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# Genital Human Papillomavirus Prevalence and Genotyping Among Males in Putuo District of Shanghai, China 2015-2019

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**Background:** Reports of human papillomavirus (HPV) infection and genotype distribution in Chinese men are limited, and HPV vaccination has not yet been recommended for men in China.


**Material/Methods:** We retrospectively reviewed the prevalence and genotyping of male genital HPV. A total of 1227 male patients (aged 17 to 81 years) attending the dermatology and sexually transmitted disease clinics at Putuo District Center Hospital in Shanghai from 2015 to 2019 were included. Genital exfoliated specimens were obtained for detection and genotyping of 27 HPV types by Luminex-based multiplex assay.

**Results:** The prevalence of any HPV was 65.5% (804/1227). The rate of multiple infection was 25.8% (317/1227). The 5 main HPV types were 6 (32.0%), 11 (23.2%), 16 (5.6%), 43 (4.3%), and 59 (4.0%). Among all detected HPV genotypes, 65.5% (875/1336) were 9-valent HPV genotypes. No significant differences were observed in the detection rate of HPV infection over 5 years ( $P>0.05$ ). Age groups  $\leq 24$  years (70.7%) and  $\geq 55$  years (72.9%) showed higher infection rates, and significant differences were detected in rates of low-risk HPV infection in different age-stratified groups ( $P<0.05$ ). Prevalence of HPV infection among patients with warts (74.4%) was significantly higher than that of patients with other clinical characteristics (40.4%) and physical examination (63.6%).

**Conclusions:** Our study suggested that more than half of Chinese male patients have detectable HPV infections, and penile-genital and anogenital warts were the most common clinical manifestations. Moreover, the available 9-valent HPV vaccine covers the most frequently observed HPV types among men.

**Keywords:** **Genotype • Papillomaviridae • Papillomavirus Vaccines • Prevalence**

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/932093>

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## Background

Human papillomavirus (HPV) is one of most common sexually transmitted viruses in men and women worldwide and can cause many types of dermatology problems and diseases, from benign warts to various cancers [1,2]. Several sexually transmitted infectious diseases, including HIV and HSV-2, are strongly correlated with HPV infection [3]. *Chlamydia trachomatis* infection contributes to the persistence of HPV and has been considered as a risk factor for cervical cancer [4]. Approximately 230 distinct types of HPV have been identified to date [5,6]. Among these genotypes, nearly 40 types can induce genital infection and even genital lesions. Most people know that HPV is strongly related to cervical cancer in women [7]. However, the fact that HPV infection in men can be associated with high morbidity is often ignored. High-risk HPV (HR-HPV) has been considered to play a key role in the development of anal, penile, and head and neck cancers [8-10]. There are 630 000 new cancer cases worldwide per year, approximately 4.5% of these are associated with HPV [11], and HPV-related diseases are increasingly prevalent in men [12]. Many studies have indicated that nearly 90% of anal cancers are mainly correlated with a persistent infection of HR-HPV, and HPV-16 was identified in 76% of cases [13,14]. Meta-analyses of previous studies revealed that HR-HPV was involved in more than 50% of penile cancers [15], and 90% of genital warts are correlated with certain low-risk HPV (LR-HPV) infections, such as HPV-6 and HPV-11 [16].

Each year, there are nearly 34 800 new HPV-related cancer cases in the United States, with 13 100 cases in men. Of these cases, 92% are caused by infection with the HPV genotype, which is covered by the 9-valent HPV vaccine [17]. Currently, there are 3 HPV vaccines that can protect against multiple HPV genotypes: bivalent (2v, HPV-16/18), quadrivalent (4v, HPV-6/11/16/18), and 9-valent (9v, HPV-6/11/16/18/31/33/45/52/58) vaccines [18]. HPV vaccinations have a great preventive potential to reduce HPV-associated diseases in men and women [19-22]. However, most countries, including China, have only administered vaccines to female patients but not to males [23]. It has been reported that 71 countries (37%) provided HPV vaccines for females, while only 11 countries (6%) provided vaccines for males in 2017 [24]. Comprehensive studies of male HPV infection and types are crucially important for developing evidence-based HPV vaccination, while relevant information in Chinese men is still scarce [25].

The HPV infection rate is an important parameter when considering the cost-effectiveness of HPV vaccines. In addition, regional differences are essential in the distribution of male HPV, and age can be the underlying cause affecting the incidence of HPV genotypes [26]. This study aimed to assess HPV infection prevalence and genotype distribution among Chinese male patients (Shanghai) who visited our dermatology and

sexually transmitted disease (STD) clinics from 2015 to 2019. To improve the understanding of HPV infection and types in Chinese men, an age-stratified analysis and theoretical vaccine coverage analysis were performed.

## Material and Methods

### Study Population

This retrospective cross-sectional study was conducted from January 1, 2015 to December 31, 2019. A total of 1227 male patients attending a dermatology department and STD clinic in Putuo District Center Hospital were included in this study. The inclusion criteria were as follows: (1) male patients; (2) patients  $\geq 17$  years old; (3) patients with signs such as anogenital wart and condyloma acuminata; (4) clinical manifestations of urethritis, rash, and dermatitis; (5) patients with discomfort or who had undergone physical examination; (6) willingness to provide adequate genital samples for the HPV DNA genotyping test; and (7) agreement to participate in our study. This study was performed in accordance with the principles of Declaration of Helsinki and was approved by the Ethics Committee of the Putuo Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (Shanghai, China) (reference no. PTEC-A-2020-24-2). Written informed consent was obtained from each patient at a previous clinical visit. To protect personal privacy, data were deidentified before analysis.

### HPV Sample Collection and DNA Extraction

Genital scraped cells from the urethral epithelium, anus, or penile epidermis were collected by physicians with a nylon flocked swab. The swab was placed in separate 3-mL tubes containing transport medium and immediately transported to a clinical laboratory affiliated with our hospital for the HPV testing. Specimens that could not be shipped for the examination in time were stored at 4°C and analyzed within 72 h. A total of 200  $\mu$ L of the specimen mixture was transferred to a new 1.5-mL centrifuge tube and centrifuged at 12 000 rpm for 10 min. The precipitate was retained, and 200  $\mu$ L of exfoliated cell nucleic acid release agent (Telgen Life Science Co. Ltd. Shanghai, China) was added, followed by incubation for 15 min at 100°C with a metal bath. Afterward, the Eppendorf tube was centrifuged at 12 000 rpm for 5 min, and the supernatant was kept and moved to a new Eppendorf tube for polymerase chain reaction (PCR) examination or stored at -20°C.

### PCR Amplification and Hybridization

A Tellgenplex™ HPV DNA genotyping test assay kit (Shanghai Tellgen Life Science, Co. Ltd.) was used for detection and genotyping 27 HPV types, including 19

HR-HPV genotypes (HPV-16/18/26/31/33/35/39/45/51/52/53/55/56/58/59/66/68/82/83) and 8 LR-HPV genotypes (HPV-6/11/40/42/43/44/61/81) by a combination of PCR and Luminex technology [26]. The detailed experimental operation protocol is included in previous studies [27,28]. In brief, PCR testing was performed in a total amplification volume of 20  $\mu$ L/reaction per sample, containing 15  $\mu$ L of the reaction solution (including 10  $\mu$ L master mix, 5.0  $\mu$ L primers, and 0.8  $\mu$ L taq DNA polymerase) and 5  $\mu$ L of the extracted DNA, according the manufacturer's instructions. The PCR reaction condition was as follows: 95°C for 5 min, 1 cycle; 95°C for 30 s, 58°C for 30 s, 72°C for 30 s, 5 cycles; 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, total of 35 cycles; and 72°C for 3 min, 1 cycle. After amplification, 3  $\mu$ L of the PCR product and 22  $\mu$ L of hybridization solution (containing carboxylated beads coupled with oligonucleotide probes for each of the 27 genotypes) were added into each well of a 96-well plate. Subsequently, hybridization was conducted at 95°C for 5 min followed by 48°C for 30 min. The hybridization product was stained by streptavidin-R-phycoerythrin (75  $\mu$ L) and incubated at 48°C for 15 min. Finally, the products were analyzed by a Luminex 200 bioanalyzer. The human  $\beta$ -actin gene was used as an internal control and assessed for each specimen to ensure that each tube contained enough cellular components for the HPV genotyping. The positive and negative controls in the reaction were HPV-16/18 genotype plasmid and ultrapure water, respectively.

### Statistical Analysis

Excel 2010 and SPSS version 22.0 (IBM Corp, Armonk, NY, USA) were used to collate and analyze the data, respectively. Figures were drawn with GraphPad Prism 6.02. We calculated the overall and specific genotype prevalences of HPV infection. A binomial 95% confidence interval (CI) was also estimated for calculation of the prevalence of each type of HPV. The distribution of categorical variables in different groups was evaluated by using the chi-squared test. Also, the positive infection rate of studied participants with different clinical characteristics was also calculated. The patients were stratified by age:  $\leq 24$ , 25-34, 35-44, 45-54, and  $\geq 55$  years. The gamma value and linear-by-linear association test were used to evaluate the trend changes in HPV infection rates by year and age. A  $P$  value  $< 0.05$  was considered statistically significant.

## Results

### Genital HPV Prevalence and Trends

In our study, 804 of the 1227 patients examined were infected with at least 1 genotype of HPV; thus, the prevalence of HPV infection was 65.5%. Specifically, the rate of HPV infection in patients with only 1 genotype was 39.69% (487/1227),

and the percentage of patients with multiple HPV infections ( $\geq 2$  genotypes) was 25.84% (317/1227) (Table 1, Figure 1). Further analysis indicated that 15.57% (191/1227) of patients had double infections, 5.79% (71/1227) had triple infections, and 3.02% (37/1227) had quadruple infections. The detection of single high-risk types and low-risk types accounted for 5.46% (67/1227) and 33.33% (409/1227), respectively. Among the mixed risk types, the detection rate of patients with 2 or more HR-HPV genotypes was 2.44% (30/1227), the multiple LR-HPV genotypes detection rate was 4.16% (51/1227), and 20.13% (247/1227) of patients had a mixture of HPV infection containing HR-HPV and LR-HPV genotypes (Table 1). In terms of the linear-by-linear association test, no significant difference was found in the rates of HPV infection from 2015 to 2019 ( $P > 0.05$ ) (Table 2, Figure 1). Interestingly, the prevalence of HR-HPV genotypes had a statistically significant decreasing trend from 2015 to 2019 ( $P = 0.001$ ), while no significant difference was observed in the detection rates of LR-HPV genotypes over this period ( $P > 0.05$ ) (Table 2).

### Distribution and Genotypes of Male HPV Infection

Overall, the rate of any HPV genotype infection was 65.5% (95% CI, 62.9-68.2%), ranging from 60.9% to 70.1%. Among the HPV-positive patients, the rate of HR-HPV infection was 28.0% (95% CI, 25.5-30.5%), and the LR-HPV genotype infection rate was 57.6% (95% CI, 54.9-60.4%). The 5 HR-HPV types with the highest detection rate among patients in the STD clinic were HPV-16 (5.6%), HPV-59 (4.0%), HPV-52 (3.4%), HPV-51 (3.3%), and HPV-56 (3.1%) (Figure 2). In addition, the 3 most frequently observed LR-HPV types were HPV-6 (32.0%), HPV-11 (23.2%), and HPV-43 (4.3%) (Table 2, Figure 2).

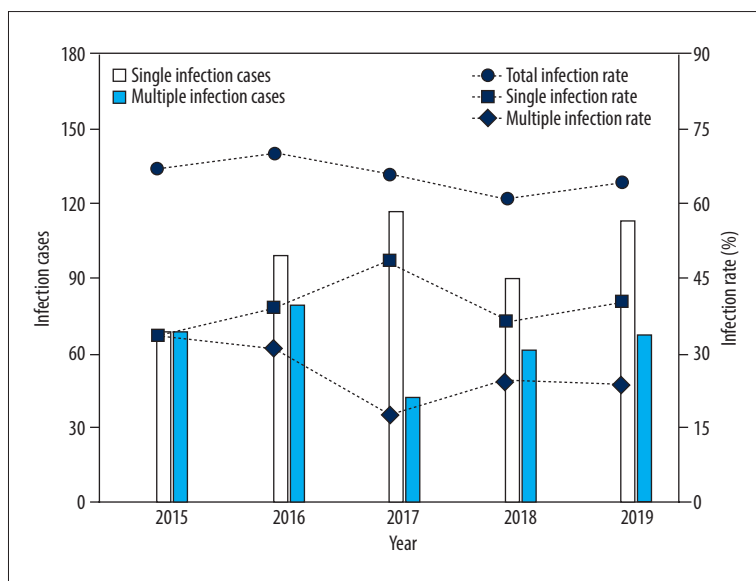
### HPV Genotype Infection in Different Age Groups

The HPV infection rates of age groups  $\leq 24$ , 25-34, 35-44, 45-54, and  $\geq 55$  years were 70.75% (116/164), 63.25% (315/498), 58.73% (148/252), 70.45% (93/132), and 72.93% (132/181), respectively. Figure 3 shows that the infection rate of the  $\leq 24$  years group exhibited a peak, with an age-specific prevalence of total HPV infection at  $\leq 24$  years old. Then, the infection rate sharply dropped with increasing age and finally surged again in patients over 45 years old. The pattern of the age-specific LR-HPV infection rate was similar to that of any type of infection and showed an increasing trend with age ( $P < 0.05$ ). However, HR-HPV infection exhibited a relatively flat curve ( $P > 0.05$ ) (Figure 3). There were significant differences in the detection rate of the single LR-HPV type among the classified age groups (HPV-6,  $P < 0.05$ ) and the 4 HR-HPV types (HPV-16, -52, -53, and -59,  $P < 0.05$ ) (Table 3). The prevalence of HPV infection exhibited a downward trend in the age group 25-34 years and an upward trend in the age group  $\geq 55$  years over the investigated 5 years (Table 4).

**Table 1.** Detection of HPV genotypes of male patients in a sexually transmitted disease clinic from 2015 to 2019.

Number of infection types	Positive cases	2015 (n=203)	2016 (n=254)	2017 (n=241)	2018 (n=248)	2019 (n=281)	$\chi^2$	P	gamma value
1	487 (39.69%)	68 (33.50%)	99 (38.98%)	117 (48.55%)	90 (36.29%)	113 (40.21%)	0.795	0.373	0.036
2	191 (15.57%)	39 (19.21%)	45 (17.72%)	25 (10.37%)	39 (15.73%)	43 (15.30%)	1.432	0.231	-0.065
3	71 (5.79%)	17 (8.37%)	18 (7.09%)	11 (4.56%)	14 (5.65%)	11 (3.91%)	4.614	0.032	-0.185
4	37 (3.02%)	10 (4.93%)	11 (4.33%)	4 (1.66%)	5 (2.02%)	7 (2.49%)	3.855	0.05	-0.228
$\geq 2$	317 (25.84%)	68 (33.50%)	79 (31.10%)	42 (17.43%)	61 (24.60%)	67 (23.84%)	7.439	0.006	-0.124
Single HR	67 (5.46%)	18 (8.87%)	3 (1.18%)	16 (6.64%)	19 (7.66%)	11 (3.91%)	0.307	0.580	-0.047
Single LR	409 (33.33%)	50 (24.63%)	86 (33.86%)	101 (41.91%)	71 (28.63%)	101 (35.94%)	2.544	0.111	0.067
HR+HR	30 (2.44%)	8 (3.94%)	13 (5.12%)	2 (0.83%)	5 (2.02%)	2 (0.71%)	9.698	0.002	-0.405
HR+LR	247 (20.13%)	49 (24.14%)	64 (25.20%)	36 (14.94%)	46 (18.55%)	52 (18.51%)	4.573	0.032	-0.107
LR+LR	51 (4.16%)	11 (5.42%)	12 (4.72%)	4 (1.66%)	10 (4.03%)	14 (4.98%)	0.052	0.820	-0.020

HR – high risk; LR – low risk.



**Figure 1.** Changes in the infection cases and infection rates of human papillomavirus (HPV) in male patients who visited our sexually transmitted disease (STD) clinic over 2015 to 2019.

**Coverage of the HPV Infection by Vaccines**

In this study, we detected a total of 1336 HPV genotype numbers. Among the 1336 HPV genotypes, 480 (35.9%) were HR-HPV and 856 (64.1%) were LR-HPV. The total number of detected genotypes targeted by 2v, 4v, and 9v vaccines were 99 (7.4%), 777 (58.2%), and 875 (65.5%), respectively. All the

detected 9v, non-9v HR-HPV, and non-9v LR-HPV genotypes and rates are shown in **Figure 4**. The prevalence by HPV type among patients by age group is shown in **Table 5**.

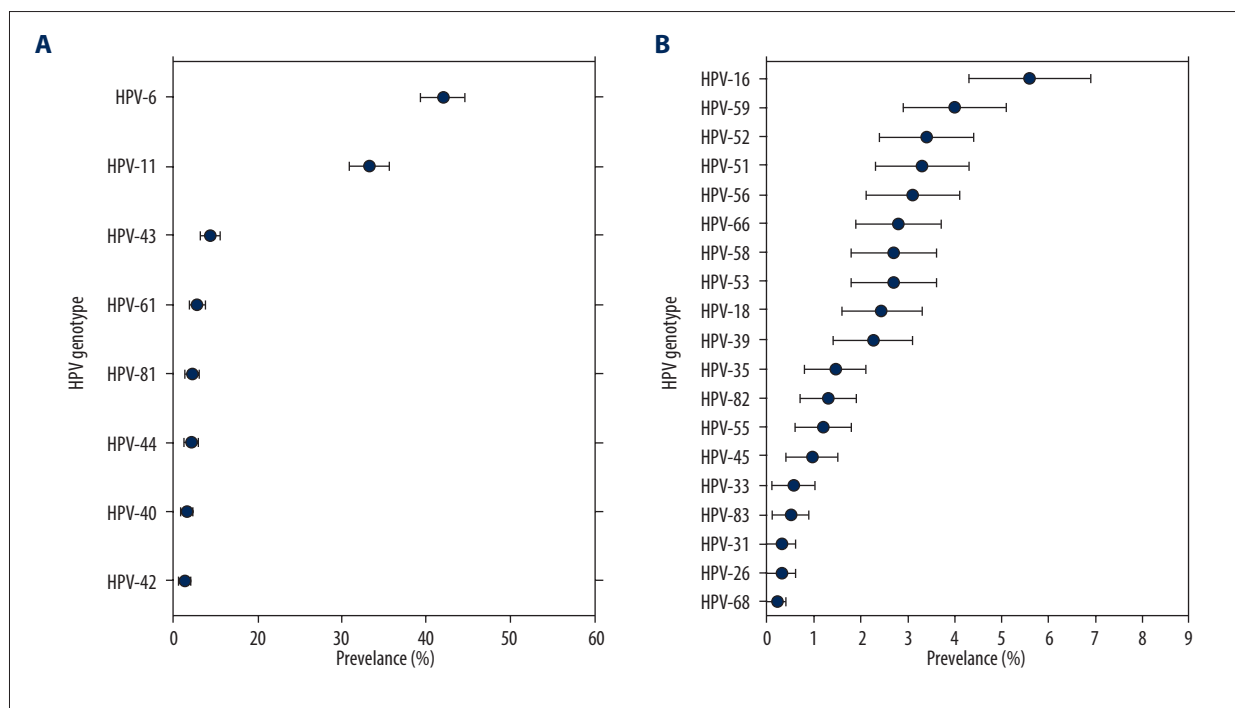
**Table 2.** Distribution of infection in patients from sexually transmitted disease clinic with 27 genotypes of human papillomavirus from 2015 to 2019.

HPV genotype	Positive cases	Percentage for whole sample (%)	95% CI for whole sample (%)	2015 (n=203)	2016 (n=254)	2017 (n=241)	2018 (n=248)	2019 (n=281)	$\chi^2$	P	gamma value
Any HPV	804	65.5	(62.9-68.2)	136 (67.0)	178 (70.1)	159 (66.0)	151 (60.9)	180 (64.1)	2.542	0.111	-0.068
Low-risk	707	57.6	(54.9-60.4)	110 (54.2)	162 (63.8)	141 (58.5)	127 (51.2)	167 (59.4)	0.093	0.760	-0.013
HPV-6	393	32.0	(29.4-34.6)	61 (30.0)	97 (38.2)	77 (32.0)	55 (22.2)	103 (36.7)	0.069	0.792	-0.01
HPV-11	285	23.2	(20.9-25.6)	41 (20.2)	57 (22.4)	54 (22.4)	69 (27.8)	64 (22.8)	1.242	0.265	0.052
HPV-40	20	1.6	(0.9-2.3)	5 (2.5)	8 (3.1)	1 (0.4)	4 (1.6)	2 (0.7)	3.989	0.046	-0.318
HPV-42	17	1.4	(0.7-2.0)	4 (2.0)	4 (1.6)	3 (1.2)	2 (0.8)	4 (1.4)	0.504	0.478	-0.119
HPV-43	53	4.3	(3.2-5.5)	14 (6.9)	12 (4.7)	7 (2.9)	9 (3.6)	11 (3.9)	2.394	0.122	-0.150
HPV-44	26	2.1	(1.3-2.9)	6 (3.0)	5 (2.0)	1 (0.4)	5 (2.0)	9 (3.2)	0.158	0.691	0.062
HPV-61	34	2.8	(1.9-3.7)	7 (3.4)	11 (4.3)	6 (2.5)	4 (1.6)	6 (2.1)	2.653	0.103	-0.200
HPV-81	28	2.3	(1.4-3.1)	6 (3.0)	5 (2.0)	4 (1.7)	6 (2.4)	7 (2.5)	0.003	0.954	-0.005
High-risk	344	28.0	(25.5-30.5)	75 (36.9)	80 (31.5)	54 (22.4)	70 (28.2)	65 (23.1)	10.626	0.001	-0.145
HPV-16	69	5.6	(4.3-6.9)	13 (6.4)	19 (7.5)	12 (5.0)	11 (4.4)	14 (5.0)	1.621	0.203	-0.111
HPV-18	30	2.4	(1.6-3.3)	10 (4.9)	6 (2.4)	3 (1.2)	6 (2.4)	5 (1.8)	3.234	0.072	-0.229
HPV-26	4	0.3	(0.0-0.6)	0 (0.0)	3 (1.2)	0 (0.0)	1 (0.4)	0 (0.0)	0.787	0.375	-0.330
HPV-31	4	0.3	(0.0-0.6)	1 (0.5)	0 (0.0)	0 (0.0)	2 (0.8)	1 (0.4)	0.290	0.590	0.193
HPV-33	7	0.6	(0.1-1.0)	1(0.5)	3 (1.2)	2 (0.8)	1 (0.4)	0 (0.0)	1.716	0.190	-0.357
HPV-35	18	1.5	(0.8-2.1)	3(1.5)	4 (1.6)	3 (1.2)	3 (1.2)	5 (1.8)	0.018	0.893	0.025
HPV-39	28	2.3	(1.4-3.1)	5 (2.5)	8 (3.1)	3 (1.2)	7 (2.8)	5 (1.8)	0.362	0.547	-0.083
HPV-45	12	1.0	(0.4-1.5)	4 (2.0)	1 (0.4)	5 (2.1)	1 (0.4)	1 (0.4)	2.377	0.123	-0.310
HPV-51	40	3.3	(2.3-4.3)	14 (6.9)	8 (3.1)	3 (1.2)	10 (4.0)	5 (1.8)	5.714	0.017	-0.265
HPV-52	42	3.4	(2.4-4.4)	10 (4.9)	7 (2.8)	9 (3.7)	5 (2.0)	11 (3.9)	0.329	0.566	-0.059
HPV-53	33	2.7	(1.8-3.6)	8 (3.9)	7 (2.8)	3 (1.2)	6 (2.4)	9 (3.2)	0.145	0.703	-0.043

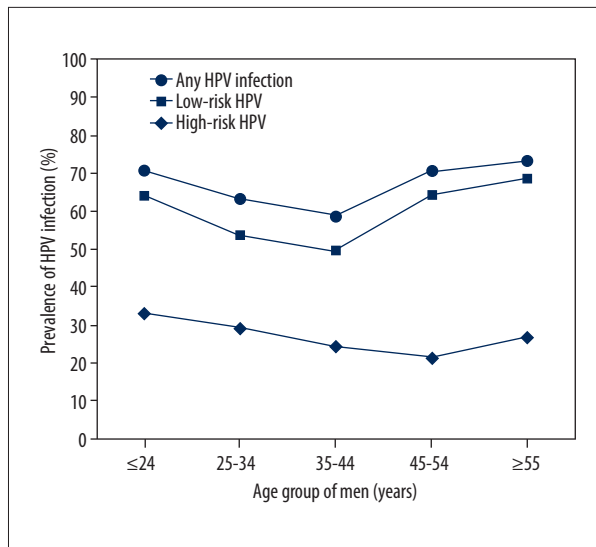
**Table 2 continued.** Distribution of infection in patients from sexually transmitted disease clinic with 27 genotypes of human papillomavirus from 2015 to 2019.

HPV genotype	Positive cases	Percentage for whole sample (%)	95% CI for whole sample (%)	2015 (n=203)	2016 (n=254)	2017 (n=241)	2018 (n=248)	2019 (n=281)	$\chi^2$	P	gamma value
HPV-55	15	1.2	(0.6-1.8)	4 (2.0)	2 (0.8)	3 (1.2)	4 (1.6)	2 (0.7)	0.503	0.478	-0.129
HPV-56	38	3.1	(2.1-4.1)	8 (3.9)	6 (2.4)	6 (2.5)	10 (4.0)	8 (2.8)	0.006	0.940	-0.008
HPV-58	33	2.7	(1.8-3.6)	5 (2.5)	10 (3.9)	8 (3.3)	6 (2.4)	4 (1.4)	1.589	0.208	-0.161
HPV-59	49	4.0	(2.9-5.1)	10 (4.9)	15 (5.9)	9 (3.7)	9 (3.6)	6 (2.1)	4.304	0.038	-0.215
HPV-66	34	2.8	(1.9-3.7)	5 (2.5)	11 (4.3)	3 (1.2)	6 (2.4)	9 (3.2)	0.020	0.886	0.017
HPV-68	2	0.2	(0.0-0.4)	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	1 (0.4)	0.145	0.704	0.198
HPV-82	16	1.3	(0.7-1.9)	2 (1.0)	3 (1.2)	2 (0.8)	5 (2.0)	4 (1.4)	0.525	0.469	0.128
HPV-83	6	0.5	(0.1-0.9)	2 (1.0)	3 (1.2)	0 (0.0)	1 (0.4)	0 (0.0)	3.848	0.050	-0.562

HPV – human papillomavirus.



**Figure 2.** Type-specific prevalence of HPV among men in sexually transmitted disease (STD) clinic, 2015-2019. (A) LR-HPV types; (B) HR-HPV types. Vertical bars represent 95% confidence intervals.



**Figure 3.** Prevalence of HR, low-risk(LR) and any HPV infection in different age groups.

### Relationship of HPV Infection and Clinical Symptoms

By analyzing the patients' files, we found that the clinical manifestations of HPV infection included penis-genital warts, anogenital warts, common warts, urethritis, rash, and dermatitis. Studied patients were divided into wart, physical examination, and "other" groups. The HPV infection rate of the wart group was 74.4% (360/484). More specifically, the positive rates of HPV infection in patients diagnosed with penis-genital warts, anogenital warts, and common warts were 78.9% (195/247), 81.0% (111/137), and 54.0% (54/100), respectively. It is worth noting that the prevalence of HPV infection in the physical examination group was 63.6% (378/594). The HPV detection rate of the "other" group, including urethritis (28.6%), rash (46.1%), and dermatitis (42.1%), was 40.4% (40/99) (**Table 6**).

**Table 3.** Changes of type-specific genital human papillomavirus detection across different age groups.

HPV type	≤24 years (n=164)	25-34 years (n=498)	35-44 years (n=252)	45-54 years (n=132)	≥55 years (n=181)	$\chi^2$	P	gamma value
Any HPV	116 (70.73%)	315 (63.25%)	148 (58.73%)	93 (70.45%)	132 (72.93%)	1.748	0.186	0.033
Low-risk	105 (64.02%)	267 (53.61%)	125 (49.60%)	85 (64.39%)	124 (68.51%)	4.711	0.030	0.062
HPV-6	56 (34.15%)	137 (27.51%)	70 (27.78%)	60 (45.45%)	71 (39.23%)	8.481	0.004	0.114
HPV-11	53 (32.32%)	112 (22.49%)	45 (17.86%)	22 (16.67%)	52 (28.72%)	0.672	0.412	-0.075
HPV-40	3 (1.83%)	10 (2.01%)	3 (1.19%)	3 (2.27%)	1 (0.55%)	1.014	0.314	-0.166
HPV-42	1 (0.61%)	7 (1.41%)	3 (1.19%)	3 (2.27%)	2 (1.10%)	0.219	0.640	0.103
HPV-43	6 (3.66%)	26 (5.22%)	6 (2.38%)	5 (3.79%)	10 (5.52%)	0.023	0.880	-0.013
HPV-44	5 (3.05%)	9 (1.81%)	1 (0.40%)	3 (2.27%)	8 (4.42%)	1.242	0.265	0.083
HPV-61	4 (2.44%)	17 (3.41%)	7 (2.78%)	3 (2.27%)	3 (1.66%)	0.893	0.345	-0.113
HPV-81	3 (1.83%)	13 (2.61%)	3 (1.19%)	5 (3.79%)	4 (2.21%)	0.058	0.810	0.020
High-risk	55 (33.54%)	147 (29.52%)	61 (24.21%)	29 (21.97%)	49 (27.07%)	3.692	0.055	-0.106
HPV-16	16 (9.76%)	35 (7.03%)	9 (3.57%)	4 (3.03%)	5 (2.76%)	11.555	0.001	-0.342
HPV-18	7 (4.27%)	13 (2.61%)	6 (2.38%)	1 (0.76%)	3 (1.66%)	3.080	0.079	-0.254
HPV-26	0 (0.00%)	3 (0.60%)	1 (0.40%)	0 (0.00%)	0 (0.00%)	0.589	0.443	-0.271

**Table 3 continued.** Changes of type-specific genital human papillomavirus detection across different age groups.

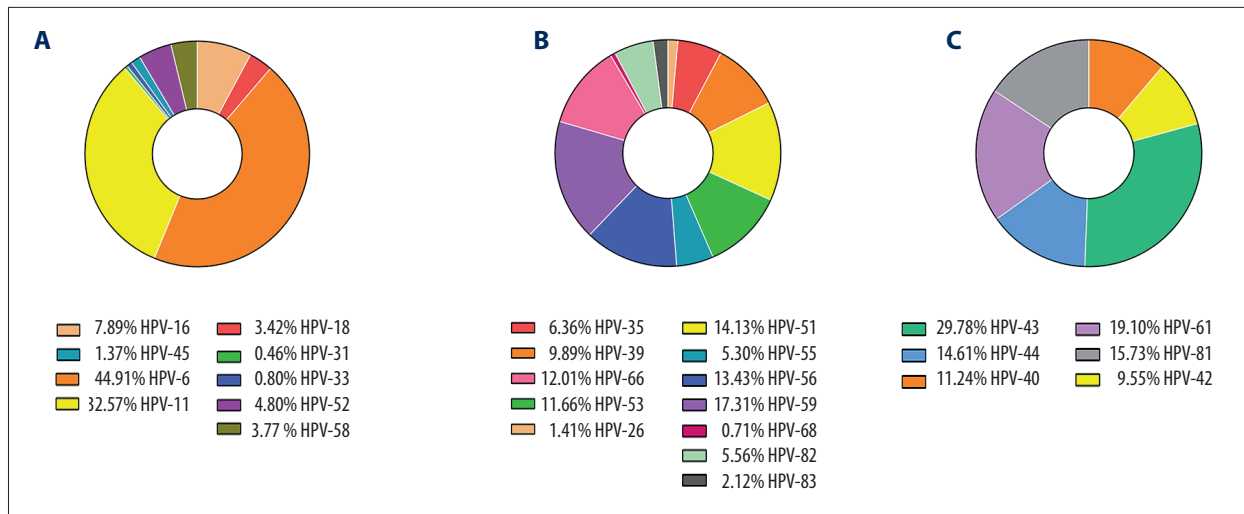
HPV type	≤24 years (n=164)	25-34 years (n=498)	35-44 years (n=252)	45-54 years (n=132)	≥55 years (n=181)	χ <sup>2</sup>	P	gamma value
HPV-31	1 (0.61%)	3 (0.60%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	2.458	0.117	-0.699
HPV-33	1 (0.61%)	2 (0.40%)	2 (0.79%)	0 (0.00%)	2 (1.10%)	0.329	0.566	0.142
HPV-35	2 (1.22%)	5 (1.00%)	4 (1.59%)	1 (0.76%)	6 (3.31%)	2.833	0.092	0.254
HPV-39	7 (4.27%)	8 (1.61%)	3 (1.19%)	3 (2.27%)	7 (3.87%)	0.155	0.694	-0.003
HPV-45	2 (1.22%)	5 (1.00%)	1 (0.40%)	2 (1.52%)	2 (1.10%)	0.003	0.954	-0.021
HPV-51	6 (3.66%)	17 (3.41%)	8 (3.17%)	4 (3.03%)	5 (2.76%)	0.288	0.592	-0.064
HPV-52	9 (5.49%)	20 (4.02%)	6 (2.38%)	5 (3.79%)	2 (1.10%)	4.896	0.027	-0.268
HPV-53	4 (2.44%)	8 (1.61%)	8 (3.17%)	2 (1.52%)	11 (6.08%)	5.698	0.017	0.270
HPV-55	0 (0.00%)	8 (1.61%)	2 (0.79%)	2 (1.52%)	3 (1.66%)	0.710	0.399	0.161
HPV-56	6 (3.66%)	20 (4.02%)	6 (2.38%)	1 (0.76%)	5 (2.76%)	1.992	0.158	-0.197
HPV-58	5 (3.05%)	15 (3.01%)	6 (2.38%)	4 (3.03%)	3 (1.66%)	0.733	0.392	-0.110
HPV-59	12 (7.32%)	21 (4.22%)	7 (2.78%)	4 (3.03%)	5 (2.76%)	4.272	0.039	-0.247
HPV-66	11 (6.71%)	12 (2.41%)	5 (1.98%)	1 (0.76%)	5 (2.76%)	3.678	0.055	-0.286
HPV-68	0 (0.00%)	1 (0.20%)	0 (0.00%)	0 (0.00%)	1 (0.55%)	0.759	0.383	0.364
HPV-82	5 (3.05%)	5 (1.00%)	2 (0.79%)	2 (1.52%)	2 (1.10%)	0.882	0.348	-0.204
HPV-83	0 (0.00%)	3 (0.60%)	1 (0.40%)	2 (1.52%)	0 (0.00%)	0.042	0.838	0.115

HPV – human papillomavirus.

**Table 4.** Changes in the rate of male human papillomavirus infection in sexually transmitted disease clinic in different age groups from 2015 to 2019.

Age (years)	2015 (n=203)	2016 (n=254)	2017 (n=241)	2018 (n=248)	2019 (n=281)	χ <sup>2</sup>	P	gamma value
≤24	14 (6.90%)	32 (12.60%)	16 (6.64%)	15 (6.05%)	39 (13.88%)	1.708	0.191	0.093
25-34	63 (31.03%)	80 (31.50%)	55 (22.82%)	54 (21.77%)	63 (22.42%)	9.006	0.003	-0.138
35-44	22 (10.84%)	30 (11.81%)	39 (16.18%)	31 (12.50%)	26 (9.25%)	0.322	0.570	-0.038
45-54	20 (9.85%)	14 (5.51%)	23 (9.54%)	21 (8.47%)	15 (5.34%)	1.217	0.270	-0.084
≥55	17 (8.37%)	22 (8.66%)	26 (10.79%)	30 (12.10%)	37 (13.17%)	4.367	0.037	0.137





**Figure 4.** The detection rates among 9v HPV genotypes (A), non-9v HR-HPV genotypes (B) and non-9v LR-HPV genotypes (C).

**Table 5.** Prevalence by human papillomavirus type in male patients by age groups over 5 years.

Age (years)	Men, No.	2vHPV*	4vHPV**	9vHPV***
Overall	1227	94 (7.66%)	690 (56.23%)	726 (59.17%)
≤24	164	20 (12.20%)	105 (64.02%)	108 (65.85%)
25-34	498	46 (9.24%)	260 (52.21%)	266 (53.41%)
35-44	252	15 (5.95%)	122 (48.41%)	125 (49.60%)
45-54	132	5 (3.79%)	80 (60.61%)	104 (78.79%)
≥55	181	8 (4.42%)	123 (67.96%)	123 (67.96%)

\* 2vHPV – includes HPV-16 and -18; \*\* 4vHPV – includes HPV-6, -11, -16, and -18; \*\*\* 9vHPV – includes HPV-6, -11, -16, -18, -31, -33, -45, -52, and -58.

**Table 6.** Detection rates of human papillomavirus in male patients with different diagnosis types.

Diagnosis	Diagnostic types	Cases	Positive cases	Rates (%)	Total positive rates (%)
Wart	Penis-genital wart	247	195	78.9	74.4
	Anogenital wart	137	111	81.0	
	Common wart	100	54	54.0	
Physical examination		594	378	63.6	63.6
Other	Urethritis	28	8	28.6	40.4
	Rash	52	24	46.1	
	Dermatitis	19	8	42.1	

## Discussion

There are limited studies on HPV distribution among men from the Asia-Pacific region, with even fewer reports from China. To better control and reduce the prevalence of HPV infection, more comprehensive information on the distribution of HPV types and the effectiveness of vaccines in a targeted male group is needed in China. This study provides supplementary

information on the distribution of genital HPV infection in Chinese male patients by year, age group, and HR-HPV and LR-HPV genotypes.

HPV is associated with a variety of diseases and is commonly detected in the oral cavity, urethra, anus, penis, and certain tumor tissues [29]. It has been reported that the average anal HPV positivity rate of any genotype among men who have

sex with men in China is 62.1% [30]. The HPV infection rate of male outpatients with urethral or penile sampling sites in the Qingyuan area of Guangzhou is 54.3% [31]. In the present clinic-based study, we investigated the genotyping of the urethral epithelium, anus, or penile epidermis from 1227 male patients in the Putuo district, and the overall HPV infection rate was 65.5%. Our study was conducted in Shanghai, a large economically developed city, with a population of nearly 20 million and location in eastern China. We observed more than half of the men who visited our dermatology and STD clinic had at least 1 HPV genotype infection. The detection rate of genital HPV infection in our study population was higher than that of previous studies in Henan province (17.5%, from the general population) [32] and Beijing (50.9%, from an STD clinic) [12]. The different geographical areas of China, study cohorts (study population from clinic or general), and HPV testing methods might account for the observed heterogeneity. In addition, in one report, the prevalence of HPV among men varied greatly in different countries, ranging from 12.9% (Spain) to 80% (Brazil) [33]. However, Sudenga et al indicated that the infection rates of any genital HPV type in men from Brazil, Mexico, and the United States were 60.2%, 49.2%, and 46.9%, respectively [34]. Recently, Matsuzawa et al revealed that the percentage of any HPV infection among Japanese men who visited the urological clinics is 24.8% [35]. The rate was much lower than our results because our study population of STD clinic outpatients may have higher exposure associated with high-risk sexual behaviors.

In our study, a total of 344 (28.0%) cases were high-risk infection, and 707 (57.6%) cases were low-risk infection. Single infections are the most common manifestation of LR-HPV infections, and LR-HPV infections are more common than HR-HPV infections. The most frequently observed LR-HPV types are HPV-6 and HPV-11. They are the predominant types that can cause condyloma acuminata in all countries [36,37]. In the present study, HPV types 16, 59, 52, and 51 were the top 4 HR-HPV types reported, while results from another study conducted in southern China showed that HPV types 16, 6, 52, and 58 are the most frequent genotypes among male homosexual participants [23]. The dominant types found in the cervical region of Chinese women are HPV types 16, 18, 52, and 58 [38]. HPV-16 and HPV-52 are the most frequently encountered types in men and women, which is consistent with our observation. Meanwhile, it was worth noting that HPV-59 and HPV-51 were also the most commonly observed HR-HPV types in the present study. Since they are still not covered in the latest 9v HPV vaccine, these 2 high-risk types should attract the attention of researchers who are developing HPV vaccines. The 9v HPV vaccine covers nearly 65.5% of the detected genotypes in this study, which means that the vaccine may be effective in preventing HPV infection in Chinese men. Men infected with HPV are usually unfamiliar with the disease and

transmit it to their sexual partners, which would increase the risk of HPV-associated diseases, including tumors. Therefore, regular pathological examination, follow-up, and strengthened treatment of infected patients should be recommended.

Different age groups can exhibit different rates and main types of HPV infection. Several studies found that the age-specific rate of any type of infections among women formed a quasi-U-shaped curve with 2 peaks in groups aged  $\leq 24$  years and  $\geq 55$  years [39,40]. One theory is that young women may have less sensitive immune systems and are more likely to have frequent sexual activity. The second peak in the older group may be due to the decline in the immune function of women in this age group and virus clearance ability, leading to an increase in HPV infection. In the present study, the HPV infection rates of the groups aged  $\leq 24$  years and  $\geq 55$  years were 70.75% and 72.93%, respectively, which were higher than those of other age groups. Therefore, the relationship between age and HPV infection rate in men in our study was similar to the pattern in women found in other studies. The prevalence of HPV in the age group  $\geq 55$  years showed an upward trend from 2015 to 2019. Considering that older men with persistent HPV infection are at high risk of anal and penile cancer, and a certain percentage of men do develop HPV-associated diseases, it is necessary and beneficial to perform comprehensive and regular screening for older men with HPV infection [34]. In addition, our results also suggested that young men ( $\leq 24$  years) were more susceptible to HPV infection.

Penis-genital and anogenital warts are also called condyloma acuminata. Similar to a previous study [31], the HPV detection rate was highest in patients with the clinical manifestation of condyloma acuminata in the present study. The main objective of genital treatment is the destruction and removal of the lesions, as well as local modification. Currently, there is no specific and established therapy to eradicate the HPV virus, but vaccines can decrease the incidence of certain HPV infections. Treatment strategies for condyloma acuminata have been summarized by Fathi et al, including combination and monotherapies [41]. It has been reported that imiquimod 3.75% medicated cream has been approved for treating anogenital warts in patients aged  $\geq 12$  years [42]. We also noticed that the positive rate of HPV infection in the population without obvious symptoms reached 63.6%. Vaccination and cervical screening have been used for the primary prevention of HPV infection in women [43]. However, whether a screening method would be also applicable to men needs further research and discussion. On the other hand, strategies and policies toward the mitigation of HPV infection, such as vaccination, should target both females and males.

There are some limitations to keep in mind when interpreting these study results. First, the results may not represent

the general male population because the study included only those patients who attended the dermatology and STD clinic and who were tested for HPV. Thus, the reported HPV infection rate might be higher than that of the general population and cannot be representative of all males in Shanghai. Second, this was a single-center pilot study including data only from one hospital. Third, the inability to obtain complete information about the sociodemographic and behavioral characteristics of recruited men and the lack of such information make it difficult to assess the effect of specific patient characteristics on HPV infection.

## Conclusions

In conclusion, this study investigated the status of genital HPV infection among men from an STD clinic in Shanghai, China, from 2015 to 2019. Results revealed the percentage of male HPV infection was high. Moreover, it also showed that HPV types 16, 59, 52, 51, and 56 were the dominant HR-HPV types,

and the most frequently observed LR-HPV genotypes were HPV types 6, 11, and 43. Since June 2016, vaccinations have been recommended for females aged 9 to 45 years in mainland China [44]. Molecular epidemiological studies and targeted HPV vaccination among high-risk Chinese males are urgently needed.

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## Conflicts of Interest

None.

## Declaration of Figures Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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