ARTIGO ORIGINAL
ISSN 1677-5090

Comparative analysis between colonization and infection strains starting from statistical methods

Análise comparativa entre cepas de colonização e de infecção a partir de métodos estatísticos

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Abstract

Isolated colonization strains of preoperative patients were compared with isolated infection strains of surgical patients, seeking to establish compatibility relationships that can suggest a possible common origin between them. Sixty preoperative patients from Hospital Universitário Pedro Ernesto, Rio de Janeiro, Brazil, that have used cephalotin as pre-surgical prophylaxis, were studied as to the intestinal, oropharyngeal and cutaneous colonization for cephalotin-resistant bacteria, using selective culture media. The infection strains were studied through antibiogram analysis of other 2005 surgical patients from the studied clinics. Statistical methods such as Qui-square and Mann-Whitney U tests were employed for comparison of strains as to frequencies of species and numbers of resistance markers to antimicrobials. The cephalotin-resistant microorganism infections represented 62,8% of the infections detected in the 2065 patients. The average number of resistance markers to antimicrobials was higher for the cephalotin-resistant strains. There were no significant differences between colonization and infection strains when compared to frequencies of species and number of resistance markers, for most of the microorganisms. There were significant differences in the number of resistance markers among susceptible and cephalotin-resistant strains of *Escherichia coli* isolated of urinary infections. Cephalotin has proved to be an effective marker for the detection of multiresistant strains. The results indicate the possibility of microorganism populations that colonize inpatients to be related to microorganisms that cause infections in these patients.

Keywords: Colonization and infection strains - Comparative analysis - Surgical patients.

Resumo

Cepas isoladas da colonização de pacientes pré-operatórios foram comparadas com cepas isoladas de infecções de pacientes cirúrgicos, para estabelecer relações que sugerissem uma origem comum. 60 pacientes pré-operatórios que usaram cefalotina como profilaxia pré-cirúrgica foram estudados quanto à colonização intestinal, orofaríngea e cutânea por bactérias resistentes à cefalotina, usando meios de cultura seletivos. As cepas de infecção foram analisadas por meio dos antibiogramas de 2005 pacientes cirúrgicos. Testes estatísticos como Qui-quadrado e U de Mann-Whitney foram utilizados para comparar as cepas quanto à frequência de espécies e números de marcadores de resistência. As infecções causadas por microrganismos resistentes à cefalotina representaram 62,8% das infecções detectadas nos pacientes cirúrgicos. A média de número de marcadores de resistência foi maior para as cepas cefalotina-resistentes. Para a maioria dos microrganismos, não houve diferenças significativas entre as cepas de colonização e de infecção quanto à frequência de espécies e número de marcadores. Foram detectadas diferenças significativas entre as cepas sensíveis e resistentes de Escherichia coli isoladas de infecções urinárias. A cefalotina mostrou-se um marcador útil para a detecção de cepas multirresistentes. Os resultados indicam a possibilidade de as populações de microrganismos que colonizam pacientes hospitalizados estarem relacionadas às populações que causam infecções nos mesmos pacientes

Palavras-chave*: colonização e infecção – análise comparativa – pacientes cirúrgicos.*

INTRODUCTION

Several studies point to the importance of taking precautions against inpatients' colonization, specially for potentially pathogenic microorganisms, considering the endogenous origin of most of the nosocomial infections, particularly for Gram-negative rods.[1]

Recebido em 08 de fevereiro de 2010; revisado em 02 de agosto de 2010.
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The multiresistance to antimicrobials can be observed in strains associated to hospital setting, so very common in patient colonization[2,3] as in the etiology of infections.[4]

The mechanisms of genetic recombination make the problem even more relevant, because of the high dissemination of resistance genes in hospital environment, favored by strong selective pressures due to the use of antimicrobials.[5]

Several authors recommend periodic bacteriological cultures of colonized sites in order to monitor inpatients, seeking to establish predictive conditions for

endogenous infections, mainly in specific hospital units.[6]

Several methods of epidemiological marking, such as biotyping, antibiotyping and use of selective media containing antimicrobials, besides molecular biology techniques, have been proposed and used in the last decades, with the objective of detecting strains of special interest, due to their prevalence or their endemic or epidemic characteristic, and/or their virulence.[7]

Some statistical methods have been used in the comparison of populations, as the non-parametric Qui-square test, Fisher exact test and Mann-Whitney U test. Observed differences can be caused by biological fluctuations or represent an effect that comes from a defined mechanism, that can't be attributed to chance.[8,9]

In the present study, we tried to compare isolated strains from the colonization of preoperative patient with those strains responsible for infectious processes in surgical patients, seeking to establish eventual relationships among populations of microorganisms that colonize inpatients and populations of microorganisms that cause infections. Statistical tests were used for the comparison of frequencies of species and of number of antimicrobial resistance markers.

METHODOLOGY

Sixty adult patients in pre-operative period, in Hospital Universitário Pedro Ernesto (HUPE), from the University of the State of Rio de Janeiro (UERJ), with 450 beds, were evaluated in 1997, with relation to the intestinal, oropharyngeal and cutaneous colonization by cephalotin-resistant microorganisms.

We used as a selective medium of feces culture the E M B medium containing 32 µg/ml of cephalotin (E M B-cephalotin) and for skin and oropharynx samples the CLED medium containing 32 µg/ml of cephalotin (CLED-cephalotin).[2]

The isolated microorganisms in the selective medium were identified [10,11,12,13,14,15,16] and submitted to the antimicrobial susceptibility test (amikacin, ampicillin, cephalotin, ceftazidime, chloramphenicol, gentamicin, oxacillin, perfloxacin, trimethoprim -sulfamethoxazole, tetracyline) by the diffusion method in agar.[17]

With the intention of finding out if the group of studied patients could be considered as belonging to a global sample of the patients submitted to surgeries in HUPE, the rates of surgical sites infections, bacteremias and urinary infections which took place in the year of 1997 were calculated for the 60 studied patients for colonization and for the other 2005 surgical patients of the sectors included in the study. We considered the proportion of cephalotin-resistant bacteria as agents of surgical site infections, urinary infections and bacteremias in 2065 patients submitted to surgeries in the clinics included in the study. We also obtained the resistance patterns of all the isolated microorganisms of blood culture, urine and surgical wound secretions of the 2065 patients in the studied clinics.

For obtaining all these data, we fell back upon the registrations of cultures and antibiograms of the Laboratory of Bacteriology of HUPE.

The frequencies with that each species occurred at each colonization site and with that each species was isolated from surgical site infections**,** urinary infections and bacteremias were calculated. The frequencies that refer to infection strains were obtained separately for cephalotin-resistant and cephalotin -susceptible strains. We used the Qui-square test and the Fisher exact test [8,9] to compare: intestinal colonization to surgical site infections and urinary infections; skin colonization to surgical site infections and bacteremias, seeking to verify probability of isolated strains could belong to the same population.

We calculated the arithmetic mean of the number of resistance markers of the colonization strains (in the total and separately for each colonization site) and of the isolated infection strains (in the total and separately for bacteremias, surgical site infections and urinary infections). Calculations of the averages were carried out separately for infections caused by cephalotin-resistant bacteria and for those caused by cephalotin-susceptible bacteria.

Additionally, the average number of resistance markers for each species or genera, referring to each colonization and infection site were calculated, with the intention of observing if the averages were compatible or not, within the same species.

The numbers of resistance markers were compared within a group of more frequent bacterial species or genera. For each selected species or gender, we analyzed possible relations between fecal colonization strains and urinary infections strains and/or of surgical site infection strains and between skin colonization strains and surgical site infection strains. The Mann-Whitney U test, a powerful non-parametric test applicable when the objective is to test the hypothesis of two independent groups being taken out from the same population or not [8], was used in this analysis.

We used the same proof to evaluate strains of *Escherichia coli* isolated of urinary infections, comparing cephalotin-resistant strains to cephalotin-susceptible strains, seeking to establish if the two groups could belong or not to the same population.

RESULTS

The rate of infections of surgical site in the six studied patients was of 5% (3 in 60), not significantly different ($p = 0.3190$; χ^2) from the total of other patients submitted to surgeries in the studied clinics (2.1% ; 43 in 2005).

When we approached the infection rates considering bacteremias, surgical and urinary infections as a whole, the results were 8.3% (5 in 60) for the patients of the group studied for colonization and of 5.6% for the total of other surgical patients (114 in 2005) ($p = 0.5579$; χ^2).

Table 1 - Frequencies and average of number of resistance markers for the main isolated microorganisms, comparing colonization and infection, separately by anatomic site.

	Colonization						Infection					
Species/genera	**Feces**		**Oropharynx**		**Skin**		**Bac**		**UTI**		**SSI**	
	F	A	F	A	F	A	F	A	F	A	F	A
K. pneumoniae	8,3%	5,7	__(*)	__	__	__	__	__	3,8%	7 (†)	7%	7 (†)
E. cloacae	70%	3,5	13,3%	2,8	1,6%	3	__	__	1,9%	10 (†)	6,3%	5,6 (†)
E. coli	8,3%	3,8	__	__	__	__	__	__	48%	4,5(†) 1,5(‡)	6,3%	2,6 (‡)
Acinetobacter spp.	3,3%	5,5	5%	6,6	10%	4,6	13,3%	8,3 (†)	1,9%	3 (†)	2,1%	10 (†)
P. aeruginosa	16,6%	6,2	1,6%	6	__	__	__	__	15,3%	7,25 (†)	7%	8,2 (†)
Enterococcus spp.	25%	9,2	__	__	__	__	__	__	1,9%	10 (†)	1%	6 (†)
Coagulase-negative *Staphylococcus* spp	1,6%	9	1,6%	10	6,6%	9,2	18,1%	9 (†) 1 (‡)	1,9%	0 (**)	25,5%	8,7 (†) 3 (‡)
S. aureus	__	__	__	__	1,6%	7	18,1%	8,5 (†) 2 (‡)	__	__	10,6%	8,7 (†) 1 (‡)

Notes: F = frequencies; A = average; Bac = bacteremias; UTI= urinary infections; SSI = surgical siteinfections; *K. pneumoniae= Klebsiella pneumoniae* ; *E. cloacae= Enterobacter cloacae*; *E. coli = Escherichia coli; P aeruginosa= Pseudomonas aeruginosa*; *S. aureus= Staphylococcus aureus*; spp= specie.
* = Non-isolation of the species or genera in the mentioned sites. † = cephalotin-resistant strains; ‡ cephalotin-susceptible strains.

The occurrence of 121 infections was detected (22 bacteremias, 47 surgical site infections and 52 urinary infections) in the universe of 2065 surgeries performed at the studied surgical clinics.

The infections caused by cephalotin-resistant microorganisms represented 62.8% (76) and those caused by cephalotin-susceptible microorganisms 37.2% (45). In the specific case of surgical site infections, 40 had as etiological agents cephalotin-resistent bacteria (85.1%) and 7 cephalotin-susceptible ones (14.9%).

In the comparison study of resistance patterns presented by the colonization strains of the 60 studied patients and by isolated bacteremias, surgical and urinary site infections strains, we found 10 phenotypic patterns of resistance markers, in different species, that also occurred in at least two isolated strains of different sites and different patients. For one patient, there were found *Morganella morganii* strains with the same resistance profile colonizing the intestinal tract and as aetiological agent of surgical site infection.

For the infection strains in general, the average was of 5.2 markers, while just considering the cephalotin-resistant strains the average went up to 7.2 and just for the cephalotin-susceptible ones the average was of 2,0 markers.

For isolated bacteremia strains the averages were of 7.6 markers (cephalotin-resistant) and 2.1 (cephalotin-susceptible). For urinary infections, they were 6.2 (cephalotin-resistant) and 1.8 (cephalotin-susceptible). As for surgical site infections the averages were 7.5 (cephalotin-resistant) and 2.1 (cephalotin-susceptible).

Table 1 presents frequencies and average of number of resistance markers for the main isolated microorganisms, comparing colonization and infection, separately by anatomic site.

When we compared intestinal colonization with urinary infections frequencies, we didn't detect any differences, using the Qui-square test**,** for strains of several species (TABLE 2).

In the approach relating intestinal colonization and surgical site infections frequencies, the results didn't indicate significant statistic differences for the strains of some species. However, for strains of *Enterobacter cloacae* and of *Enterococcus* spp., the data point out a significant difference (TABLE 2).

Table 2 - Values of probability for the qui-square and Fisher exact tests, in the comparison of the frequencies of 8 species or genera analyzed separately in the colonization of feces and in the aetiology of urinary infections; in the colonization of feces and in the aetiology of surgical site infections; in the skin colonization and in the etiology of surgical site infections; in the skin colonization and in the bacteremia etiology**.**

Species	Feces x UTI	Feces x SSI	Skin x SSI	Skin x Bac
K. pneumoniae	p= 0,5572*	p= 0,9427*	__†	__
E.cloacae	p< 0,0001*	p< 0,0001*	p= 0,4455*	__
E. coli	p= 0,8025*	p= 0,9917*	__	__
Acinetobacter spp.	p= 0,8999*	p= 0,8297*	p= 0,2148*	p= 0,6945*
P. aeruginosa	p= 0,9413*	p= 0,8323*	__	__
Enterococcus spp.	p= 0,0013*	p= 0,0025*	__	__
Coagulase-negative *Staphylococcus* spp.	__	__	p= 0,0282*	p= 0,6521†
S. aureus	__	__	p= 0,2289*	p= 0,3561*

Notes: UTI= urinary tract infections; SSI= surgical site infections; Bac = bacteremias; spp = species; p = values of probability;*K. pneumoniae = Klebsiella pneumoniae*; *E. cloacae = Enterobacter cloacae*; *E. coli = Escherichia coli*; *P. aeruginosa = Pseudomonas aeruginosa*; *S. aureus = Staphylococcus aureus*;- p value using the qui-square test; ‡ - p value using the Fisher exact test; † = not_accomplished due to the non-isolation of the species in the site of colonization and/ or of the mentioned infection.

Table 3- Values of probability of a common origin for a group of 4 species, results of the comparison of the numbers of resistance markers of intestinal colonization strains with the numbers of resistance markers of strains of same species or genera, isolated of cases of surgical site and/or urinary infections, and of skin colonization strains with strains of same species or genera, isolated of surgical site infections, using the Mann-Whitney test**.**

Species	Feces x UTI	Feces x SSI	Skin x SSI
P. aeruginosa	p= 0,2031	p= 0,2844	__*
K. pneumoniae	__	p= 0, 1898	__
E. coli	p= 0,5368	__	__
Coagulase-negative *Staphylococcus* spp.	__	__	p= 0,6923

Notes: p = values of probability; spp = species; UTI = urinary tract infections; SSI= surgical site infections; *P. aeruginosa = Pseudomonas aeruginosa*; *K. pneumoniae = Klebsiella pneumoniae*; *E. coli = Escherichia coli*; * = test not accomplished due to non-occurrence of the mentioned species**.**

For skin colonization and surgical site infections, the results didn't suggest significant differences in *Enterobacter cloacae* and *Staphylococcus aureus* frequencies. However, the results indicated differences in coagulase-negative *Staphylococcus* spp. frequencies (TABLE 2).

Relating frequencies of skin colonization with those of bacteremias, we didn't detect significant differences for the frequencies of isolation of coagulase-negative *Staphylococcus* spp., however the results suggested differences in the frequencies of isolation of *Staphylococcus aureus* (TABLE 2).

Table 3 presents the values for a group of microorganisms, reflecting the comparison of the number of resistance markers of intestinal colonization strains with the numbers of antimicrobial resistance markers of strains of same species or genera isolated from surgical site and/or urinary infections, and of strains of skin colonization with strains of same species or genera isolated from surgical site infections, using the Mann-Whitney test. [8]

In the analysis of number of markers comparing *E. coli* isolated from urinary infections, the results pointed significant statistic differences (p = 0,0056) between populations of cephalotin-resistant and cephalotin-susceptible strains.

DICUSSION AND CONCLUSIONS

Bacterial colonization of skin and mucous membranes by microorganisms related to hospital environment begins in the first days after admission, progressing as the factors linked to the patient, as the compromising of the immune system, or to the assistance, as antimicrobial use, leading to a change for a nosocomial_microbiota[18]. It can be observed, therefore, the coexistence of two microbial populations (one of in-hospital origin, with multiresistance, and another of extra-hospital origin).[3]

Once recognized the studied colonized sites as important reservoirs of infection agents, specially for certain infectious processes to the which they are respectively related, it seemed important to us the approach of the subject of the impact of selective processes in the context of the prophylaxis, what would be similar to the selection in a culture media. Those selective processes would be reflected in some measure in the occurrence of infections.

Cephalotin acts on some Gram-positive and Gram-negative microorganisms [19], but it can select relevant microorganisms in a hospital setting. Our findings suggest that the cephalotin could be used as " indicator " of bacterial resistances with a higher numbers of markers, including ESBL-producing strains.

A clear difference was observed among the average numbers of resistance markers comparing cephalotin-resistant and susceptible strains to this antimicrobial isolated from infections in the period of the study. In this way, the occurrence of infections could reflect the microbial populations selective process.

Even if *Enterobacter* spp., *Pseudomonas* spp. and *Serratia* spp. are intrinsically cephalotin-resistant[19], it is not a waste of time to investigate them in relation to antimicrobial prophylaxis, since they cause an important portion of nosocomial infections, mainly respiratory and urinary ones. On the other hand, if subpopulations more capable of causing infections occur, in general, the intrinsic resistance of *Enterobacter* spp. could explain significant differences of infection frequencies, when compared to their colonization frequency.

It is possible that particularities of each microorganism in relation to virulence factors and selection by drug resistance, besides inherent aspects to the immunity of the host, orient the opportunity of a microorganism that colonizes a certain anatomic site to cause infections in that or in other sites, taking the highest or smallest proportions among colonization and infection frequencies.

Enterococcus spp. and coagulase-negative *Staphylococcus* appear in the studied group of surgical patients in infection frequencies significantly smaller than in the colonization frequencies. This fact could, hypothetically, be due to the occurrence of multiresistance even among the eventually less pathogenic strains.

In the analysis regarding the strains of *Escherichia coli* isolated from urinary infections, comparing cephalotin-resistant and susceptible strains, the differences were significant ($p = 0.0056$), what suggests that there could be different subpopulations, causing infections within the same species. We emphasized that the Mann-Whitney U test is a powerful non-parametric proof for that kind of approach.

In reference to the use of the U test in the comparison of number of markers between colonization and infection strains, for some species, as *Pseudomonas aeruginosa* and *Escherichia coli*, the fecal colonization and urinary infections strains could not be considered as belonging to distinct populations, the same happening for fecal and surgical infection strains for *P. aeruginosa* and *K. pneumoniae*, and for skin colonization and surgical infection strains in coagulase-negative *Staphylococcus* (TABLE 3). We cannot affirm that the microorganism population that colonized the studied patients are, in its totality or in great proportion, the same that caused infections in the surgical patients, however the results point to that direction. Additional studies, involving a higher number of samples could allow to a stronger evidence. However, we pointed out that the absence of significant differences suggests that the isolated strains in the colonization can participate, in an important way, in the infectious processes.

The use of selective culture media containing antimicrobials can allow, with high accuracy, the supply of data for more appropriate choices of prophylactic agents, in each case, place, situation and time. The method is also useful to make it evident agents such as multiresistant Gram-negative in neonatal intensive-care units, methicillin-resistant *Staphylococcus aureus* and vancomicin-resistant *Enterococcus* spp. with the objective of isolating the patients.

The previous evaluation of the inpatient's microbiota could allow, in the case of isolation of resistant microorganisms, the possibility of the use of drugs of larger action spectrum in the prophylaxis, capable of covering multiresistance hospital populations.

The indications obtained by statistical methods and by the comparisons of resistance profiles used in this study allow us to maintain the hypothesis of correlations among strains of different sites. Even not having been used molecular methods (of a larger analytic power), the fact that we have detected infections by microbial agents resistant to the prophylactic agents seems to permit us to: recommend larger attention to the problem and **t**o establish proposals of investigation, seeking larger adaptation of the therapeutic and prophylactic procedures.

ACKNOWLEDGMENT

This work was supported by CNPq, FINEP and FAPERJ.

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