The global prevalence ptxP3 lineage of Bordetella pertussis was rare in young Children with the Co-purified aPV vaccination: a 5 years retrospective study

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Abstract

Background: The global prevalent ptxP3 strains varies from about 10% to about 50% of circulating B. pertussis population in different areas of China. Methods To investigate the difference of vaccination status between different genotypes in the circulating B. pertussis after 10 years of acellular pertussis vaccine (aPV) used in China. The nasopharyngeal swabs and isolates of B. pertussis from these patients were used to perform genotyping of antigen genes. We use antibiotic susceptibility test against erythromycin and sequencing methods for site 2047 of 23S rRNA to determine the resistance status. Results The ptxP1 allele with erythromycin resistant strains infection (total of 449 samples) consisted of 84.70% to 96.70% from 2012 to 2016. Only 2 of the 21 ptxP3 strains infected in children vaccinated with co-purified aPV, that showed a significant difference between the ptxP1 strains does (χ 2 =6.87, P=0.032). Conclusions The ptxP1 allele with erythromycin resistant B. pertussis was steadily increased in Xi'an, China from 2012 to 2016, where co-purified aPV was prevalence used . We assumed that the co-purified aPV might protect against ptxP3 strains more efficient, which generated a rare chance for ptxP3 strains to be under the antibiotic pressure and further developed to be erythromycin resistance. A further cohort study and the mechanisms of the additional antigen proteins of co-purified aPV protected against B. pertussis should be consideration.

Introduction

Pertussis is a respiratory disease mainly caused by *Bordetella pertussis*. The incidence of pertussis marked decreased after the whole cell pertussis vaccine (wPV) has introduced all over the world. Since the 1990s, a resurgence of pertussis has emerged in many countries, especially when the acellular pertussis vaccine (aPV) has replaced from the wPV. Furthermore, the circulating *B. Pertussis* has evolved mainly changed in the vaccine antigen genes proposed by the vaccine-driven, such as the pertactin deficient strains and the *ptxP1* lineage to *ptxP3* lineage and also pertactin deficient [1]. Nowadays, the *ptxP3* lineage with/or without pertactin deficient strains, which has been proved to be more virulent and reflect selective advantage under the high coverage of aPV vaccination, has emerged globally and raised an important public issue toward an alternative vaccine in pertussis prevention [2].

However, the *ptxp1* lineage was still prevalence in some countries used wPVs. We have reported the *ptxp1* strains further shown erythromycin resistance (ER) that emerged in China since 2012. Furthermore, we found that all the *ptxP1*-ER strains originated from a *fhaB3* lineage, which seems to be selected from the wPV or antibiotic pressure [3]. Interestingly, although the ER/*ptxP1* strains expanded all over the countries of China, the proportions of *ptxP3* strains varied from less than 10% to about 50% in different areas of China, especially occurred much higher in developed areas [4, 5].

The aPV came in two varieties according to the producing procedures: one is obtained through co-purified procedures so called co-purified aPV, which was used mainly in China and Japan. The other one with purification of each one to five components individually antigen and then blending them in an appropriate ration called purified aPV, which was used in lots of areas all over the world [6]. In China, the

co-purified aPV was free and predominated used since 2006. The purified aPV (Sanofi) was imported and rechargeable since 2011 and supplied much more in developed areas in China. Thus, we assumed that despite the clonal expansions of *ptxp1*-ER strains, the *ptxP3*-ES strains were adapted to the purified aPV much more than to co-purified aPV, which results for the different proportions of the clinical strains in different areas.

In this study, we conducted a 5-year retrospective study to survey the dynamic changes in genetic makeup & resistance status of the circulating *B. pertussis* and further the difference in demographic characteristics between different genotypes in Xi'an China, where co-purified aPV was still prevalence used. We hope that studies such as this can give more information in consideration of the modified vaccine for global pertussis prevention.

Patients And Methods

Study populations, strains and samples

All the patients admitted to Xi'an Children Hospital for suspected of pertussis from 2012 to 2016 were sampled of nasopharyngeal swabs (NPs) and diagnostic by culture and special PCR for *B. pertussis*. The demographic characteristics were collected if culture and/or special PCR for *B. pertusssis* was positive. All of 204 *B. pertussis* strains and 702 NPs with culture-negative but positive of special PCR were stored at -80℃ until to use.

Antibiotic susceptibility test

In-vitro sensitivity of clinical strains against erythromycin was performed and reported as previously[7].

23S rRNA sequencing and antigen gene typing

The nucleotide position 2047 of the 23S rRNA was performed by DNAs of strains and/or NPs by our previously reported sequencing methods [7]. Cause the A2047G of 23S rRNA was associated with erythromycin resistance, if the nucleotide position 2047 of the 23S rRNA was the wild type as adenine (A), we defined as an erythromycin sensitive *B. pertussis* infection. A mutation type as guanine (G) of site 2047 was taken for erythromycin resistance *B. pertussis* infection strain [8, 9]. The allele of *ptxP, fim3* and *prn* was performed by DNAs of strains and/or NPs as previously reported when successful sequencing of 23S rRNA [10].

Statistical analysis

Data were statistically analyzed with SPSS 17.0. Comparisons were performed using one-way analysis of variance (ANOVA).

Results And Discussion

In total, 4 strains have the MICs against erythromycin lower than 0.023 ug/ml, which refers to sensitive to erythromycin in vitro. The rest of the 200 strains were all resistant to erythromycin with the MICs≥256 ug/ml. All the resistant strains posed an A2047G mutation in 23S rRNA and no mutation occurred this site of the sensitive strains.

Among the 702 NPs for sequencing, 480 obtained both the available sequencing results of 23 rRNA and *ptxP* . All the sequencing results from the strains were as same as from the related NPs. Combined with the results from strains, there were 449 in 480 specimens (93.5%) shown the allele G in 2047 site of 23 rRNA that defined as erythromycin resistant *B. pertussis* infection, which also shown the allele of *ptxP1*. The dynamic changes of proportions of circulating *B. pertussis* from 2012 to 2016 as shown in figure 1.

Furthermore, 47 patients were excluded when analysis the difference among demographic characteristics cause of unclear vaccination status. Only 2 of the 21 *ptxP3* strains infected in children vaccinated with co-purified aPV, that showed a significant difference between the *ptxP1* strains does (c^2=6.87, P=0.032). All the vaccinated subjects were administrated with co-purifid aPV (Table 1).

Within our study, we discovered that *ptxP1*-ER strains have been steadily increased to the circulating *B. pertussis* population from 2012 to 2016 in Xi'an, China. Moreover, unlike what happened to purified aPV has been administrated that *B. pertussis* could not only infect the infants that were too young to be vaccinated, but also the infants vaccinated with the purified aPV [11, 12], the *ptxP3* strains rarely infected the infants administrated with co-purified aPV from our study.

The increasing incidence of pertussis was also emerged in China from 2013 according to the national infectious diseases case reported system. Besides the A2047G mutation in 23S rRNA occurred in *ptxP1*-ER *B. pertussis* strains, a novel *fhaB* C5330T was also founded in all these strains. This *fhaB3* lineage has been proved to be prevalence among China via expansions most likely due to antibiotic pressure[3]. This study also illustrated that the *ptxP1/fhaB3*-ER strains might be adapted to the co-purified aPV. Whether the *fhaB* C5330T contributed to this adaption need to be further investigated.

According to this study, *ptxP3* strains with the decreased proportions have observed from 2012 to 2016 in Xi'an, the western of China. In China, the co-purified aPV was free and predominated used since 2006 while the purified aPV (Sanofi) was available by paid since 2011with rarely market supplied, especially in undeveloped regions of western China, such as Xi'an. The rare of *ptxP3* strains in Xi'an after 10 years of co-purified aPV used also indicated that the co-purified aPV did not give the adaption as purified aPV did in developed countries where *ptxP3* was quickly predominant worldwide [13].

The co-purified aPVs have more protein antigen than purified aPVs [14]. Therefore, this study further supported the hypothesis that the small antigen targets of purified aPV could induce the vaccine pressure and vaccine adaption more easily than the more antigen targets vaccine, such as wPV, even the co-purified aPV [15]. Furthermore, among the additional protein antigens of co-purified aPV, most was the out membrane proteins such as BipA and SphB1. Such membrane proteins containing in the out membrane vesicle (OMV) of *B. pertussis* have been suggested as an attracting candidate component of

the possible new modified vaccine against pertussis [16, 17]. The latest study further proved that the OMVs can protect against *B. pertussis* with long term duration, even the global popular *ptxP3* and pertactin deficient strains [17].

Japan was the first country to develop aPV (co-purified) in 1981 and to adopt for use in the general population. It has been reported that both of the two types of aPVs was used recently [18]. However, the *ptxP3* lineage still holds lower than 50% from 2006 to 2010 until the period of 2011-2014which reached close to 80% [19].

Most of the cases of this study were from the west of China. Otherwise, it was reported that the *ptxP1*-ER strains contributed to 75.4%, 50.7% and 48.6% in the circulating strains in Zhejiang province (Southern of China, 2016), Shanghai (Southern of China, 2016-2017) and Shenzhen (Southern of China,2015-2017), while the rest ES strains were almost *ptxP3* strains [4, 20, 21]. No details of the vaccine type were described in these relative high proportion of *ptxP3* areas of China. Liking what happened to Japan, we assumed that the purified aPV used was much more in these developed areas of China than in Xi'an, which generate a relative low level of vaccine protection from co-purified aPV in general population. As a result, the proportions of *ptxP3* strains were much more.

Consistent with reports in these areas of China, the erythromycin resistant strains were almost *ptxp1* allele while the *ptxP3* strains were all sensitive to erythromycin. As shown in this study, though the average age of *ptxP3*- ES strains infection group is lower than in *ptxP1*- ER groups, there is no significant difference. Furthermore, more than 85% of subjects have taken antibiotics before sampling and detection, no difference was observed between the *ptxP3*- ES and *ptxP1*- ER groups (data not shown). Therefore, despite the antibiotic pressure which seems to provide the selective advantage for expansion of erythromycin resistant strains, we suggested that the co-purified aPV protect against *ptxP3* strains more efficient, which generated a rare chance for *ptxP3* strains to be under the antibiotic pressure and further developed to be erythromycin resistance.

However, it is a limitation that the cases of *ptxP3* strains were relative too small to give strong evidence about the protection against *ptxP3* lineage by co-purified aPV. Furthermore, the *ptxP3* with the pertactin (PRN) deficient isolates were widely appeared in some industries countries [22], if the *ptxP3* isolations in this study expressed of PRN were unknown in this study. Lastly, the age of the patients in our study was mainly the infant but not many children after at least 5 years of vaccination of co-purified aPV. Thus we can not give powerful support about the protection duration against *ptxP3* linage of the co-purified aPV.

Conclusions

In conclusion, this study revealed that the erythromycin resistant *B. pertussis* have been steadily increased from 2012 to 2016 in Xi'an, western of China. We also assumed that the co-purified aPV containing more antigens has the possibility to protect the infant from being ill of pertussis infected by global popular *ptxP3* lineage *B. pertussis*. To be better understanding the effect of co-purified aPV, an

international multicenter cohort study should be performed. This will support a progressive insight into global pertussis prevention in a possible aPV2.0 era of the future.

Declarations

Ethical Approval an Consent to participate: This study was approved by the institutional Review Board of Xi'an Children Hospital, Xi'an, Shannxi Province, China.

Consent for publication: Not applicable.

Availability of supporting data: Xi'an Children's hospital is the custodian of the data for this study. The data are not accessible online, but may be made available upon written request to the authors, if in line with the Ethical Review Board guidelines.

Competing interests: All the authors declare that they have no competing interests.

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Author' contributions: Zengguo Wang, Xiaoguai Liu and Yarong Li contributed to the study design. Yang Luan and Quanli Du contributed to carry out the experimental work, writing and data analysis. Chang Shu, Xiaokang Peng and Huijing Wei contributed to acquisition of data. Ying liu and Tiejun Hou collected the data and samples. All authors reviewed and approved the final approved the final manuscript as submitted.

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Table

Table 1. The demographic characteristics of children suffering from pertussis with different genetic makeup & erythromycin resistance status of *B. pertussis*

	ER/*ptxP1* n=403(%)	ES/*ptxP3*[a] n=21(%)	ES/Non-*ptxP3*[b] n=9(%)	c^2	P
Vaccinated with co-purified aPV[c]	133 (33.0)	2 (9.5)	1 (11.1)	6.87	0.032
Age (Months)[d]	3 (2-5.5)	2 (1-3.5)	3 (2-5)	1.479[e]	0.225

[a] The *ptxP3*-ES with proportions of 8.93%, 9.38%, 6.19%, 2.65% and 3.09% from 2012 to 2016 in this study.

[b] Including 8 of *ptxP1/fim3-4/prn1* and 1 of *ptxP1/fim3-1/prn3.*

[c] The cases of unclear vaccination status were not enrolled, what was 46 and 1 in *ptxP1*-ER and *ptxP3*-ES group each.

[d] The ages were represented as Med, $x._{5}$ (Q1, $x._{25}$- Q3, $x._{75}$) .

[e] Refers to the F value with ANOVA test between *ptxP1*-ER and *ptxP3*-ES group.

Figures

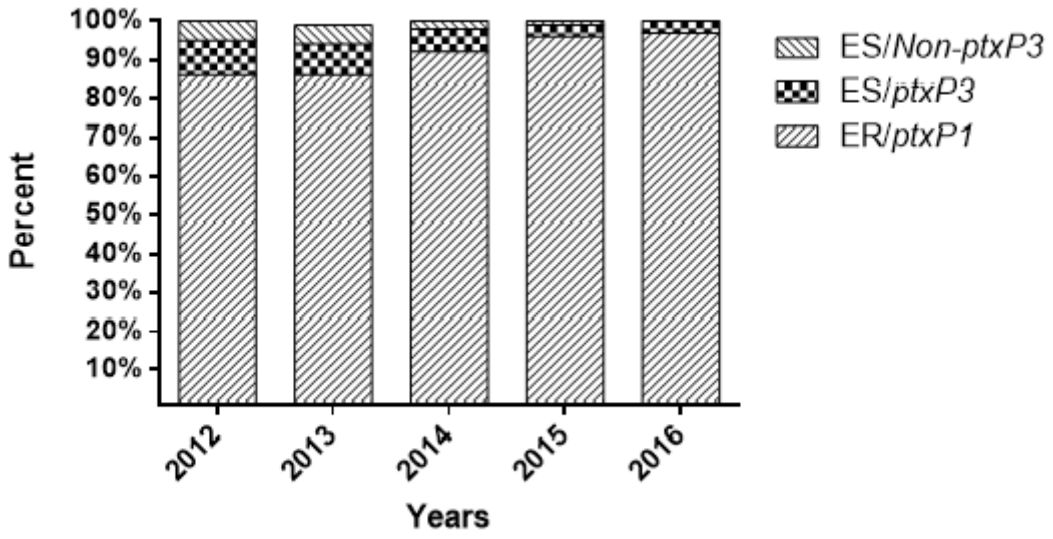

Figure 1

The proportions of different B. pertussis lineage in B. pertussis population from 2012 to 2016 in Xi'an, China