

ORIGINAL ARTICLE

Diagnosis of pertussis in vaccinated children of Khairpur, Sindh, Pakistan by Cough Plate Method

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ABSTRACT

Objectives: Pertussis or whooping cough is a communicable infection of upper respiratory tract that mainly affects children. Reports regarding resurgence of pertussis in vaccinated children mainly motivated us to document pertussis in the children. The aim of the study was to explore pertussis in vaccinated children using an alternative method for pertussis diagnosis.

Materials and methods: A total of 700 clinical samples were collected during study period (2006-2009), from suspected whooping cough cases of Diphtheria-tetanus- whole cell pertussis (DTwP) vaccinated children both male and female aged from 6 months to 84 months. The classical 'Cough Plate Method' instead of Nasopharyngeal Swab was used for sampling to find out its potential in diagnosis of pertussis.

Results: Present study reports the presence of pertussis in vaccinated children using Cough Plate Method. The method successfully isolated *Bordetella pertussis* from suspected patients of pertussis. A total of 28 culture confirmed cases were detected among 700 samples tested. (Total Isolation rate: 4%). The peak incidence age under risk was 48 months. However, pertussis was detected in children aged as young as 6 and 12 months.

Conclusion: The 'Cough plate' method used for isolation proved successful and simple instead of nasopharyngeal swabs that is difficult to perform and children may be reluctant to this sampling method when tried. *J Microbiol Infect Dis 2011;1 (2) : 68-72*

Key words: *B.pertussis*. Whooping cough, Vaccination, Children

Pakistan'ın Sind eyaletinde aşılanmış çocuklarda boğmacanın öksürük plak yöntemi ile tanılanması

ÖZET

Amaç: Boğmaca veya boğucu öksürük başlıca çocuklarda görülen üst solunum yollarının bulaşıcı bir hastalığıdır. Aşılanmış çocuklarda Boğmacanın ortaya çıkışı hakkındaki raporlar bizleri çocuklardaki boğmacayı bildirmeye motive etti. Bu çalışmanın amacı aşılanmış çocuklarda alternatif bir yöntem kullanarak boğmaca durumunu araştırmaktır.

Gereç ve yöntem: Çalışma süresi boyunca (2006-2009 arası) yaşları 6-84 arasında değişen erkek ve kız çocuklardan difteri-tetanoz-tam hücre boğmaca aşısı uygulanan toplam 700 klinik örnek toplandı. Çalışmada nazofarengeal sürüntü yerine klasik "öksürük plak yöntemi" kullanılarak yöntemin boğmaca tanısındaki etkinliği araştırıldı.

Bulgular: Bu çalışma, öksürük plak metodu kullanarak, aşılanmış çocuklarda boğmacanın varlığını bildirmektedir. Bu yöntem ile boğmaca şüpheli çocuklarda *B.pertussis* başarıyla izole edildi. Toplam 700 örnek arasında 28'inde (% 4) kültürde *Bordetella pertussis* varlığı doğrulandı. En yüksek risk altındaki yaş grubu olarak 48 ay saptandı. Ancak 6-12 aylık küçük çocuklarda da boğmaca saptandı.

Sonuç: Uygulanması zor ve çocukların isteksiz olabileceği nazofarengeal sürüntü yerine uygulanan öksürük plak yönteminin bakteriyi göstermede basit ve başarılı bir yöntem olduğu kanıtlandı.

Anahtar kelimeler: *B.pertussis*, boğucu öksürük, aşı, çocuklar

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INTRODUCTION

Whooping cough or pertussis is a non-invasive, highly communicable acute human infection of ciliated cells of upper respiratory tract, which mainly affects infants and young children. It is caused by bacterium *Bordetella pertussis*. Symptoms of pertussis include having a cough lasting 14 or more days often accompanied by a gasping sound during coughing. The World Health Organization has reported in 2008, 16 million cases, 195000 children deaths with 95 % in developing countries.¹ This epidemiological data calls attention to the requirement for sensitive and speedy methods for diagnosis of pertussis.

Pertussis, because of the classic feature of whooping cough is not often experienced in adults and young infants² and it is generally suspected after an extended cough with no paroxysms particularly in adults. The infection spreads from person to person in respiratory droplets either directly inhaled or via contaminant surface from which organisms are transmitted to the nose by hands.³

This study describes the success of classical methods of diagnosis of pertussis from vaccinated children.

MATERIALS AND METHODS

Study Design

This study was carried out in Diagnostic and Research Center (DRC) Department of Microbiology, Shah Abdul Latif University Khairpur and samples were collected from DRC and OPD (outpatient department) of District Civil Hospital Khairpur, Sindh Pakistan.

Study Population

A total of 700 clinical samples were collected during study period (2006-2009), from suspected whooping cough cases of Diphtheria-tetanus-whole cell pertussis (DTwP) vaccinated children both male and female aged from 6 months to 84 months, visiting to DRC or OPD of District Civil Hospital were enrolled in this study. The vaccination history of patients was ascertained by inspecting the vaccine card from the parents.

The study population was divided in 14 groups (aging 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, and 84 months). Each group com-

prised of 50 children. The District Health Committee, Khairpur approved this study. The possible cases of pertussis were identified on the bases of clinical history and case definitions recommended by WHO.⁴ The patients were selected based on classical signs of persistent cough with paroxysms, vomit and whoop. Parents were requested to cooperate by filling consent form and providing cough samples. Clinical information and medical history of the children was taken on report form. All children received three doses of vaccine.

The samples were collected by Cough Plate Method⁵. The plate (90 mm agar Petri plate containing Charcoal agar (Oxoid) with 15% v/v sheep blood and 40 mg/L cephalexin (Oxoid) to minimize the growth of unwanted bacteria) was held 4-5 inches from the patient's mouth during expulsive coughs. Maximum care was taken to minimize the exposure of plate by replacing the lid immediately after each cough bout. Maximum three bouts per plate were collected. After exposure, the Petri plates were incubated at 35°C for 72 hours in a moist atmosphere (Humidified box). Samples were taken in duplicates.

The positive cultures of *B. pertussis* were selected on the basis of cultural characteristics, motility and biochemical tests (Sugar fermentation and other tests (Table 1). characteristics isolates

Table 1. Biochemical characteristics of *B. pertussis* isolates

TEST	RESULT
Sugar Fermentations	Negative
Simmon's Citrate test	Negative
Oxidase test	Positive
Catalase test	Negative
Nitrate Reduction	Negative
Urease Production	Negative
Indole Production	Negative
Methyl Red Test	Negative
Voges Proskovar test	Negative

Clinical cough samples were collected by cough plate method (n=700). 22 samples out of 700 showed typical biochemical reaction for *B. pertussis*.

Motility test was performed by hanging drop technique. Motility test of all isolates shown non-

motile result and for further confirmation, isolates were tested biochemically. For further confirmation on the basis of hemolysis, the isolates were grown on BG medium. The identified colonies were sub-cultured on Bordet- Gengou (BG) medium⁶ and charcoal agar to observe hemolysis and to prepare pure culture. The percentage of age wise distribution of positive pertussis cases in vaccinated children (n=50 per group) was calculated.

RESULTS

B.pertussis was detected in 28 suspected child patients ages from 6 months to 84 months among 700 subjects included in this study. Despite vaccination, pertussis was culture-confirmed in 28 children. Since the culture medium used for isolation of *B.pertussis* by cough plate method was selective, containing cephalixin, *B.pertussis* gave characteristics colonies that were smooth, raised, pin pointed with light metallic luster, resembling a drop of mercury (Fig.1).

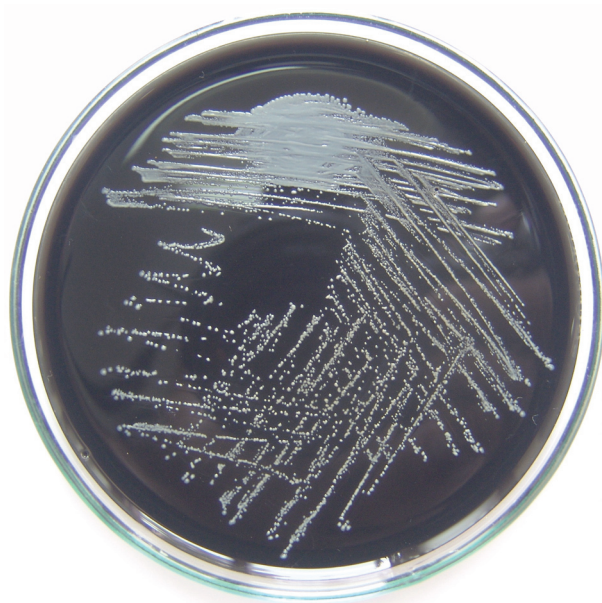


Figure 1. *B.pertussis* colonies on cough plate.

The Gram stain smear showed tiny coccobacillary bacteria that occurred singly or in pairs and stained Gram negative with bipolar appearance. The isolates were found Oxidase positive, and all other tests were negative (Table 1), confirming that the 28 isolates were *B.pertussis*. Typical tiny mercury drop colonies with diffused zone of

hemolysis were observed on BG medium indicating the growth was *B.pertussis* (Fig. 2).

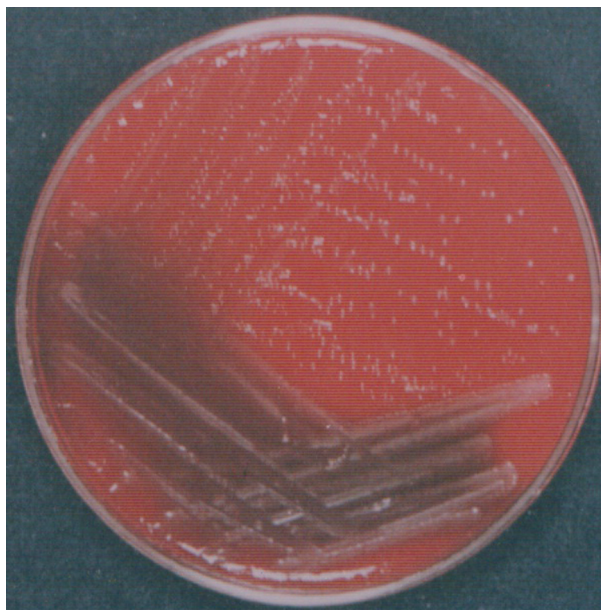


Figure 2. *B.pertussis* on BG medium.

Frequency distribution data shown the highest frequency of pertussis i.e. 10% in age group 8 (48 months) followed by 6% in age groups 2 and 7 (12 and 42 months respectively), 4% in age groups 3, 5, 10 (18, 30 and 60 months respectively), 3% in age group 4, 6, 9, 11, 13 and 14 (24, 36, 54, 72, months respectively) and 2% in age groups 1, 11, 13 and 14 (6, 66, 78 and 84 months respectively) as shown in Fig.3. The results indicate the trend that pertussis was common in all age groups tested but the peak risk group was 48 months.

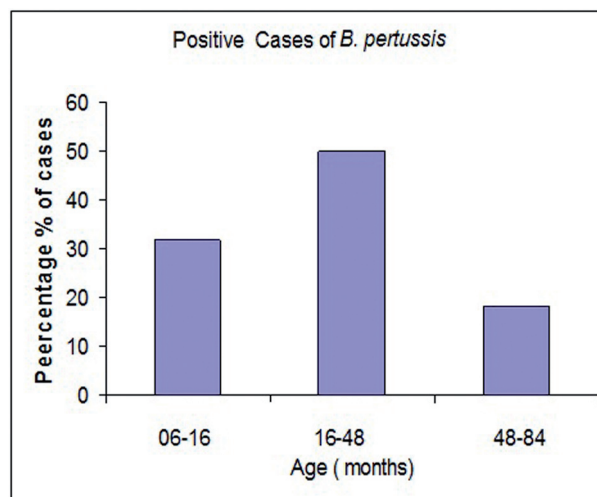


Figure 3. The age distribution of positive cases of pertussis.

DISCUSSION

Whooping cough, a disease of respiratory tract is frequently a life-threatening illness in early infancy.⁷ The diagnosis of pertussis is a key step in reducing the disease burden therefore present study was undertaken to find out the efficiency of classical method for isolation of *B.pertussis* from the study population. Identification of *B.pertussis* by culture from nasopharyngeal secretions is still considered to be a reference method. Other diagnostic methods used for diagnosis of pertussis include polymerase chain reaction (PCR) and Enzyme linked immunosorbant assay (ELISA).⁸

Here we report the isolation and identification of *B.pertussis* from vaccinated children who received three shots of DTwP, at the age of 6, 12 and 14 weeks (immunization schedule by Expanded program on immunization in Pakistan). The classical and simple procedure 'Cough Plate Method' was used for diagnosis of pertussis. This method appeared more suitable as the In vivo organism did not hamper due to delays in transport and direct contact with the growth medium enhanced the chances of recovery of *B.pertussis*.

A total of 700 cases of suspected pertussis reported by local pediatricians among children of Khairpur city were included in this study.

Our study reveals a trend toward higher attack rates in the 48 months age group; It has been reported that vaccine-induced immunity wanes over time, leading to increased susceptibility however, the present study also establish cases of pertussis in as young as 6 and 12 months that contradicts this hypothesis and reflects the altered immune status probably due to antigenic variation of circulating strains as compared to vaccine strains.

The vaccine coverage in Pakistan for DTwP reached the 80% reference point for the first time in 2005 and 2006, and remained at 80% until 2007.⁹ In this context, the isolation of *B.pertussis* from vaccinated children is alarming. This suggests that current immunization practices may not be adequate in protecting infants and children less than 5 years of age against pertussis. Isolation of *B.pertussis* form vaccinated children of 6 to 84 months may represent the shift of the typical pertussis to atypical (less severe clinical symptoms) in the children to which the physician may not recognize. Pertussis largely appears to

be undiagnosed and therefore unreported infection in Pakistan, where culturing for diagnosis of pertussis is not a routine practice and only advanced laboratories in big cities diagnose pertussis by PCR. Recently we have reported the low isolation rate of *B.pertussis* as compared to *B. parapertussis* despite the PCR was performed.¹⁰ This shows that the PCR may have limited, if not insignificant impact on the isolation rate. Our report suggest that the cough plate method appeared to be an easy to perform sampling method and fulfills the Gold standard of diagnosis of pertussis recommended by WHO4 i.e. culturing and growth of *B.pertussis* from clinical samples.

In the literature, the rate of culture-positivity among patients with pertussis has been shown to vary markedly (e.g., 20%–83%).¹¹ The low isolation rate in the present study might be due to impediment in suspecting of the disease on account of patients or physician. It has been reported that culture is not sensitive in many routine laboratories, and isolation rate decreases rapidly after the onset of paroxysms.¹² Furthermore, delay in transport of samples reduces the chances of recovery of *B.pertussis* that could only be overcome by using methods where In vivo *B.pertussis* is directly inoculated onto the growth medium without any delay.

Other explanation for reduced recovery could be the use of antibiotics, because according to the history, two third patients received treatment prior to participating in this study.

PCR developed for direct identification of *B.pertussis* 13 uses a repetitive sequence from *B.pertussis*. Although rapid, sensitive and specific, PCR uses the sequences that may vary within strains of *B.pertussis*.^{14,15} Due to the presence of this sequence in some *B. bronchiseptica*, this cannot be regarded as totally specific for *B.pertussis* provided species specific PCR targets are used. Moreover, PCR needs a great deal of expertise and care; requires costly reagents and equipment. These facilities are not present in majority of resource -limited laboratories of developing countries like Pakistan. This leaves the choice open for the classical methods such as Cough Plate Method, to be revived and implemented in routine laboratory practices for diagnosis of pertussis.

The isolation of *B.pertussis* from cough samples of DTwP – vaccinated children is noticeable.

Numerous studies have exposed that important shifts have occurred in the *B. pertussis* resulting in antigenic difference between vaccine strains and circulating strains and signifying pathogen variation.¹⁴ Resurgence of pertussis is being reported from several countries despite high vaccination coverage.¹⁶⁻²⁴

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