Analysis of epidemiological trends in human papillomavirus infection among gynaecological outpatients in Hangzhou, China, 2011–2015

Lili Qian¹, Yu Zhang¹, Dawei Cui², Bin Lou², Yimin Chen¹, Ying Yu¹, Yonglin Liu¹ and Yu Chen²*

Abstract

Background: HPV infection is the major pathogenic factor underlying cervical cancer and precancerous lesions. The cervical HPV infection rates in gynaecological outpatients from Hangzhou, China, were studied in the period from January 2011 to December 2015.

Methods: Exfoliated cervical cells were harvested from gynaecological outpatients in Hangzhou from January 2011 to December 2015. Twenty-one HPV subtypes were detected using flow-through hybridization. The HPV infection rates in various disease groups were compared using the Chi-square test. The infection rates of different HPV subtypes in different calendar years and in different age groups were analysed using the linear-by-linear association test and gamma value.

Results: A total of 43,804 patients were recruited, of whom 9752 (22.3%) were infected with HPV. The top five among the 21 HPV subtypes detected in terms of infection rates were HPV-16, −52, −58, −53 and −18. No significant differences (linear-by-linear association test) were found in the HPV infection rates when compared over the studied years (P > 0.05). However, the 15–24-year-old age group showed the highest HPV infection rate, and significant differences (linear-by-linear association test) were detected among the different age groups (P < 0.05). The HPV infection rates exhibited an upward trend in the 15–24-year-old and >24–34-year-old groups over the past five years. There were significant differences in the HPV infection rates among the disease groups (P < 0.05).

Conclusions: HPV-16, −52 and −58 were the major HPV infection subtypes in Hangzhou, China. The 15–24-year-old age group had a relatively high HPV infection rate with an upward trend over the past five years and thus represented a population susceptible to HPV infection.

Keywords: Human papillomavirus, Genotype, Flow-through hybridization, Epidemiology
Cancer (IARC, 2007), the top five HPV subtypes in terms of infection frequency are HPV-16, −53, −52, −18 and −39 in North America, HPV-16, −18, −31, −33 and −58 in Europe and HPV-16, −52, −58, −18 and −56 in Asia.

The latest research showed that HPV-52, −16 and −58 were the HPV subtypes with the highest infection frequencies in China [6]. Vaccines against four HPV subtypes (HPV-6, −11, −16 and −18) have been used in clinics in developed countries. In the present study, we analysed the cervical HPV infection rates in gynaecological outpatients from Hangzhou, China, over the past five years. Additionally, we performed an age-stratified analysis and an analysis by disease type. The results provide objective evidence for epidemiological studies of HPV infection and the application of HPV vaccines in the study region.

Materials and methods
Subjects
Clinical specimens were collected from 43,804 gynaecological outpatients at the First Affiliated Hospital of Zhejiang Chinese Medical University and the First Affiliated Hospital of the Medical School of Zhejiang University from January 2011 to December 2015. A woman was considered eligible to enter the study if she a) had current or past sexual activity, b) was not pregnant at the time of enrolment, c) had never been screened or treated for cervical cancer, d) had not undergone a total uterus or cervix resection, e) agreed to undergo an HPV test and f) agreed to participate in the present study. This study was conducted in accordance with the Declaration of Helsinki and a protocol approved by the First Affiliated Hospital of Zhejiang Chinese Medical University and the First Affiliated Hospital of the Medical School of Zhejiang University (Hangzhou, China). There were 5001 cases in 2011, 6410 cases in 2012, 7863 cases in 2013, 11,402 cases in 2014 and 13,128 cases in 2015. The recruited patients were 17–88 years old, with a median age of 44 years. In accordance with a prior overseas study, [7] we organized the subjects into the following five age groups: 15–24 years, >24–34 years, >34–44 years, >44–54 years and >54 years. Regarding the disease type, the subjects were assigned to the following five groups: cervical cancer, cervical intraepithelial neoplasia grade 1 (CIN1), CIN2, CIN3 and other diseases. The other diseases included gynaecological diseases other than cervical cancer and CIN, such as uterine fibroids, ovarian cysts, endometriosis, endometrial cancer and choriocarcinoma.

Methods
HPV sample collection
The opening of the cervix was exposed using a vaginal dilator. Excess secretions at the opening of the cervix were wiped away using a cotton swab. A cervical brush was inserted into the opening of the cervix and rotated clockwise 3–5 times to acquire exfoliated cervical cells. The cells were placed into a sample tube containing cell preservation solution, stored in a refrigerator at 4 °C and analysed within 3 days of collection.

HPV genotype testing
Samples that tested positive for β-globin were analysed by PCR amplification of HPV DNA. HPV-positive samples were confirmed by PCR with universal L1 primer MY09/11 and GP5/6 systems. DNA from HeLa and Caski cell lines was used as positive controls, and mixtures without sample DNA were used as negative controls. HPV genotypes were determined using an HPV GenoArray Test Kit (Hybribio, Chaozhou, China), according to the manufacturer’s instructions [8, 9]. Geno-Array is an L1 consensus primer-based PCR assay that is capable of amplifying 21 HPV genotypes, including six low-risk HPV subtypes including six low-risk HPV subtypes (HPV-6, −11, −42, −43, −44 and CP8304) and 15 high-risk HPV subtypes (HPV-16, −18, −31, −33, −35, −39, −45, −51, −52, −53, −56, −58, −59, −66 and −68) (Table 1).

The test was conducted in four steps as follows: (1) HPV DNA extraction, (2) PCR amplification, (3) flow-

| HPV subtype | Probe Sequence |
|-------------|----------------|
| CP8304      | 5’-gtgctctggacagctatcag-3’ |
| HPV-6       | 5’-gctgctctggacagctatcag-3’ |
| HPV-11      | 5’-gctgctctggacagctatcag-3’ |
| HPV-44      | 5’-gctgctctggacagctatcag-3’ |
| HPV-42      | 5’-gctgctctggacagctatcag-3’ |
| HPV-43      | 5’-gctgctctggacagctatcag-3’ |
| HPV-16      | 5’-gctgctctggacagctatcag-3’ |
| HPV-52      | 5’-gctgctctggacagctatcag-3’ |
| HPV-58      | 5’-gctgctctggacagctatcag-3’ |
| HPV-53      | 5’-gctgctctggacagctatcag-3’ |
| HPV-18      | 5’-gctgctctggacagctatcag-3’ |
| HPV-39      | 5’-gctgctctggacagctatcag-3’ |
| HPV-33      | 5’-gctgctctggacagctatcag-3’ |
| HPV-31      | 5’-gctgctctggacagctatcag-3’ |
| HPV-51      | 5’-gctgctctggacagctatcag-3’ |
| HPV-68      | 5’-gctgctctggacagctatcag-3’ |
| HPV-66      | 5’-gctgctctggacagctatcag-3’ |
| HPV-56      | 5’-gctgctctggacagctatcag-3’ |
| HPV-59      | 5’-gctgctctggacagctatcag-3’ |
| HPV-45      | 5’-gctgctctggacagctatcag-3’ |
| HPV-35      | 5’-gctgctctggacagctatcag-3’ |
through hybridization (a hybridized membrane coated with genotype-specific probes was placed into a hybridizer for rapid nucleic acid hybridization, and the amplification products were tested by reverse dot blotting with an enzyme label to yield a coloured reaction) and (4) result interpretation. Positive test results appeared as bluish violet dots. Samples that were positive for only one HPV subtype were defined as single HPV infections, and samples that were positive for more than one HPV subtype were defined as multiple HPV infections.

Statistical analysis
The data were analysed using SPSS 22.0 statistical software. The positive infection rate is expressed as a percentage. For multiple infection patients, the HPV-positive rate was calculated repeatedly for each genotype. The infection rates were compared based on disease group using the Chi-square test. The linear-by-linear association test and gamma value for trends were used to evaluate changes in HPV prevalence by calendar year and by age group. P-values were two-sided, and statistical significance was defined as \( P < 0.05 \).

Results
HPV infection rates and trends
In this study, 9752 of the 43,804 women examined were infected with HPV, resulting in an infection rate of 22.3%. Specifically, the rate of single infections was 17.1% (7496/43,804), and the rate of multiple infections was 5.2% (2256/43,804). Among the 21 HPV subtypes detected, the infection rate for the low-risk subtypes was 4.5% (1987/43,804), and the infection rate for the high-risk subtypes was 20.1% (8802/43,804). Regarding the infection rate, the top five HPV subtypes were HPV-16 (4.8%, 2108/43,804), HPV-52 (4.7%, 2056/43,804), HPV-58 (3.9%, 1712/43,804), HPV-53 (2.3%, 995/43,804) and HPV-18 (1.9%, 831/43,804); the top three low-risk HPV subtypes were HPV-8304 (1.9%, 822/43,804), HPV-6 (1.3%, 585/43,804) and HPV-11 (1.2%, 524/43,804). The HPV-positive rate in 2011 was 22.7% (1133/5001), with single infections in 16.5% (823/5001) and multiple infections in 6.2% (310/5001) of the subjects. The HPV-positive rate in 2012 was 21.6% (1383/6410), with single infections in 15.8% (1015/6410) and multiple infections in 5.7% (368/6410) of the subjects. The HPV-positive rate in 2013 was 22.2% (1745/7863), with single infections in 16.4% (1291/7863) and multiple infections in 5.8% (454/7863) of the subjects. The HPV-positive rate in 2014 was 22.6% (2575/11,402), with single infections in 16.2% (1846/11,402) and multiple infections in 6.4% (729/11,402) of the subjects. The HPV-positive rate in 2015 was 22.2% (2916/13,128), with single infections in 15.4% (2015/13,128) and multiple infections in 6.9% (901/13,128) of the subjects (Fig. 1). No significant differences (linear-by-linear association test) were found in the HPV infection rates based on the year \( (P > 0.05) \) (Table 2).

Infection of different age groups with the HPV subtypes
The HPV infection rates of the 15–24-, >24–34-, >34–44-, >44–54- and >54-year-old groups were 34.7% (324/933), 23.5% (2155/9159), 21.3% (2712/12,717), 20.6% (2932/14,245) and 24.1% (1629/6750), respectively. The highest infection rate appeared in the 15–24-year-old group, and
significant differences (linear-by-linear association test) were found among the age groups ($P < 0.05$). Additionally, significant differences (linear-by-linear association test) were found among the age groups in the infection rates for the 11 high-risk subtypes (HPV-18, −35, −51, −52, −53, −58, −59 and −68; $P < 0.05$) and the four low-risk subtypes (CP8304, HPV-6, −11 and −42; $P < 0.05$) (Table 3). The HPV infection rates exhibited an upward trend in the 15–24- and >24–34-year-old age groups over the past five years (Fig. 2) (Table 4).

**HPV infection in the different disease groups**

The HPV infection rates of the different disease groups were as follows: HPV-16 (46.7%, 415/889), HPV-58 (19.8%, 176/889), HPV-52 (17.5%, 155/889), HPV-18 (14.2%, 126/889) and HPV-53 (7.8%, 70/889) in the cervical cancer group; HPV-16 (44.6%, 128/287), HPV-52 (18.3%, 53/287), HPV-58 (17.1%, 49/287), HPV-33 (15.9%, 46/287) and HPV-53 (14.6%, 42/287) in the CIN3 group; HPV-16 (29.4%, 42/143), HPV-58 (23.0%, 33/143), HPV-52 (15.4%, 22/143), HPV-33 (14.1%, 20/143) and HPV-53 (11.9%, 17/143) in the CIN2 group; HPV-16 (24.9%, 243/978), HPV-58 (23.3%, 228/978), HPV-52 (22.0%, 215/978), HPV-33 (9.3%, 91/975) and HPV-53 (8.5%, 83/978) in the CIN1 group and HPV-52 (21.6%, 1611/7455), HPV-16 (17.2%, 1280/7455), HPV-58 (16.5%, 1226/7455), HPV-53 (10.5%, 783/7455) and HPV-8304 (10.0%, 745/7455) in the other disease group (Fig. 3).

**Discussion**

HPV is a group of DNA viruses that specifically infect human skin and the mucosal epithelium. HPV infection can cause abnormal proliferation of the skin and mucosal epithelial cells, leading to verrucous lesions and

**Table 2** Infection of gynaecological outpatients with 21 subtypes of human papillomavirus (HPV) in different years

| HPV subtype | Positive cases | 2011 ($n = 5001$) | 2012 ($n = 6410$) | 2013 ($n = 7863$) | 2014 ($n = 11,402$) | 2015 ($n = 13,128$) | $\chi^2$ | $P$ | gamma value |
|-------------|----------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------|-----|-------------|
| Low-risk    |                |                   |                   |                   |                   |                   |        |     |             |
| CP8304     | 822            | 112 (2.2%)        | 118 (1.8%)        | 114 (1.5%)        | 197 (1.7%)        | 281 (2.1%)        | 0.228  | 0.633 | 0.028       |
| HPV-6       | 585            | 45 (0.9%)         | 76 (1.2%)         | 140 (1.8%)        | 150 (1.3%)        | 174 (1.3%)        | 2.209  | 0.137 | 0.300       |
| HPV-11      | 524            | 42 (0.8%)         | 83 (1.3%)         | 83 (1.1%)         | 127 (1.1%)        | 189 (1.4%)        | 7.433  | 0.006 | -0.001      |
| HPV-44      | 127            | 4 (0.1%)          | 12 (0.2%)         | 19 (0.2%)         | 43 (0.4%)         | 49 (0.4%)         | 15.253 | <0.001| -0.248      |
| HPV-42      | 119            | 13 (0.3%)         | 37 (0.6%)         | 48 (0.6%)         | 10 (0.1%)         | 11 (0.1%)         | 36.303 | <0.001| 0.410       |
| HPV-43      | 35             | 2 (0.04%)         | 5 (0.08%)         | 4 (0.1%)          | 6 (0.1%)          | 18 (0.1%)         | 4.019  | 0.045 | -0.279      |
| High-risk   |                |                   |                   |                   |                   |                   |        |     |             |
| HPV-16      | 2108           | 257 (5.1%)        | 311 (4.9%)        | 453 (5.8%)        | 567 (5.0%)        | 520 (4.0%)        | 15.769 | <0.001| 0.073       |
| HPV-52      | 2056           | 248 (5.0%)        | 304 (4.7%)        | 328 (4.2%)        | 508 (4.5%)        | 668 (5.1%)        | 0.612  | 0.434 | -0.020      |
| HPV-58      | 1712           | 227 (4.5%)        | 265 (4.1%)        | 293 (3.7%)        | 454 (4.0%)        | 473 (3.6%)        | 7.423  | 0.006 | 0.046       |
| HPV-53      | 995            | 113 (2.3%)        | 129 (2.0%)        | 148 (1.9%)        | 272 (2.4%)        | 333 (2.5%)        | 5.674  | 0.017 | -0.062      |
| HPV-18      | 831            | 91 (1.8%)         | 92 (1.4%)         | 148 (1.9%)        | 291 (2.4%)        | 209 (1.6%)        | 0.685  | 0.408 | -0.007      |
| HPV-39      | 765            | 56 (1.1%)         | 74 (1.2%)         | 78 (1.0%)         | 288 (2.5%)        | 269 (2.1%)        | 52.680 | <0.001| -0.193      |
| HPV-33      | 733            | 86 (1.7%)         | 113 (1.8%)        | 150 (1.9%)        | 187 (1.6%)        | 197 (1.5%)        | 2.691  | 0.101 | 0.049       |
| HPV-31      | 611            | 71 (1.4%)         | 70 (1.1%)         | 141 (1.8%)        | 139 (1.2%)        | 190 (1.5%)        | 0.103  | 0.748 | -0.008      |
| HPV-51      | 476            | 11 (0.2%)         | 16 (0.3%)         | 32 (0.4%)         | 129 (1.1%)        | 288 (2.2%)        | 221.134 | <0.001| -0.554      |
| HPV-68      | 458            | 61 (1.2%)         | 75 (1.2%)         | 72 (0.9%)         | 97 (0.9%)         | 153 (1.2%)        | 0.315  | 0.575 | 0.005       |
| HPV-66      | 373            | 44 (0.9%)         | 51 (0.8%)         | 55 (0.7%)         | 98 (0.9%)         | 125 (1.0%)        | 1.168  | 0.280 | -0.049      |
| HPV-56      | 270            | 35 (0.7%)         | 14 (0.2%)         | 43 (0.6%)         | 75 (0.7%)         | 103 (0.8%)        | 8.898  | 0.003 | -0.147      |
| HPV-59      | 197            | 11 (0.2%)         | 32 (0.5%)         | 42 (0.5%)         | 46 (0.4%)         | 66 (0.5%)         | 2.259  | 0.133 | -0.068      |
| HPV-45      | 122            | 15 (0.3%)         | 17 (0.3%)         | 33 (0.4%)         | 20 (0.2%)         | 37 (0.3%)         | 0.666  | 0.415 | 0.053       |
| HPV-35      | 93             | 14 (0.3%)         | 16 (0.3%)         | 12 (0.2%)         | 25 (0.2%)         | 26 (0.2%)         | 0.865  | 0.352 | 0.059       |
Table 3 Infection of gynaecological outpatients with 21 subtypes of human papillomavirus (HPV) in different age groups

| HPV subtype | 15–24 (n = 933) | >24–34 (n = 9159) | >34–44 (n = 12,717) | >44–54 (n = 14,245) | >54 (n = 6750) | χ² | P | gamma value |
|-------------|-----------------|-------------------|---------------------|---------------------|---------------|-----|-----|-------------|
| Low-risk    |                 |                   |                     |                     |               |     |     |             |
| CP8304      | 35 (3.8%)       | 134 (1.5%)        | 208 (1.6%)          | 274 (2.0%)          | 173 (2.6%)    | 11.683 | <0.001 | 0.100 |
| HPV-6       | 57 (6.1%)       | 231 (2.5%)        | 137 (1.1%)          | 99 (0.7%)           | 64 (1.0%)     | 185.419 | <0.001 | -0.382 |
| HPV-11      | 62 (6.7%)       | 208 (2.3%)        | 109 (0.9%)          | 86 (0.6%)           | 61 (1.0%)     | 186.174 | <0.001 | -0.393 |
| HPV-42      | 1 (0.1%)        | 22 (0.2%)         | 33 (0.3%)           | 34 (0.2%)           | 29 (0.4%)     | 3.940 | <0.001 | 0.127 |
| HPV-43      | 1 (0.1%)        | 7 (0.1%)          | 5 (0.04%)           | 11 (0.1%)           | 12 (0.2%)     | 3.832 | <0.001 | 0.243 |
| HPV-44      | 3 (0.3%)        | 32 (0.4%)         | 28 (0.2%)           | 45 (0.3%)           | 19 (0.3%)     | 0.088 | <0.001 | -0.014 |
| High-risk   |                 |                   |                     |                     |               |     |     |             |
| HPV-16      | 74 (8.0%)       | 477 (5.2%)        | 547 (4.3%)          | 638 (4.5%)          | 381 (5.6%)    | 0.498 | 0.481 | -0.006 |
| HPV-18      | 31 (3.3%)       | 224 (2.5%)        | 217 (1.7%)          | 233 (1.6%)          | 132 (2.0%)    | 13.148 | <0.001 | -0.089 |
| HPV-31      | 20 (2.1%)       | 127 (1.4%)        | 170 (1.3%)          | 189 (1.3%)          | 105 (1.6%)    | 0.002 | 0.962 | 0.003 |
| HPV-33      | 34 (3.6%)       | 154 (1.7)         | 183 (1.4%)          | 221 (1.6%)          | 143 (2.1%)    | 0.025 | 0.874 | 0.015 |
| HPV-35      | 4 (0.4%)        | 27 (0.3%)         | 32 (0.3%)           | 26 (0.2%)           | 6 (0.1%)      | 10.705 | <0.001 | -0.248 |
| HPV-39      | 30 (3.2%)       | 173 (1.9%)        | 217 (1.7%)          | 232 (1.6%)          | 121 (1.8%)    | 3.529 | 0.060 | -0.045 |
| HPV-45      | 7 (0.8%)        | 29 (0.3%)         | 30 (0.2%)           | 43 (0.3%)           | 16 (0.2%)     | 1.817 | 0.178 | -0.072 |
| HPV-51      | 39 (4.2%)       | 140 (1.5%)        | 105 (0.8%)          | 127 (0.9%)          | 73 (1.1%)     | 32.258 | <0.001 | -0.166 |
| HPV-52      | 66 (7.1%)       | 459 (5.0%)        | 639 (5.0%)          | 603 (4.2%)          | 297 (4.4%)    | 15.420 | <0.001 | -0.065 |
| HPV-53      | 45 (4.8%)       | 183 (2.0%)        | 244 (1.9%)          | 317 (2.2%)          | 215 (3.2%)    | 7.704 | 0.006 | 0.076 |
| HPV-56      | 10 (1.1%)       | 68 (0.7%)         | 63 (0.5%)           | 81 (0.6%)           | 51 (0.8%)     | 0.286 | 0.593 | -0.017 |
| HPV-58      | 60 (6.4%)       | 320 (3.5%)        | 437 (3.4%)          | 531 (3.7%)          | 372 (5.5%)    | 17.682 | <0.001 | 0.082 |
| HPV-59      | 14 (1.5%)       | 53 (0.6%)         | 47 (0.4%)           | 52 (0.4%)           | 34 (0.5%)     | 6.424 | 0.011 | -0.113 |
| HPV-66      | 15 (1.6%)       | 97 (1.1%)         | 86 (0.7%)           | 94 (0.7%)           | 83 (1.2%)     | 0.254 | 0.615 | -0.014 |
| HPV-68      | 24 (2.6%)       | 116 (1.3%)        | 113 (0.9%)          | 127 (0.9%)          | 80 (1.2%)     | 5.572 | 0.018 | -0.070 |

Fig. 2 Changes in the rate of human papillomavirus infection in gynaecological outpatients in different age groups, 2011–2015
Changes in the rate of human papillomavirus infection in gynaecological outpatients in different age groups, 2011–2015

| Age (years) | 2011 (n = 5001) | 2012 (n = 6410) | 2013 (n = 7863) | 2014 (n = 11,402) | 2015 (n = 13,128) | \( \chi^2 \) | \( P \) | \( \gamma \) value |
|------------|----------------|----------------|----------------|------------------|------------------|----------------|---------|----------------|
| 15–24      | 5 (0.1)        | 20 (0.3)       | 36 (0.5)       | 83 (0.7)         | 180 (1.4)        | 111.4         | <0.001  | 0.464**       |
| >24–34     | 207 (4.1)      | 273 (4.3)      | 394 (5.0)      | 579 (5.1)        | 702 (5.4)        | 16.8          | <0.001  | 0.064*        |
| >34–44     | 332 (6.6)      | 389 (6.1)      | 482 (6.1)      | 723 (6.3)        | 786 (6.0)        | 1.159         | 0.282   | −0.015**      |
| >44–54     | 404 (8.1)      | 441 (6.9)      | 523 (6.7)      | 776 (6.8)        | 788 (6.0)        | 20.372        | <0.001  | −0.031**      |
| >54        | 185 (3.7)      | 260 (4.1)      | 310 (3.9)      | 414 (3.6)        | 460 (3.5)        | 2.587         | 0.108   | −0.032**      |

Linear-by-linear Association test and Gamma Value for trend to evaluate changes in HPV prevalence by calendar year and by age group. *<p < 0.05;**<p > 0.05,

Cervical cancer cases have long plagued the majority of women’s health care in China, particularly for rural women; every year, there are approximately 130,000 new cervical cancer cases [21]. The best choice for the

Single and multiple infections in human papillomavirus (HPV)-positive patients in different disease groups

| Disease Group | Cervical cancer\(a\) (n = 915) | CIN3\(b\) (n = 324) | CIN2\(c\) (n = 270) | CIN1\(d\) (n = 1924) | Other diseases\(e\) (n = 40,371) | \( \chi^2 \) | \( P \) |
|---------------|---------------------------------|---------------------|---------------------|----------------------|---------------------------------|----------------|---------|
| HPV-positive  | 889 (97.2)                      | 287 (88.6)          | 143 (52.7)          | 978 (50.8)           | 7455 (18.5)                     | 4557.239       | <0.001  |
| Single infections | 616 (69.3)                   | 203 (70.7)          | 109 (76.2)          | 764 (78.1)           | 5970 (80.1)                     | 63.613         | <0.001  |
| Multiple infections | 273 (30.7)                        | 84 (29.3)           | 34 (23.8)           | 214 (21.9)           | 1485 (19.9)                     |                |         |

Pairwise comparisons were performed using the Bonferroni method; \( \alpha \) was adjusted with \( \alpha \) considered significant. In comparing the rates of single and multiple infections, the following results were found: the difference was significant for \( a + d \) (\( \chi^2 = 18.820, P < 0.001 \)); the difference was significant for \( a + e \) (\( \chi^2 = 55.598, P < 0.001 \)); the difference was significant \( b + e \) (\( \chi^2 = 14.978, P < 0.001 \))

Changes in the rate of human papillomavirus infection ingynaecological outpatients in different age groups, 2011

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Pairwise comparisons were performed using the Bonferroni method; \( \alpha \) was adjusted with \( \alpha \) considered significant. In comparing the rates of single and multiple infections, the following results were found: the difference was significant for \( a + d \) (\( \chi^2 = 18.820, P < 0.001 \)); the difference was significant for \( a + e \) (\( \chi^2 = 55.598, P < 0.001 \)); the difference was significant \( b + e \) (\( \chi^2 = 14.978, P < 0.001 \))

Cervical cancer cases have long plagued the majority of women’s health care in China, particularly for rural women; every year, there are approximately 130,000 new cervical cancer cases [21]. The best choice for the
prevention of HPV infection and treatment of cervical cancer is preventive vaccination. The US Food and Drug Administration has approved two preventive vaccine products that mainly target HPV-6, -11, -16 and -18. Clinical data have shown that the specific effect is unclear despite the protective effect of the two vaccines against infection by other HPV subtypes [22–24]. Cervarix was recently approved in China mainly against HPV-16 and -18. In the current results, the top three HPV types were HPV-16, -52 and -58 in Hangzhou, which was similar to most previous surveys in China. [6] HPV-52 and -58 were more prevalent than the vaccine type HPV-18. We expect to provide objective evidence to enhance the hypothesis that second-generation HPV prophylactic vaccines including HPV-52 and -58 may offer higher protection for women in Hangzhou and other parts of China.

Age is an important factor associated with HPV infection. One point of view is that young women have frequent sex. Reports in the literature suggest that young women are more prone to have multiple partners [25]; additionally, their immune systems are susceptible to HPV infection because they are non-sensitized. Consequently, the HPV infection rate is high in women in the 15–24-year-old group, and the rate decreases with age [26, 27]. From another perspective, women in the menopausal period have reduced immune functions, with decreased viral clearance rates and increased HPV infection rates. Therefore, there are two peaks in the age distribution of HPV infection: 15–24 year olds and women >54 years [28, 29]. In the present study, the HPV infection rates in the different age groups showed the highest HPV infection rate of 34.7% in the 15–24-year-old group, followed by an infection rate of 24.1% in the >54-year-old group. The HPV infection rate in the 15–24-year-old group showed an annual upward trend over the past five years. This result suggested that HPV infection exhibited a trend towards younger patients and that the 15–24-year-old group was the population most susceptible to HPV infection. The prevalence in the >54-year-old group should also arouse attention and suggest that HPV testing is clinically valuable for perimenopausal women in cervical cancer screening programmes.

In the present study, the HPV infection rate varied in different disease groups. The highest HPV infection rate appeared in the cervical cancer group (97.2%). This rate was close to the rate reported in the literature of 99.7% [30]. Whether multiple infections with HPV can increase or promote the development of cervical cancer is currently a controversial issue. In the present study, differences were detected in the rates of single and multiple infections among the HPV-positive patients in the different disease groups. The cervical cancer group showed the highest rate of multiple infections. This result is in agreement with previous results [18, 19]. Among the five disease groups, the top three HPV subtypes in terms of infection were HPV-16, -52 and -58. The rate of HPV-16 infection was 2.7 times the rate of HPV-52 infection (46.7%/17.5%) and 2.4 times the rate of HPV-58 infection in the cervical cancer group (46.7%/19.8%).

The present study evaluated HPV infection in gynaecological outpatients in Hangzhou, China, from 2011 to 2015. This study was an investigation of HPV infection with the largest sample size in this region to date. The
results showed that HPV-16, −52 and −58 were the dominant HPV infection subtypes in Hangzhou, which provided objective evidence for the application of preventative vaccines in this region. Moreover, we found that the HPV infection rate progressively increased every year in the age groups below 34 years. HPV infection exhibited a trend towards younger patients, and the 15–24-year-old group was a population susceptible to HPV infection. These findings provide objective evidence for the selection of this age group for vaccination in the study region.

Abbreviations
CIN: Cervical intraepithelial neoplasia; HPV: Human papillomavirus; IARC: International Agency for Research on Cancer; ISO: International Standard Organization

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Availability of data and materials
All data supporting the presented findings are contained within the manuscript.

Authors’ contributions
All authors participated in interpretation of the findings. LQ and DC designed the study and drafted the manuscript. YZ, BL and YC were responsible for collection and patient follow-up. YL and YL performed the HPV subtype detection. YC was the principal investigator, provided all facilities necessary to complete this work and was involved in editing the manuscript. All authors read and approved the final manuscript.

Competing interests
The HPV test kit for the manuscript has a patent certificate. The WIPO-SIPO Award is bestowed upon Xie Longxu. For his invention of Human Papillomavirus Genotyping Diagnostic Kit and Its Gene Chip Preparation Method. Chinese Award is bestowed upon Xie Longxu. For his invention of Human Papillomavirus (HPV) test kit for the manuscript has a patent certificate. The WIPO-SIPO Standard Organization.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The study was approved by the Ethics Review Board of the First Affiliated Hospital of Zhejiang Chinese Medical University and the First Affiliated Hospital, School of Medicine, Zhejiang University.

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Author details
1Department of Laboratory Medicine, the First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China. 2Department of Laboratory Medicine, Center of Clinical Laboratory, the First Affiliated Hospital, School of Medicine, Zhejiang University, No. 79, Qianchu Road, Hangzhou 310003, China.

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