Phenotypic and Molecular Characterization of Community-acquired Bacterial Pneumonia in Khartoum State

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ABSTRACT

Community-acquired pneumonia (CAP) due to bacterial agents is a major medical problem worldwide. The disease is a leading cause of death especially in elderly and immunocompromised patients. Bacteriological methods are used traditionally for isolation and identification of the causative agents as well as molecular-based techniques. The objectives of this study were to characterize community-acquired bacterial pneumonia and to determine the prevalence of the disease among Sudanese population.

One hundred and eighty sputum specimens were collected from patients with CAP attending four leading hospitals in Khartoum State. The specimens were cultured on agar media. Identification of the causative agents was done using conventional microbiological methods. Antibiotic sensitivity test was carried out using modified Kirby-Bauer disc diffusion method. DNA was extracted from sputa by kits from Jena Bioscience. PCR amplification of the target sequences CpsA gene of *S. pneumoniae, Cap* gene of *H. influenzae* and RpoB gene of *K. pneumoniae* 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. Traditional culture methods revealed 38 (21.1%) bacterial isolates. These were *S. pneumoniae* 20 (11.1%), *K. pneumoniae* 12 (6.6%) and *H.influenzae* 6 (3.4). The genotypic investigation showed that 70 (38.8%) bacterial agents were detected. These were 45 (25.0%) *S. pneumoniae*, 15 (8.3%) *K. pneumoniae* and 10 (5.5%) *H. influenzae*.

The differences between traditional culture methods and PCR were statistically significant ($p<0.00$). The results showed that the prevalence of the disease in males is more than females, but the relation between gender and CAP was statistically insignificant ($p>0.005$). PCR is a rapid test for the detection and differentiation of community- acquired bacterial pneumonia; it is more sensitive and specific than the conventional culture method. *S. pneumoniae* and *H. influenzae*. *S. pneumoniae* are the major causative agents of CAP in Khartoum State.

Keywords: Community-acquired pneumonia (CAP), *Streptococcus pneumoniae*, *Haemphillus influenzae*, *Klebsilla pneumoniae*, PCR, Sudan.

INTRODUCTION

Community-acquired pneumonia (CAP) is one of several diseases in which individuals who have not been hospitalized develop an infection of the lungs (pneumonia) [1]. In many countries CAP is considered to be a major medical problem and potentially life-threating illness [2]. The disease is caused by different types of bacteria such as *Streptococcus pneumoniae* (*S. pneumoniae*), *Haemophilus influenzae* (*H. influnzae*), *Klebsilla pneumoniae* (*K. pneumoniae*), *Mycoplasma pneumoniae* (*M. pneumoniae*), *Chlamydia pneumoniae* (*C. pneumoniae*) and less commonly *Legionella pneumophila* (*L. pneumophila*) [3]. In children CAP is more often caused by virus and bacteria is secondary invader to a viral infection [3, 4]. In the pre antibiotic era, *S. pneumoniae* caused 95% of cases of pneumonia, and remains the most commonly identified agent [5]. Early initiation of antimicrobial therapy increases the likelihood of a good outcome depending in part on the diagnostic techniques that are used. Pathogen-directed therapy greatly decreases the cost of care and reducing the risk of developing complications [6]. Microscopic examination of pulmonary secretions may provide immediate information about possible causative organisms. Results of Gram staining and culture of sputum are positive in more than 60% of cases of pneumococcal pneumonia when a good quality of sputum specimen can be obtained before, or within 6 to 12 hours after, the initiation of antibiotics [7, 8]. In addition culture methods are time consuming and less sensitive for the isolation of the causative agents [8].

Polymerase chain reaction based diagnosis of CAP is used worldwide for rapid detection of the infectious agents [9]. The detection of specific types of bacteria in sputum samples

using PCR is a promising approach for the rapid diagnosis of the disease [10]. The method is a remarkably sensitive and type specific than other methods that used routinely for identifying respiratory pathogens [11].

In Sudan, CAP due to bacterial agents is a true health problem. Patients with CAP are diagnosed clinically, and then clinicians prescribed antibiotics for the treatment. To our knowledge no published data concerning assessment of the etiology of this disease.

This is a prospective study undertook to characterize community-acquired bacterial pneumonia among Sudanese population as well as detection of antibiotics resistant genes *TEM* & *SHV* genes in *K. pneumoniae* isolates [12]. The study was approved by the College Research Committee, Sudan University of Science and Technology.

MATERIALS AND METHODS

Microbiology: This study included 180 Sudanese patients with CAP attended Chest Unit at Khartoum North Teaching Hospital, AL-Shaab Teaching Hospital, Omdurman Teaching Hospital and Abu Anga Hospital during the period 2013 - 2015. Sputa were collected from each patient then cultured on bacteriological media Chocolate blood agar (CBA), Blood agar (BA), and MacConkey agar (MACC). Identification of the causative agents was done using Optochin disc for *S. pneumoniae*, X and V factor for *H. influenzae* and API 20E for *K. pneumoniae.* The isolated pathogens were subjected to biochemical tests and antibiotic sensitivity test using modified Kirby-Bauer Disc diffusion method.

The later was judged by the National Committee for Clinical Laboratories Standards (NCCLS).

DNA isolation: DNA was extracted from sputa using nucleic acids extraction kits (Jena Bioscience, Germany). The DNA quality was checked on agarose gel electrophoresis and the DNA yield was determined by Nanophotometer.

PCR amplification: Molecular characterization of the extracted DNA was carried out by conventional PCR technique using Maxime PCR premix kit (I-Taq). Three sets of primers were used. These were *CpsA* gene of *S. pneumoniae*, *Cap* gene of *H. influenzae* and *RpoB* gene of *K. pneumoniae* (Table1). PCR conditions were programed as follows; initial denaturation at 95°C for 5 min., followed by 30 cycles each at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. The final extension step was held at 72°C for 7 min. PCR success was examined on 1.5% agarose gel electrophoresis stained with Ethidium bromide. Data were recorded and then were analyzed using chi-square test.

Table 1. Primers sequences used for detection of genes for *S. pneumoniae, H. influenzae* and *K. pneumoniae*

Species	Gene	Amplicon size (bp)	Primer sequence	Reference
S. pneumoniae	*CpsA*	653	F 5- AGTGGTAACTGCGTTAGTCCT-3 R 5- GTGGCGTTGTGGTCAAGAG-3	[13]
H. influenzae	*Cap*	177	F 5- ATGTTAGATCGTGCGGATACTC-3 R 5- GCGAGGAACAGAACCATCAG-3	[13]
K. pneumoniae	*RpoB*	108	F 5- CAA CGG TGT GGT TAC TGA CG-3 R 5-TCT ACG AAG TGG CCG TTT TC-3	[14]

RESULTS

The one hundred eighty patients were enrolled in this study. Of them 105(58.3 %) were males and 75(41.7%) females. The mean age of the patients was 41.9 years; the high prevalence was in the age group 30-60 years. Cultivation of the specimens on an agar medium revealed that 38(21.1%) bacterial isolates were recovered. These were *S. pneumoniae* 20(11.1%), *K. pneumoniae* 12 (6.6%) and *H. influenzae* 6 (3.4). The genotypic investigations revealed that 70 (38.8%) bacterial agents were detected. These were 45 (25.0%) *S. pneumoniae*, 15 (8.3%) *K. pneumoniae* and 10 (5.5%) *H. influenzae*. The differences between traditional culture method and PCR technique

were statistically significant (p<0.00). The results showed that the prevalence of the disease in males was more than females, but the relation between gender and CAP was statistically insignificant (p>0.005).

Table 2. 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 13.1%	75 41.3%	98 54.4%
61-91	5 2.8%	21 11.6%	26 14.4%
Total	40 22.5%	140 77.5	180 100.0%

P= 0.727

Table 3. Relationship between age group and *H. influenzae*

Age group	*H. influenzae*		Total
	Positive	Negative	
1-30	6 (3.0%)	50 28.1%	56 31.1%
31-60	8 4.4%	90 50.0%	98 54.4%
61-91	3 1.7%	23 12.7%	26 14.4%
Total	17 9.2%	163 90.8%	180 100.0%

P=0.724

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

Age group	*K. pneumoniae*		Total
	Positive	Negative	
1-30	3 1.9%	53 29.2%	56 31.1%
31-60	13 7.2%	85 47.2%	98 54.4%
61-91	5 2.8%	21 11.7%	26 14.4%
Total	21 11.9%	159 88.1%	180 100.0%

P=0.041

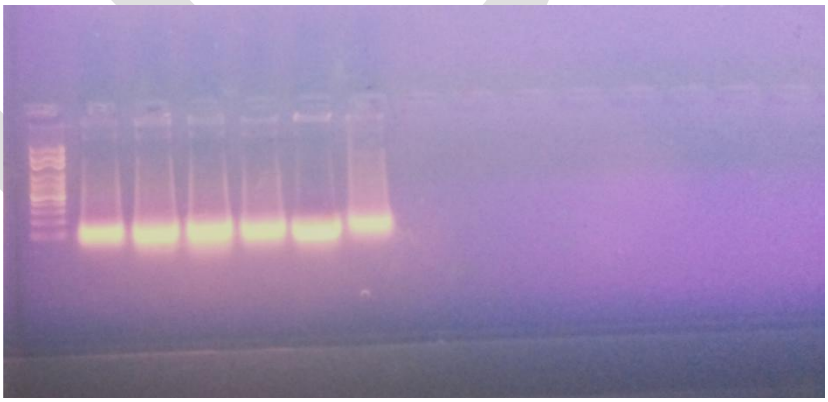

Figure1. Analysis of the extracted genomic DNA in 1.0% agarose gel with 1X TBE buffer.

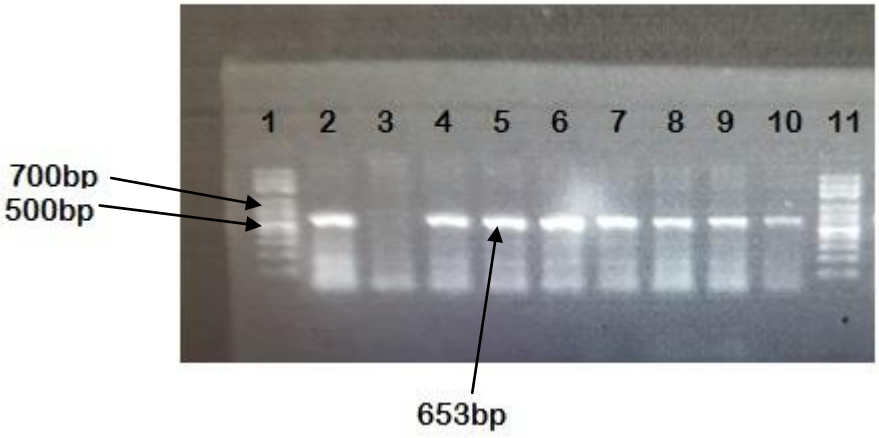

Figure 2. PCR product for CpsA gene of *S. pneumoniae* 653 bp PCR product: 1.5 % agarose gel electrophoresis of *S. pneumoniae* by PCR and they have land one and eleven M. Mw 100 – 1000 bp fragments – lane two control Positive. The pictorial showed all (7) isolates (4, 5, 6, 7, 8, 9, 10), with a band 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 a significant disease due to its high burden of morbidity, mortality, and cost of treatment and its significant problem of antibiotic resistance globally [15]. Knowledge about the epidemiology, etiology and pathogenesis of CAP is essential for patient management in order to achieve the optimum patients' outcomes [16].

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 [17] and atypical [18] bacteria.

The present study showed insignificant relationship between hospitals and CAP (*P*= 0.325). The prevalence of CAP in males was more than in females. The results showed insignificant relationship between gender and CAP (*P*= 0.211). Similarly the results showed insignificant relationship between age group and *H. influenzae*, *S. pneumonia*e (*P*= 0.724) but significant with *K. pneumoniae* (*p* = 0.041). Cultivation specimens revealed that 38 (21.1%) of the sputa were found to be positive for CAP and 142 (78.9%) were negative. The isolates were *S. pneumoniae* 20 (11.1%), *K. pneumoniae* 12 (6.6%) and *H. influenzae* 6 (3.4). The genotypic investigations revealed that 70 (38.8%) of the specimens were positive for CAP agents. These were 45 (25.0%) *S. pneumoniae*, 15 (8.3%) *K. pneumoniae* and 10 (5.5%) *H. influenzae. S. pneumoniae* was found to be the most common causative organism. This result is in consistent with other studies carried out elsewhere [19]. Youning Liu *et al*., (2009) reported that the most common pathogen isolated from patients with CAP was *S. pneumoniae*. Niclas *et al*., (2010) from Sweden showed that *S. pneumoniae* 38%, *H. influenzae* (5%). Our result confirmed their finding. The PCR method was found more sensitive than cultures. This is in agreement with Kristoffer *et al*., (2006) from Denmark who showed that the sensitivity of culture was 50% and PCR was 76%. *K. pneumoniae* was the most common etiology of CAP in Southeast Asia mostly 76% as a single pathogen. This fact was reported in 10 studies (Hara *et al*., 2011). In the present study *K. pneumoniae* account only 10 (5.5%) of the

positive specimens. This finding disagrees with Ryota *et al*., (2015) from Japan and Yi from Taiwan whom reported *K. pneumoniae* as 46% and 51% respectively. This may be due to different environmental conditions such as temperature and humidity and may be due to differences in food quality. On the other hand, PCR confirmed that 110 (61.2 %) of the samples were negative for bacterial infection. This might be of either viral infections, other bacterial cause or non-infected [15, 16].

The study results showed that *S. pneumoniae, H. influenzae and K. pneumoniae* were the major causative bacterial agents for CAP in Sudanese population. The PCR technique is a rapid test for the detection and differentiation of community acquired bacterial pneumonia in sputum samples. The results can be available within 24 hours of specimen collection. PCR is more sensitive and specific than the conventional sputum culture and it is more useful for fastidious microorganisms. 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. Further studies addressing the assessment of antibiograms and resistance genes of the bacteria causing community acquired pneumonia are also needed.

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