World Journal of Pharmaceutical research

Volume 1, Issue 3, 874-882. Research Article ISSN 2277 – 7105

ASSESSMENT OF DIFFERENT RESTRICTION ENDONUCLEASES: (SAL 1 AND XBA 1) ON PURIFIED CHLAMYDIA PNEUMONIA DNA

Otoikhian C.S.O.*, Okoror, L.E. Ekakitie, A.O.

Novena University, College of Natural and Applied Sciences, Department of Biological Sciences, Delta State, NIGERIA.

Article Received on
13 April 2012,

Revised on 20 May 2012,
Accepted on 28 June 2012

***Correspondence for Author:**

* **Otoikhian C.S.O**
NOVENA UNIVERSITY, COLLEGE OF NATURAL AND APPLIED SCIENCES, DEPARTMENT OF BIOLOGICAL SCIENCES, P.M.B. 2, KWALE-OGUME, DELTA STATE, NIGERIA
megabrosy2k@yahoo.co.uk

ABSTRACT

Two restriction endonucleases (Sal 1 and Xba l) where compared to ascertain if same strain of Chlamydia pneumonia that is responsible for respiratory tract infection of the patients isolated from Delta State of Nigeria. Results revealed that 95 samples (76.61%), where positive while 29 samples (23.38%) were negative using the culture method and staining with giemsa technique. The restriction endonucleases Sal l and Xba 1 cleaved the DNA at different point as evidenced on agrose gel electrophoresis and also the DNA could not be cleaved by both restriction endonucleases indicating strain variation in Chlamydia pneumonia species.

INTRODUCTION

Chlamydia pneumonia (TWAR) is a recently recognized third species of the genus Chlamydia that causes acute respiratory disease including pneumonia, bronchitis, sinusitis and phanyngitis. The organism was first isolated in 1965 from the conjunctiva of a Taiwanese child participating in a trachoma vaccine trial. The isolation was in the yolk sack of an embryonated chicken egg. The only method then available for growth of Chlamydiae. [1] Chlamydia pneumonia is a species of Chlamydia bacteria in the phylum Chlamydia in the order Chlamydiales and in the family chlamydiaceae belonging to the genus chlamydophila that infects humans and is a major cause of pneumonia has a complex life cycle and must infect another cell in order to produce and is thus classified as an obligate intracellular pathoge.[2].

The organism role as a human pathogen was not defined until 1983, when the first respiratory isolate (AR-39) was obtained in Seattle wash, from a university student with pharyngitis[3]. The isolation was accomplished because serologic evidence in our laboratory suggests that the orphan TW-183 organism was associated with pneumonia [4]. The strain name TWAR was derived from the laboratory designation of the first conjunctival and respiratory isolate (TW- 183 and AR-39).

Addition to its role in pneumonia, there is evidence associating chlamydia pneumonia with Arteriosclerosis and Asthma in addition to pneumonia less commonly causes several other illnesses among these are Meningoencephalitis, (infection and inflammation of the brain and spinal cord). Arthritis, Mycocaditis (inflammation of the heart) and Gullain – Barre syndrome (inflammation of the nerves)[5]. It has also been associated with dozen of other conditions such as Alzheimer's, Fibromyalgia, Chronic fatigue syndrome, Prostatitis and many others. Other species of Chlamydia include Chlamydia muridarum, Chlamydia trachomatis and Chlamydia psittaci[6]. Chlamydia pneumonia is a typical coccoid or rod – shaped measuring 0.5cm. They are gram negative (or at least are classified as such, they are difficult to stain but are more closely related to gram-negative bacteria) aerobic intracellular pathogen Chlamydia cannot synthesize its own Adenosine tri phosphate (ATP) and can also not be grown on an artificial medium and was once thought to be a virus[7]

AIMS AND OBJECTIVE

The aim of this research work is to compare the two restriction endonucleases (Sal 1 and Xba l) and ascertain if it is the same strain of Chlamydia pneumonia that is responsible for respiratory tract infection of the patients isolated from Delta State of Nigeria.

Therefore, this research work is designated to access different restriction endonucleases especially Sal 1 and Xba 1 on purified Chlamydia pneumonia DNA.

MATERIALS AND METHODS

SOURCES OF SAMPLES

Sputum were collected from different hospitals within Edo and Delta State 27 sputum sample were collected from General Hospital Warri, 27 sputum samples were collected from University of Benin teaching Hospital 37 sample were collected from Central Hospital Benin

and 33 sputum samples were collected from Irrua specialist teaching hospital. In total 124 sputum samples were collected.

MEDIA USED

The culture medium used for culturing Chlamydia pneumonia is a 5-10 days old embryonic egg. The embryonic eggs were incubated to allow the development of blood capillaries and embryo and were subsequently candled.

PROCEDURE FOR INNOCULATION

The samples were emulsified with PBS (phosphate buffer saline) and streptomycin was added to it before inoculation, the embryonic eggs were sterilized with formalin and a hole made to it using a sterile needle from there, 1m1 of emulsified sample was used to inoculate the yolk sac of embryonic eggs. The hole was then sealed with candle wax, before incubating at 37^0c for 10 days.

HARVESTING CHLAMYDIA PNEUMONIAE

After ten days of incubation, the cultured embryonic eggs were harvested.

PROCEDURE FOR HARVESTING

The seal of candle wax on the embryonic eggs were opened and a sterile syringe was used to harvest yolk sac fluid from the eggs then Romanawsky Geimsa Stain was used to stain the harvested samples.

PROCEDURE FOR GEIMSA STANING

Smears were made from materials collected from the sac of the embryonated eggs. The smear were allowed to dry (air drying) and fixed in absolute methanol for 1 minute. The smears were air dried again and stained with enough giemsa stain to cover the smears and left for 1 minute. Deionized water was added such that ratio of water to stain was 2:1. The stain was allowed to act on the smear for 15-20mins the slides were washed with de-ionized water gently. The slides were allowed to dry in air observed under oil immersion objective for Chlamydia and Chlamydia elementary bodies (EB)

EXTRACTIONS AND PURIFICATION OF CHALMYDIA PNEUMONIA DNA

Emppendorf tubes were sterilized in an autoclave (Prestige Series 2100) at 121^0c for 15mins. The harvested Chlamydia pneumonia samples were placed in different eppendof tubes[8].

Differential centrifugation was carried out on all samples at 3,000 RPM(Revolution Per Minute) for 5mins to remove the cell debris from the supernatant. The resulting supernatants was centrifuged at 16,000rpm for 10min to separate the DNA (pellet) from the cell and the supernatant layer was removed using a micropipette.

2.5µml of Proteinase K enzyme was pipetted into the DNA(Proteinase K was added in other to digest the cellular enzyme). 10% of SDS (sodium dodecyl sulphate) was added to the DNA To ease the extraction of pure DNA. The DNA was incubated in a water bath at 50^0c for Ihr 30mins. After incubation 50µl of buffer (phenol) was added to the Eppendorf tube containing the DNA(This process is called phenol extraction of DNA).

The content of the tube were homogenized, using a vortex mixer. The Eppendorf tube turned hundred times (100), then centrifuged for 3mins at 16, 000rpm. The DNA was pipetted out of the Eppendorf tube with aid of a micropipette.

The DNA was precipitated by the addition of equal volume of cold isopropanol and refrigerated at 4^0 C for 5mins. The tubes were centrifuged for 3mins at 16, 00rpm and redissolved in 50µml of distilled water. The contents in the tubes were homorgenised with a vortex mixer (to make the DNA visible). 5uml of Sodium Acetic acid, (NaAc), was added and 2x volume of absolute ethanol, and kept the pellet (DNA) at -20^0 c for 20mins. The contents (DNA) were centrifuged for 5mins at 16,00rpm. The supernatant layer was discarded leaving the pure DNA extracts. The DNA was rinsed with 70% ethanol. The DNA was centrifuged at 16, 16,000 rpm for 3 minutes the supernatant were then discarded. The DNA was incubated in a water bath at 37°C for 15mins to evaporate the 70% ethanol. 500µl of sterilized distilled water was added to the Eppendorf tube and votexed. The two enzymes Sal l and Xbal were dissolved.Sal 1 was dissolved in 500µl of distilled water while Xbal was dissolved in 1000µl of distilled water. 5µl of DNA was pipetted into new Eppendorf tubes. 3µl of digestion buffer were added to the different tubes. 1µl of the restriction enzyme (Xbal) and Sal1) was added to the Eppendorf tubes. Each enzyme having its own Eppendorf tubes containing the DNA. 11µl of distilled water was added to the Eppendorf tubes to make 20l of liquid content[9].

The tubes containing the DNA were centrifuged at 16,000 rpm for 1min they were incubated in a water bath containing a thermometer at 37°C for 1hr. A blue dye was pipetted onto an

aluminium foil, 15µl of each DNA containing the different enzyme were piptted separately onto the dye.

Agarose gel (Benchmate) was made and poured into an electrophoretic tank. The DNA containing the enzymes (Sal1and Xbal1) (Roche) were pipetted into the walls of the gel in the electrophoretic tank. A dye containing no enzyme and DNA was also pipetted into the walls of the agarose gel as a control. The movement was monitored on the electrophoretic tank. The agrose gel was then placed on an ultraviolet illuminator (Clifton-Scie-Plas)[10]

AGROSE GEL PREPARATION

- Mix 1 g of agrose in 100ml 1x TAE solution
- Place in a mircro-wave ovum (National) for 2mins
- Allow to cool to 50°c
- Add 30µl of Ethydium Bromide dye to 100ml of the TAE buffer.
- Pour gel into electrophoretic tank

RESULTS

From the total 124 sputum sample collected from patients attending clinic at General Hospital Warri, University of Benin Teaching Hospital (UBTH), Central Hospital Benin and Irrua Specialist Teaching Hospital Irrua screened for Chlamydia pneumonia only 95 samples (76.61%), where positive while 29 samples (23.38%) were negative using the culture method and staining with giemsa technique (Krivoshein, 1989).

Table 1a : Location of Sample Collected for the Isolation of Chlamydia pneumonia Within Edo and Delta State of Nigeria

Location of sample	No of sample collected	Positive sample for Chlamydia pneumonia	Negative sample for Chlamydia pneumonia	Percentage of positive sample	Percentage of negative sample
GHW	27	20	7	74.0	25.9%
UBTH	27	20	7	74.0	25.9%
CHBC	37	30	7	81.0%	18.95
ISTH	33	25	8	75.7%	24.2%
TOTAL	124	95	29	76.6%	23.3%

UBTH-University of Benin Teaching Hospital
CHB-Central Hospital Benin
GHW-General Hospital Warri
ISTH-Irrua Specialist Teaching Hospital

DNA was extracted and characterized using enzyme Xba l and Sal 1. The two enzymes cleaved samples at different point. Two of the isolated DNA could be elevated by any restriction endonucleaeses. From this DNA were excreted and characterized using enzyme Xba 1 and Sal1. The two enzymes cleaved samples at different point. Two of the isolated DNA could be cleaved by of the restriction endonucleases from this DNA were extracted and characterized using enzyme Xba 1 and Sal 1. The two enzymes cleaved sample at different point. Two of the isolated DNA could be elevated by any restriction endonucleases. From this result it shows that the prevalence rate of Chlamydia Pneumonia is high with percentage of 76.61%.

In GHW(General Hospital Warri) 27 sputum samples were collected from patients attending clinic after culture method and gram staining with Giemsa staining technique 20 samples came out positive and 7 samples came out negative for Chlamydia Pneumonia, which implies 74.0% of the patients from GHW came out positive with Chlamydia pneumonia while 25.9% came out negative with Chlamydia pneumonia taking a look at the percentage result it obvious that the incidence of Chlamydia pneumonia in this hospital is on the highest side. UBTH 27 sputum samples were also collected from patients in the hospital, 20 samples were found positive with Chlamydia pneumonia and 7 samples were found negative after culturing and gram staining (Geimsa). By percentage patients positive were 74.0% and 25.9% negative patients also implying that there is a high rate of Chlamydia pneumonia existence in these patients, compared to patients in GHW the incidence rate of this organisms doesn't differs. 37 samples were collected from (CHBC) Central Hospital Benin City, after the application of the culture method and gram staining technique (Geimsa) 30 samples were observed to be positive with Chlamydia pneumonia and 7 samples were seen and 18.95% were negative of Chlamydia pneumonia. It was observed that the occurrence of this organism in this clinic was at a very high rate.

In Irrua Specialist Teaching Hospital (ISTH) a total of 33 samples were collected, after which the samples were cultured and stained using Geimsa stain technique. 25 samples came out positive for Chlamydia pneumonia. And 8 came out negative of Chlamydia pneumonia, b

percentage 75.7% were positive and 24.2% came out negative. Chlamydia pneumonia came out positive at a very high rate according to the result and this implies the organisms is also at high prevalence rate. Looking at the table result its observable that the organisms rate of infection is highest in central hospital Benin city (CHBC) compared to other hospitals that samples were collected from. 81.0% were positive for Chlamydia pneumonia and 18.95% were negative this implies that the prevalence rate of Chlamydia pneumonia was highest in Central hospital Benin City (CHBC).

DISCUSSION

The study of Chlamydia pneumonia varies depending upon the criteria for diagnosis, location (endemic versus non endemic areas) and whether an epidemic is occurring at the time the study is evaluated this indicates that approximately 10% of all cases of community acquired pneumonia requiring hospitalization and associated with Chlamydia pneumonia. [11-14] this fact was established in this work looking at the tables results.

The estimated number of cases of Chlamydia pneumonia in Nigeria is 300,000 cases per year and this organism is estimated to cause 10-2-% of Community Acquired Pneumonia (CAP) cases among adults. Although Chlamydia pneumonia infections occur every year, epidemiologic studies suggest a 4 – years cycle in the incidence of Chlamydia pneumonia. This disease sis more common in males (60 – 90%) that infection, a difference is possibly due to cigarette smoking and the incidence of Chlamydia pneumonia is highest among elderly persons. Although primary infection pneumonia is more common in persons aged 7-40 years, re-infection pneumonia is more common in elderly persons. Approximately 50% of young adults and 75% of elderly persons have serologic evidence of a previous infection.

Pharyngeal erythema without exudates occurs in various a typical pneumonia; however sinus percussion tenderness is more common with Chlamydia pneumonia that with other species of pneumonia. Histological findings note that intra – alveolar inflammations with a milder degree of interstitial reaction is a characteristic pathologic findings in the lungs of patients with Chlamydia pneumonia (Alveolar – lining cells contains intracytoplasmic inclusions) [14-17]. Based on the result the incidence rate of Chlamydia pneumonia is higher in Edo state. Central hospital Benin had the highest incidence rate with a percentage of 81.0% from 37 samples collected. Irrua Specialist Teaching Hospital followed with percentage of 75.7% from 33 samples collected while the University of Benin Teaching Hospital and General hospital Warri had the same incidence rate of 74.0% each from 27 samples collected.

Chlamydia pneumonia is seen as a common cause of infection worldwide, based on the result the prevalence rate was very high in all the four hospitals. Due to the fact that Chlamydia pneumonia causes on infection at least once in a life time by 20 years of age. The restriction endonucleases Sal l and Xba 1 cleaved the DNA at different point as evidenced on agrose gel electrophoresis. Two the DNA could not be cleaved by both restriction endonucleases indicating strain variation in Chlamydia pneumonia species from our samples. According to Valassina et al., enzyme Sal 1 and Xba 1 cut at the same point on the same DNA sequence, hence the difference this study could have been due to strain variation. This means that a different strain of the organism may exist in Nigeria.

CONCLUSION AND RECOMMENDATION

From this research work it shows that Chlamydia pneumonia has a high prevalence or incidence rate in Niger Delta sregion of the country. It also shows that the restriction endonuleases Xba 1 and Sal 1 cleaved the purified Chlamydia pneumonia DNA at different points. The two Chlamydia pneumonia that could not be cleaved showed strain variation. Therefore continued work should be done specifically on the two Chlamydia pneumonia, which could not be cleaved by Xbal 1 and Sal 1 endouncleases enzyme. This work also goes to shows that there could be another strain of Chlamydia pneumonia that causes pneumonia.

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