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Research Article

Microbial Aetiology and Risk Factors of Community-Acquired Pneumonia in Security-Challenged Communities in the Northeast Nigeria

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Abstract

Background: Community-acquired pneumonia is still a major cause of hospital admission in the sub-Saharan Africa. It is primarily due to gram positive organisms and atypical pathogens as well as gram negative organisms.

The aim of the study was to identify the common risk factors of community-acquired pneumonia and the causative pathogens of community-acquired pneumonia among adults in the Northeastern States, Nigeria.

Methods: This was a hospital-based cross-sectional analytical study conducted on adult patients seen at the general outpatient clinics and the medical outpatient clinics of a referral Teaching Hospital in Gombe, Northeast Nigeria between June 2017 and January 2018.

Hundred patients with clinical and or radiological diagnosis of community-acquired pneumonia who presented to the General Outpatient Clinic and Medical Outpatient clinic of the hospital were recruited. A chest radiograph, sputum culture and multiplex PCR analysis was conducted on all the hundred sputa.

Findings: 45 (45%) of the patients had one or more identified risk factors of CAP. Of these, HIV 22 (22%), smoking 11 (11%), alcohol consumption 2 (2%) while 7 (7%) of these patients smoked cigarette and consumed alcohol. Culture diagnosis of CAP was negative in 58 (58%) of cases. The culture positive sputa included gram positive organisms 9 (9%) and gram-negative pathogens 33 (33%). However, with PCR analysis; *Streptococcus pneumoniae* 23 (23%) and *Hemophilus influenzae* 6 (6%) were the typical pathogens detected, *Legionella pneumophila* 5 (5%), *Chlamydia pneumoniae* 3 (3%) and legionella/ chlamydia co-infection 1(1%) were the atypical pathogens identified.

Conclusion: The risk factors of pneumonia identified among patients with community-acquired pneumonia in these insurgent facing communities were immunosuppressive illness, smoking and alcohol consumption. The identified microbial aetiologies were Streptococcus pneumoniae, *Hemophilus influenzae*, *Staphylococcus aureus* and gram-negative pathogens like *Klebsiella pneumoniae*, and

Pseudomonas aeruginosa. Additionally, *atypical pathogens such as the Legionella pneumophila* and *Chlamydia pneumoniae* were also detected.

Keywords: Community-acquired pneumonia (CAP); Incidence, Risk factors; Atypical pneumonia; Aetiologic agents; Africa; North-east; Nigeria

Introduction

Pneumonia is an inflammatory infectious disease of the lung parenchyma as a result of invasion by microbial pathogens. It is often characterized by clinical and or radiological evidence of consolidation of one or more parts of the lungs and filling of the alveolar airspaces with exudate and inflammatory cells [1,2].

Globally, it is a significant cause of morbidity and mortality in adults and as well as among children especially those who live in the low- and middle-income nations [1,3]. In addition, it is the sixth leading cause of death in the United States [4]. Pneumonia remains the most common reason for adult hospitalization in sub-Saharan Africa with an estimated [4] million episodes and 200,000 deaths occurring each year [5]. Sofowara and Onadeko found a 20% death rate among Nigerians with pneumonia [6].

Pneumonia that develops in the outpatient setting or within 48 h of hospital admission is termed community-acquired pneumonia (CAP) [7]. It is also defined as an infection of the lung parenchyma with clinical and or radiologic evidence of pneumonia in persons outside of a health care facilities or in a person who has not been hospitalized in the preceding 14 days [8,9] CAP is a serious public health problem worldwide, and an important cause of morbidity and mortality in the tropics [10]. In spite of the new and potent antibiotics, mortality rate still ranges between 5-10% [11,12]. In sub-Saharan Africa, it ranks consistently high as a cause of admission in hospital based series, [13] accounting for about 10% of all adult medical hospital admissions in sub-Saharan Africa [14]. As a form of lower respiratory tract infection, it causes death in 76.2 per 100,000 people in the Sub-Saharan Africa [15]. CAP is associated with an in-hospital mortality of 6–15% among adults in Africa [16]. However, in Malawi, it accounts for a crude mortality rate of 14.6% [17].

The most common aetiologies of CAP in the outpatient setting, in descending order of frequency are as follows: *Streptococcus pneumoniae* (*S. pneumoniae), M pneumoniae, Hemophilus influenzae (H influenzae), C pneumoniae* and respiratory viruses while in the non-ICU inpatient settings, *S pneumoniae*, *M pneumoniae, C pneumoniae, H influenzae, Legionella* species and respiratory viruses as well as aspiration are known aetiologies [18]. These pathogens are classified into atypical pathogens and typical pathogens based on the ease of culturing these microbes and the ability to be detected on gram staining [19]. These typical pathogens account for about 85% of CAP cases, [20] and include *S pneumoniae, H influenzae and M catarrhalis* while the prevalence of the atypical pathogens of CAP ranges from 8% to 63% [5] and across the continents, these atypical pathogens account for 22%, 28%, 21% and 20% of patients with CAP in North America (USA and Canada), Europe, Latin America as well as Asia and Africa, respectively [6].

This study was aimed to determine the risk factors and aetiology of CAP in communities ravaged by the insurgents in the North-East, Nigeria.

Many patients have areas of gingival recession that are stable and require no specific intervention [1]. Common associated problems compromised esthetics, hypersensitivity, root caries and mucogingival problems.

Methods

Study Design

This study was a hospital-based cross-sectional analytic study conducted on adult patients who presented to the emergency room and the medical outpatient units of the Federal Teaching Hospital Gombe, North East, Nigeria between June 2017 and January 2018. The hospital is a tertiary health institution serving various communities from all the North Eastern states in Nigeria.

1. Eligible patients were those that fulfilled the criteria for clinical and or radiological criteria for community-acquired pneumonia that include the following:
    i. Presence of at least one of the major clinical criteria (cough, sputum production, fever > 37.9°C or hypothermia < 35.0° C) or two of the minor criteria (pleuritic chest pain, dyspnea, altered mental state, sign of pulmonary consolidation on examination or total leukocyte count of 12000/µl) [21].
    ii. Presence of a new pulmonary infiltrate/ shadow on chest X-ray suggestive of pneumonia at/ within 48 h of hospitalization.
    iii. Patient residing in the community.

Exclusion Criteria

1. Patients aged <18 years
2. Patients who declined consent
3. Patients exposed to antibiotics within 30 days of current illness.
4. Patients that developed symptoms of pneumonia after 48 h of hospitalization.
5. Patients with previous hospitalization for two or more days in an acute care facility within 90 days of current illness.
6. Patient diagnosed with pulmonary tuberculosis as confirmed by sputum GeneXpert.

The study was approved by the ethics committees of the hospital and a written informed consent was obtained from each patient before the study was conducted. Demographic and relevant clinical information were obtained from the patients. A thorough general and physical examination with emphasis on the respiratory system was performed and a digital axillary thermometer was used to measure and monitor the patients' temperature. A chest radiography was carried on all the patients and a quality sputum (as assessed by the Bartlett scoring system) was collected and handled according to international best practices and based on the recommendation of the Vircell speed-oligo bacterial pneumonia

combi kit manufacturer in order to ensure the safety and adequacy of the specimen. A diagnosis of community-acquired pneumonia was made based on the clinical and or radiological features as stated above while Mycobacterium tuberculosis infection was excluded with the aid of GeneXpert.

To ensure good and quality sputum, Barttlet score [22] of each sputum was done and sputum with Bartlett score less than zero was rejected. The sputa collected were transported immediately in the containers sealed at the top to the microbiology laboratory where the sputa were immediately analyzed. Firstly, GeneXpert test was conducted on the sputa, then negative test samples were then analyzed as follows;

I. Gram staining – done at a section of the lab where the reagents are arranged in the necessary order of use close to a running tap after smear fixing the sputa on a specimen slide. The slides were viewed with the oil immersion lens.

II. Some portion of the sputa was dropped in a culture disc plate and incubated for minimum of 48hrs or until growth of pathogens became obvious after which sensitivity testing were carried out. These were done in order to identify or isolate typical pathogens of CAP. Sheep blood agar, Mueller-Hinton agar, Chocolate agar and MacConkey agar were employed for this purpose.

III. The remaining portions of the sputa were stored and refrigerated at between 0^{o} -4^{o}C before sending it for PCR analysis. This molecular analysis was carried out at the clinical research laboratory of College of Medicine, University of Lagos, Nigeria.

PCR Analysis [23]

a. DNA was extracted directly from specimen samples using Norgen Genomic DNA isolation kit (product NO 24700, 2475, Canada) designed for the rapid preparation of genomic DNA from various tissue samples, cultured cells, body fluids, nasal or throat swabs and sputa.

Using protocol specifically designed for DNA extraction; from up to 150µl of sputum from frozen specimen was collected.

b. Lysate Preparation from sputum

150 µl of the sputum was transferred to a 1.5ml microfuge tube using micropipette. The volume was adjusted to 300 µl by adding digestion buffer A. The solution was vortex gently and incubated for 5 min at room temperature. This was mixed, then 12µl of proteinase K was added to the suspension. This was also mixed by gentle vortexing with subsequent incubation at 55 ^{o}C for 1 h. This was followed by adding 300µl of buffer SK to lysate and subsequently vortexing the mixture. Additional 300 µl of 98% ethanol was added, mixed by vortexing.

Exactly 600 µl of the lysate solution in spin column assembly was centrifuged in a micro-centrifuge at 5,200xg (8000RPM) for 3 min after which, the flow-through was discarded and the spin column reassembled with its collection tube. This process was repeated to ensure that all lysate mixture has passed through the

column to allow the DNA to bind to the column.

Then about 500 µl DNA wash buffer, was added into the spin column in a new collection tube and centrifuged at 14,000xg for 1 min. This was followed by addition of 500µl DNA wash buffer into the spin column and centrifuged at 14,000xg for 2 min. The spin column was transferred into a clean 1.7ml micro-centrifuge tube and 200µl of elution buffer B was added directly to the centre of the resin bed. This was later centrifuged at 3000xg (in 6000RPM) for 1 minute to elute the DNA. Then, this was further centrifuged at 14,000xg (in 14000RPM) for an additional 2 min to collect the total elution volume. The purified genomic DNA was stored at 2-8 °C prior to amplification

DNA Amplification

Seeplex Pneumobacter ACE detection kit (v 3.0, cat no PB16104) designed for the simultaneous detection of six targets pneumonia causing pathogens: *Streptococcus pneumoniae, Bordetella pertussis, Mycoplasma pneumoniae, Hemophilus influenzae, Chlamydia pneumoniae and Legionella pneumophila* was used for the DNA amplification.

An Aliquot of 3 µl of template DNA was added to 17 µl of PCR mastermix (that is made up of: 4 µl 5x pneumobacter ACE primer, 3 µl methoxysoralen (8-MOP) solution and 10µl 2x multiplex mastermix) giving a total volume of 20 µl. The mixture was subjected to amplification for 40 PCR cycles. The program was set at an initial temperature of 95 °C for 15 min, followed by annealing at 60°C for 30 sec and 1.5 min for elongation at 72°C. The last lap of the elongation process was extended to 10 minutes at 72°C.

Gel Electrophoresis/Analysis

The amplicon obtained from the multiplex PCR were analyzed by agarose gel electrophoresis containing 2% agarose and 0.5 x TBE. 5 µl of the amplicon and same DNA marker were loaded on gel walls respectively and subjected to a gel electrophoresis at 150v for 45 min. The size of the amplification was situated by comparing it with a standard ladder of 100bp molecular size. 0.5 µl/ml of ethidium bromide stain was added to the gel to aid visualization of the amplicons. This was visualized under an ultra-violent trans-illuminator (EDVOTEK USA).

Result/Interpretation of Gel Images

As specified by the manufacturer, the amplicon sizes characteristics of each of the organisms are as follows: *S. pneumoniae* 349, *B. pertussis* 230, *M. Pneumoniae* 583, *H. influenzae* 259, *C pneumonia* 154 and *L. pneumophila* 472 base pairs (bp) respectively. Hence gel images were compared with typical result preview as shown below provided by the manufacturer and results interpreted as follows: All positive bands corresponding to expected band sizes for each organism were recorded as positive while no corresponding bands recorded as negative. Internal control bands were also observed for all the runs.

All these reactions were carried out in A|E Thermocycler model CY 005 UK. Every run of the amplification reaction included an internal control, a negative control and a positive control (composed of plasmids of the 6 pneumonia causing pathogens as DNA ladder).

Data Analysis

Data collected were transferred into Microsoft excel spreadsheet then exported into SPSS version 20 (statistical package for social sciences Chicago Ilinois). Continuous variables were expressed as means and standard deviation (S.D), while categorical data were expressed as proportions. Chi square test (χ^2) was used to compare proportions, to determine association between two categorical variables and Fischer exact test was used in cells variables less than five. Independent t-test and ANOVA analysis were done to determine statistical significance between means among two or more groups. In all cases, p value of <0.05 or likelihood of <0.05 was considered statistically significant.

Results

4.2 Socio-demographic Characteristics of study subjects

Out of the hundred patients who fulfilled the inclusion criteria, 57 (57%) of the patients were males while 43 (43%) were females with a male: female ratio of 1.32: 1. The mean age of the patients was 48.91 ± 14.51 years and their ages ranged from 22 – 76 years.

Table 1: Distribution of subjects by age and gender.

Age (years)	Gender		Total N (%)
	Male n (%)	Female n (%)	
< 20	0 (0)	0 (0)	0 (0)
20 – 40	18 (18)	15 (15)	33 (33)
41 – 60	24 (24)	17 (17)	41 (41)
61 – 80	15 (15)	11 (11)	26 (26)
Total	**57 (57)**	**43 (43)**	**100 (100)**

The age and gender distribution of the study is depicted in Table 1. The mean ages of both the males and the females were 49.79 ± 14.86 years and 47.74 ± 14.13 years respectively (t=0.696, p=0.488).

Age group with the highest proportion was 41-60 years 41% while age group 61-80 had the lowest proportion (26%).

Risk Factors

Fifty-five (55%) of the total studied subjects had no identifiable risk factors, 18 (18%) of the subjects smoked cigarettes, 9 (9%) took alcohol before and or during the study period, 22 (22%) had been diagnosed of HIV before and or at the onset of symptoms, while 1 (1%) each had hepatitis C and malignancy. This is shown in Table 2.

Table 2: Risk factors for community-acquired pneumonia.

Risk factors	Gender		Total N (%)
	Male n (%)	Female n (%)	
Smoking	11 (11)	0 (0)	11 (11)
Alcohol	2 (2)	0 (0)	2 (2)
Steroids	0 (0)	1 (1)	1 (1)
Smoking/ Alcohol	7 (7)	0 (0)	7 (7)
HIV	9 (9)	13 (13)	22 (22)
None	27 (27)	28 (28)	55 (55)
Others	1 (1)	1 (1)	2 (2)
Total	**57 (57)**	**43 (43)**	**100 (100)**

X^2 = 22.221, p = 0.002

[x]Others include hepatitis C virus infection and Malignancy.

Microbial aetiology of community-acquired pneumonia

Typical pathogens of community-acquired pneumonia tested multiplex PCR kit

isolated 29 (29%) typical pathogens of community-acquired pneumonia. These include *Streptococcus pneumoniae* 23 (23%), and *Hemophilus influenzae* 6(6%). None of the samples isolated *Bordetella pertussis* as shown in Table 3. Two samples had *Streptococcus pneumoniae* and *Legionella pneumophila* confection, 3 (3%) had *Streptococcus pneumoniae* and *Hemophilus influenzae* co-infections.

Atypical pathogens accounted for a total of 9% of the total pathogens of community-acquired pneumonia. They comprise *Legionella pneumophila* 6(6%), *Chlamydia pneumoniae* 3(3%). No case of *Mycoplasma pneumoniae* was identified. Also,1(1%) of the patients had *Legionella pneumophila* and *Chlamydia pneumoniae* coinfection. This is also displayed in Table 3.

Table 3: Microbial aetiology of community-acquired pneumonia.

Organisms	Gender		Total N (%)
	Male n (%)	Female n (%)	
Typical			
Streptococcus	15 (15)	8 (8)	23 (23)
H. influenzae	5 (5)	1 (1)	6 (6)
	20 (20)	**9 (9)**	**29 (29)**
Atypical			
Legionella	4 (4)	2 (2)	6 (6)
Chlamydia	2 (2)	1 (1)	3 (3)
	6 (6)	**3 (3)**	**9 (9)**
Negative	31 (31)	31 (31)	62 (62)
Total	**57 (57)**	**43 (43)**	**100 (100)**

X^2 = 12.948, p = 0.073

Prevalence of Community-Acquired Pneumonia by sputum culture

Sputum culture identified some microbes (typical pathogens) of community-acquired pneumonia. Aetiological diagnoses were made in 42(42%) samples, 58 (58%) sputa had negative culture. This is shown in fig 1. Of the 42 (42%) typical pathogens identified; gram negative organisms were isolated in 23 (23%), *Streptococcus pneumoniae, Staphylococcus aureus* and *Moraxella catarrhalis* were identified in 2 (2%), 7 (7%) and 10 (10%) respectively as displayed in Table 4.

Figure 1. Culture diagnosis of community-acquired pneumonia.

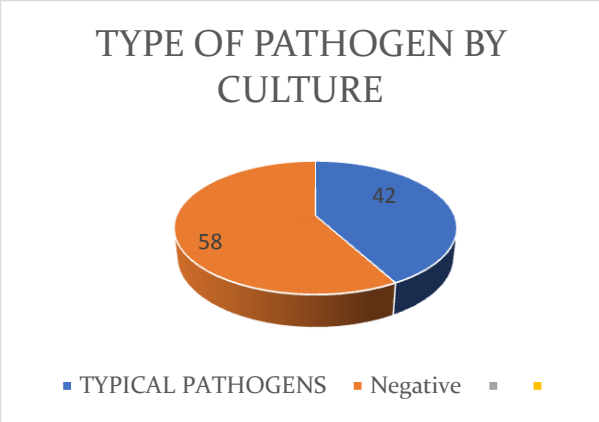

Table 4: Prevalence of typical pathogens of CAP with sputum culture.

Organisms	Frequency	Percentage (%)
Streptococcus pneumoniae	2	2
Staphylococcus aureus	7	7
Klebsiella pneumoniae	15	15
Pseudomonas aeruginosa	4	4
Moraxella catarrhalis	10	10
Proteus species	2	2
Klebsiella and ***Pseudomonas***	1	1
None	58	58
Total	**100**	**100**

Discussion

A positive culture for the diagnosis of CAP has been reported to range from 50-97% in several studies [24] while risk factors of CAP have been reported in about 70% of patients according to Sinata Koulla -Shiro et al. [25].

An aetiological diagnosis of CAP was made by the sputum culture in (42%) of the total sputum collected while negative sputum culture was obtained in 58% of the total sputum cultured. This positive culture diagnosis reported in this study was similar to what was documented by Ibrahim et al. [26] Belayneh Regasa, [27] and Almeral J et al. [28] but slightly lower than 52% reported by Sinata Koulla-Shiro et al. [26]. Gram positive organisms accounted for 9% of the total pathogens of CAP (*Streptococcus* 2%, *Staphylococcus aureus* 7%) while gram negative pathogens were responsible for 33% of the infection. The low detection rate of *Streptococcus pneumoniae* may be due to antibiotic use prior to hospital visit and fastidious nature of this microbe.

Staphylococuus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and proteus species in sputum culture are documented causes of CAP as was reported in a study by Yunusa et al. [29,30].

Diagnosis of atypical pathogens of community-acquired pneumonia with the aid of a multiplex PCR found 9 (9%) of the 100 samples to be positive for atypical pathogens. Of these; 6 (6%) was *Legionella pneumophila*, 3 (3%) was *Chlamydia pneumoniae*, and 1 (1%) was *Legionella and Chlamydia* co-infection. This was similar to 2.5% reported for each of *Chlamydia* and *Legionella* by Yun-Fong Ngeow et al. [29] using similar molecular investigative tool, but had higher detection rate for mycoplasma of 8.7%. This difference in mycoplasma detection rate may be due to seasonality of mycoplasma infection. However, with PCR, the detection rate of *Streptococcus pneumoniae* rose to 23 (23%) comparable to the 21.4% reported by Aston SJ et al. using similar investigative tool [17]. Additionally, *Hemophilus influenzae* was detected in 6 (6%) of the patients. This may be due to the higher sensitivity rate of PCR for detection of these pathogens.

Identified risk factors in this study include smoking (18%), alcohol (9%), steroid use (1%) and in about 55% of the study subjects, no risk factor was identified. This was quite lower to what Sinata Koulla-Shiro reported in Cameroon, where he documented 46.2% of his study subjects took alcohol, 41.8% were smokers and in about 29.7% risk factors were not identified [25]. These differences may be explained by differences in cultural practices and religious beliefs as practiced in the Northern part of Nigeria.

Conclusion

The etiological diagnosis of community-acquired pneumonia in this study was 42% and the pathogens identified with culture and or PCR were *Streptococcus pneumoniae*, *H. influenzae*, gram -negative organisms. Additionally, *Legionella pneumophila* and Chlamydia pneumoniae were the atypical pathogens of community-acquired pneumonia detected among patients in the Northeastern part of Nigeria. HIV, smoking and alcohol ingestion were the commonest risk factors of community-acquired pneumonia identified in this study.

Furthermore, this study also showed that etiological diagnosis of community-acquired pneumonia may still be a daunting task in the developing nations as many of these pathogens are fastidious. However, with the high sensitivity and specificity rate of molecular methods, detection rate of these microbes will be increased using this method of diagnosis.

Consent

Written Informed consent was obtained from all participants in the study.

Ethical Approval

The design of the study was approved by the Research and Ethics Committee of the Federal Teaching Hospital, Gombe.

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Competing Interests

None declared.

Authors' Contributions

This work was carried out in collaboration between all authors. Authors GOO, FN, YAA, MWA designed the study. Author GOO performed the project work, analyzed, interpreted the data and wrote the first draft of the manuscript. Authors designed, analyzed and interpreted the data, also contributor in writing the manuscript. All authors read and approved the final manuscript.

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