



# Human papillomavirus genotypes 68 and 58 are the most prevalent genotypes in women from quilombo communities in the state of Maranhão, Brazil



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## SUMMARY

**Objectives:** To determine the frequency of human papillomavirus (HPV) types and behavioral characteristics related to cytological abnormalities in women descendants of slaves, who live in isolated communities known as quilombos in the state of Maranhão, Brazil.

**Methods:** Cervicovaginal specimens of 353 women were analyzed by conventional cytology and genotyping. HPV detection and genotyping was performed using a linear array HPV genotyping test kit. Behavioral factors and their association with cytological abnormalities were analyzed, as well as the association between cytological abnormalities and HPV infection.

**Results:** The frequency of HPV infection was 13%, and infection with high-risk HPV types was more frequent than with low-risk types (10.2% vs. 2.8%). The most prevalent genotypes were HPV 68 (3.1%) and HPV 58 (2.6%). HPV-positive women were 6.5 times more likely than HPV-negative women to be diagnosed with cytological abnormalities. There was a significant association between HPV infection and the presence of cytological abnormalities in women 31–40 years of age and in women 51–60 years of age.

**Conclusions:** A distinct profile of high-risk HPV genotypes was detected, with predominance of types 68 and 58. It is possible that the results of the present study are due to specific characteristics of the population, which is geographically isolated and maintains conservative sexual habits.

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## Introduction

Genital tract infection by human papillomavirus (HPV) is highly prevalent worldwide and is considered the most common sexually transmitted disease (STD). HPV infection is typically acquired at the beginning of an individual's lifetime sexual activity period.<sup>1,2</sup> It is believed that the great majority of sexually active women contract one or more HPV infections during their lives,<sup>2</sup> and it is suggested that the frequency of infection depends on age and is higher in women under 25 years of age.<sup>3,4</sup> The observed decrease in

the average HPV infection rate associated with increasing age is most likely the result of a combination of decreased HPV exposure, the self-limiting nature of most infections, and resistance to reinfection.<sup>4</sup>

A meta-analysis of a systematic literature review, which included 78 studies involving 157 879 women in populations from 15 areas of the world, found that HPV 16 is among the five most common HPV types in HPV-positive women, followed by HPV 18, HPV 31, HPV 58, and HPV 52; together, these five types represent 50% of all HPV infections. In Japan, Taiwan, and East Africa, HPV 52 is the most common type. HPV 18 is the most frequent type everywhere else except in Southern Europe, and HPV 53 and HPV 58 are among the most common HPV types found in women in East Africa and North and Central America.<sup>5</sup> In Brazil,

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HPV 16 is the most prevalent type in all regions of the country, whereas HPV 18 is frequently detected in the north, southeast, and south regions of Brazil. In the midwest and northeast regions of the country, HPV 31 and HPV 33 are also reported with high frequencies.<sup>6–10</sup>

In Brazil, many slaves who escaped or were released began to live in communities isolated from urban centers. These communities, which were called quilombos, still exist. The current inhabitants of these communities, the quilombolas, represent ethnic and racial groups with characteristic behavioral features, including geographic isolation, specific territorial relationships, conservative habits, and monogamous relationships only between quilombo members. In addition, the quality of life in these communities is generally poor, and poverty, low educational level, difficulty in accessing general services, hunger, malnutrition, occupational and social violence risks, morbidity, and mortality occur at higher rates than are recorded at the national level.<sup>11,12</sup>

The literature includes limited data on the health of quilombo communities, especially on the frequency of specific HPV types and cytological abnormalities among women in these communities. Thus, the importance of conducting research involving this social group has been emphasized, given that there is a disparity in local healthcare attention to this population compared to the general population of Brazil.<sup>11</sup> The present study aimed to determine the frequency of HPV genotypes and relate them to clinical and socio-demographic behavioral characteristics of women from quilombo communities in the state of Maranhão, Brazil.

## Materials and methods

### Study design and sample collection

This was a cross-sectional study involving the rural quilombola community of the east coast, a remnant of the quilombos formed by the municipalities of Alcântara, Bequimão, Central do Maranhão, and Mirinzal, in the State of Maranhão, Northeastern Brazil. The communities were certified by the Palmares Foundation and are located approximately 30 km from the capital. In total, 395 women were selected, of whom 42 were excluded due to DNA degradation or unsatisfactory cervicovaginal smears. The inclusion criteria were women 15 to 75 years of age from the municipalities of the east coast of Maranhão in the mentioned communities and living and registered in the areas covered by family health teams. Pregnant women, those who had undergone a hysterectomy, and women who had any mental impairment that would prevent their understanding and answering the specific questionnaire were excluded from the study. The women were informed of the purpose of the project and invited to participate. Those who agreed to participate answered a standardized questionnaire and signed an informed consent form. Women with abnormal cytological results were treated in accordance with the guidelines for management standardized by the Ministry of Health in Brazil.

### Cytological examination

The smears were stained using the Papanicolaou method and analyzed at the Center for Clinical Analysis Rômulo Rocha (Centro de Análises Clínicas Rômulo Rocha) of the School of Pharmacy of the Federal University of Goiás (Universidade Federal de Goiás – UFG). All smears were subjected to the strict quality control procedures adopted by the aforementioned laboratory; these were performed through a quick review of all negative smears and a detailed review of unsatisfactory smears and of smears that were suspected of or positive for cytological abnormalities. The results regarding the suitability of samples and degree of cervical

abnormalities were interpreted according to the cytological criteria defined in the Bethesda system.<sup>13</sup>

### Sample processing

DNA extraction was performed using commercial reagents; the method is based on the selective binding of DNA to a silica membrane in the presence of chaotropic salts (PureLink; Invitrogen). HPV amplification and genotyping was performed using the Roche PCR-based Linear Array HPV Genotyping Test (Roche Molecular Systems, Branchburg, NJ, USA). HPV DNA was amplified using the L1 consensus biotinylated PGMY09/PGMY11 primer set in a thermal cycler at 95 °C for 13 min, followed by denaturation for 1 min at 95 °C, annealing for 1 min at 55 °C, and extension at 72 °C for 1 min for a total of 40 cycles. Amplification was followed by a 5-min terminal extension step at 72 °C. Biotinylated GH20 and PC04 primers to the  $\beta$ -globin gene were used as amplification control. PCR products were denatured in 1.6% sodium hydroxide (NaOH) and hybridized to an immobilized probe array containing probes for  $\beta$ -globin at two concentrations plus 37 HPV genotypes. One of the groups included the HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, and 82. The other group included the HPV types 6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 70, 71, 72, 81, 83, 84, IS39, and CP6108. Positive hybridization was detected by streptavidin–horseradish peroxidase-mediated color precipitation on the membrane at the probe array. Each experiment included controls for extraction and PCR contamination, as well as known positive HPV samples to control the performance of the methods.

### Statistical analysis

According to the recommendations of the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística – IBGE), the estimated total population was 53 332 inhabitants; the sample size to be evaluated was set at 353 women, which was calculated as described previously.<sup>14</sup> The data were analyzed using the statistical software BioEstat 5.0 by non-parametric Chi-square independence test to verify the association of cytological abnormalities with age groups. The odds ratio (OR) and 95% confidence interval (CI) were also calculated. The significance level was set at 5% for all tests.

## Results

In total, 353 women were included in the study. The demographic data, including age, education, and marital status, are presented in Table 1. Most of the women were less than 40 years old, had completed elementary school, and were married.

The total frequency of HPV was 13% (46/353). Of the 353 subjects, 328 had an inflammatory cytology. Among 25 women who exhibited cytological abnormalities, 44% (11/25) were positive for HPV. Detection rates of HPV ranged from 10.7% (inflammatory cytology) to 75% (high-grade squamous intraepithelial lesion, HSIL) (Table 2).

With regard to the analysis of cytological abnormalities associated with HPV infection, it was found that HPV-positive women were 6.5 times more likely than HPV-negative women to be diagnosed with cytological abnormalities (OR 6.58, 95% CI 2.55–16.91;  $p=0.001$ ). None of the other variables analyzed (age at onset of sexual activity, number of partners, age at menarche, number of gestations, contraceptive use, or Papanicolaou test in the last 3 years) showed a significant association with the detection of cytological abnormalities (Table 3).

**Table 1**

Demographic data of 353 quilombola women from Maranhão, Brazil.

Variable	No. (%)
Age (years)	
<30	99 (28.0)
31–40	88 (25.0)
41–50	72 (20.4)
51–60	59 (16.7)
>60	35 (9.9)
Education level	
Elementary	227 (64.3)
High school	62 (17.6)
Higher education	9 (2.5)
Illiterate	55 (15.6)
Marital status	
Single	111 (31.4)
Married	212 (60.1)
Divorced	6 (1.7)
Widowed	24 (6.8)

High oncogenic risk genotypes were more frequent (10.2%, 36/353) than low oncogenic risk genotypes (2.8%, 10/353). HPV genotypes 68 (3.1%, 11/353) and 58 (2.5%, 9/353) were the most frequently detected. Other high-risk genotypes were detected at low frequencies (0.3–0.9%), including genotypes 16, 18, and 31. In general, single infections were more frequent than multiple infections (Table 4).

Single or multiple infections of type 68 were exclusively detected in women whose smears were considered inflammatory. Nine other high oncogenic risk HPV types (31, 18, 45, 70, 39, 56, 59, 66, and 53) were also found in women whose smears were classified as inflammatory. Another six low oncogenic risk HPV types (54, 61, 62, 72, and 84) were detected in women with inflammatory smears. Women diagnosed with low-grade squamous intraepithelial lesions (LSIL) exhibited infections with low oncogenic risk HPV types (HPV 62), and those with atypical squamous cells of undetermined significance (ASC-US) and LSIL were infected with high oncogenic risk HPV types (HPV 52 and HPV 31). High-risk HPV types 16, 18, and 58 were detected in quilombola women with a cytological diagnosis of HSIL.

## Discussion

The total frequency of HPV in women, regardless of age, may vary according to the technique used and the population and region studied. In the present study, only 13% of quilombola women were infected with HPV. This result differs from the results reported by other authors who have studied isolated communities. A higher frequency of HPV (64%) was found in sexually active indigenous Guarani women in the Misiones region in Argentina.<sup>15</sup> In a recent study involving indigenous populations of the Amazonian Rainforest, in Brazil, the overall frequency of HPV was 39.7%, but in women from the Yanomami District it reached

**Table 2**

HPV infection and cytological results for quilombola women from Maranhão-Brazil.

Cytological result	Number	HPV-positive	%
Inflammatory	328	35	10.7
ASC-US	7	1	14.3
LSIL	6	2	33.3
ASC-H	4	2	50.0
HSIL	8	6	75.0
Total	353	46	13.0

HPV, human papillomavirus; ASC-US, atypical squamous cells – undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

**Table 3**

Association of clinical and behavioral variables with cytological abnormalities in quilombola women.

Variables	Cytological abnormality		OR	95% CI	p-Value
	Positive, n (%)	Negative, n (%)			
Onset of sexual activity					
≤15 years	9 (6.6)	128 (93.4)	0.878	0.377–2.048	0.93
>15 years	16 (7.4)	200 (92.6)			
Number of partners throughout life					
1	10 (6.6)	141 (93.4)	1.13	0.46–2.80	0.93
>1	15 (7.4)	187 (92.6)			
Age at menarche					
≤12 years	14 (9.7)	129 (90.3)	1.96	0.864–4.458	0.15
>12 years	11 (5.2)	199 (94.8)			
Number of gestations					
Up to 1	2 (3.6)	54 (96.4)	2.57	0.50–14.3	0.40
>1	23 (7.7)	274 (92.3)			
Use of contraceptives					
Yes	5 (7.9)	58 (92.1)	1.16	0.37–3.46	0.98
No	20 (6.9)	270 (93.1)			
Papanicolaou test (last 3 years)					
No	3 (6.1)	46 (93.9)	0.84	0.19–3.11	0.98
Yes	22 (7.2)	282 (92.8)			
HPV infection					
Positive	11 (23.9)	35 (76.1)	6.58	2.55–16.91	<0.001
Negative	14 (4.6)	293 (95.4)			

OR, odds ratio; CI, confidence interval; HPV, human papillomavirus.

45.9%, whereas in women from the Eastern Indigenous District HPV infection was found in 34.5% of women.<sup>16</sup>

Of the clinical and behavioral variables analyzed and their association with cytological abnormalities, HPV infection was the only significant factor. Despite the low number of women included in the study, which could have influenced some of the conclusions, individuals living in quilombo communities are geographically isolated and display conservative habits. In the present study, of the HPV-positive participants, 9.6% had single infections and 3.4% had infections by multiple HPV types. In a previous study in women from Costa Rica, the frequency of HPV was 26.5%, but 8.2% represented infections by multiple HPV types.<sup>17</sup>

Of the 25 HPV types found in the present study, there was a greater frequency of HPV 68 (3.1%), followed by HPV 58 (2.6%).

**Table 4**

Frequency of HPV genotypes in 353 quilombola women.

HPV types	Number	%
High-risk	36	10.2
68	11	3.1
58	9	2.6
31	3	0.9
45	2	0.6
18	2	0.6
73	1	0.3
70	1	0.3
66	1	0.3
59	1	0.3
56	1	0.3
52	1	0.3
39	1	0.3
33	1	0.3
16	1	0.3
Low-risk	10	2.8
62	3	0.9
61	2	0.6
IS39	2	0.6
84	1	0.3
72	1	0.3
54	1	0.3
HPV-positive	46	13.0
Single infection	34	9.6
Multiple infection	12	3.4

HPV, human papillomavirus.

Although there are geographical variations in the distribution of some HPV types, HPV 16 and HPV 18 are the most prevalent genotypes in both women with a normal cervical cytology and those with cervical cancer worldwide.<sup>5,18,19</sup> The same pattern of genotype frequency has also been observed in women from indigenous isolated communities in South American countries, such as Argentina, Brazil, and Paraguay.<sup>15,16,20,21</sup> Although the number of samples was low, it can be hypothesized that one of the reasons for the difference in the genotype profile is that individuals living in quilombos maintain conservative sexual habits, without having sex with people in the general population. Thus, the dynamics in the circulation of HPV types might have been influenced by their sexual isolation over the years.

In the quilombola population studied here, HPV 58 was found only in women without cytological abnormalities. Of the HPV 68 infections found in the present study, five were single and six were multiple; of the multiple infections, three were associated with genotype 58. Goldman et al. observed that the rate of infection with HPV 68 in multiple infections was four times higher than expected, suggesting the existence of an interaction between high oncogenic risk HPV types, especially between types 31/68, 51/68, and 33/58.<sup>22</sup> These authors suggested that some genotypes of the virus act as cofactors in infection by other types.

The frequency of HPV infection ranged from 14.2% in women with a cervical cytology classified as ASC-US to 33.4% in LSIL, 50% in atypical squamous cells where high-grade squamous intraepithelial lesions cannot be excluded (ASC-H), and 75% in HSIL. Guan et al. found similar values, reporting 12% HPV infection in participants diagnosed with ASC-US, 52% in women with LSIL, and 76% in women diagnosed with HSIL.<sup>23</sup> The viral types found in women with a cytological diagnosis of HSIL were 16, 18, 58, IS39, and 73. Three of these are considered carcinogenic in humans, and type 73 is considered possibly carcinogenic in humans.<sup>19</sup> In contrast, type 68, which is considered most likely carcinogenic in humans, was not present in women with cytological abnormalities in the present study. In this context, it is important to consider the regional differences as well as the type of population studied and its habits.

To the authors' knowledge, the present study is the first to provide an estimate of the frequency of HPV infection among quilombola women in Northeastern Brazil. Another important result of the study is the distinct genotype profile, showing a higher frequency of HPV genotypes 68 and 58. Genotype 68 is uncommon in epidemiological surveys worldwide and has not been associated with the presence of cytological abnormalities. These data might be of importance for public health authorities regarding vaccine formulation for isolated communities like quilombos.

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## Ethical approval

The study was approved by the Ethics and Research Committee of the University Hospital of the Federal University of Maranhão

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## Conflict of interest

The authors declare that they have no competing interests.

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