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Moleculer Detection and Characterisation of Bordetella Pertusis and Presence of Pertactin Gene Among Patients with Symptoms of Respiratory Tract Infection in Kaduna State

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Abstract

This study detected B. pertussis gene from sputum of respiratory infected patients. Eighty-nine (89) sputum samples were collected and stained using gram staining technique. Gram negative cocco-bacilli were seen in 19 of the samples which were then subjected to DNA extraction by phenol chloroform method. 16SrRNA primer specific for *B. pertussis* was used for the amplification of *B. pertussis*. The positive samples were subjected to Dye terminator cycle sequencing with quick start kit. Four 4 (4.5%) of the samples were positive for *B. pertussis* with an amplicon of 998bp. The sequence was analyzed using basic local alignment search tool (BLAST) from National Centre for Biotechnology Information NCBI. The query sequence showed 90% identity with *Bordetella pertussis* strain VITSBSTV04 16SrRNA gene partial sequence. The four positive samples for *B.pertussis* subjected to PCR for the amplification of pertactin gene, showed negative amplicon of the gene. These indicate that pertactin gene is not present in the *B.pertussis* detected in this study. A total of 92.1% of the case patients responded positively to having at least one dose of vaccination. The significant proportion of pertactin negative strains analyzed in this study indicates that adequate measures are needed to curtail the spread of *B.pertussis*.

Keywords: B. pertussis, Acellular vaccine, pertactin, Whole cell vaccine, amplicon, virulence

Introduction

The genus Bordetella in the family Alcaligenaceae, comprises five species, four of which cause infections of the upper respiratory tract in different host organisms. *Bordetella pertussis* is an obligate human pathogen and is the etiological agent of whooping cough (pertussis), an acute respiratory infection more serious among infants. Annually, more than 50 million cases of pertussis, including 600,000 deaths are reported worldwide (Mir-Cros *et al*., 2022). During the last 15 years, the incidence of pertussis has increased again in different regions of the world despite intensive programs of infant vaccination (Cordova *et al*., 2000; De Melker *et al*., 2000; Khetsuriani *et al*., 2001; Gonzalez Moran *et al*., 2002; De Schutter *et al*., 2003). Prior to the implementation of pertussis immunization programs, this disease, caused primarily by the bacterium *Bordetella pertussis*, was a scourge of childhood, causing about 73 000 deaths from 1922 to 1931 in the USA, most of whom were infants; in contrast, the toll was reduced to 56 deaths between 1983 and 1992 (Mir-Cros *et al*., 2022). In 1999, there were an estimated 48.5 million cases in children and 295 000 deaths attributed to pertussis, with disability-adjusted life years exceeding that of lung cancer in 2000 (Crowcroft *et al*., 2003). Pertussis appears to be resurgent in a number of countries in the developed world that boast of high immunization coverage. Starting in the 1980s, increased incidence rates were reported in the USA (Romani and Drake 2011), Australia (Scheil, 1998, Quinn and McIntyre, 2011), and Poland (Gzyl *et al*.,

2004), along with other high vaccine coverage countries (Bamberger and Srugo, 2008). In addition to increases in mean incidence rates, shifts in age-specific incidence have been observed, with a general trend towards higher representation by adolescents and adults (Wood *et al*., 2008).

Although cases of pertussis have dropped dramatically in the United States since the introduction of the pertussis vaccine, the disease has caused widespread outbreaks in recent years. In 2012, nearly 50,000 cases of pertussis were reported in the United States, the most cases since 1955. Twenty pertussis deaths were reported in 2012. Fifteen of those deaths occurred in infants too young to have received the vaccine (CDC, 2013). Jackson and Pejman (2012) studied the pattern of pertussis from 1990-2010 using a country specific pertussis case counts and vaccination coverage estimates for the third dose of diphtheria toxoid, tetanus toxoid, and pertussis vaccine (DTP3), for the years between 1990 and 2010 which was obtained from WHO database. Statistically significant increases or decreases in pertussis were detected. The data shows a story of substantial epidemiological heterogeneity across countries. Based on the trend analysis, 10 of the 54 countries had a significantly increasing trend in pertussis incidence during the period, 27 had a significantly decreasing trend and 17 did not exhibit any significant trend. According to the same data, there was lack of geographical pattern in the trends (Jackson and Pejman, 2013). Pertussis is being increasingly found in previously immunized subjects. In Nigeria, the immunization coverage rates are low. A hospital based retrospective study from the University of Uyo teaching hospital Nigeria from 2007 to 2011 showed that Fifty-three patients were diagnosed with pertussis during the five-year period, with majority of cases in the year 2011 (Oloyede *et al*.,2015). The Virulence Determinants and Molecular Pathogenesis is that during the course of infection, *Bordetella pertussis* adheres to the ciliated epithelium, invades alveolar macrophages, multiplies rapidly on the mucous membrane and expresses an array of virulence factors that help colonize the upper respiratory tract by specific adhesion to the ciliated cells. These factors include cell surface proteins and several extracellular toxins that inhibit host defenses and induce damage to host tissues.

Like many other bacterial pathogens, the expression of the virulence factors in *Bordetella pertussis* is controlled by growth conditions. Two important phenomena in the regulation of the virulence genes are phase variation and phenotypic modulation. Phase variation indicates a reversible alteration in the genotype caused by frame shift mutations in which the virulent bacteria simultaneously lose the ability to synthesize toxins and other factors associated with pathogenicity (Mir-Cros *et al*., 2022). The natural emergence of phase variants in the later stages of infection implies that phase change could be a defense mechanism to escape immune detection, like Salmonella flagellar phase variation where the change of antigenic type helps the bacteria to evade the immune system (Safarchi *et al*., 2019). The other phenomenon termed phenotypic modulation was first observed by Lacey in 1960, it implies repression of the expression of virulence factors except the Tracheal cytotoxin at lower temperature (25^0C) or in the presence of in vitro modulators like SO4 2-, ClO4- and Nicotinic acid (Weigand *et al*., 2019). Using transposonal mutagenesis, a single gene locus responsible for both the phenomena was identified (Polak *et al*., 2019). This was termed the bvg (Bordetella virulence gene) locus which shares homology with a family of prokaryotic regulatory proteins that respond to environmental stimuli (Polak *et al*., 2019).

The BvgAS two component system *Bordetella pertussis* employs a two-component signal transduction system comprising BvgS and BvgA, to regulate cellular functions in response to environmental conditions, which are not properly understood so far. BvgS is a 135 kDa periplasmic sensor histidine kinase. It consists of a periplasmic input domain and several cytoplasmic domains, the linker, transmitter, receiver and the C-terminus. The BvgS periplasmic domain senses external stimuli (perhaps via dimerization) and triggers a series of phosphorylation steps starting with auto-phosphorylation in the transmitter domain at a conserved histidine residue. This is followed by the transfer of the phosphate group to a conserved aspartic acid in the receiver domain which transfers it to a histidine at the C-terminal domain (Zomer *et al*., 2018). BvgA, the response regulator, is a 23-kDa cytoplasmic protein comprising an N-terminal receiver and a C-terminal output domain, which contains a helix-turn-helix (HTH) DNA binding motif. BvgA is activated by the transfer of the phosphate group from the C-terminal of BvgS to a conserved aspartic acid in the BvgA N-terminal receiver domain. Upon phosphorylation, BvgA positively regulates the bvgAS and other virulence

factor promoters by binding to the target sequence TTTCCTA (Safarchi *et al*., 2019). It has also been shown that BvgA is able to dimerize in solution as a possible means to bring about its DNA binding property (Zomer *et al.*, 2018). Filamentous haemagglutinin B. pertussis confers infection by adhering to host tissues of the upper respiratory tract implicating several adhesins in the process, especially the filamentous haemagglutinin or FHA (Safarchi *et al*., 2019).

Statement of the Research Problem

As at 2012 11,628 cases of pertussis was reported in Nigeria (WDA, 2012). Nearly 50,000 cases of pertussis were reported in the United States, the most cases since 1955. Twenty pertussis deaths were reported in 2012. Fifteen of those deaths occurred in infants too young to have received the vaccine (CDC, 2013). The re-emergence of pertussis in several countries with high vaccine coverage (Weigand *et al*., 2019, De Meker *et al.*,2000) has led to renewed interest in whooping cough and in pertussis vaccine efficacy. Looking at 2023 until late November, data from the UK Health Security Agency (UKHSA) has revealed there were 1,141 suspected cases in England and Wales, compared with 450 for the same period of 2022 and 454 for that period in 2021- about 250% increase.

Pertactin, a 69 kDa nonfimbrial outer membrane protein, under the control of the bvg locus, is partly responsible for the adhesion of the bacteria to the host cells. It also functions as a protective antigen in animal models (Leininger *et al.,* 1992, Gotto *et al.,* 1993). 11 prn alleles showing a variable number of GGXXP repeats have been identified (GenBank database). As demonstrated in isolates collected in The Netherlands and Finland (Mooi *et al.,* 1998, Mooi *et al*., 1999), all isolates from the pre-vaccine period harbored the vaccine pertactin type prn1. However, these types were replaced during the 1990s by the nonvaccine prn types prn2 (72 and 27%, respectively, in these two countries), prn3 (12 and 63%, respectively), and prn4 (0 and 9%, respectively). For the same period, high prevalence of non-vaccine strains was detected as well in other countries such as Italy (6% prn1, 41% prn2, 51% prn3, and 2% prn5), the United States (30% prn1 and 70% prn2), and the United Kingdom (53% non-prn1) (Mastrantonio *et al.,* 1999 Cassiday *et al.,* 2000, Fry *et al.,* 2001). Vaccine-induced immunity may select among circulating strains for antigenic variants that are divergent from those of the vaccine strains (Weigand *et al*., 2019).

Pertactin gene plays an important role in the adhesion of the bacterium to its host and it has been used in the constitution of acellular vaccine for the prevention of pertussis. Different variants of the gene have emerged over the years with a global distribution, at the same time some strains have been found not to contain the gene pertactin. This is a threat to the efficacy of the vaccine and a major concern about the lethality of the organism. Hence this study is necessary in order to detect the presence and variant of pertactin gene in *Bordetella pertussis* isolated in patients with symptoms of respiratory tract infection in Kaduna state.

Aim of the Study

The aim of the study is to detect *Bordetella pertussis* and presence of pertactin gene in sputum of patients with symptoms of respiratory tract infection.

Objectives of the study

To study the demographic studies of patients and record their clinical symptoms, to detect *Bordetella pertussis* using polymerase chain reaction (PCR), to characterize the *Bordetella pertussis* organism using DTCS sequencing technique and bioinformatics analyses using the NCBI tools and finally to detect the presence and variants of pertactin gene in *Bordetella pertussis*

Inclusion and Exclusion Criteria

This involves all patients referred to the medical microbiology department of Barau Dikko teaching hospital with symptoms of respiratory tract infection particularly whooping cough and the exclusion criteria excludes all patients without signs and symptoms of respiratory tract infection.

Materials and Methods

Ethics statement

Ethical clearance was requested from ethical and human research committee of Kaduna state ministry of health. Approval was granted after finding the research worthy; relevant and timely (see appendix).

Description of the Study Area

This Prospective and detection study was conducted in Barau Dikko Teaching Hospital; Kaduna State, Nigeria from 24th August, 2015 and continued till the year 2017 to when the sample size was obtained and duly analyzed. Kaduna State is located at Northwest of Nigeria; it has a total area of 46,053 km2 (17,781 sq mi) and an area rank of 4th of the 36 states of Nigeria. There are about 9,000,000 million people going by 2022 census leaving it the 3rd of the 36 states of Nigeria in rank and a density of 130 km2 (340/sq mi). Its coordinates are 10º201 N and 7º451 E; this indicates connection to the major routes reaching most of the states of the Nation. Kaduna State, north central Nigeria, is politically classified as belonging to the now 'North - West' zone of the current six Geo - political zones. It is populated by about 59 to 63 different ethnic groups; where Gwari, Hausa and Fulani are the dominant ethnic groups. Its water supply is sourced through damping of rivers and digging of wells and boreholes. Kaduna State consists of twenty-three (23) Local Government Areas (Maryam *et al.,* 2019).

Sample Size Determination

Sample size determination for this study was calculated based on the prevalence reported in a previous study by Oloyede *et al.,* (2015). The formular for determination of sample size is as follows;

$n = \frac{Z^2pq}{d^2}$

Where n= number of samples (sample size) Z= standard normal deviation

P= degree of confidence q=1-p

d= precision of allowable error From the values obtained n=95%=0.95

p=54.7=0.547 d=5%=0.05

Substituting in the formula

-; $n = \frac{(0.95)^2 \times 0.547(1-0.547)}{(0.05)^2}$

$$n = \frac{0.9025 \times 0.547 \times 0.453}{0.0025}$$

$$n = \frac{0.2236}{0.0025}$$

= 89.45 that is approximately **89** samples

Sample Collection

Eighty-nine (89) sputum samples were collected randomly from patients suspected to have respiratory tract infections from Barau Dikko teaching hospital. The sample were collected aseptically in a sterile universal container, labeled and placed in a transport medium containing an icepack. Samples were immediately transported to DNA laboratory Kaduna, for investigations.

Screening of samples using Gram staining technique

Samples were screened for gram negative cocco bacilli, diplococci arrangement; which is the morphological characteristic of *Bordetella pertussis*. The gram stain which is basically used to differentiate bacteria based on their cell wall was carried.

DNA Extraction

This was carried out using the phenol chloroform extraction method

Amplification of 16SrRNA for the detection of *B.pertussis*

Primer Sequence

16S rRNA gene Primer sequence for the confirmation of *Bordetella pertussis* was obtained from a paper by Benoit *et al.,* (2007). While the primer sequence for the amplification of pertactin gene was obtained from the probe section of National Centre for biotechnology information. Both primers were procured from Bioneer Corporation China. Table 1 shows the primer sequence and their product sizes.

Positive samples for gram negative cocco-bacilli were subjected to polymerase chain reaction for the confirmation of *Bordetella pertussis*.

Table 1: primer sequences and product sizes

Gene	Primer Sequence 5'-3'	Product Size (bp)
16SrRNA-Forward	[59-T(A/T)ACACATGCAAGTCGA(A/G)CG-39	998
16SrRrna-Reverse	[59-CACCTGTGTTCCGGTTCTCT-39	
PRN- Forward	TGCCGACTGGAACAACCA	2736
PRN- Reverse	GTCGGAGCCCTGGATATGG	

Key: 16SrRNA F: 16SrRNA forward, 16SrRNA R: 16SrRNA reverse, PRN F: Pertactin forward, PRN R: Pertactin reverse

PCR assay was performed on programmable thermal controller (PTC-100), PCR 3 with oil by MJ Research Incoporation. Accupower hot start PCR premix by Bioneer Corporation was used.

Agarose Gel Electrophoresis

The separated DNA fragments bands were viewed by illumination with gel imaging system and images recorded by photography after agarose gel electrophoresis.

Dye Terminator Cycle Sequencing with Quick Start Kit

PCR amplicons were sequenced using the DTCS where all reagents were kept on ice during the sequencing reactions according to manufacturer's instructions.

Amplification of Pertactin Gene

The Amplification of pertactin gene was performed on a programmable thermal controller (PTC- 100), PCR 3 with oil by MJ Research Incoporation. Accupower hot start PCR premix by Bioneer was used; it contained 1 unit of hotstart DNA polymerase, 1×PCR Buffer, 250 uM of each dNTP.

Results: Gram Stain Screening, Sign and Symptoms

Nineteen 19(21.3%) out of the 89 samples collected were positive for gram reaction of gram-negative coccobacilli (Table 2). The symptoms recorded include; Cough, Paroxysmal, Non-paroxysmal, Post tussive vomitting, Fever, Whooping cough, Catarrh, Difficulty breathing, Weight loss, Red eye, Apnea, Nasalflaring. Ninety-two point one percent, 92.1% of the case patients received at least one dose of DPT or the Pentavalent vaccine, both vaccine contain the whole cell pertussis vaccine (Table 6).

PCR and Sequencing of 16S rRNA gene of *Bordetella pertussis*

Four (4) out of the nineteen (19) samples positive for gram negative cocco bacilli, shows a positive amplicon of 998bp (Plate 1) which is the expected band size. The nucleotide sequence of 237 letters (Figure 1) was analyzed using Basic local alignment search tool (BLAST) from National Centre for Bioinformatics information. A sequence identity of 90% with *Bordetella pertussis* strain VITSBSTV04 16SrRNA gene partial sequence, with a total score of 313 and an expected value (E value) of 3e-84 (Table 4) was obtained. Pertussis incidence of 4.5% was observed(Table 5) with the age group of 3-5 having the highest incidence of 6.7% while age group 6-9 has no incidence of pertussis. The majority of the participants came from the age group of 3-5(44.4%). While the lowest age group participants was 2-4(21.3%). Females have the highest incidence of 5.2%; while male have the incidence of 3.2% (Table 5).

Table 2: Sign and symptoms of study patients

Sign and symptoms	Patients (n=89)	GS+(n=19)	Percentage of GS+ strains symptoms (%)
Cough	68	19	27.90
Paroxysmal	37	5	13.50

Non-paroxysmal	53	12	22.60
P.vomitting	21	19	90.40
Fever	79	11	13.90
Whoop	25	4	16.00
Catarrh	85	15	17.60
D.breathing	82	11	13.40
Weight loss	56	10	17.90
Red eye	42	2	4.80
Apnea	56	8	14.20
Nasalflaring	79	9	11.3

Key notes: GS+; Gram stain positive for cocco bacilli, n; number of patients, p.vomitting; post tussive vomiting, D. breathing; difficulty in breathing.

Table 3: Sign and Symptoms of Patients with *B.pertussis*

Sign and symptoms	**GS+(n=19)**	**BP+(n=4)**	**Percentage of BP+ symptoms (%)**
Cough	19	4	21.10
Paroxysmal	5	3	60.00
Non-paroxysmal	12	1	8.30
P.vomitting	19	3	15.80
Fever	11	1	9.10
Whoop	4	2	50.00
Catarrh	15	4	26.70
D.breathing	11	2	18.20
Weight loss	10	3	30.00
Red eye	2	0	0.00
Apnea	8	3	37.50
Nasalflaring	9	4	44.40

Key notes:GS+; Gram stain positive for cocco bacilli, n; number of patients, p.vomitting; post tussive vomiting, D.breathing; difficulty breathing.

Table 4: Blast Results of 16SrRNA Specific for *Bordetella pertussis*

Q. S	Expected (E-Value)	Bits	1st Organism	%homology
Query Sequence	3e-84	313	*Bordetella pertussis* strain VITSBSTV04	90

Table 5: Demographic Study of Participants and Incidence of *Bordetella pertussis*

Age Range	**No of Participant by Age (%)**	**Study Participants (Males) (%)**	**Study Participants (Females) (%)**	**BP+ Incidence (Male) (%)**	**BP+ Incidence (Female) (%)**	**Immunization Coverage(IC) (%)**
0-2	19 (21)	6	13	0	1	Male: 29
3-5	44 (49)	17	27	1	2	Female: 53
6-9	26 (29.2)	8	18	0	0	-
Total	89	31 (34.8)	58 (65.2)	1 (25)	3 (75)	82 (92.1)

Freq(n=89): frequency of age and gender that participated in the study.

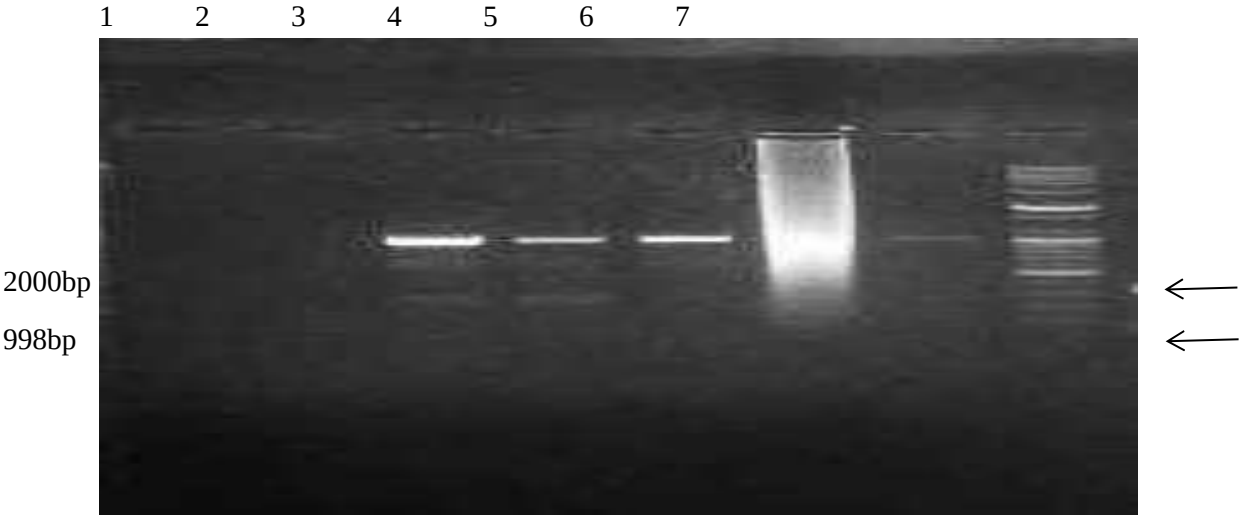
Freq(BP+, n=4): frequency of age and gender of *Bordetella pertussis* positive patients that participated in this study.

Figure 1: First Sequence Obtained from DTCS

TGTCAAGCGTAGGTAAGGTTTTTCGCGTTGCATCTAATTAATCCACNTCATCCACCG
CTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGG
CGGTCTACTTCACGCGTTATCTGCCTTACCAAGGAAATTAATCCCCGACAACTATTA
CAGTCGTTTAGGGTGTGGACTACCAAGGTATCTAATCCTGCTTGCTCCCCACGCTTTC
GTGCATG

NB: Fasta format of 16S rRNA sequence specific for *Bordetella pertussis*

Plate 1. Amplification of 16SrRNA specific for *B.pertussis*.

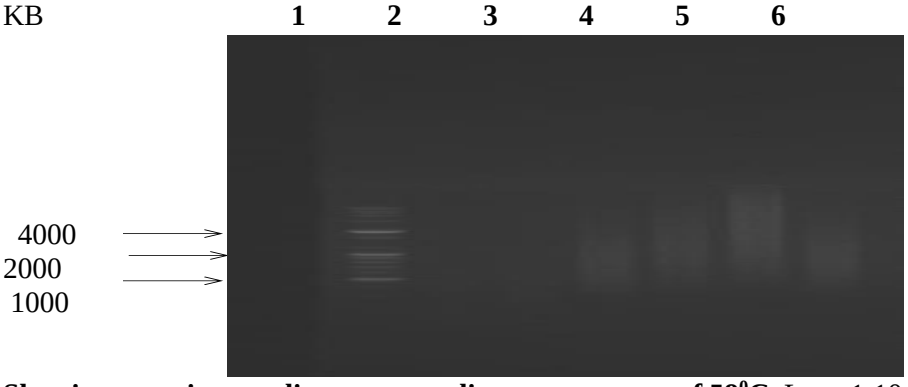


pLane 1: negative control, lane 2,3,4 and 6: positive samples, lane 7: 10kb molecular marker

Polymerase Chain Reaction and sequencing of Pertactin Gene

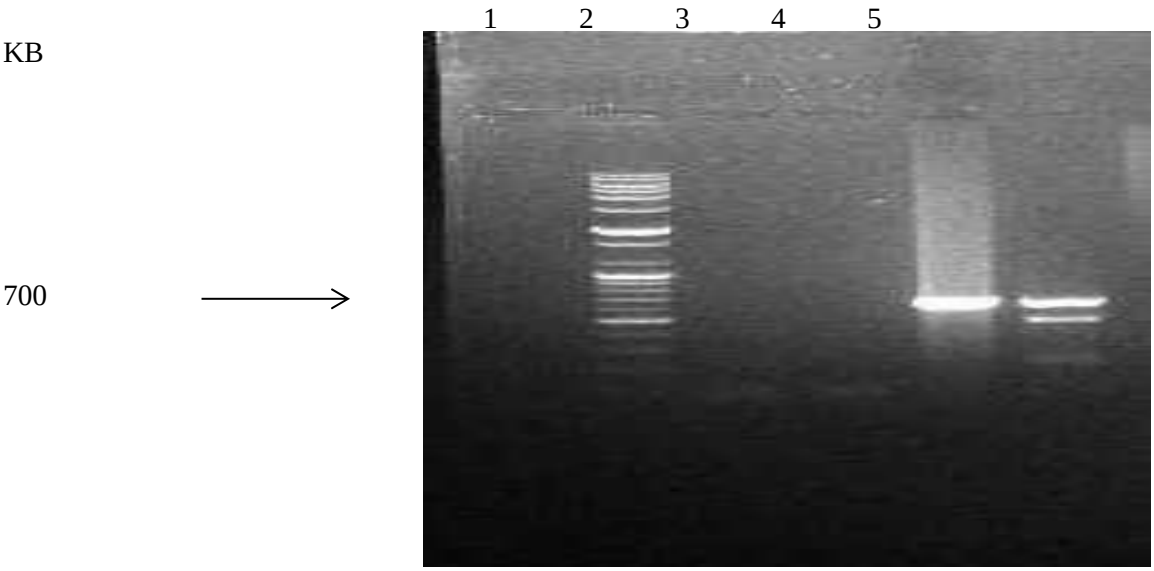
The four positive samples for *B.pertussis* subjected to PCR for the amplification of pertactin gene showed no positive amplicon of the gene at an annealing temperature of 58^0C (Plate 2). The annealing temperature was optimized to 56^0C, at this temperature an amplicon size of 700bp instead of 2736bp was observed in two of the samples (Plate 3). The nucleotide sequence of 370bp and 68bp was retrieved for the samples respectively (Figure 2 and 3). This was analyzed using the BLAST from NCBI which gave a sequence similarity of 100% with a homosapien chromosome 19 clone CTC-503J8 complete sequence, a total score of 126 and Evalue of 3e-26 (Table 4.6) accession number AC011471.6 and 95% identity with homosapien chromosome 19 clone CTC-503J8 complete sequence, an Evalue of 2e-161 and a total score of 579 for the two samples respectively. This indicates that the amplicon recovered was not that of the pertactin gene.

Plate 2: Negative Results for Amplification of Pertactin gene



Showing negative amplicon at annealing temperature of 58^0C. Lane 1:10 kb molecular marker, lane 2: negative control, lane 3-6: samples positive for *B.pertussis*.

Plate 3: Amplification of supposed pertactin gene



Lane 1: 10kb molecular marker, lane 2: negative control, lane 4-5: samples positive for *B.pertussis*.

After optimization at 56^{0}C that turned out to be that of homosapien chromosome 19 clone CTC-503J8 complete sequence after blasting**.** The expected size of 2736 was not obtained for the pertactin gene even after optimization.

Figure 2: 2nd sequence obtained from the DTCS

AGTAAGTAAAGTGGCATTGTAGTACATGCACAGGGTTGTACTACTGCCGTCTCCTTC
TAGTTCCAGAGCAGTTTCATCACCCCGGAAGGAAACCTTGGTGCCAGTAAGCCGCTG
TTTCCCGTTCCCCCCCCATCGCCACGGTCCCGGGCACCAACTCTTCTTTTTGGTGTCT
GGGTGGATGGGCCTATTCTGGCAGTTTCATATAAATGGAGTCATATAGTACGTGACG
TTTGTGTCTCGCTTCTTTCCTTCTTTCTGCCGTATTTGTGGAGTTCATCCGTGTTGTTG
CATGTATAAGAACTGCATGCCTTTTCACGGCCAAGTAACATTCTGCTGTATGGCTGT
CCCACACTTTATCCATTCATCCATT

Fasta format sequence (370 letters)

Figure 3: 3rd sequence obtained from the DTCS

TGGTACTCAAACACATACACGCACAGCCATGTTCAAAGCCGTACTTATTCACAATAG
CCAAGAGGTGG

fasta format sequence (68 letters)

Table 7 BLAST Results of pertactin gene

Query Sequence (Q. S)	Expected (E)-Value	Bits	Organism	% Homology
Sample 3	3e-26	126 CTC-503J8 Complete sequence	homosapien chromosome 19 clone	100
Sample 4	2e-161	579 CTC-503J8 Complete sequence	homosapien chromosome 19 clone	95

Discussion

This study presents the incidence of *Bordetella pertussis* and the detection of pertactin gene among patients with symptoms of respiratory infection. An incidence of 4.5% cases of pertussis was detected this was less than what was reported by Oloyede *et al.,* (2015) where 54.7% cases were reported in 2011 in a retrospective study conducted in Nigeria. Pertussis appears to be resurgent in a number of countries in the developed world that boast high immunization coverage. Pertussis is being increasingly found in previously immunized subjects. Females were found to have high incidence with three (5.2%) being infected and only one male (3.2%) was infected. This is also similar to the findings of Oloyede *et al.,* (2015) were females have the highest prevalence of 60.4% against 39.6% for males.

Four (4.5%) of the 19 samples analyzed was positive for *B.pertussis* but lacks pertactin. Prn- negative strains have been isolated in several countries that have high coverage for vaccination but have not been shown to constitute such a high proportion of circulating *B. pertussis* (Otsuka *et al.,* 2012, Barkoff *et al.,* 2012, Bodilis and Guiso, 2013; Queenan *et al.,* 2013). Nigeria is still among the countries using whole cell vaccine (WCV), until 2015 no published report on pertactin negative isolates was reported. In comparison, until 2001 and 2009, respectively, Russia (Kurova *et al.,* 2010) and Senegal (Weigand *et al*., 2019), which currently use only WCVs, have not reported Prn-negative isolates. The United States introduced accelular vaccines (ACVs) as booster vaccinations in 1991, but not until 1997 were all 5 primary doses replaced with ACVs (Schmidtke *et al.,* 2012). Although Finland replaced WCVs with ACVs at a later time (booster vaccinations in 2003 and primary vaccinations in 2005), both countries detected Prn-negative isolates during 2011–2012(Martin *et al.,* 2015). Long-term temporal analysis has not been performed to determine whether such isolates are increasing over time (Martin *et al.,* 2015).

Japan was the first country to implement ACVs against pertussis in 1981, and the proportion of Prn-negative isolates reported from countrywide surveillance during 2005–2009 was 32%. In France, where ACVs have been used since 1998, originally as booster vaccinations, and then for all doses since 2002 (Bouchez *et al.,* 2009), Ptx-negative and Fha-negative isolates were first obtained in 2003, although only Prn-negative isolates have increased and were reported to make up 13.3% of 120 isolates analyzed in 2011 (Hegerle *et al.,* 2012). The Prn-negative *B. pertussis* isolates have also been identified in Finland and the United States (Barkoff *et al.,* 2012, Queenan *et al.,* 2013, Pawloski *et al.,* 2013). Acellular vaccines against *Bordetella pertussis* were introduced in Australia in 1997. By 2000, these vaccines had replaced whole-cell vaccines. During 2008–2012, large outbreak of pertussis occurred and, in this period, 30% of *B. pertussis* isolates did not express the vaccine antigen pertactin (Prn) (Connie *et al.,* 2014). In a 20-year study of isolates in Canada (1994-2013), a prevalence of 5.4% of pertactin negative pertussis was detected, however no such isolate was found prior to 2011 and 17.8% of isolate examined in 2012 were also pertactin negative (Raymond *et al.,* 2014). In another study conducted in the united states, determining whether pertactin deficient *B.pertussis* is evading vaccine –induced immunity or altering severity of illness,85% of the isolates were pertactin negative. All the case patients positive for prn-ve *B.pertussis* in this study had at least one dose of vaccination this is in accordance with a study conducted in the United States where case patients having received at least 1 pertusis vaccine dose had higher odds of having prn-ve *B.pertussis* compared with unvaccinated case patients (Martin *et al.,* 2015).

The mechanism of loss of pertactin in this study was not determined, but similar studies done in Canada suggested that pertactin negative isolates contained a significant mutation in there pertactin gene. IS481 was found in the pertactin genes of eight isolates, while a single point mutation occurred either in the coding region (resulting in a premature stop codon) or in the promoter region (preventing gene transcription) in two other isolates (Mir-Cros *et al*., 2022). PFGE analysis also showed multiple profiles suggesting that

several independent genetic events might have led to the emergence of these pertactin-negative strains rather than expansion of a single clone (Raymond *et al.,* 2014). Insertion of IS*481* into the *prn* gene in either the forward or reverse direction was still the main mechanism of disruption. This disruption occurred at the same conserved site identified in 3 isolates from the United States (nt position 1613) (Queenan *et al.,2013*) and 9 isolates from Japan (not position 1598) (Otsuka *et al.*, 2012). In another study in australia The mechanism of lack of expression of Prn in (17%) of the isolates could not be determined at the sequence level. These findings suggest that *B. pertussis* not expressing Prn arose independently multiple times since 2008, rather than by expansion of a single Prn-negative clone (Connie *et al.*, 2014). The multiple origins of Prn-negative isolates also point strongly to selective pressure on the bacterium. Therefore, it is conceivable that these Prn-negative isolates are more likely to evade a vaccine-induced immune response. However, the relative contribution of Prn to pertussis disease has not been clearly established (Mir-Cros *et al*., 2022). Various studies using *prn* mutants have shown that mutants that do not express Prn do not colonize mouse lungs as well as isolates that express Prn (Vangent *et al.,* 2011) but were more invasive in epithelial cells and persist for a longer period (Bassinet *et al.,* 2000). The Prn-negative strains have a greater growth advantage in vitro than their Prn-positive counterparts (Otsuka *et al.,* 2012).

This growth advantage can be beneficial in maintaining a high level of transmissibility between hosts, which is consistent with increasing numbers of infections with Prn-negative isolates identified in Australia and elsewhere. Whether these isolates have greater or lesser virulence than Prn-positive strains is unclear. In contrast to lack of production of Ptx, loss of Prn does not seem to affect *B. pertussis* lethality in mice, possibly because of the range of autotransporters within *B. pertussis* that can compensate for the role of Prn (Hergerle *et al.,* 2012). In a retrospective study, no differences were found in severity of symptoms or duration of hospitalization between infants infected with Prn-positive and Prn- negative strains in France (Bodilis *et al.,* 2013); the only major difference observed was the longer period from onset of pertussis symptoms to time of hospitalization among infants whose *B. pertussis* isolate was Prn negative. Regardless of Prn expression, vaccination reduced the severity of disease and the likelihood of being admitted to intensive care, which suggests that even an incomplete course of primary vaccination provides some protection against severe pertussis (Bodilis *et al.,* 2013).

Conclusion

In conclusion, an incidence of 4.5% analyzed in this study suggests that pertussis is still a disease of concern in Kaduna state, Nigeria despite positive attitude to immunization and that the isolates obtained are PRN- *Bordetella pertussis* strain. The emergence of pertactin-negative strains is a new dimension globally which has been partially addressed and poses a serious threat to the general populace which will require newer vaccine strategies to address the issue.

Recommendation

The significant proportion of pertactin negative strains analyzed in this study indicate that adequate measures are needed to curtail the spread of *B.pertussis*. The reason behind Loss of pertactin could not be determined but is assumed to arose independently multiple times rather than expansion of a single pertactin-negative clone. As such, vaccine formulation using the indigenous strain isolated is highly recommended for better management of the condition.

Limitations of the Research

A prevalence study on pertactin-negative *Bordetella pertussis* was not conducted as this study only gave an idea and the first to detect the emergence of PRN^- *Bordetella pertussis* in Kaduna; carrying it out will aid in determining the prevalence and number of cases in Nigeria as a whole, again further studies should be done to ascertain the cause of loss of the gene.

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Conflict of Interest
All authors declared no conflict of interest towards the conduction of research and writing out the complete project of this original research article.

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