Weber, 2007).

Identifying microorganisms and their susceptibility patterns to antimicrobial drugs and nosocomial disinfectants could be useful to trace origins and determine the persistence of bacteria potentially associated to hospital infections. This study aimed to evaluate bacterial persistence in the nosocomial environment of the University Hospital, University of Juiz de Fora, Brazil, in the rinse water after mopping by the cleaning staff and to determine the susceptibility patterns of the isolated bacterial strains to antimicrobial drugs and disinfectants commonly used in health care routine protocols.

## MATERIALS AND METHODS

### Isolation and identification of bacterial samples

One hundred aliquots of 2 mL of the water used to rinse mops at the University Hospital of Juiz de Fora were collected and processed for the selective isolation of GNR, NFGNR, CNS and ENT. The water aliquots were collected in two sets of fifty, as duplicates within a six months interval, from different critical areas of the hospital, which were: surgical and intermediary surgical centre, intensive care unit, bone marrow transplant unit, pediatric isolation unit, low birth weight newborn infirmary, lactarium, male and female infirmary of infectious diseases, pharmacy, clinical analysis and pathological anatomy laboratories, kitchen, laundry and sterilized material centre.

The routine wet swapping is usually made with a mop wetted with water and neutral detergent, under the supervision of the 'hygiene and cleaning service'. The collected material was serially diluted and inoculated into different culture media. From 3 to 5 bacterial colonies were isolated from each culture medium.

Hypertonic Manitol Agar (Himedia Laboratories, Mumbai, India) was used for the isolation and presumptive identification of CNS. The isolated bacteria were identified according to established methodology through the morphotinctorial and biochemical characteristics (MacFaddin, 1977).

Bile Esculin Agar (Himedia Laboratories, Mumbai, India) containing 0.1% sodium azide was used for selective isolation of ENT. Isolated strains were presumptively identified by

morphotinctorial characteristics, ability to hydrolyze esculin and catalase production (Mondino et al., 2003).

Eosin Methylene Blue Agar (Himedia Laboratories, Mumbai, India) was used to identify GNR and NFGNR, which were identified by established morphotinctorial and biochemical characteristics by using a commercial semi-automated system BACTRAY3 (Laborclin, Parana, Brazil) according to instructions of the manufacturer.

### Antimicrobial susceptibility assays

The antimicrobial susceptibility assay was performed on Mueller-Hinton agar (Himedia Laboratories, Mumbai, India) by the disc-diffusion method and growth inhibition zones were interpreted according to the Clinical Laboratory Standards Institute (CLSI, 2009). The antimicrobial disks were of commercial grade (Laborclin, Parana, Brazil). The drugs tested were: (i) for GNR strains: ampicillin, cephalothin, amikacin, ampicillin-sulbactam, cefepime, cefoxitin, ciprofloxacin, imipenem, sulfamethoxazole-trimethoprim, ceftazidime, piperacillin-tazobactam and gentamicin; (ii) for NFGNR strains: amikacin, ampicillin-sulbactam, cefepime, imipenem, sulfamethoxazole-trimethoprim, ticarcillin-clavulanic acid, meropenem, aztreonam, ceftazidime, ciprofloxacin, piperacillin-tazobactam and gentamicin; (iii) for CNS strains: rifampicin, penicillin, oxacillin, clindamycin, gentamicin, chloramphenicol, azithromycin, vancomycin, sulfamethoxazole-trimethoprim, ciprofloxacin and erythromycin; (iv) for ENT strains: ampicillin, penicillin, vancomycin, azithromycin, rifampicin and linezolid. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. To determine the level of antibiotic resistance of the isolated bacteria populations, the multiple antibiotic resistance (MAR) index of different bacterial groups were calculated according to Krumperman (1983), by the equation  $a/(b.c)$ , where 'a' represents the aggregate antibiotic resistance score of all isolates within the same microbial group, 'b' is the number of tested antimicrobial drugs, and 'c' is the number of isolated strains within the same group (GNR, NFGNR, CNS and ENT).

### Disinfectants susceptibility assays

Minimal inhibitory concentrations to disinfectants commonly used in hospitals (sodium hypochlorite, benzalconium chloride, and 4% peracetic acid/26% hydrogen peroxide) was determined for the isolated bacterial strains by agar dilution method according to the (CLSI, 2007).

Disinfectants used were of commercial grade, stored in normal conditions and used within the validity periods. *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as controls.

### Statistical analysis

The non-parametric Wilcoxon test was applied to compare the number of microorganisms isolated from the 14 critical areas at the two periods of collection, and the chi-square test was applied to the individual areas to verify frequency significant differences among microbial groups. The significance level was set as  $p < 0.05$ .

## RESULTS

Five hundred and forty seven (547) bacterial samples were isolated from the rinse water of cleaning mops used at the critical areas of the hospital. Strains representative

**Table 1.** Distribution of microbial groups recovered from critical areas of the tertiary care hospital evaluated.

Critical areas	Frequency of recovery (%)			
	GNR	NFGNR	CNS	ENT
Surgical center	29.1	25.6	45.3	-
Intermediate surgical unit	21.4	21.4	35.8	21.4
Sterilized material center	31.7	29.7	38.6	-
Bone marrow transplant unit	26.4	24.2	45.0	4.4
Intensive care unit	25.0	37.5	37.5	-
Infectious parasitic diseases infirmary	18.3	20.0	46.7	15
Low birth weight newborn infirmary	22.2	27.8	50.0	-
Pediatric isolation infirmary	21.7	6.52	56.5	15.2
Pediatric infirmary – lactarium	28.1	12.5	40.6	18.8
Clinical analysis laboratory	29.4	17.6	47.1	5.9
Pathological anatomy laboratory	30,8	61.5	7.7	-
Hospital kitchen	24.2	14.5	37.1	24.2
Hospital laundry	30.8	23.1	46.2	-
Pharmacy (dosage preparing sector)	-	33.3	66.7	-

GNR: Gram negative rods from the *Enterobacteriaceae* family; NFGNR: non-fermenting Gram negative rods; CNS: coagulase-negative staphylococci; ENT: enterococci.

of CNS were the most prevalent (n = 239) accounting for 43.7% of the isolated bacteria. Representatives of the GNR were the second most prevalent (n = 138, 25.2%), followed by NFGNR (n = 125, 22.9%). The least frequent bacterial group was ENT (n = 45) accounting for 8.2% of the total bacteria isolated.

Only bacteria representative of CNS and NFGNR were recovered from all sampled areas. Representatives of GNR were not found in the pharmacy and ENT strains were absent in the surgical centre, sterilized material centre, intensive care unit, low birth weight newborn infirmary, pathology laboratory and pharmacy. Statistical analysis suggested that, when present, microorganisms were uniformly distributed at the two moments of collection (Table 1).

The results of the antimicrobial drug susceptibility tests are shown in Table 2, and are presented in terms of resistance, intermediate resistance and susceptibility. The drug susceptibility patterns for the quality control strains *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were in accordance with CLSI (CLSI, 2009). Overall, for the GNR, antimicrobial resistance was observed against all tested drugs, being rates higher than 10% of resistance observed against ampicillin, ampicillin-sulbactam, cephalothin, ceftazidime and sulfamethoxazole-trimethoprim, whereas the most effective antimicrobials were piperacillin-tazobactam and imipenem for which the resistance were 2.5 and 1.7%, respectively. For the NFGNR, antimicrobial resistance was also observed against all tested drugs with rates higher than 10% for aztreonam, ampicillin-sulbactam, cefepime ceftazidime and trimethoprim-sulfamethoxazole. Resistant rate

against meropenem was 9.2% among these bacteria. Piperacillin-tazobactam and gentamicin were the most effective drugs with sensitivity rates higher than 95% and no intermediate resistance was observed.

Considering the antimicrobial susceptibility patterns for the Gram positive bacteria, among CNS strains antimicrobial resistance levels higher than 10% were observed against penicillin, oxacillin, erythromycin, azithromycin, clindamycin, chloramfenicol and sulfamethoxazole-trimethoprim, whereas lower levels of resistance were observed for the other antimicrobials with exception for vancomycin to which all bacteria were susceptible. For the ENT strains, azithromycin was the less effective drug being observed resistance rate of 57.1% and intermediate resistance rate of 23.8%. Resistance higher than 10% was also observed against rifampicin and vancomycin. The beta-lactamic drugs penicillin and ampicillin were the most efficient antimicrobials with sensitivity rates of 97.8%.

Bacteria isolates resistant to three or more of the antimicrobial drugs tested were designated as multiple antimicrobial resistant and this multiresistance ranged from 3 to 12 antimicrobials for the GNR and NFGNR, or 3 to 11 for CNS, or 3 to 6 for ENT. The phenomenon was observed within all bacterial groups at high frequencies such as 72.8% for NFGNR, 72.4% for CNS, 70.3% for GNR and 13.4% for ENT. With exception for NFGNR strains for which the MAR index was 0.16, for the other bacterial groups the indexes were > 0.2 (GNR = 0.22, CNS = 0.26 and ENT = 0.28). Evaluation of the susceptibility patterns from the isolated bacteria to disinfectant solutions showed sensitivities to different products which are routinely used in hospitals in

**Table 2.** Antimicrobial susceptibility patterns of bacteria strains isolated in this study.

<b>Microorganisms and antimicrobial drugs</b>	<b>S (%)</b>	<b>IR (%)</b>	<b>R (%)</b>
<b>Gram negative rods – <i>Enterobacteriaceae</i></b>			
Ampicillin	34.2	7.5	58.3
Ampicillin-sulbactam	72.5	5.0	22.5
Piperacillin-tazobactam	97.5	0	2.5
Cephalothin	37.5	2.5	60.0
Cefepime	93.4	3.3	3.3
Cefoxitin	59.2	2.5	38.3
Ceftazidime	88.4	0.8	10.8
Ciprofloxacin	92.5	4.2	3.3
Amikacin	94.2	1.7	4.1
Gentamicin	94.2	0.8	5.0
Imipenem	98.3	0	1.7
Sulphamethoxazole-trimethopim	75.0	0	25.0
<b>Non-fermenting Gram negative rods</b>			
Aztreonam	52.0	20.4	27.6
Ampicillin-sulbactam	66.3	0	33.7
Tircacillin-clavulanic acid	93.9	0	6.1
Piperacillin-tazobactam	96.9	0	3.1
Cefepime	81.6	3.1	15.3
Ceftazidime	73.5	1.0	25.5
Ciprofloxacin	94.9	3.1	2.0
Amikacin	94.9	1.0	4.1
Gentamicin	96.9	0	3.1
Imipenem	93.9	0	6.1
Meropenem	90.8	0	9.2
Sulphametoxazole-trimethopim	72.5	0	27.5
<b>Coagulase negative <i>Staphylococcus</i></b>			
Penicillin G	40.4	0	59.6
Oxacillin	56.6	0	43.4
Rifampicin	93.4	0	6.6
Azithromycin	50.0	6.1	43.9
Erythromycin	42.6	9.6	47.8
Gentamicin	92.1	0	7.9
Ciprofloxacin	91.7	2.6	5.7
Clindamycin	67.1	12.3	20.6
Chloramfenicol	87.9	1.3	10.8
Vancomycin	100.0	0	0
Sulphamethoxazole-trimetropim	87.8	1.3	10.9
<b><i>Enterococcus</i> sp.</b>			
Penicillin G	97.8	0	2.2
Ampicillin	97.8	0	2.2
Rifampicin	44.4	6.7	48.9
Azithromycin	19.1	23.8	57.1
Vancomycin	88.9	6.7	4.4
Linezolid	86.7	0	13.3

S: sensitivity, IR: intermediate resistance, R: resistance.

**Table 3.** Minimum inhibitory concentrations (MIC) of disinfectant solutions against bacteria strains isolated in this study.

Microorganisms and disinfectants	MIC (% of aqueous solutions)		
	50%	90%	Range
<b>Gram negative rods – <i>Enterobacteriaceae</i></b>			
Quaternary ammonium	0.125	0.125	0.125 to 0.5
Sodium hypochlorite	0.25	0.5	0.125 to 1.0
Peracetic acid/ Hydrogen peroxide	0.125	0.25	0.125 to 0.25
<b>Non-fermenting Gram negative rods</b>			
Quaternary ammonium	0.125	0.125	0.125 to 0.5
Sodium hypochlorite	0.25	0.5	0.125 to 0.5
Peracetic acid/ Hydrogen peroxide	0.125	0.125	0.125 to 0.5
<b>Coagulase negative <i>Staphylococcus</i></b>			
Quaternary ammonium	0.125	0.125	-
Sodium hypochlorite	0.125	0.25	0.125 to 1.0
Peracetic acid/ Hydrogen peroxide	0.125	0.125	-
<b><i>Enterococcus sp.</i></b>			
Quaternary ammonium	0.125	0.125	-
Sodium hypochlorite	0.5	1.0	0.125 to 1.0
Peracetic acid/ Hydrogen peroxide	0.125	0.125	-

concentrations ranging from 0.125 to 1%. Sodium hypochlorite was the compound which showed the highest inhibition concentrations (0.125 - 1%) and the CNS were the less susceptible bacteria to this disinfectant. Gram negative rods were less susceptible to quaternary ammonium and to the association peracetic acid/ hydrogen peroxide in concentrations varying from 0.125 to 0.5%. However, for Gram positives a 0.125% concentration of these compounds was able to inhibit growth (Table 3).

## DISCUSSION

Identification of bacterial strains of clinical-microbiological relevance in critical areas of the hospital after the mopping by cleaning staff confirms the persistence of putative microorganisms in nosocomial environment, which are potentially related to hospital infections. The similar bacterial distribution, confirmed by statistical analysis, may suggest that the occurrence is not casual, but, indeed, it represents the microbiota associated to surfaces the institution.

Hospital surfaces are contaminated by factors inherent to the presence of patients, such as biological fluids, sometimes associated to assistance techniques and hygiene. Another contamination factor would be the circulation of vectors as carrier agents for fungi and bacteria resistant to antimicrobials (Prado et al., 2006; Rodovalho et al., 2007). The occurrence and distribution

of vectors associated to dissemination of microorganisms were not evaluated in this study, but it should be noted that the bacterial groups associated with these vectors were observed in all sampled critical areas.

Although, surfaces are not directly connected to transmission in most hospital infections, the impact of hygiene and cleaning procedures in microbial control is evident. It is suggested that microorganisms associated to hospital infections are able to survive during large periods of time, thus being a continuous source of contamination in cases where population control is not efficiently conducted (Kramer et al., 2006; Rossi et al., 2008). It is believed that hospital fluxes contribute to the distribution of microorganisms in the nosocomial environment, mainly in critical areas. Two great sets of fluxes may be outlined: the interfunctional fluxes between functional units; and the intrafunctional fluxes that occur inside a single functional unit and may be characterized as contaminated or free of contamination risks. These observations confirm results in this study where GNRs and ENTs were not isolated, respectively, in the hospital pharmacy, surgical centre, sterilized material centre, intensive care unit, low birth weight newborn infirmary and in the laboratory of pathological anatomy, which are critical areas of intrafunctional flux.

Occurrence of contaminated surfaces by different microbial groups in the nosocomial environment is described in the literature (Carvalho et al., 2007), but it should be emphasized that in this study microorganisms have been isolated from rinse water of cleaning mops,

eventually discarded without treatment into the general sewer system. These isolates are representative of local persistent microbiota and may be disseminated inside the hospital and from the hospital to the environment. According to the literature, the high level of antimicrobial resistance to drugs used in hospitals and in the community constitutes an important alert to this severe phenomenon, which is considered one of the great challenges to science and medicine in the 21st century (ASM, 2009). The high levels of resistance shown by CNSs against penicillin, oxacillin, erythromycin, azithromycin and clindamycin are relevant since these antimicrobials are used in the hospitals and in the community. However, the low levels of resistance to gentamicin, chloramphenicol, rifampicin, sulfamethoxazole-trimethoprim and ciprofloxacin might indicate that these compounds are carefully controlled in our health system. Considering the susceptibility to oxacillin and other drugs, our resistance rates are lower and do not confirm other studies related to Brazilian and international institutions (Mendes et al., 2002; Bernardi et al., 2007). However, the resistance rates of these microorganisms to gentamicin and chloramphenicol are similar to that registered in Europe, USA and Latin American countries, including Brazil (Mendes et al., 2002; Bernardi et al., 2007; Biedenbach and Jones, 2009).

Drug susceptibility patterns of ENT strains showed high resistance levels to azithromycin, rifampicin and linezolid. Intermediate resistance was also shown against azithromycin. Rifampicin, among other drugs, has been proposed as a therapeutic option to the treatment of endocarditis by vancomycin-resistant ENT (Khan et al., 2002; Murray, 2000). This is usually followed by resistance to other antimicrobials, like aminoglycosides, tetracycline and ampicillin. It is interesting to note that the low levels of resistance among enterococci of hospital origin to the  $\beta$ -lactams observed in this study are similar to that reported by other Brazilian authors (Mondino et al., 2003; Horner et al., 2005) although, these resistance rates are significantly lower than the detected in Europe and Asia (Qu et al., 2006). Ampicillin is the antibiotic of choice in less severe ENT infections and resistance to this compound by *Enterococcus faecalis* is not frequent in contrast to *Enterococcus faecium*, which is resistant to most antimicrobials especially ampicillin (Murray, 2000).

As shown in this study GNRs are resistant to ampicillin in similar levels to the reported recently in European countries but lower than USA rates (Bouchillon et al., 2005; Rodloff et al., 2008). Resistance was lower to  $\beta$ -lactams and inhibitors of  $\beta$ -lactamase, as expected, but the association of amoxicillin-clavulanic acid showed 27% whereas resistance rate to piperacillin-tazobactam was 3%. These results suggest production of beta-lactamase type enzymes as the main resistance mechanism. Literature data confirm the results in this study, especially the efficacy of association of piperacillin-tazobactam as a therapeutic strategy (Andrade et al., 2006; Dipersio and Dowzicky, 2007; Rodloff et al., 2008). Resistance to

cephalothin and ceftazidime agrees with European literature (Koch et al., 2008). Considering third generation compounds like ceftazidime, resistance is low but still indicative of strains producing extended spectrum beta-lactamases (ESBL) however, this phenotype was not investigated in enterobacteria isolated in this study. Low levels of resistance and intermediate resistance were observed with cefepime, but lower than the values reported for hospitals in Brazil and other countries. Imipenem, one of the options for the treatment of infections caused by ESBL producing bacteria, was an effective antimicrobial to 98% of the enterobacteria in this study. Even that low, carbapenems resistance is a cause of concern and the literature indicate rates of 1% among hospital related bacteria (Sader et al., 2005; Andrade et al., 2006; Deshpande et al., 2006). The low levels of resistance to ciprofloxacin and aminoglycosides, observed, when compared to literature, would probably be associated to the limited prescription of these drugs in our region (Sader et al., 2005; Andrade et al., 2006; Dipersio and Dowzicky, 2007; Koch et al., 2008).

Several NFGNR are known for their resistance to all classes of antimicrobials and for their easily acquiring new resistance mechanisms (Enoch et al., 2007). Considering the carbapenems evaluated, imipenem and meropenem, the resistance levels were lower than reported in the literature also for bacteria of nosocomial origin in Brazil (Pellegrino et al., 2002; Kokis et al., 2005). Resistance to monobactams like aztreonam agrees with other Brazilian reports (Figueiredo et al., 2007). As observed by others, the associations of ticarcillin-clavulanic acid or piperacillin-tazobactam were more efficient against the NFGNR than ampicillin-sulbactam (Sader et al., 2005; Fritsche et al., 2008; Pillar et al., 2008). Comparing the cephalosporins, cefepime and ceftazidime, a lower resistance detected to the first named, confirms national and international reports although, results from Brazil indicate a resistance of 45% to cephalosporins of second and third generation by non-fermenting Gram negative rods (Sader et al., 2005; Fritsche et al., 2008; Pillar et al., 2008). Peculiar results were obtained for the susceptibility testing to ciprofloxacin and the association sulfamethoxazole-trimethoprim in which resistance levels were far below values reported by other investigators (Sader et al., 2005; Fritsche et al., 2008; Pillar et al., 2008).

According to the literature, routine cleaning of articles and surfaces does not remove the entire microbial load suggesting that an additional disinfection procedure is necessary (Rossi et al., 2008). All the tested strains in this study were susceptible to sodium hypochlorite up to 1%, quaternary ammonium and to the association peracetic acid/hydrogen peroxide at 0.5%. Reference values of bacterial susceptibility or resistance to disinfectant agents are not available, but concentrations ranging from 0.125 to 1% are within the limits recommended by the Health Ministry of Brazil. Attention should be given to the susceptibility of all strains to 1%

sodium hypochlorite. This substance is widely used in hospitals. It is a fast reactant of low cost and should be indicated for medium level disinfection of articles and surfaces, for 10 min in concentrations ranging from 0.2 to 1% (Kramer et al., 2006; Rutala and Weber, 2007; Rossi et al., 2008).

Overall, the levels of resistance to antimicrobials detected in this study are lower than the reported in the literature, but worrisome. However, all bacterial groups evaluated showed high frequencies of multiple resistances to antibiotics. The MAR index is an excellent tool that permits us to analyze the dissemination of bacteria resistance in a given population. When isolates are exposed to high risk sources of contamination where antibiotics are often used, a MAR index value  $> 0.2$  is observed. When antibiotics are seldom or never used, a MAR index value less than or equal to  $\leq 0.2$  is observed (Krumperman, 1983; Pontes et al., 2009). MAR indexes for the different bacterial populations isolated from critical areas in this tertiary care hospital  $> 0.2$  (GNR, CNS and ENT), even considering the MAR = 0.16 observed for the NFGNR, indicate and might alert us for the high levels of antimicrobial drugs routinely used and should stimulate professional discussion on the rational use of such drugs in the nosocomial environment.

This is true not only for antimicrobials of the same chemical group but also for chemically diverse compounds. The data is relevant to the discussion of resistance transference between exogenous and endogenous bacteria in different environments. Factors involved in the selective pressure of multiple resistances were not evaluated but a co-selection phenomenon may be considered based on phenotypic evidence presented by others (Akwar et al., 2008).

Microbial resistance to antimicrobials has been frequently associated to indiscriminate use of antibiotics, therapeutic or prophylactic, emphasizing the fact that scientific criteria are not respected in the prescription of these medicines. Rather, a rational use of antibiotics should be exercised in order to prevent selective pressure originated by indiscriminate use of these compounds.

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