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Prevalence of *Streptococcus pneumoniae* in Pneumonia Patients that Attend Madonna Catholic Hospital Umuahia, Abia State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author IUN designed the study wrote the protocol. Authors IUN and KCE wrote the first draft of the manuscript. Authors CNN and MOI managed the collection of data and analyses of the data used in the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the prevalence of *Streptococcus pnenumoniae* in pnenumonia patients attending Madonna Catholic Hospital, Umuahia; Abia State.
Methods: The study was a cross-sectional study on the prevalence of *Streptococcus pnenumoniae* among patients in Madonna Catholic Hospital, Umuahia. It lasted for a period of three months (September 2019 to January 2020). Standard microbiological techniques were used to evaluate 60 sputum samples collected from pneumonia patients and the modified Kirby-Buar disk diffusion technique was used to test the sensitive pattern of the isolates to some antibiotics.
Results: A total of thirty-three (33) *Streptococcus pneumoniae* isolates were recovered from sixty (60) sputum samples from pneumonia patients. 17(51%) were gotten from male and 16 (49%) of isolates were gotten from female. The highest frequency of occurrence among the age groups was observed with adults (19-59 years) (34.4%) followed by adolescence (13-18 years) (33.3%), while

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the least frequency was from those of senior adults (≥60) (9.1%). However, the antibiotic susceptibility pattern of the *Streptococcus pneumoniae* isolates from the sputum samples shows varying degrees of sensitivity and resistance to the antibiotics. From the study, Streptomycin and Cotrimoxazole showed a high percentage of sensitivity against *Streptococcus pneumoniae* isolates at 78.8% and 72.7% respectively. The highest percentage of resistance was observed with Gentamicin and Tetracycline at 42.4% each.

Conclusion: This study highlights that there was no distinct variation in occurrence of *Streptococcus pneumoniae* in relation to gender and age. As most patients were hospitalized in separated wards, this suggests a role for local dissemination of this bacterium in the respective wards rather than age or gender specific predilection. This study also suggests that streptomycin and Cotrimoxazole could be a drug of choice in the treatment of pneumonia.

Keywords: Streptococcus pneumonia; prevalence; pneumonia; patients; hospital.

1. INTRODUCTION

Pneumonia refers to a pathogen-initiated acute inflammation of the lower respiratory trait, characterized by inflammation of the lung parenchyma [1]. It is a leading cause of death among children worldwide; however, in developing countries the greatest burden of the disease is among the under - 5 years of age [1]. Pneumonia can be classified as either community acquired when the presumed pathogen is acquired outside the health facility or health care associated-when the antecedents of the disease and etiological agents can be traced to a health facility or hospital [2].

Pneumonia is a common lung infection that affects millions of people worldwide [3]. Worldwide infection rates are highest in developing nations, particularly in countries in Southeast Asia and Africa [4,5]. Children are particularly susceptible. Pneumonia occur throughout the year but are more prevalent during the colder months, presumably disease direct transmission of infected droplets is enhanced by indoor crowding [6].

A large number of microorganisms have been implicated as etiologic agents of pneumonia. The agent commonly responsible vary according to age and the setting in which the infection is acquired [7]. The spectrum of possible pathogens of acute pneumonia varies widely. Lung aspirate studies from several countries have shown that bacterial agents account for over 60% of Pneumonias in the developing world [8]. Fungal agents like *candida, Aspergillus, Cryptococcus, Histoplasma, Nocardia* species and *Pneumocystis jiroveci* also account for a significant proportion of non-bacterial Pneumonia in the immune compromised host [9]. The etiological importance of viral pathogens, sometimes as multiple agents was highlighted in some earlier reports emanating from Asia and Sub-Sahara in Africa [10]. With respiratory syncytial virus (RSV), and parainfluenza (PIV) constituting the top two, the spectrum of the viral agents and their aetiological ranking appeared comparable to those reported by workers in the developed world [3]. Important bacterial causes of Pneumonia include *Streptococcus pneumoniae*, *Haemophilus influenza* type b, nontypeable *Haemophilus Influenza, Moraxella catarrhalis*, *Staphylococcus aureus*, *Streptococcus* pyogenes, and atypical bacteria [11]. In prospective, microbiology-based studies, their leading bacterial cause is pneumococcal, being identified in 30-50% of pneumonia cases [12]. The second most common organism isolated in most studies is *H. influenza* type b (Hib; 10-30% of cases), followed by *S. aureus* and *Klebsiella pneumoniae*. In addition, lung aspirate studies have identified a significant fraction of acute pneumonia cases to be due to *Mycobacterium tuberculosis*, which is notoriously difficult to identify in children [2]. Controversy surrounds the role of three important organisms non typeable *H. influenza* (NTHI), *S. aureus* and non typhoid *Salmonellas specie* [2].

Determining the bacterial etiology of pneumonia is a challenge, since access to the infection site (lung tissue) is complex and samples are difficult to collect. Samples from a sterile site are the “gold standard” in the diagnosis of invasive disease, but airway samples are more easily obtained in nonsterile sites [13,14]. A database should be established to support decisions making in terms of sample collection, considering the broad range of bodily fluid and tissue samples that could yield relevant data, the available clinical and laboratory resources in developing countries, patients safety, case control studies and pathogen identification [13].

Laboratory diagnosis is the corner stone of any study on the etiology of pneumonia. The routine laboratory evaluation of pneumonia patients still depends on methods that have been used for decades. Microscopy of lower respiratory tract (LRT) samples, blood cultures, antigen detection in urine and respiratory samples, and the detection of specific antibiotics in blood (serology), [15,3]. Nucleic acid detection methods, such as the polymerase chain reaction (PCR) have been available for over 2 decades and are now standard tools in tertiary level diagnostic laboratory [15].

Pneumonia accounts for approximately one-fifth (19%) of the two million deaths with 90% of these occurring in the developing world; 50% of these deaths occur in Africa alone. The explanation for the disproportionately higher share of the global mortality burden of pneumonia in Africa has been linked partly on the reportedly higher incidence of bacterial etiology of the disease and as suggested by the bindings of the comprehensive study, the African regional disease severity may also be associated with the possible role of multiple pathogens [2]. *Streptococcus pneumoniae* accounts for over 60% of bacterial pneumonia of adults who required hospitalization [16]. *S. pneumoniae* is a gram positive diplococcus with thick capsule, which contributes to the organism's virulence. Incubation period is about 1-3 days. The typical symptoms include cough, fever, chest pain and sputum production. Among the factors that predisposes to *S. pneumoniae* infections are: immune suppression, alcoholism, smoking, influenza, chronic disease of the lung or heart etc. Pneumonia develops when *S. pneumonia* is inhaled into the alveoli of a susceptible host, multiply rapidly and cause an inflammatory response. When this happens, fibrous edema fluid and phagocytic cells streams into the air sacs of the lung, causing difficulty in breathing and sputum production. The increase in fluid produces abnormal shadows on x-ray films on the chest in patients [17,18]. Surveillance data on *S. pneumoniae* infection are few if available for health care provider in Umuahia and its environs. Hence, the relevance of accurate and current data on the infection trend of *Streptococcus pneumoniae* cannot be over emphasized. This study was therefore designed to determine the prevalence of *Streptococcus pneumoniae* in pneumonia patients attending Madonna Catholic hospital Umuahia, Abia State. Nigeria and also to determine the antibiotic sensitivity pattern of the *S. pneumoniae* isolate recovered from the pneumonia patients.

2. METHODS AND MATERIALS

Study Location: This study was carried out in Umuahia, Abia State, Nigeria with focal point on different patients (male and female), with pneumonia infection, attending Madonna Catholic Hospital Umuahia.

Duration of Study: The study lasted for a period of 3 months (Sept. 2019-Jan. 2020).

Study Design: The study was a cross-sectional study on the prevalence of *S. pneumoniae* among pneumonia patients in Madonna Catholic Hospitals Umuahia.

Sample Size: A total of 60 samples from pneumonia patients attending Madonna Catholic Hospital were collected for the study. Samples were collected from all the pneumonia patients irrespective of age and sex, admitted in the hospital within the period of the study. Seven (7) samples were gotten from children, 20 from adolescence, 30 from adults and3 from senior adults.

2.1 Specimen Collection

Sputum samples were collected using disposable wide-mouth, screw-cap, leak-proof sputum container. All the participants were instructed to properly rinse their mouth before producing the sputum, and then to inhale deeply 2-3, times cough up deeply from the chest and spit in the sputum container by bringing it close to the mouth. This was done in a well-ventilated environment, very close to the window for biosafety purpose. Adequate precaution was taken to avoid the spread of aerosols and contamination of the outside of the container with sputum. When spillage occurred, participants were instructed to properly clean up outside of the container with the tissue paper soaked in phenol containing disinfectant provided before submission of the specimen was made.

Lastly, each specimen was labeled accordingly with the patient identify number. Salivary samples were rejected as they are unsuitable for microbiological investigations. And due to the fragility of *S. pneumoniae* purulent part of the sputum was transferred to a cotton wool swab and place inside Aimes transport medium delivered promptly to the laboratory and cultured

without any delay. The Aimes transport medium supports *S. pneumoniae* (if present) to survive while restricting avoid over growth of fast multiplying commensals.

2.2 Microbiological Analysis of Sputum Sample

Sputum macroscopy: The sputum specimens were examined and the physical appearance which involves observing whether sputum is purulent, mucopurulent, mucoid, mucosalivary or bloody), were noted.

Sputum Microscopy: Gram stained sputum smears were examined microscopically for the detection of *S. pneumoniae* as described by Cheesbrough [19]. If *S. pneumoniae* was present appeared as gram positive dipolocci.

2.3 Sputum Culture

Using a sterile applicator stick, each sputum sample was inoculated on a chocolate agar and MacConkey agar (Hi-media, India) as described by Joon et al. [20]. The chocolate agar plates were incubated as 37^0C for 24 hours in a candle jar with a piece of wet, sterile cotton placed in it to provide a 5-10% Co_2 atmosphere and humid environment. Also an optochin disc (5µg) (Oxoid, UK) was placed on the inoculated blood agar and incubated aerobically. This help to provide rapid presumptive identification of *S. pneumoniae* which is sensitive to optochin (ethylhydrocupreiene hydrochloride). The zone of inhibition was at least 14mm. Suspected colonies with zone of inhibition less than 14mm were later tested for bile solubility before been discarded.

2.4 Characterization of Isolates

The morphological appearance of the bacterial colonies were examined based on size, colour, opacity, surface, shape consistency, haemolysis, edge, elevation, and pigmentation. Suspected isolates of *S. pneumoniae* were characterized using standard biochemical tests including catalase test. Bile solubility test and optochin sensitivity test as described by Cheesbrough [19]. The other three bacteria were identified thus: *S. aureus*: Pinkish raised colonies on MacConkey agar, Cocci in cluster, catalase (+), Coagulase (+); *K. pneumoniae:* Mucoid colonies with a vedic consistency on MacConkey agar, Methyl red (-), voges proskauer (-), citrate (+); S. *pyogenes*: catalase (+) pyrrolidonyl (PRY) test.

2.5 Determination of the Antibiotic Sensitivity Pattern of *S. pneumoniae* Isolates

The modified Kirby-Bauer disc diffusion technique as described by Cheesbrough [19] and Joon et al. [21] was used to determine the sensitivity pattern of *S. pneumoniae* isolates to some antibiotics. The zones sizes of each antibiotic were interpreted with the aid of standard interpretative chart and the isolate reported as either resistant or susceptible.

2.6 Operational Definition

Sensitivity: The quality of reacting quickly or more than usual to drugs (Antibiotics).
Resistance: The power not to be affected by drugs (Antibiotics).

2.7 Data Analysis

Statistical analysis was carried out using Statistics Package for Social Science (SPSS) (version 18.0). One-way analysis of variance (ANOVA) and Turkey-Kramer multiple comparison tests was used to compare the prevalence of *S. pneumoniae* among pneumonia that attend Madonna Catholic Hospital Umuahia, Abia State according to their gender and age characteristics. P-value $\leq$ 0.05 was considered statically significant.

3. RESULTS

A total of sixty (60) samples were collected from 60 patients with signs and symptoms of pneumonia.

Table 1 shows the frequency of occurrence of bacterial species associated with pneumonia. A total of 60 isolates of different bacterial species were obtained of which *Streptococcus pneumoniae* had the highest frequency of occurrence 33(55%). It was followed by *Klebsiella pneumoniae* 19 (15%) and *Streptococcus pyogenes* and *Staphylococcus aureus* 4(6.7%) respectively.

Table 2 presents the frequency of occurrence of *Streptococcus pneumoniae* in relation to age group of patients examined. *Streptococcus pneumoniae* was mostly isolated from adults (19-59 years) (36.4%), followed by adolescence (13-18 years) (33.3%). The isolation rate from children (1-12 years and senior adults ($\geq$60 years) are 21.1% and 9.1% respectively. There was no significant difference in the rate at which

Streptococcus pneumoniae was isolated from the different age groups ($p \geq 0.05$).

Fig. 1 depicts the frequency of occurrence of *Streptococcus pneumoniae* in relation to gender. Out of the 33 isolates, 52 % of it were gotten from male patients while 48% were isolated from female patients. There was no significant difference in the rate of isolation of *S. pneumoniae* among gender ($p \geq 0.05$).

The antibiotic sensitivity pattern of the *Streptococcus pneumoniae* recovered from the sputum of pneumonia patients is presented in Fig. 2. 1.63.6%, 66.7%, 69.7%, 57.6%, 60.6%, 57.6% of the recovered *Streptococcus pneumoniae* were sensitive to ciprofloxacin, erythromycin, cotrimoxazole, streptomycin, amoxicilin, gentamicin, penicillin and tetracycline respectively while 36.4%, 33.3%, 27.3% 21.2%, 30.3%, 42.4%, 39.4% and 42.4% of the isolates were resistant to ciprofloxacin, erythromycin, cotrimoxazole, streptomycin, amoxicillin, gentamicin, penicillin and tetracycline respectively.

4. DISCUSSION

The community acquired pneumonia (CAP) caused by the bacterium *Streptococcus pneumoniae* is one of the most lethal public health problem in developing countries including Nigeria. It is widely reported that carriage of *S. pneumoniae* is a precursor for developing any invasive pneumococcal disease [22]. From this study, four species of bacteria were isolated namely, *Streptococcus pneumoniae, Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes*. Among these four species of bacteria, *Streptococcus pneumoniae* had the highest preponderance (55%) followed by *Klesiella pneumoniae*. This finding is contrary to the findings of Jokinen *et al* from Eastern Finland [23], who reported *Klebsiella pneumoniae* (35) to be predominant in pneumonia samples than *Streptococcus pneumoniae* (12). Also, in a study carried in China by Fan et al. [24] where they analysed the distribution and drug sensitivity of pathogens in severe community acquired pneumonia, *Klebsiella pneumoniae* was found to be of higher percentage than *Streptococcus pneumoniae.*

Furthermore, this study reveals that 36.4% adults (19-59 yrs.), had the highest preponderance followed by 33.3% adolescence (13-18 yrs.), 21.2% children (1-12 yrs.) and 9.1% senior adults ($\geq$ 60 yrs.). Reports made by Holter et al [25] and Feikin et al. [26] says that *Streptococcus pneumoniae* is among the most important etiologies of hospitalized community- acquired *pneumonia* among adults.

Table 1. Frequency of occurrence of bacterial species associated with pneumonia

Gender	Age	No. Examined	St.pn.	K.pn.	S.a	St.py
Male	1-10	6	3	2	1	-
	11-22	7	5	1	1	-
	23-32	5	2	2	-	1
	33-43	5	2	2	1	-
	44-54	3	2	1	-	-
	≥ 55	4	3	1	-	-
Total		30	17	9	3	1
Female	1-10	5	4	1	-	-
	11-22	8	6	1	-	1
	23-32	6	2	2	1	1
	33-43	5	2	3	-	-
	44-54	3	1	2	-	-
	≥ 55	3	1	1	-	1
Total		30	16	10	1	3
Ground Total N(%)		60	33(55)	19(15)	4(6.7)	4(6.7)

Key: St.pn = Streptococcus pneumoniae, k.pn= Klebsiella pneumonia; S.a = Staphylococcus aureus, St.py= Streptococcus pyogenes

Table 2. Frequency of Occurrence of *Streptococcus pneumoniae* in relation to age

Age	No. of Patients with Pneumonia	No of Patients with *S. pneumoniae* infection	Percentage of *S. pneumoniae* infection
1-2 yrs (Children)	7	7	21.2
13-18yrs (Adolescence)	20	11	33.3
19-59 yrs (Adults)	30	12	36.4
≥60yrs (Senior Adults)	3	3	9.1
Total	60	33	100

P-value = 0.377. P-value <0.05 is considered statistically significant

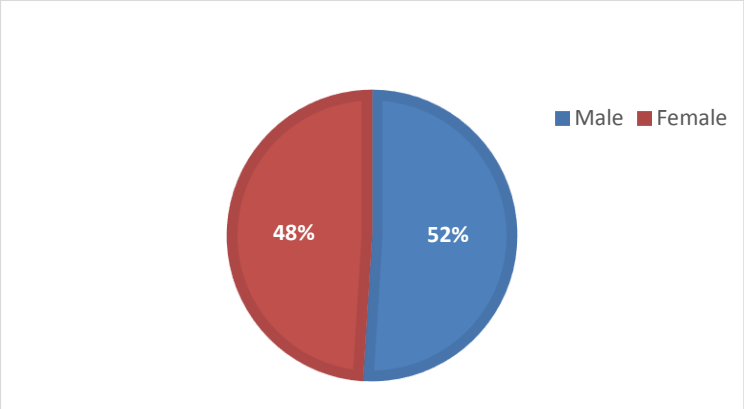

Fig. 1. Frequency of Occurrence of *S. pneumoniae* in relation to gender

p-value = 0.188. p-value <0.05 is considered statistically significant

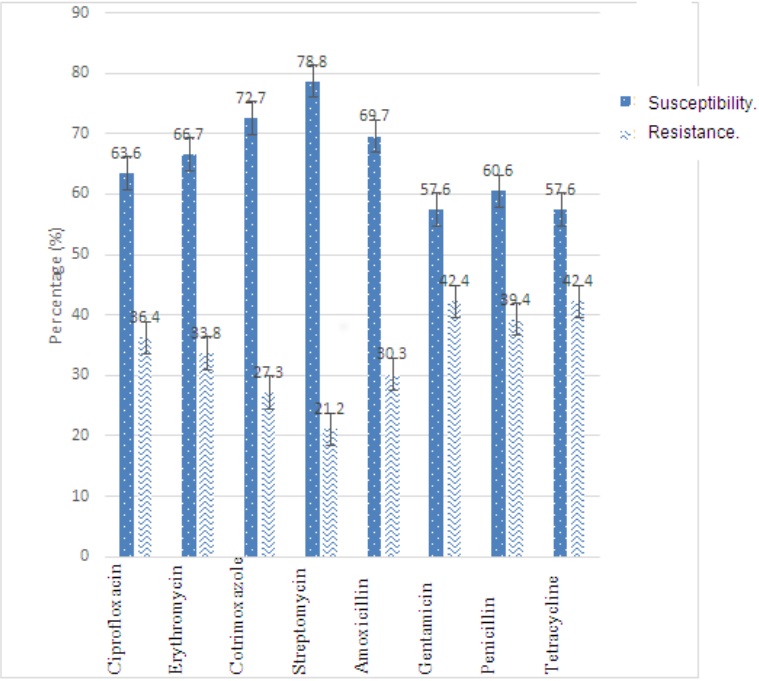

Fig. 2. Antibiotic Sensitivity pattern of the recovered *S. pneumonia*

However, there was no significant difference in the rate of isolating of *S. pneumoniae* from either male or female patients (p≥, 0.05). There was 52% isolation from male and 48% isolation from female. According to [27], males are more likely to develop lower respiratory trait infection by *S. pneumoniae* and the greater resistance found in female can be explained by their enhanced TH1 immune response. These findings resemble what was reported by El-Shaymaa et al [28], after their investigation on the prevalence of *Streptococcus pneumoniae* in Egyptian children in Assiut, Egypt where 56% of *S. pneumoniae* were isolated from male and 44% were isolated from female. As most patients were hospitalized in separate wards, this suggested a role in the dissemination of this bacterium in the respective wards rather than age or gender specification.

The antibiotic sensitivity pattern of the *Streptococcus pneumoniae* isolates recovered from the sputum of the pneumonia patients in this study is partly comparable with the work of [29], who tested isolates of *S. pneumoniae* recovered from sputum of patients in Ogun State, Nigeria against the following antibiotics: ciprofloxacin, erythromycin, cotrimoxazole, ampicillin-cloxacillin, amoxicillin and gentamicin. The outcome of their work shows that 69.5%, 69.5%, 78.3%, 65.2%, 56.5% and 73.9% were sensitive to the antibiotics mentioned above respectively while 30.5%, 30.5%, 21.7%, 34.8%, 43.5% and 26.1% were resistant to the antibiotics mentioned above respectively. Similarly, in a study carried out in Northwest Nigeria by Iliyasu et al [30], a total of 117 *Streptococcus pneumoniae* isolates were recovered from sputum samples. 72.0% were sensitive to penicillin and 28.0% were resistant, only 3.8% were sensitive to septrin while 78.8% were sensitive to amoxycillin and 21.4% were resistant.

Generally, when a pathogen is being reported as resistant, it implies that the infection it has caused will not respond to treatment with the antibiotic to which it is resistant irrespective of dose or site of infection. On the other hand, a pathogen reported as susceptible is an indication that the infection it has caused is likely to respond to treatment when the antibiotic to which it is susceptible was used in normal recommended doses and administered by an appropriate route [31,19].

5. CONCLUSION

This study demonstrated a high prevalence of *Streptococcus pneumoniae* in patients with pneumonia more than other bacteria isolated. *Streptococcus pneumoniae* is of more preponderance in adult patients (19-59 yrs.). There was no distinct variation in occurrence of *Streptococcus pneumoniae* with regards to gender and age. *Streptococcus pneumoniae* shows more susceptibility to streptomycin (78.8%), followed by cotrimoxazole (72.7%). The highest resistance was observed with gentamicin and tetracycline (42.4% each).

CONSENT

Informed consent was obtained from each participating patient before commencing the study. The purpose and nature of the study was properly explained to them and thereafter, the intended participants were requested to complete a consent form which they endorsed by a signature indicating their willingness to participate without any form of coercion. For children, the consent of their parent/guardian and children's assent were sought before hand.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Ethical Committee of Madonna Catholic Hospital Umuahia, Abia State. Administrative clearance for this study was also obtained from the management of Madona Catholic Hospital, Umuahia, Abia State.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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