

Prevalence of gram-positive bacteria in patients with HIV in specialized services

Prevalência de bactérias gram-negativas em portadores de HIV internados em serviço especializado

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Abstract

Objective: To identify the prevalence of gram-positive bacteria in patients with HIV and who are hospitalized in specialized services.

Methods: The present cross-sectional study approached 365 patients admitted in two specialized units of a teaching hospital located in the countryside of the state of São Paulo. The population was composed of 220 subjects. Sociodemographic and clinical data were obtained by means of individual interviews and medical record analysis. Saliva and nasal secretion were collected in the first 24 hours of the hospitalization process.

Results: The prevalence of gram-negative bacteria in patients with HIV reached 14.5%, regardless of the site of their isolation. *Pseudomonas aeruginosa* was the most frequently isolated microorganism, followed by *Klebsiella pneumoniae*.

Conclusion: The identification level of gram-negative bacteria was higher in the saliva (11.8%) than in the nasal secretion (3.6%), thus indicating that the collection of samples in more than one site may favor the identification of colonized and/or infected individuals.

Resumo

Objetivo: Identificar a prevalência de bactérias gram-negativas em portadores de HIV internados em serviço especializado.

Métodos: Trata-se de um estudo de corte transversal, foram abordados 365 indivíduos internados em duas unidades especializadas, de um hospital escola do interior paulista, sendo a população composta por 220 sujeitos. Os dados sociodemográficos e clínicos foram obtidos por meio de entrevista individual e consulta aos prontuários. Coletaram-se também amostras de saliva e secreção nasal nas primeiras 24 horas de internação.

Resultados: A prevalência de bactérias gram-negativas em portadores de HIV foi de 14,5 % independente do sítio onde foi isolado. *Pseudomonas aeruginosa* foi o microorganismo mais frequentemente isolado, seguida por *Klebsiella pneumoniae*.

Conclusão: A identificação de bactérias gram-negativas foi maior na saliva (11,8%) que na secreção nasal (3,6%), indicando que coletar amostras de mais de um sítio pode favorecer a identificação de indivíduos colonizados e ou infectados.

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Introduction

Healthcare associated infections (HAIs) have become a reason for serious concern, especially due to microorganisms that are multi-resistant against available antimicrobials. The World Health Organization recently recognized antimicrobial resistance as one of the three major human health problems.⁽¹⁾

In the last decade, gram-negative bacilli were highlighted as generators of HAIs. From this time onwards, epidemiologic monitoring processes started being addressed as a relevant strategy toward the creation of prevention measures.^(2,3) The lack of therapeutic alternatives for such microorganisms justifies the measures toward minimizing cross-transmission processes.⁽⁴⁾ It should be highlighted that the identification of gram-positives within the community and in other non-hospital institutions points out that individuals may serve as a reservoir of these bacteria, which can be reinserted into the hospital environment.⁽⁵⁾

The most frequent pathogens related to HAIs were grouped into an acronym and named after ES-KAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species),⁽⁶⁾ of which most are gram-negative.

Patients with HIV/Aids have higher risks of developing HAIs as a result of the vulnerability of their immunologic system, prolonged neutropenia, repeated hospitalizations, therapeutic procedures and invasive diagnostics, and frequent treatments with antibiotics and steroids.⁽⁷⁾

In face of the issue of multi-resistant microorganisms, above all in patients with a compromised immunologic system, allied to the lack of studies on the prevalence of gram-negative bacteria in patients with HIV/Aids, the objective of the present study is to identify the prevalence of gram-negative microorganisms in individuals with HIV/Aids hospitalized in a teaching hospital in the countryside of the state of São Paulo.

Methods

This cross-sectional study was carried out in hospitalization units specialized in infectious diseases at a

teaching hospital located in the countryside of the state of São Paulo.

Between August 1, 2011 and February 28, 2013 a total of 365 patients with HIV/Aids were hospitalized in the units. The population of the present study is comprised of 220 patients, as 58 refused to participate and 87 did not comply with the inclusion criteria, namely, being 18 years old or over, not belonging to the prison system, and presenting clinical conditions to understand and make a decision regarding whether or not to participate in the research. Each subject was enrolled in the study only once.

Sociodemographic and clinical data were obtained by means of interviews and medical record analysis. The applied instrument was assessed by five specialists concerning its form and content, and was deemed to be adequate to the reach of the objectives.

The study collected saliva and nasal secretion samples. A pre-test - comprised of the collection of saliva and nasal secretion of 15 patients who were not included in the study - was carried out, aiming to standardize the collection technique and assess the time appointed to such procedure.

The data collection procedure involved the researcher and four previously selected research assistants, who were trained to the sample and data collection process.

The collection of the material was carried out in the first 24 hours of the hospitalization process. The patient was required to deposit 3-5 ml of saliva in a graduated, dry, sterile test tube with a cover. A swab was used to collect secretion at the frontal part of the nose. The tube contained a transport culture medium (Stuart's semisolid agar). The collection process resulted in 440 collected samples, being 220 saliva and 220 nasal secretion.

Collected materials were sent to the Microbiology Lab of the referred hospital. The sample seeding process employed the blood-Agar and MacConkey Agar culture mediums in order to isolate gram-negative bacteria. Next, the Vitek-Biomérieux automated system, as well as the GN Test Kit Vitek 2 and AST-N105 for sensitivity tests were employed, aimed at the identification of microorganisms.

Following data collection, data were double typed and inserted into a Microsoft Office Excel sheet for Windows 2011, in order to verify the internal consistency analysis. Later, after the correction of typing errors, the definite sheet was transferred to the Statistical Package for the Social Sciences (SPSS), version 17.0 for Windows. The final database was then formatted, based on which definite managing operations were carried out, such as the creation of new variables, the definite categorization of variables in specific intervals, the grouping process of variables, and all other operations included in the study. The research employed a descriptive statistical analysis.

The development of the study complied with national and international ethical guidelines for research involving human subjects.

Results

From the 220 participants in the present study, 32 (14.5%) presented a positive culture for gram-negative microorganisms, regardless of the site they were isolated from. The majority of the subjects, 23 (67.6%), were men; 16 (47.0%) were in the 30-39 age group; and 12 (35.3%) had not completed the elementary school (Table 1).

Data indicate that the amount of individuals with viral load > 100,000 copies/ml and T CD4 counts < 200 cells/mm³ was higher than those displaying positive samples for gram-negatives. As for hospitalization in the previous six months, most of patients had been hospitalized at least once, were making use of antimicrobials, were not undergoing antiretroviral therapy, and showed an invasive procedure at the time of the collection process (Table 2).

It should be highlighted that two patients displayed positive gram-negatives at both sites, resulting in 34 samples. The most frequently isolated microorganism was the *Pseudomonas aeruginosa*, both in the nasal secretion (03, 37.5%) and in the saliva (13, 50.0) (Table 3).

None of the isolated bacteria presented resistance against antimicrobials.

Table 1. Sociodemographic variables

Variables	Gram-negatives	
	Present (n=32) n (%)	Absent (n=188) n (%)
Gender		
Female	11(34.4)	79(42.0)
Male	21(65.6)	109(58.0)
Age group (years)		
19 — 29	02(6.2)	25(13.3)
30 — 39	15(46.9)	53(28.2)
40 — 49	09(28.1)	73(38.8)
50 — 59	06(18.7)	25(13.3)
≥60	0(0)	12(6.4)
Educational level		
Illiterate	01(3.1)	15(8.0)
Incomplete Elementary School	11(34.4)	78(41.5)
Complete Elementary School	07(21.9)	31(16.5)
Incomplete High School	05(15.6)	24(12.8)
Complete High School	07(21.9)	29(15.4)
Incomplete College Degree	0(0)	05(2.6)
Complete College Degree	01(3.1)	06(3.2)

Table 2. Clinical variables

Variables	Gram-negatives	
	Present (n=32) n(%)	Absent (n=188) n(%)
Length of time of HIV diagnosis (years)		
≤ 5	11(34.4)	67(35.6)
5 - 10	04(12.5)	37(19.7)
11 - 15	09(28.1)	38(20.2)
≥ 15	04(12.5)	29(15.4)
Not informed	04(12.5)	17(9.0)
Viral loads (copies/ml)		
< 50	06(18.7)	41(21.8)
50 – 10,000	04(12.5)	22(11.7)
10,001 – 100,000	07(21.9)	37(19.7)
> 100,000	09(28.1)	36(19.1)
Not informed	06(18.7)	52(27.6)
T CD4 (cell/mm ³)		
>350	08(25.0)	37(19.7)
200 - 350	05(15.6)	25(13.3)
< 200	15(46.9)	88(46.8)
Not informed	04(12.5)	38(20.2)
Number of hospitalizations in the previous 6 months		
None	13(40.6)	64(34.0)
One	08(25.0)	64(34.0)
Two or more	10(31.2)	55(29.2)
Not informed	01(3.1)	05(2.6)
Use of Antimicrobial		
Yes	21(65.6)	129(68.6)
No	11(34.4)	59(31.4)
Use of Antiretroviral		
Yes	16(50.0)	92(48.9)
No	16(50.0)	96(51.1)
Invasive procedures		
Yes	22(68.7)	131(69.7)
No	10(31.2)	57(30.3)

Table 3. Isolated gram-negative bacteria

Species	Gram-negatives	
	Nasal (n=8) n (%)	Saliva (n=26) n (%)
<i>Pseudomonas aeruginosa</i>	03(37.5)	13(50.0)
<i>Klebsiella pneumoniae</i>	0(0)	08(30.7)
<i>Achromobacter dentrificans</i>	01(12.5)	0(0)
<i>Brevudimonas diminuta</i>	01(12.5)	0(0)
<i>Stenotrophomonas maltophilia</i>	01(12.5)	01(3.8)
<i>Citrobacter koseri</i>	0(0)	01(3.8)
<i>Escherichia coli</i>	0(0)	01(3.8)
<i>Pseudomonas putida</i>	01(12.5)	01(3.8)
<i>Klebsiella oxytoca</i>	0(0)	01(3.8)
<i>Enterobacter aerogenes</i>	01(12.5)	0(0)

Discussion

The limits of the present research are related to the specific descriptive approach of the cross-sectional study, which did not assess the development of a possible infection in patients who were colonized by gram-negative microorganisms. Nevertheless, the knowledge about the prevalence of colonized patients supports the implementation of measures to be taken into account in the practice of healthcare professionals, aiming at both decreasing the risk of colonizing other patients and making them aware of the importance of those prevention measures.

Healthcare services have been experiencing a considerable increase of infections caused by gram-negative bacteria including the *Enterobacteriaceae*, which produce beta-lactamases, *Pseudomonas aeruginosa* and multi-resistant *Acinetobacter baumannii*, thus creating a new profile of clinical conditions to which only few therapeutic alternatives are available.⁽⁸⁾

Other studies have pointed out that gram-negative bacteria have been frequently isolated in patients with HIV/Aids and in patients with other chronic diseases.^(9,10) A research carried out with 508 patients submitted to hematopoietic stem cell transplants showed that the most frequently isolated gram-negative microorganisms in blood samples were the *Enterococcus* spp. and the *Escherichia coli*. At the time of the study, there was an increase of carbapenemics and beta-lactamics resisting gram-negative microorganisms (34%).⁽¹¹⁾

A comparison between the presence of *Staphylococcus* spp., *Enterobacteriaceae* and *Pseudomonas*

spp. in the oral cavity of patients with both positive and negative HIV showed a higher growth of gram-negatives in the group of positive HIV patients ($p=0.001$); additionally, there was no statistically significant difference between the groups regarding the *Staphylococcus* spp.⁽¹²⁾

A research carried out in Thailand assessed positive blood cultures of 140 patients with HIV undergoing antiretroviral therapy. The study found that most of the patients were men (65%), with a mean age of 38 years, and showed a predominance of gram-negatives (40%).⁽¹³⁾ The study also stressed out the relevance of research on this type of microorganism in this clientele.

In this present study, 35.3% of participating patients with positive samples for gram-negatives informed that they were aware of the HIV diagnosis for a period lower than five years. Similar data were identified in a study that analyzed hemocultures of 201 patients with HIV/Aids admitted in a hospital in Nigeria. Most of the patients presenting blood stream infections had already been aware of the HIV diagnosis for nearly six years.⁽¹⁴⁾

The T CD4 lymphocyte count has been employed in the follow-up of patients with HIV/Aids in order to indicate the onset of the treatment, control the progression of the disease, and monitor the administration of antiretrovirals.

Patients with lower counts of T CD4 present a higher risk of developing infections. As observed, 15 (46.9%) patients who displayed positive cultures for gram-negatives had T CD4 counts < 200 cells/mm³. Low counts for T CD4 lymphocytes were also observed in a study that assessed the clinical, etiological and inflammatory characteristics of sepsis in HIV-positive patients, compared with HIV-negative patients.⁽¹⁵⁾ A study that compared a group of healthy men with a group of men with HIV using antiretroviral therapy showed that gram-negative bacteria were more frequently isolated in the second group. Enterobacteria and *Pseudomonas* spp were more commonly found in patients with low T CD4 counts ($p=0.011$), but there was no statistically significant difference concerning the viral load.⁽¹²⁾

In Los Angeles, a study that assessed 4,825 patients with HIV observed a strong correlation be-

tween low T CD4 counts and the presence of *Pseudomonas aeruginosa*.⁽¹⁶⁾

The viral load count in most of the patients with positive samples for gram-negative bacteria was above 100,000 copies/ml. Most of the individuals were not undergoing antiretroviral therapy and were using antimicrobials, being the sulfamethoxazole/trimethoprim the most frequent antimicrobial agent used. The viral load count may be assigned as a risk factor to the diminishment of T CD4 cells, and the antiretroviral therapy aims at restoring the patient's immunologic system and suppress the viral replication, thus generating a positive impact in the progression of the HIV/Aids status and decreasing mortality rates.⁽¹⁷⁾

The use of antiretrovirals had a protective effect against pneumonia caused by the *Pseudomonas aeruginosa* in patients with HIV-1; moreover, a significant reduction of sepses/bacteremias was observed following the introduction of the HAART.^(18,19)

It must be highlighted that 68.7% of patients who presented gram-negative microorganisms were undergoing some sort of invasive procedure. A study conducted in long-stay institutions showed that patients undergoing invasive procedures were five times more likely of being colonized by multi-resistant microorganisms, and the most frequently identified were the *Escherichia coli*, *Acinetobacter baumannii* and the *Enterobacter aerogenes*.⁽⁵⁾ The study brought light on the importance of monitoring and surveilling such microorganisms in all sorts of healthcare scenarios.

A study on the epidemiology of blood stream infections in patients with HIV pointed out that gram-negative bacteria (39.6%) were more frequently isolated in cultures, followed by fungi (24.3%).⁽¹³⁾

As per the site of the collection, the *Pseudomonas aeruginosa* and the *Klebsiella pneumoniae* were more frequently isolated in the saliva than in the nasal secretion, thus indicating that future studies capable of identifying the need for collections in one or more sites will be required.

Although gram-negative bacteria isolated in the saliva and the nasal secretion in the first 24 hours of hospitalization of patients with HIV/Aids do not

present any resistance against the tested antimicrobials, several resistance mechanisms may be developed by such microorganisms. Therefore, surveillance may favor the advanced identification of colonized individuals and consequently the adoption of prevention and control measures. The manual on the management of multi-resistant microorganisms in the healthcare service recommends that interventions be grouped into seven categories, namely administrative support, accurate use of antimicrobials, active surveillance, use of standard and contact precautions, environmental and educational measures, and decolonization.⁽²⁰⁾

Although standard and contact precautions stand out as very well defined norms, the engagement on the part of healthcare professionals remain below the ideal in the different healthcare institutions.^(21,22)

The nursing team is responsible for a significant parcel of the direct care provided to hospitalized patients and plays a crucial role in the prevention and control of microorganisms that may cause healthcare associated infections. Leading nurses impersonate a critical duty toward the safety of patients.

Safety culture research has been on the rise in the last two decades. They show that whenever safety-related technology is aligned with qualified leadership, such equation is able to promote efficient care processes and improved quality.⁽²³⁾ The compliance with protocols contributes to minimize colonization and consequently the transmission of multi-resistant microorganisms.

Conclusion

The prevalence of gram-negative bacteria in patients with HIV who are hospitalized in specialized services reached 14.5%. Regarding the collection site, a higher number of gram-negatives were isolated in the saliva in comparison with those isolated in the nasal secretion.

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Collaborations

Lopes AER; Canini SRMS; Reinato LAF; Lopes LP and Gir E confirm their contributions regarding the conception and development of the research, as well as data interpretation, wording, relevant critical review of the intellectual content, and final approval of the version to be published.

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