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NASOPHARYNGEAL GRAM-NEGATIVE BACILLI COLONIZATION IN BRAZILIAN CHILDREN ATTENDING DAY-CARE CENTERS

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ABSTRACT

This study aimed at determining prevalence and resistance profile of Gram-negative bacilli isolated from nasopharynx of children attending day-care centers in Goiânia (Brazil). *P. aeruginosa* (100.0%), *E. coli* (50.0%), *K. pneumoniae* (35.3%), and *E. aerogenes* (16.7%) were the most frequent multi-drug resistant microorganisms isolated. No production of ESBL was detected.

Key words: Gram-negative bacilli, nasopharyngeal colonization, infants.

The nasopharynx constitutes the primary ecological reservoir or source of dissemination of microorganisms such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, as well as Gram-negative bacilli (GNB). Asymptomatic nasopharyngeal carriage of pathogens is prevalent in young infants and precedes the development of invasive diseases. Children in day-care centers may be important vectors for horizontal spread of respiratory pathogens and GNB within the community (1, 4, 8, 14). Infants are susceptible to being GNB carriers and play a fundamental role in the epidemiology of respiratory infections.

The nasopharyngeal flora is established during the first year of life and comprises a broad variety of microorganisms, including commensal bacteria and potential pathogens that may cause infections (3, 5, 7). Nasopharyngeal carriage with GNB is frequent in warm-climate countries and associated with community-acquired pneumonia in children (13, 14). Epidemiologic studies demonstrate that many risk factors influence nasopharyngeal colonization by GNB, such as age, gender, season, acute respiratory illness, exposure to other children, socio-economic status, family size, warm climate and antibiotic therapy (5, 6, 9, 10, 14).

In a study developed with Angolan, Brazilian and Dutch children, the authors compared the nasopharyngeal flora prevalence in industrialized and warm-climate developing countries (12). A higher carriage rate of GNB was found in healthy children from Angola and Brazil than in children from the Netherlands, suggesting that GNB isolated from nasopharyngeal cultures might be associated with high family size and community-acquired pneumonia in warm-climate developing countries (12). In Brazil, few studies have been conducted to evaluate the nasopharyngeal colonization of children aged less than 5 years.

This study aimed to determine the prevalence and resistance pattern of GNB isolated from nasopharynx of children less than 5 years old attending day-care centers in the municipality of Goiânia, located in the Midwestern Region of Brazil.

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The protocol of this study was approved by the Ethical Committee of the Universidade Federal de Goiás. The investigation was carried out from August to December 2005 as part of an ongoing prospective surveillance of *S. pneumoniae, H. influenzae*, and *S. aureus* in children that attend day-care centers in the city. Nasopharyngeal specimens from 1192 children were obtained after written consent of their parents, collected with a transwab placed in Stuart transport medium tubes (Transwab; Medical Wire & Equipment, Corsham, United Kingdom), and transported to the Laboratory of Bacteriology of the Instituto de Patologia Tropical e Saúde Pública, at the Universidade Federal de Goiás, within 4 h after collection. All samples were cultured on MacConkey agar plates and sodium thioglycollate broth and incubated at 37°C for 24 h. The isolates were stored in nutrient agar at 4°C and in broth Todd Hewitt Bacto supplemented with 20% of glycerol at -20°C. The isolates were identified by colony morphology, Gram staining technique and standardized tests (sugar fermentation, indole production, methyl red test, citrate test, urea hidrolysis, hydrogen sulfide production, and motility) (11). Susceptibility tests were performed using the disk diffusion method with Oxoid disks (Basingstoke, United Kingdom): amikacin, amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, cefpodoxime, ceftazidime, ceftriaxone, ciprofloxacin, imipenem, meropenem, nalidixic acid, piperacillin-tazobactam, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole. The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (2).

The GNB nasopharyngeal prevalence was 8.9% (106/1192), and 13 species were identified. The more prevalent species were (Table 1): 26 *Enterobacter aerogenes* (24.5%), 17 *Klebsiella pneumoniae* (16.0%), and 11 *Escherichia coli* (10.4%). Wolf *et al*. (14) demonstrated the nasopharyngeal colonization by GNB in 912 children up to 5 years old and the more prevalent species were: 45 *Klebsiella* spp. (4.9%), 34 *Enterobacter* spp. (3.7%), and 28 *Escherichia* spp. (3.0%). Although the authors isolated the same microorganisms herein detected, the overall prevalence of GNB was lower than the results observed in this study. We isolated only 9 *Pseudomonas* spp. (8.7%), but Wolf *et al*. (14) found a higher prevalence of nonfermentative species (29.7%); nevertheless, the prevalence of *Pseudomonas* spp. (7.0%) was similar in both experiments.

During our study, 41 out of 106 GNB carriers (38.7%) received antibiotics for a period ranging from 7 to 90 days. However, no significant difference was observed in the nasopharyngeal GNB carriage distribution between the group of children that used antibiotics and the group that did not receive this treatment. The frequency of GNB isolated in the day-care centers participating in the present study ranged from 0 to 7.5%.

The susceptibility of GNB to 17 antibiotics was determined and we detected that were resistant only to ampicillin (57.3%), amoxicillin-clavulanic acid (24.0%), trimethoprim-sulfametoxazole (26.7%), and tetracycline (12.0%), as presented in Table 1. Our results are in accordance with those found by Wolf *et al*. (14), who reported *Klebsiella* spp. and *Enterobacter* spp. resistant only to trimethoprim-sulfametoxazol (24.0% and 22.0%, respectively). Nonetheless, the authors also observed that *Escherichia* spp. presented high resistance to ampicillin (61.0%) and trimethoprim-sulfametoxazol (64.0%). In our study, although the extended-spectrum beta-lactamase production was not detected in GNB isolates, we observed GNB resistant to two or three antibiotic classes: *P. aeruginosa* (100.0%), *E. coli* (50.0%), *K. pneumoniae* (35.3%), and *E. aerogenes* (16.7%) were the most frequent multi-drug resistant microorganisms.

According to the results herein obtained, we conclude that nasopharyngeal colonization with GNB is common in young children attending day-care centers in Goiânia. These children might be GNB carriers, which could explain why such pathogens are a common cause of community-acquired pneumonia in Brazil. The clinical implications of our findings remain unclear and further studies are necessary to determine the relation between GNB nasopharyngeal colonization and development of invasive infections.

Table 1. Susceptibility patterns of Gram-negative bacilli isolated from the nasopharynx of children attending day-care centers of Goiânia from August to December 2005.

Microorganism	N = 106	%	S*	%	Non-susceptible GNB									
					AMP	%	AMC	%	SXT	%	TET	%	MDR	%
Enterobacter aerogenes	26	24.5	8	30.7	13	50.0	7	26.9	4	15.4	1	3.8	3	16.7
Klebsiella pneumoniae	17	16.0	0	0.0	17	100	4	23.5	5	29.4	4	23.5	6	35.3
Escherichia coli	11	10.4	5	45.4	0	0.0	1	9.0	5	45.4	3	27.3	3	50.0
Enterobacter agglomerans	8	7.5	4	50.0	4	50.0	1	12.5	0	0.0	1	12.5	1	25.0
Pseudomonas aeruginosa	4	3.8	0	0.0	4	100	4	100	4	100	0	0.0	4	100
Enterobacter cloacae	3	2.8	0	0.0	3	100	1	33.3	0	0.0	0	0.0	0	0.0
Citrobacter freundii	2	1.9	2	100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Enterobacter gergoviae	1	0.9	0	0.0	1	100	0	0.0	1	100	0	0.0	1	100
Klebsiella oxytoca	1	0.9	1	100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Enterobacter sakazakii	1	0.9	1	100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Shigella sonnei	1	0.9	1	100	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Enterobacter sp	5	4.7	_	_	_	_	_	_	_	_	_	_	_	_
Pseudomonas sp	5	4.7	_	_	_	_	_	_	_	_	_	_	_	_
GNB**	21	19.8	_	_		_	_	_	_	_	_	_	_	_

S*: Susceptibility to the antibiotics analyzed in the study (amikacin, amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, cefpodoxime, ceftazidime, ceftriaxone, ciprofloxacin, imipenem, meropenem, nalidixic acid, piperacillin-tazobactam, tetracycline, tobramycin, trimethoprim-sulfamethoxazole).
GNB**: Gram-negative bacilli isolated but not identified.
AMP = ampicillin, AMC = amoxicillin-clavulanic acid, SXT = trimethoprim-sulfamethoxazole, TET = tetracycline, MDR = multi-drug resistant microorganisms.

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