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Streptococcus pneumoniae in patients with community- acquired pneumonia in Khartoum State: A cross-sectional Study

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Abstract:
`Knowledge of pathogens causing community-acquired pneumonia (CAP) constitutes the basis for selection of empirical antimicrobial treatment, which has a substantial impact on the prognosis of the patient. Despite the development of improved microbiological methods during the past few years, the etiology of CAP has still not been well characterized. Streptococcus pneumoniae (S. pneumoniae), is a major cause of CAP in every community. The objectives of this study were to detect S. pneumoniae in patients with pneumonia as well as to determine the prevalence of the disease in Khartoum State hospitals.

One hundred and eighty patients with symptoms of pneumonia were enrolled in this study. The patients attended different Chest Units in Khartoum State hospitals. Sputum specimen was collected from each patient. The sputa were cultured on Chocolate Agar Medium.

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Identification of S. pneumoniae was carried out using conventional microbiological methods. DNA was extracted from sputa by kits from Jena Bioscience. PCR amplification of the target sequences CpsA gene of S. pneumonia were carried out and then analyzed on agarose gel electrophoresis. Out of 180 patients enrolled in this study, 105 (58.3 %) were males and 75 (41.7%) females. The mean age of the patients was 41.9 years; the high prevalence was found in the age group 30-60 years. 20(11.1%) S. pneumoniae were recovered by traditional culture method compare with 40(22.2%) S. pneumoniae when using PCR technique. The relation between culture technique and PCR was statistically significant (P=0.000). The prevalence of CAP in males was more than females but the relationship between S. pneumoniae and gender was found insignificant (P= 0.990). There is insignificant relation between S. pneumoniae and age groups (P<0.727).

It is concluded that PCR technique is a rapid, more sensitive and specific than the conventional culture method for the detection of S.pneumoniae.

Key words: Community-acquired pneumonia (CAP), *Streptococcus pneumoniae* (*S.pneumoniae*), PCR, Sudan.

INTRODUCTION

Community acquired pneumonia (CAP) is defined as a lower respiratory tract infection that acquired from community not as nosocomial infection (Andrews *et al*., 2003). The etiology of community acquired pneumonia differs around the world however *Streptococcus pneumonia* is the most common cause in almost every community (Mandell *et al*., 2003).

Prevalence of CAP ranges from 4 to 5 million cases per year, and about 25% involve hospitalization. The disease is a significant cause of morbidity and mortality and the significance is increased due to the increased global burden of antibiotic resistance (Steel *et al*., 2013).

CAP most frequently affects individuals at extremes of age and those with any type of parallel illness (Torres *et al*., 2013). Pneumococcal community-acquired pneumonia most commonly presents as non-bacteremic disease. Invasive pneumococcal disease, which involves infection of normally sterile sites, occurs in approximately 25% of cases (Said *et al*., 2013). Conventional detection of *S. pneumoniae* is based on culture. However, culture methods are time consuming and relatively low sensitive, especially during ongoing antibiotic treatment (Morozumi *et al* 2006). Recently, polymerase chain reaction (PCR) based techniques have improved sensitivity of bacteria detection (Luo *et al*., 2012). In the present study, we concluded PCR assay for simultaneous detection of *S. pneumoniae.* The performance of the assay was evaluated using clinical sputum specimens.

MATERIALS & METHODS

Study area

The Khartoum State consists of three locality with a total population 5274321 peoples were distributed as fellows; Omdurman Locality (2215330), Al-Khartoum Locality (1582027) and Bahri Locality (1247745). The people residence in Khartoum is from different ethnic groups. From the total population of Khartoum State about 61% of the population is aged 15–64 years.

Samples collection

The specimens for this study were collected from three major Localities in Khartoum State including Al-Khartoum Locality, Bahri Locality and Omdurman Locality.

Patients: This study included **180** Sudanese patients with CAP attended the outpatient Chest Units in Khartoum State

Hospitals. The patients were both males and females. Their ages ranged from 13 to 91 years.

Specimens: Sputa specimens were collected from each patient and transferred to the laboratory under the standard conditions following the guidelines of the National Committee for Clinical Laboratories Standards (NCCLS).

Conventional culture method: Sputa were collected from each patient then cultured on bacteriological medium Chocolate blood agar (CBA). Identification of the *S. pneumoniae* was done using Optochin disc and other biochemical tests.

Nucleic acid isolation: DNA extraction was done by DNA Preparation Kit (Jena Bioscience, Germany). All specimens were extracted according to the manufacturer's instructions. Briefly 1 ml of sputum sample was transferred into a 1.5 ml micro tube, centrifuged at 15,000 rpm for 1 min and discarded the supernatant. Resuspend the pellet in 300 µl of Cell Lyses Solution. Added 1.5 µl of RNase a Solution and mix by inverting, Incubated at 37 °C for 15-30 min and cool on ice for 1 min, added 100 µl of Protein Precipitation Solution and vortex vigorously for 20-30 sec, centrifuged at 15,000 rpm for 5 min. Transferred the supernatant to a clean 1.5 ml micro tube containing 300 µl Isopropanol >99 %, mixed the sample by inverting gently for 1 min, centrifuged at 15,000 rpm for 1 min. Discarded supernatant on clean absorbent paper, added 500 µl Washing Buffer and centrifuged at 15,000 rpm for 1 min, discarded the ethanol carefully, air driers at room temperature for 10-15 min. the last step by added 50-100 µl of DNA Hydration Solution to the dried DNA pellet incubated at 65 °C for 60 min. Stored the DNA at 4 °C. For long time the samples were stored at -20°C or -70°C.

PCR: Using Maxime PCR premix kit (I-Taq) (Introgen Korea), positive control and a negative control, 2µl of DNA extract was added to PCR tube and the following solutions were placed in a total volume of 20 µl; 10X Taq buffer, 2.5 mM 4dNTP stock (final concentration 200 µmol), 10 pmol/µl primer F, 10 pmol/µl primer R, 100 ng of genomic DNA template, $MgCl_2$ (final concentration 1.5µm), H_2O (up to the total volume of 20µl) and 2.5uu Taq Polymerase. PCR amplification was performed using primers described by Luo *et al*., (2013) for *S. pneumoniae*. Cycling conditions of conventional PCR technique of *S. pneumoniae* were initial denaturation at 95°C for 5 , 30 cycles each at 95°C for 30 sec, at 54°C for 30 sec and 72°C for 30 sec followed by 5- min hold at 72°C. PCR success was examined on 1.5% agarose electrophoresis stained with Ethidium bromide.

Ethical consent: This study was approved by the College Etheical Committee. Consent was obtained verbally for each patient. All information regarding risk factors was explained to all patients under the study.

Statistical data analysis: Data were analyzed using statistical package for social studies(SPSS) software, version 11.0 for Windows (SPSS). *P* value of <.05 was considered statistically significant. chi-square ($\chi 2$) test was used to compare the differences between patient groups.

Table 1. Primer sequences used for detection of gene for *S. pneumoniae*

Species	Gene	Amplicon size (bp)	Primer sequence	Reference
S. pneumoniae	*CpsA*	653	F 5- AGTGGTAACTGCGTTAGTCCT-3 R 5- GTGGCGTTGTGGTCAAGAG-3	(Luo*et al*., 2012)

RESULTS:

Out of one hundred eighty screened patients, 105(58.3%) were males and 75(41.7%) females. Median age of the patients was 42.1 years (range 13–91 years). They categorized into four age groups. The patients were attended to four teaching hospitals in Khartoum State. Sputa were collected from patients in each hospital. Sputa specimens investigated by conventional culture method revealed 20(11.1%) positive and 160(88.9) were negative. PCR technique showed 40(22.2%) of *CPS*gene *S. pneumoniae*, the relation between culture technique and PCR statistically significant (*P*=0.000) (Table 2).

The prevalence in males was more than females but the relationship between *S. pneumoniae* and gender was found insignificant (*P*= 0.990) (Table 3). There is insignificant relation between *S. pneumoniae* and age groups (*P*<0.727) (Table 4).

Table 2. Relationship between *S. pneumoniae* PCR and culture

*S. pneumoniae*PCR		*S. pneumoniae*PCR		Total
		Positive	Negative	
Positive	Count % of Total	32 17.8%	17 4.7%	81 22.5%
Negative	Count % of Total	0 0%	279 77.5%	279 77.5%
Total	Count % of Total	32 17.8%	148 82.2%	180 100.0%

P= 0.000

Table 3. Relationship between *S. pneumoniae* and gender

Sex		*S. pneumoniae*PCR		Total
		Positive	Negative	
Male	Count % of Total	24 13.6%	84 46.9%	108 60.6%
Female	Count % of Total	16 8.9%	56 30.6%	72 39.4%
Total	Count % of Total	40 22.5%	140 77.5%	180 100.0%

P= 0.990

Table 4. Relationship between age group and *S. pneumoniae*

Age group	*S. pneumoniae*		Total
	Positive	Negative	
1-30	12 (6.7%)	44 (24.4%)	56 (31.1%)
31-60	23 (12.8%)	75 (41.6%)	98 (54.4%)
61-91	5 (2.8%)	21 (11.6%)	26 (14.4%)
Total	40 (22.2%)	140 (77.8)	180 (100.0%)

P= 0.727

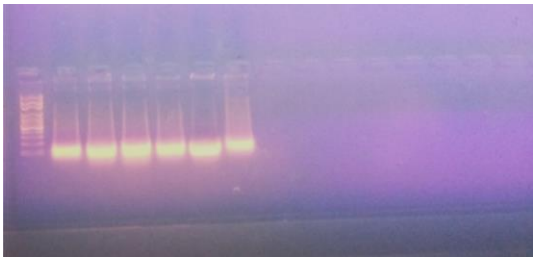

Figure1. Analysis of the extracted genomic DNA in 1.0% agarose gel with 1X TBE buffer.

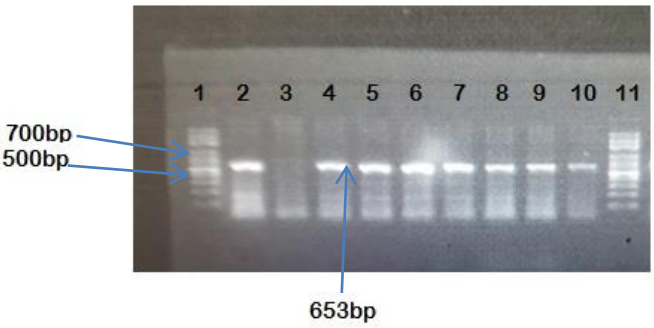

Figure 2. PCR product for CpsA gene of *S. pneumoniae* 653 bp PCR product:

1.5 % agarose gel electrophoresis of *S. pneumoniae* by PCR and they have land one and eleven M. Mw 100 – 1000 bp fragments – lane two control Positive. The pictorial showed all (7) isolates (4, 5, 6, 7, 8, 9, 10), with a band typical in size (653bp) which are positive for Cps gene, (3) negative control.

Key: First lane, Marker; second lane, Positive control, Lanes (4, 5, 6, 7, 8, 9, 10) were *Cps* gene, lane (3) negative control, lane (11) marker.

DISCUSSION

Community-acquired pneumonia (CAP) is an acute infection of the pulmonary parenchyma in a patient who has acquired the infection from the community (Feldman *et al*., 2007). The disease is a common and sometimes severe disease with an annual incidence of about 1%, and a mortality rate of 0–30% (Sharma *et al*., 2007).Molecular diagnostics hold much promise for detection of the typical and atypical bacterial pathogens that cause CAP. Analysis can be completed in hours, rather than days, for detection of typical pathogens and weeks for detection of atypical pathogens (Nolte *et al*., 2008). Sputum remains the respiratory sample of choice for isolation of the etiology of CAP, as it is well studied for the identification of both typical (Bartlett*et al*., 2004) and atypical(Kuoppa *et al*., 2002) bacteria.

The present study was designed essentially to detect *S. pneumoniae* in patients with CAP. The organism has been frequently observed in patients suffering with respiratory illness and is also reported to be related with acute exacerbation of bronchial asthma, chronic obstructive pulmonary disease (Zhang *et al*., 2011) and acute respiratory distress syndrome (Chaudhry *et al*., 2003). The PCR technique in our study revealed that 40(22.2%) of the enrolled patients were positive for *S. pneumoniae* compared with only 20(11.1%) when using traditional culture method. This result is in agreement with Kristoffer *et al*., (2006) from Denmark who reported that 50% and 76% were positive for *S. pneumoniae* when using culture and PCR methods respectively. Also our study is in line with Youning Liu *et al*., (2009) who reported that the most common pathogen isolated from patients with

CAP was *S. pneumoniae* and Niclas *et al*., (2010) from Sweden showed that *S. pneumoniae* 38%, *H.influenzae* (5%).

The present study showed insignificant relationship between *S. pneumoniae* and hospitals (*P*= 0.325). This may be due to random selection of patients from different hospitals. The prevalence of the disease in males (65%) was more than females (35%) but the relationship between *S. pneumoniae* and gender was found insignificant (*P*= 0.990). This may be due to host factors and bacterial virulence factors. There is insignificant relation between *S. pneumoniae* and age groups (*P*<0.727). This may be due to random selection of patients.PCR confirmed that 140 (77.8 %) of the samples were negative for *S. pneumoniae*. The reason of this result may be attributed to viral infections, other bacterial cause or non-infected (Hirani *et al*., 1997, Charles *et al*., 2008).

PCR technique is a rapid test for the detection of *S. pneumoniae* in sputum samples. The results can be available within few hours of specimen collection. The technique is more sensitive and specific than the conventional sputum culture and it is more useful for fastidious microorganisms. PCR detection of bacteria in respiratory samples is also problematic. In most instances, bacteria that cause pneumonia reach the lungs after colonizing the upper airways, so a positive PCR result may reflect colonization or infection (Stralin *et al*., 2008).

Further studies are recommended for the detection of the other bacterial species in sputum samples coupled with viral detection using PCR, especially for bacterial PCR-negative samples.

REFERENCE:

1- **Andrews, J; Nadjm, B; Gant, V; Shetty, N. (2003).**Community-acquired pneumonia**.** CurrOpinPulm Med. 2003; 9:175 - 80.

2- **Mandell L, Wunderink R, Anzueto A, Bartlett J, Campbell G and Dean N (2007).** Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis*; **44**: S27–S72

3- **Steel, H. C; Cockeran, R; Anderson, R; and Feldman, C. (2013).** Overview of Community-Acquired Pneumonia and the Role of Inflammatory Mechanisms in the Immunopathogenesis of Severe Pneumococcal Disease. *Med of Inflamm* v: 2013, 1 – 18.

4- **Torres, A; Peetermans, W.E; Viegi, G and Blasi, F. (2013).** Risk factors for community-acquired pneumonia in adults in Europe: a literature review. *Thorax*. (68):1057–1065.

5- **Said, M. A; Johnson, H. L andNonyane, B. A**. **(2013).** Imating the burden of pneumococcal pneumonia among adults: a systematic review and meta-analysis of diagnostic techniques. *PLoS One* 8(4): 60273.

6- **Morozumi, M. and Nakayama, S.** Simultaneous detection of pathogens in clinical samples from patients with community acquired pneumonia by real time PCR with pathogens. J clin Micro. 2006; 44: 1440 – 1446.

7- **Luo, Y. C; Du, P; Zhao, J. Z.** A multiplex touchdown PCR for detection of Streptococcus pneumoniae, Haemophilus influenzae type b and Mycobacterium tuberculosis complex in sputum samples. *Trop Bio*. 2012; 29: 422 – 428.

8- **Feldman C., A. J. Brink, G. A. Richards, G. Maartens, and E. D. Bateman (2007).** "Management of community-acquired pneumonia in adults. *South African Medical Journal*; 97: 1296–1306.

9- **Sharma, S; Maycher, B; and Eschun, G**. Radiological imaging in pneumonia: recent innovation. *Current Opinion in Pulmonary Medicine*. 2007; 13: 159 – 69.

10- **Nolte, F.S.**Molecular diagnosis for detection of bacterial and viral in community acquired pneumonia. Cliinf dis. 2008; 47: 123 – 126.

11- **Bartlett, J. G**. Diagnostic test for etiologic agents of community-acquired pneumonia. *Infect. Dis. Clin*. N. Am. 2004; 18: 809–827.

12- **Kuoppa, Y; J. Boman; L. Scott. U; Kumlin. I; Eriksson, A**. Quantitative detection of respiratory Chlamydia pneumoniae infection by real-time PCR. .*J. Clin. Microbiol*. 2002; 40: 2273–2274.

13- **Zhang L, Zong Z-Y, Liu Y-B, Ye H and Lv X-J.** PCR versus serology for diagnosing Mycoplasma pneumoniae infection: A systematic review & meta-analysis. *Indian J Med Res*. 2011; 134: 270-80.

14- **Chaudhry R, Nisar N, Malhotra P, Kumar A and Chauhan V.S.** Polymerase chain reaction confirmed Mycoplasma pneumoniae arthritis: a case report. *Indian J PatholMicrobiol* 2003; 46: 433-6.

15- **Kristoffer, S; Eva, T; Margit, S. K; Per, O; and H, H.** Etiologic diagnosis of adult bacterial pneumonia by culture and PCR applied to respiratory tract samples. *J of ClinMicrobiol*. 2006: 643–645.

16- **Liu, Y; Chen, M; Zhao, T; Wang, H; Wang, R; Cai, B; Cao, B.** Causative agent distribution and antibiotic therapy assessment among adult patients with community acquired pneumonia in Chinese urban population. *BMC Infect Dis*. 2009; 18: 9 - 31.

17- **Niclas, J; MatsKalin, A. T; Christian, G. G; and Jonas, H.** Etiology of community-acquired pneumonia: Increased microbiological yield with new diagnostic methods. *Clin Infect Dis*. 2010; 50: 202 – 9.

18- **Hirani, N. A; and Macfarlane, J. T.** Impact of management guidelines on the outcome of severe community acquired pneumonia. *Thorax*.1997; 52: 17-21.

19- **Charles, P. G. P; Whitby, M; Fuller, A. J; Stirling, R; and Wright, A.** The etiology of community-acquired pneumonia in Australia: Why penicillin plus doxycycline or a macrolide is the most appropriate therapy. *Clin Infect Dis*. 2008; 46: 1513 – 1527.

20- **Strålin K.** Usefulness of aetiological tests for guiding antibiotic therapy in community-acquired pneumonia. *Int J Anti microb Agents*. 2008; 31:3-11.