FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY OF PATHOGENS AT TERTIARY PUBLIC HOSPITAL, SAO PAULO, BRAZIL

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Abstract. Nosocomial infections are one of the leading causes of morbidity and mortality. This study determined both prevalence and antimicrobial susceptibility of microorganisms identified during January to December 2012 at a tertiary public hospital, Sao Paulo, Brazil. Data, hospital length of stay, age, identity of microorganisms, and antimicrobial susceptibilities were obtained from patients' records. A total of 724 positive strains were obtained from different body sites. Gram-negative microorganisms are significantly more prevalent than gram-positive microorganisms ($p = 0.001$). In all clinics analyzed, coagulase-negative *Staphylococcus* (CoNS) was the most prevalent microorganism isolated (21.6%), followed by *Pseudomonas aeruginosa* (12.4%). Extended spectrum β-lactamase *Klebsiella pneumoniae* was present in 62.7% of the strains and 18.9% were resistant to carbapenem/meropenem. *Acinetobacter baumannii* showed multidrug resistance. The majority of *Escherichia coli* isolates were obtained from positive urinary tract cultures (63.4%), with 27.5% resistant against cefepime. Elderly patients, long periods of hospital stay and continuous usage of a single antibiotic should be kept in mind of possible causes for infection of *A. baumannii,* ESBL and carbapenem-resistant *K. pneumoniae* and the worrisome *E. coli* with increased resistance to cefepime. The data allowed us to implement monitoring programs as part of the prevention strategy against pathogens prevalence and antibiotic resistance burden at Ipiranga Hospital, Sao Paulo, Brazil.

Keywords: antimicrobial susceptibility, pathogens frequency, tertiary public hospital, Brazil

INTRODUCTION

The worldwide rise in the incidence of antibiotic resistance in bacterial pathogens, and in the last decades, the disappointingly low rate of emergence of new, clinically useful antibiotics, are worrisome trends. Nosocomial infections are one of the leading causes of morbidity and mortality, and the incidence is globally

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growing, depending on environmental factors and differences in clinical practices (Nicholson *et al*, 2009).

Nosocomial microorganisms vary greatly in different continents, regions, countries, and even in hospitals in the same region and, furthermore, in different locations within the same hospital. Different factors pertaining to the patients also are involved, such as age, severity of infection, immune response, and period of hospitalization. Also factors related to the hospital, such as availability and use of broad-spectrum antibiotics, and the diagnostic and therapeutic procedures employed (Hawkey and Jones, 2009). In the treatment of severe diseases, such as multi-resistant infections, tertiary public hospitals play a key role in the Unified Public Health System (SUS) throughout Brazil. They support smaller hospitals and perform more complex treatment procedures (Prestes-Carneiro *et al*, 2012).

Both knowledge of the profile and susceptibility of microorganisms to antibiotics enable knowledge-based decisions to be made, which diminish their frequency and resistance burden, improve the empirical choice of antibiotics, and prevention of outbreaks (Spellberg *et al*, 2013). This study determined both the prevalence, and antimicrobial susceptibility of microorganisms identified from January to December 2012, in a tertiary public hospital, Sao Paulo, Brazil.

MATERIALS AND METHODS

Clinical samples

A retrospective study of the records from January to December, 2012 of all positive pathogenic microorganism cultures and susceptibility patterns was conducted at Ipiranga Hospital, Sao Paulo, Brazil. Microbiology cultures from blood, central line catheters, abscesses/surgical site infections (A/SSI), bronchoalveolar lavage (BAL), and positive urinary tract cultures (PUTC), and other sites (OS) (ocular secretion, diabetic foot, peritoneal, and pleural fluid), were obtained. In the case of duplicate cultures, only the first isolate cultured from a single patient was included. Colonization-site cultures (*eg*, nasal swabs) and culture reports identified by the laboratory as contaminant organisms were excluded from the study. Cultures were obtained from hospitalized patients in neonatal intensive care unit (NICU), adult intensive care unit (ICU), internal medicine ward (IM), general surgery ward (GS), and gynecology/obstetrics ward (GO). Ipiranga Hospital does not have oncologic and transplantation units. Hospital length of stay and median age of the patients were determined in IM ward and data were extended to other locations analyzed. The study was approved by the ethics committee of Ipiranga Hospital (protocol no. 35635).

Microorganism identification

All pathogens were identified employing routine methods, which were confirmed using automated or other methods if needed. Antimicrobial susceptibility test was performed using broth micro dilution methods as described by the Clinical Laboratory Standards Institute (CLSI, 2013). Extended spectrum β-lactamase (ESBL) patterns were defined for *Escherichia coli*, *Klebsiella* spp according to CLSI (2013) criteria as the minimum inhibitory concentration (MIC) ≥ 2 mg/l for aztreonam, ceftriaxone and ceftazidime. Carbapenem-resistant Enterobacteriaceae (CRE) was detected based on a MIC ≥ 2 mg/l for imipenem and meropenem. Isolates with reduced susceptibility to imipenem or meropenem were tested with the modified Hodge test (MHT) (Gupta *et al*, 2012). Quality control was performed utilizing

strains from the American Type Culture Collection (ATCC), including *Streptococcus pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, *Enterococcus fecalis* ATCC 29212, *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Statistical analysis

Data were analyzed using Epi-info version 5.0, and when indicated, chi-square, Fisher exact test. Correlation tests were applied using Instat 3 (San Diego, CA) and GraphPad software (V4.0) (Richmond, CA). Statistical significance (5%) of differences among groups was assessed by testing regression coefficients.

RESULTS

Study population

Ipiranga Hospital is a 185-bed tertiary public university hospital located in Ipiranga, one of Sao Paulo's neighborhoods, and acts as a reference center for SUS. The hospital serves a population of about 600,000 inhabitants, representing 4.9% of the Sao Paulo's whole population. The Ipiranga neighborhood consists basically of a residential area with an increasing elderly population. In the IM ward and ICU, about 58% of the patients are older than 60 years and remain hospitalized for periods longer than 10 days.

Distribution of infections in different body sites and microbiological features

A total of 724 positive strains were obtained from different body sites. Blood and PUTC shared the higher number of positive cultures (219/724, 30.2% and 215/724, 29.7%, respectively) (Table 1). Of these, a total of 289 (39.9%) strains were identified as gram-positive organisms, 352 (48.6%) as gram-negative organisms and 83 (11.5%) as *Candida* spp. A number of samples of other gram-negative bacteria, namely, *Citrobacter* spp (3), *Providencia stuartii* (9) and *Burkholderia cepacia* (1), associated with gram-negative strains,were isolated. Gram-negative microorganisms are significantly more prevalent than gram-positive microorganisms (p=0.001). Blood yielded the highest number of gram-positive cultures, accounting for 148/289 (51%) of isolates. PUTC yielded the highest number of gram-negative isolates, 109/352 (31%). PUTC also yielded the majority of *Candida* spp isolates, 63/83 (76%). As regards the distribution patterns of microorganisms in different body sites, blood and BAL accounted for 50% of *Acinetobacter baumannii* positive strains, 32/58 (55%), whereas BAL and PUTC accounted for 59/90 (65%) of *P. aeruginosa* strains. Blood represented 20/65 (31%) of *Klebsiella pneumoniae* strains, whereas PUTC had the highest level of *E. coli*, representing 50/79 (63%) of positive cultures. As for the distribution of gram-positive microorganisms, blood accounted for 31/61 (51%) of *S. aureus* and 99/157 (63%) of Coagulase-negative Staphylococcus (CoNS)-positive strains.

Antimicrobial susceptibility

Antimicrobial susceptibility rates of gram-negative were obtained from the four most frequent microorganisms. *E. coli* accounted for 27.5% of bacteria resistant against cefepime, whereas 63.4% of the isolated samples were PUTC (Table 2). Among the gram-positive microorganisms, CoNS reached 84.3% of those resistant against methicillin, 1.8% against vancomycin, but showed complete susceptibility to teicoplanin. Methicillin-resistant *S. aureus* (MRSA) constituted 55.7% of the isolates, but vancomycin intermediate/resistant *S. aureus* (VRSA/VISA) was not found, or resistance against teicoplanin (Table 3). Vancomicin resistance to *Enterococcus* (VRE) was found in 20.0% of the strains.

Table 1
Distribution of microorganisms in different body sites from patients of a public tertiary hospital between January-December 2012, Sao Paulo, Brazil.

Microorganism	Blood (*n*)	Cat (*n*)	A/SSI (*n*)	BAL (*n*)	PUTC (*n*)	OS (*n*)	Total (*n*)
Gram-positive							
CoNS	99	30	9	9	6	4	157
S. aureus	31	6	7	8	6	3	61
S. viridans	9	0		0	0	2	11
Group D streptococci	2	0	1	0	0	0	3
E. faecalis	5	6	6	2	26	1	46
E. faecium	2	1	2	0	5	1	11
Total	148	43	25	19	43	11	289
Gram-negative							
P. aeruginosa	9	8	12	30	29	2	90
Enterobacter spp	4	0	2	4	1	4	15
E. coli	4	1	19	4	50	1	79
A. baumannii	16	13	8	16	5	0	58
K. pneumoniae	20	4	6	16	15	4	65
P. mirabilis	4	4	6	13	5	0	32
S. marcescens	3	1	1	3	4	1	13
Total	60	31	54	86	109	12	352
Candida spp	11	3	0	6	63	0	83
Total	219	77	79	111	215	23	724

A/SSI, abscess/surgical site infection; BAL, bronchoalveolar lavage; Cat, central line catheter; OS, other site (ocular secretion, diabetic foot, peritoneal, and pleural fluid); PUTC, positive urinary tract culture.

Distribution of pathogens most frequently isolated in clinics

CoNS was present in all the analyzed clinics, and was the most prevalent in NICU, ICU, and IM ward (Table 4). Higher levels of *E. coli* were found in GS and GO wards. *P. aeruginosa* strains were found in 4/5 clinics analyzed, whereas ICU yielded 35/58 (60%) of *A. baumannii* strains.

Distribution of infections among different clinics and numbers of beds

Cultures were obtained from patients distributed among 148 available hospital beds distributed as follow: NICU 10 (7%), ICU 9 (6%), IM 45 (30%). GS 46 (31%), and GO 38 (26%). There is a negative correlation between low number of available beds in ICU compared to the high number of positive cultures in the clinic (251, 34.6%) ($p = 0.0001$), whereas there is an inverse correlation between the number of beds in GS compared with the low number of positive cultures found (100, 13.8%) ($p = 0.001$). Similar results were obtained for number of beds in GO compared with the number of positive strains (118, 16.3%) ($p = 0.009$). No correlation is found between IM beds compared to number of positive strains (220, 30.4%) ($p = 0.99$), and NICU beds compared to number of positive strains (35, 4.9%) ($p = 0.44$) (Fig 1A). The most prevalent body site infected in each clinic was blood, being most prevalent in

Table 2
Rates of antimicrobial susceptibility among gram-negative microorganisms isolated from patients of a public tertiary hospital between January-December 2012, Sao Paulo, Brazil.

Antimicrobial agent	*K. pneumoniae*		*A. baumannii*		*P. aeruginosa*		*E. coli*	
	n	% resistant	*n*	% resistant	*n*	% resistant	*n*	% resistant
Amp-Sulb	8	50	38	87	ND	ND	ND	ND
Pip-Tazo	14	29	102	46	88	32	62	5
Ceftriaxone	58	67	60	87	ND	ND	59	5
Ceftazidime	41	63	58	81	84	37	23	0
Cefepime	47	57	57	84	88	40	40	27
Imipenem	40	12	57	81	88	33	31	0
Meropenem	58	19	57	81	87	34	52	2
Ciprofloxacin	63	62	58	88	87	45	82	32
Gentamicin	63	36	57	7	85	29	81	11
Amikacin	57	5	57	16	85	18	81	0

Amp-Sulb, ampicilin-sulbactan; Pip-Tazo, piperacilin-tazobactam; ND, not determined.

Table 3
Rates of antimicrobial resistance among gram-positive microorganisms most frequently isolated from patients of a public tertiary hospital between January-December 2012, Sao Paulo, Brazil.

Antimicrobial agent	*S. aureus*		CoNS		*Enterococcus* spp	
	n	% resistant	*n*	% resistant	*n*	% resistant
Methicillin	61	56	157	84	ND	ND
Vancomycin	60	0	156	2	57	20
Teicoplanin	22	0	73	0	56	18

ND, not determined.

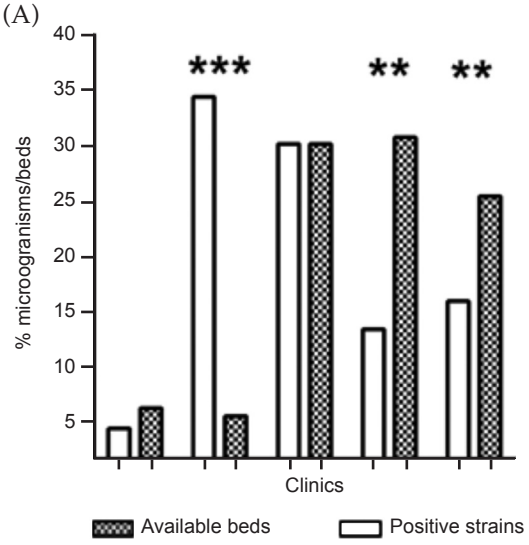
NICU, 15/35 (43%), ICU, 99/251 (39%) and IM, 84/220 (38%), whereas A/SSI was the most prevalent in GS, 55/100 (55%) and PUTC in GO, 56/118 (47%) (Fig 1B).

DISCUSSION

In developing countries and particularly in Brazil, there is a shortage of public hospital beds. All over the country the wards are filled up with patients harboring a huge number of infected diseases posing increased risks for nosocomial infections.

In the present study, worrisome multidrug resistance rates of *A. baumannii* were found, and blood and BAL accounted for 50% of positive cultures. One hypothesis for these phenomena is that adult ICU

(A)



(B)

Clinic	Site	(%)	OS (%)
NICU	blood	42.8	57.2
ICU	blood	39.4	60.6
IM	blood	38.1	61.9
GS	A/SSI	55.0	44.5
GO	PUTC	47.4	52.6

Fig 1–Distribution of infections in different wards and the number of available beds (A), and the most prevalent infected body site in various wards (B). GO, gynecology/obstetrics ward; GS, general surgery ward; ICU, adult intensive care unit; IM, internal medicine ward; NICU, neonatal intensive care unit; OS, other sites. $^{**}p = 0.001$ comparing GS and GO positive strains with available beds; $^{***}p = 0.0001$, comparing ICU positive strains and available beds.

yields *A. baumannii* strains, most of which are from ventilator-associated pneumonia and bacteremia. It is well-known that the emergence of antibiotic-resistant microorganism appears first in ICUs, where patients are debilitated, very often attached to multiple lines and tubes, and have been exposed to a wide array of antibiotics (Machado *et al*, 2011; Marra *et al*, 2011). In our case, this was aggravated by an increased number of older individuals remaining hospitalized for long periods, with higher susceptibility to severe infections. The frequency of the pathogens was in accordance with the rate determined in a Prospective Brazilian Nationwide Surveillance Study (PNSS) (16.4% compared to 11.4%, $p > 0.05$). Nevertheless, resistance against carbapenem (80.8% and 80.8%) are significantly higher than PNSS (56.1% and 22.4%) ($p < 0.0001$ and < 0.0001, respectively) from studies conducted at two tertiary hospitals, southern Brazil (Machado *et al*, 2011; Marra *et al*, 2011).

Worldwide, *P. aeruginosa* is one of the leading causes of nosocomial infections (Rossi, 2011). *P. aeruginosa* multidrug-resistant to cephalosporins, carbapenem and fluoroquinolones was found in four of the five sites analyzed, with BAL and PUTC accounting for 65.5% of the strains. *P. aeruginosa* resistance against fluoroquinolonas is extremely high in most Latin American countries, with rates > 70% (Casellas, 2011). However, in Brazil there is a significant trend that fluoroquinolones usage be withheld until culture data are available. The reduction in ciprofloxacin use may be a means of controlling multidrug-resistant *P. aeruginosa*.

In this study, ESBL-producing strains of *K. pneumoniae* were > 60% and carbapenem resistance is significantly high, with rate of 12.5% and 18.9% for imipenem and meropenem, respectively. Mechanism proposed for the high resistance rates of *A. baumannii* may be the same as for *K. pneumoniae*. The resistance rate of ESBL (ceftriaxone, ceftazidime and cefepime) *K. pneumoniae* (62.7%) was similar to those published by SENTRY (52.9%) ($p = 0.23$), and by PNSS (53.3%) ($p = 0.23$), and for carbapenemases-producing strains as

Table 4
Distribution of pathogens in wards and the most frequently isolated microorganisms at a public tertiary hospital between January and December 2012, Sao Paulo, Brazil.

Ward / microorganism	*n* (%)
NICU	
CoNS	14 (44)
K. pneumoniae	5 (16)
P. aeruginosa	4 (12)
Candida spp	5 (6)
ICU	
CoNS	57 (25)
P. aeruginosa	38 (16)
A. baumannii	35 (15)
Candida spp	24 (29)
IM	
CoNS	59 (25)
S. aureus	30 (13)
P. aeruginosa	28 (12)
Candida spp	24 (29)
GS	
E. coli	23 (23)
CoNS	12 (12)
P. aeruginosa	11 (11)
S. aureus	11 (11)
GO	
E. coli	26 (27)
CoNS	14 (14)
P. mirabilis	11 (11)
Candida spp	23 (28)

GO, gynecology/obstetrics ward; GS, general surgery ward; ICU, adult intensive care unit; IM, internal medicine ward; NICU, neonatal intensive care unit.

well (15.7% compared to 11.2%, $p = 0.12$) (Machado *et al*, 2011; Marra *et al*, 2011; Gales *et al*, 2012).

In the last decade, carbapenemases conferring resistance to virtually all beta-lactam agents have appeared worldwide. In the USA recent evidence suggests that the incidence is increasing at an alarming rate (Prabaker and Weinstein, 2011). It is worth noting and worrisome that higher rates of *E. coli* resistance are found against the fourth-generation cephalosporin cefepime. PUTC accounted for 63.4% of *E. coli* samples. As expected, the continuous usage of a single antibiotic produces a selective pressure, increasing the resistance burden.

There is a progressive increase in infections caused by gram-positive bacteria during the last decade all around the world (Casellas, 2011). In our study, CoNS was the most prevalent microorganism isolated, with 84.2% being resistant against methicillin, but completely susceptibility to vancomycin and teicoplanin. CoNS was present in all the analyzed clinics, being most prevalent in NICU, ICU and IM ward. These microorganisms usually are more resistant to antimicrobial agents than *S. aureus* (Gales *et al*, 2009). Our data are closely related to the methicillin resistance rates reported from Latin American nations and USA (Farrel *et al*, 2011; Jones *et al*, 2011).

MRSA is currently of great concern regarding the resistance challenge among gram-positive pathogens. In the USA, annual deaths due to MRSA are comparable to that caused by HIV/AIDS, viral hepatitis and tuberculosis (Rossolini *et al*, 2010). Our rates are higher than rates obtained by SENTRY in Brazilian hospitals, both during 1997-1999 and 2005-2008 (55.7% compared to 22.8%, and 31%, respectively, $p = 0.0001$) (Sader *et al*, 2003; Marra *et al*, 2011), but similar to the rate (60%) obtained in Hospital das Clinicas in Sao Paulo, one of the major university hospitals in the country (Rossi, 2011). One possible explanation for the high number of positive cultures for CoNS and *S. aureus* in our study is the fact that most of the patients hospitalized in the ICU and CM

ward were admitted through the emergency unit, mostly in critical conditions, and have undergone attachment to central line catheters, and remained catheterized for long periods.

As expected, ICU, containing 6.0% of the available hospital beds, yielded 34.6% of the positive strains. On the other hand, an inverse correlation was obtained in GO and GS in which a significant number of available beds contributes to a moderate number of positive strains. One possible explanation is that in these wards the majority of patients remained hospitalized for short periods, thereby have reduced risk of acquiring nosocomial infections. The correlation between the number of available beds and infections prevalence in IM ward was a surprise due to the similarities of patients with those in ICU. In the latter ward, there is a higher number of nurses and interns compared to other wards, and there is a weekly visit of a specialist in infectious diseases who holds discussion involving interns and preceptors. This measure certainly improved the correct choice of antibiotics, reinforced preventive measures, which might have been decisive for the results.

Sputum culture is a useful test to detect and identify bacteria or fungi that infect the lungs or breathing passages. However, the routine at Ipiranga Hospital, as recommended by the Brazilian Health Ministry, sputum culture is indicated mainly when *Mycobacterium tuberculosis* infection is suspected and the sputum smear microscopy is negative (Brazil Ministry of Health, 2011).

In conclusion, increased ESBL *A. baumannii*, carbapenem-resistant *K. pneumoniae* and the worrisome cefepime-resistant *E. coli* were found at Ipiranga Hospital, Sao Paulo, Brazil. High number of elderly patients, long periods of hospital stay and the continuous use of a single antibiotic should be kept in mind as possible causes. The data indicate the need to implement monitoring programs as a part of the prevention strategy against antibiotic resistance burden.

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