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Research Article

The Prevalence of Potential Pathogens of Pneumonia in Sputum Specimens from Adult Patients at the University Teaching Hospital in Lusaka, Zambia

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Abstract

Background: Pneumonia is a leading cause of morbidity and a significant cause of mortality worldwide. Although information is available on pneumonia in children in Zambia, the incidence in adults in many parts of Africa including Zambia is unknown. Knowledge of the aetiological agents of pneumonia in low-income countries is critical for making rational decisions regarding treatment as aetiology may differ to that of high income countries and result in poor response to therapy.

Objective: The purpose of this study was to identify aetiological agents of pneumonia in adult patients who sought health care at the University Teaching Hospital in Lusaka, Zambia.

Methodology: We conducted a cross-sectional study from March 2014 to August 2014. Conventional cultured methods and real-time Polymerase Chain Reaction (PCR) were employed in identifying aetiological agents. Demographic data were collected from patients' laboratory request forms and all data were analysed using SPSS version 16.

Results: A total of 312 samples were received and cultured, 52.9% (165/312) yielded potential pathogens with the most common being *Moraxella catarrhalis* [26.7% (47/176)], *Pseudomonas aeruginosa* [25.6% (45/176)], and *Klebsiella pneumoniae* [18.2% (32/176)]. Using PCR, 146 samples were analysed and the most common organisms were Human cytomegalovirus [24.3% (44/181)], *Klebsiella pneumoniae* [17.7% (32/181)], *Haemophilus influenzae* non-type b [16.0% (29/181)], *Streptococcus pneumoniae* [9.4% (17/181)] and *Staphylococcus aureus* [9.4% (17/181)]. Other agents, mostly viruses, were also detected. More than one agent was detected in 42% of the specimens analysed by PCR. Detection rates of probable pathogens by Culture and PCR methods were about 30.1% and 69% respectively.

Conclusion: Our study showed a wide variety of potential pathogens including; bacteria, viruses and fungi in sputum specimens obtained from patients attending the University Teaching Hospital. Polymerase Chain Reaction detected more organisms than culture. Some of the specimens yielded multiple organisms which reflects the possibility of multiple causative agents for pneumonia. These data show the importance of employing better diagnostic methods, such as molecular tools, for identifying potential pathogens associated with pneumonia.

Keywords: Pneumonia; Culture; Polymerase Chain Reaction; Sputum; University Teaching Hospital; Zambia

Abbreviations

AIDS: Acquired Immunodeficiency Syndrome; CMV: Cytomegalovirus; DNA: Deoxyribonucleic Acid; FTD: Fast Track Diagnostics; MRSA: Methicillin Resistant *Staphylococcus aureus*; PCR: Polymerase Chain Reaction; UTH: University Teaching Hospital; SPSS: Statistical Package for Social Scientists; WHO: World Health Organisation.

Background

Pneumonia is a disease affecting lung parenchyma distal to the terminal bronchioles [1], and is caused by infection with bacteria, viruses, fungi and less commonly by parasites [2]. Occasionally, inhaled chemicals can cause lung inflammation and lead to pneumonia [3]. The most recent estimate by the Global Burden of Disease Study revealed that lower respiratory tract infections, including pneumonia, are the fourth most common cause of death globally, exceeded only by ischaemic heart disease, stroke and chronic obstructive pulmonary disease [4,5]. According to the World Health Organisation (WHO), 1.6 million deaths per annum in adults are attributed to pneumonia and will be amongst the leading four causes of death by the year 2030 [6], with the highest mortality rates seen in low income countries [4].

There are differences in geographical distribution of agents of pneumonia and this poses a challenge in establishing empirical treatment. This may have led to increased morbidity and mortality, and probable contribution to the mismanagement of pneumonia patients as there is limited local information hence relying on information from developed countries to develop local treatment guidelines [7]. Most developed countries have identified aetiological agents of pneumonia, and newer antimicrobial agents are available for therapy. In Zambia, a large multi-country project, PERCH (Pneumonia Etiology Research for Child Health) case-control study was able to determine the etiology of and risk factors for severe and very severe pneumonia in children 1-59 months of age [8]. Several pathogens were identified, which included *Streptococcus pneumoniae* (54.8%), *Moraxella catarrhalis* (46.2%) and *Haemophilus influenzae* (40.7%). Among the viruses, cytomegalovirus was the most commonly detected followed by respiratory syncytial viruses. However, there is limited information on the incidence and aetiological agents of pneumonia in adults in the country. It is therefore important to determine and have knowledge of the local aetiological agents of pneumonia as this is critical for making rational decisions about treatment as response to therapy is context specific and may result in poor response when generic treatment options derived from high income settings are used [9].

Therefore, the purpose of this study was to describe the incidence of potential pathogens of pneumonia from adult patients presenting at a large tertiary hospital in Lusaka, the capital city of Zambia. This is the University Teaching Hospital (UTH) with approximately 2000 bed capacity which also serves as a referral centre. The study employed both traditional and modern diagnostic tests in order to address the challenges associated with the conventional methods. The information from the study may be valuable in reviewing the treatment protocols for adult pneumonia patients seen at this facility.

Materials and Methods

Study site

The study was conducted at the University Teaching Hospital (UTH) that consists of approximately 2000 bed capacity and serves as a referral centre.

Study design

This was a laboratory-based cross-sectional study conducted at UTH microbiology department from March-August 2014. Sputum specimens from suspected pneumonia patients submitted using sputum specimens from adult patients sent to the microbiology laboratory for diagnosis of pneumonia. Using a convenient sampling approach, all sputum specimens from adult patients suspected of having pneumonia were screened for inclusion using demographic and clinical details on laboratory request forms. Three hundred and twelve (312) sputum specimens from adult patients submitted to the Bacteriology Laboratory at UTH for routine bacteriological examination were included in the study. Patients' details were abstracted from available electronic records, and laboratory request forms recording relevant clinical and demographic data. Sputum specimens collected from patients aged less than 18 years of age and those obtained from patients with co-morbidities such as lung cancer, cardiac failure, alcoholics, asthma, diabetes, stroke and chronic obstructive pulmonary disease were excluded from the study.

Specimens were received in the laboratory in labeled plain sterile containers at room temperature. The quality of samples were assessed prior to culture and real-time Polymerase Chain Reaction (PCR) assays. We also compared the diagnostic yields of the methods applied, especially the potential benefit of PCR over conventional culture methods.

Microscopic determination of the quality of sputum using the bartlett score

Gram-stained smears were made from visually purulent portions of each sputum specimen and the quality was assessed by

using the Bartlett score. The quality of specimen was assessed by determining the number of squamous epithelial cells (SECs) and polymorphonuclear neutrophils (PMNs) within the following categories; <10, 10-25 and >25 cells per representative (100x) low power fields (LPF). The presence of PMN was graded as +1 and +2, whereas SECs were graded as -1 and -2 after observing a minimum of 20 LPF. The scores were added and the specimens with zero or less scores were classified as being of poor quality.

Phenotypic identification of pathogens

Culture

The most purulent portion of each sputum specimen was inoculated onto sheep blood, MacConkey, Sabouraud's and Chocolate agar plates (Oxoid Ltd, Basingstone, Hampshire, England). The Sheep blood, and Chocholate agar plates were incubated at 37ºC in 5% Carbon dioxide for up to 48 hours while the MacConkey and Sabouraud plates were incubated at 37ºC aerobically. Identification of the organisms was done using conventional methods.

Molecular detection of respiratory pathogens

Multiplex PCR

Detection of pathogens using PCR was done on 146 specimens using Applied Biosystems 7500 Real-Time PCR cycler (Life Technologies, California, USA). A commercial multiplex PCR kit, FTD Respiratory Pathogens 33 Kit (Fast Track Diagnostics, Junglinster, Luxembourg) was used which included respiratory viruses, bacteria and fungi. These included influenza A (H1N1), influenza B, rhinovirus, coronavirus (NL63, 229E, OC43, HKU1), parainfluenza (1, 2, 3, 4), human metapneumovirus A/B, bocavirus, *Mycoplasma pneumoniae*, respiratory syncytial virus A/B, adenovirus, enterovirus, parechovirus, *Chlamydia pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, cytomegalovirus, influenza C, *Pneumocystis jirovecii*, *Haemophilus influenzae* type B, *Bordetella* species (except *Bordetella parapertussis*), *Moraxella catarrhalis*, *Klebsiella pneumoniae*, *Legionella* species and *Salmonella* species.

Sputum specimens were first subjected to digestion with freshly prepared 0.1% Sputasol (Oxoid Ltd, Cambridge, UK) in a 1:1 ratio at room temperature until completely dissolved. DNA was extracted on the easyMag instrument (bioMérieux, Marcy I'Etoile, France) according to the manufacturer's instructions using the "on-board lysis" protocol. DNA was eluted in a final volume of 110µl. The concentration of DNA was estimated by ultraviolet spectroscopy at 260nm. A DNA sample with an optical density (OD) of 1 at 260nm corresponds to a DNA concentration of 50µg/ml of double-stranded DNA. The purity of the DNA was determined by a DNA/protein absorbance ratio of 260nm/280nm. The extracted DNA was then stored at -20ºC until required.

Statistical analysis

Raw data were entered and cleaned in Microsoft excel and exported to SPSS version 16.0 Software (IBM, Armonk, New York, USA) for final coding and analysis. Frequency and percentages distribution were generated to describe the relative proportions of relevant variables. The outcome variable was defined as sputum positive for pneumonia associated pathogen(s) by PCR and/ or culture and stratified according to demographic data. Data were presented in tables and graphs. Chi square was calculated to ascertain association between patients' demographic characteristics and the outcome, a *p*-value at a level of 0.05 considered statistically significant.

Results

Demographic information of patients

Out of a total of 312 sputum specimens from adult patients clinically suspected to have pneumonia at UTH, 51.9% (162/312) were specimens from male patients while 48.1% (150/312) were from female patients. Most of the specimens were from patients from the 18-34 (43%), and 35-44 (27%) age groups. The median age of the patients was 38 years (interquartile range: 18 years) (Table 1).

Variables Frequency (Percentage)	Variables Frequency (Percentage)
Gender	
Male	162 (51.9%)
Female	150 (48.1%)
Age	
18-34yrs	134 (42.9%)
35-44yrs	83 (26.6%)
45-54yrs	43 (13.8%)
>54yrs	52 (16.7%)

Table 1: Demographic characteristics of study patients (n = 312).

Phenotypic identification of potential pathogens by culture methods

Out of the 312 sputum samples analysed, 52.9% (165/312) yielded bacteria or yeast cells while 47.1% (147/312) had no significant organisms. A total of 176 individual isolates comprising of 7 different bacterial species and 1 fungal species, were identified. These included the following microorganisms: *Moraxella catarrhalis* 26.7%, (47/176), *Pseudomonas aeruginosa* 25.6% (45/176),

Candida albicans 21%, (37/176), *Klebsiella pneumoniae* 18.2% (32/176), *Staphylococcus aureus* 5.6% (10/176), *Haemophilus influenzae* 11.7% (3/176), and *Streptococcus pneumoniae* 1.1% (2/176) (Figure 1). There was no statistical significance difference in culture positivity with respect to sex ($X^2 = 1.125$, $p = 0.289$).

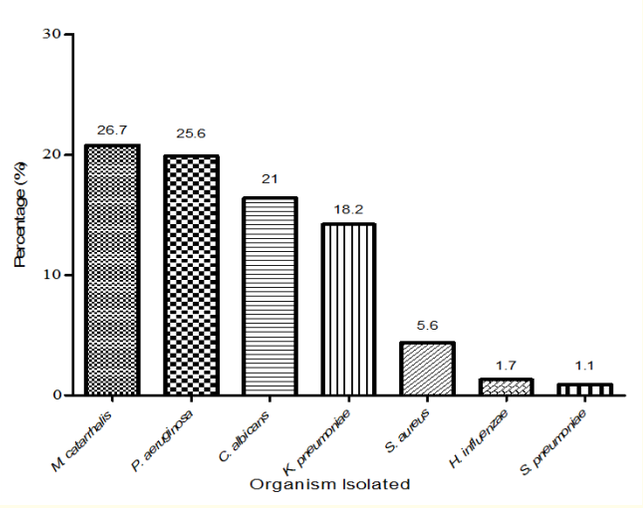

Figure 1: Different types of microorganisms isolated from sputum specimens.

Detection of respiratory pathogens by multiplex PCR

Only 146 specimens were analysed using PCR due to limited reagents. Of these 79.5% (116/146) were bacterial agents, 52.7% (77/146) viral agents and 4.8% (7/146) fungal agents.

There were 181 single organisms detected from the specimens. The distribution for the organisms was as follows; Cytomegalovirus (24.3%, 44/181), *K. pneumoniae* (17.7%, 32/181), *H. influenzae* non-type B (16.0%, 29/181), *S. aureus* (9.4%, 17/181), *S. pneumoniae* (9.4%, 17/181), *H. influenzae* type B (6.1% ,11/181), Rhinovirus (5.5%, 10/181), *M. catarrhalis* (4.4%, 8/181), *Pneumocystis jirovecii* (3.9%, 7/181), Respiratory Syncytial Virus (RSV) A/B (3.9%, 7/181), Adenovirus (1.7%, 3/181), Human bocavirus (1.1%, 2/181), Human metapneumoviruses A/B (1.1%, 2/181), Parainfluenzae type 1 (1.1%, 2/181), Parainfluenzae type 2 (1.1%, 2/181), Parainfluenzae type 4 (1.1%, 2/181), *Salmonella species* (0.6%, 1/181), *Mycoplasma pneumoniae* (0.6%, 1/181), Influenza virus type B (0.6%, 1/181), Human coronavirus 63 (0.6%, 1/181), and Parainfluenzae type 2 (0.6%, 1/181) (Figure 2).

Forty-four of the 116 PCR positive specimens (79.5%) harboured more than one type of organism (coinfections). About

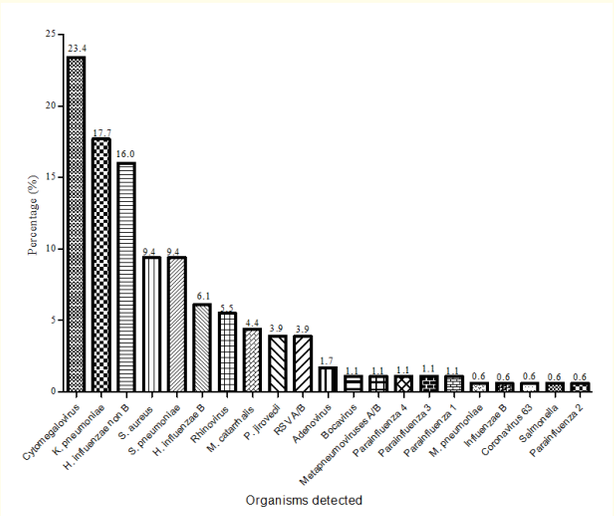

Figure 2: Distribution of organisms detected by PCR from sputum.

29.5% (13/44) of these specimens harboured *K. pneumoniae* and *H. influenzae* non-type B. The majority of the specimens had three to five different organisms. More than half of the specimens (56.8%, 25/44) harboured both bacteria and viruses, followed by those with bacteria only (27.3%, 12/44), bacteria and fungi only (9.1%, 4/44) and viruses only (4.5%, 2/44) (Table 2).

Mixed isolates	Frequency (%)
K .pneumoniae + H. influenzae non type B	13(8.9)
Cytomegalovirus *+ K. pneumoniae*	7(4.8)
S. pneumoniae + K .pneumoniae	7(4.8)
Cytomegalovirus *+ H. influenza non type B*	6(4.1)
S. aureus+ H. influenzae non type B	6(4.1)
S. pneumoniae + H. influenza non type B	5(3.4)
H. influenza non type B+ H. influenza type B	4(2.7)
Cytomegalovirus*+ S. aureus*	4(2.7)
S. pneumoniae + K. pneumoniae+ H. influenza non type B	4(2.7)
S. aureus+ P. jiroveci	3(2.1)
Cytomegalovirus+ Respiratory syncytial virus A/B	3(2.1)
Cytomegalovirus *+ M. catarrhalis*	3(2.1)
Rhinovirus *+ S. aureus*	2(1.4)
S .pneumoniae + H. influenza type B	2(1.4)
Cytomegalovirus *+ S. pneumoniae*	2(1.4)
Cytomegalovirus+ Rhinovirus*+ K .pneumoniae*	2(1.4)

S. pneumoniae+ S. aureus	2(1.4)
Cytomegalovirus + Adenoviruses *+ H. influenza non type B*	2(1.4)
S. pneumoniae +S. aureus+ P. jiroveci	1(0.7)
Cytomegalovirus+ Coronavirus *63+K.pneumoniae*	1(0.7)
Cytomegalovirus+ Rhinovirus + Parainfluenza 3	1(0.7)
Respiratory syncytial virus A/B *+ K. pneumoniae+ H. influenza non type B*	1(0.7)
Metapneumoviruses A/B+ *H. influenza type B+* Parainfluenza 1	1(0.7)
Influenza type B+ K. pneumoniae	1(0.7)
K .pneumoniae+ P. jiroveci	1(0.7)
K .pneumoniae + Parainfluenza 1	1(0.7)
S. pneumoniae + K. pneumoniae + S. aureus	1(0.7)
K. pneumoniae + Parainfluenza 3	1(0.7)
Cytomegalovirus *+ K. pneumoniae + H. influenza non type B*	1(0.7)
Cytomegalovirus+ Parainfluenza 2	1(0.7)
K. pneumoniae+ H. influenza non type B+ H. influenza type B	1(0.7)
Cytomegalovirus + Bocavirus *+ K .pneumoniae+ S. aureus*	1(0.7)
Cytomegalovirus+ Rhinovirus*+ S. pneumoniae*	1(0.7)
Cytomegalovirus+ Adenoviruses*+ S. aureus + H. influenzae non type B+ M. catarrhalis*	1(0.7)
H. influenzae non type B + H. influenzae type B + M. catarrhalis	1(0.7)
K. pneumoniae + Salmonella	1(0.7)
Adenoviruses *+ M. pneumoniae+ S. aureus+ H. influenza non type B+ H. influenza type B*	1(0.7)
Cytomegalovirus+ Bocavirus*+ M. catarrhalis*	1(0.7)
K .pneumoniae + S. aureus + H. influenza non type B	1(0.7)
S. pneumoniae + M. catarrhalis	1(0.7)
Cytomegalovirus *+ S. pneumoniae + H. influenza non type B+* Parainfluenzae 4	1(0.7)
Respiratory syncytial virus A/B *+ S. aureus + M. catarrhalis*	1(0.7)
Cytomegalovirus+ Rhinovirus*+ S. aureus+ H. influenza non type B+ P. jiroveci*	1(0.7)
K. pneumoniae + H. influenza non type B+ P. jiroveci	1(0.7)

Table 2: Mixed aetiological agents of pneumonia detected in sputum specimens using PCR.

Comparison between PCR and culture methods

An attempt was made to determine the congruence between PCR and culture methods in the identification of bacteria in the 146 specimens subjected to PCR. As observed in table 3 below, the PCR technique detected more bacteria than the culture method.

Organism	PCR positive n (%)	Culture positive n (%)
K. pneumoniae	32(21.9)	17(11.6)
H. influenzae	29(19.9)	2(1.4)
S. aureus	17(11.6)	6(4.1)
S. pneumoniae	17(11.6)	1(0.7)
H. influenzae B	11(7.5)	0(0.0)
M. catarrhalis	8(5.5)	5(3.4)
Salmonella species	1(0.7)	0(0.0)

Table 3: Comparison between PCR and Culture and methods.

Discussion

Pneumonia can be caused by a wide range of organisms. Previous studies have attributed isolation of different organisms to differences in patient groups, presence of epidemic organisms and scope of investigation [10]. Culture methods allow phenotypic identification of the causative agents and provides an opportunity for performing antimicrobial susceptibility tests, which in turn, allows modification of empirical treatment to a more focal approach [8]. In this study, 57.1% of sputum specimens yielded potential pathogens associated with pneumonia using culture methods. This was higher than what was obtained in other studies conducted in Malaysia, Egypt, Nigeria and Ethiopia [11-14]. The difference was attributed in part to variations in sensitivity of culture methods employed in different studies. The data also showed that there was no significance difference in sputum positivity between men and women, but this was not consistent with data from countries such as Nigeria where a higher sputum positivity was seen in samples were obtained from women [15,16]. This difference may be due to differences in sampling.

Culture results showed that *Moraxella catarrhalis* was the most commonly isolated organism, followed by *P. aeruginosa, C. albicans*, *K. pneumoniae, K. oxytoca* and *E. coli*. These observations are contrary to findings from other studies conducted in Nigeria and Ethiopia which reported *K. pneumoniae* and *S. pneumoniae* as the most predominant organisms, respectively [11,14]. Some studies have indicated that *M. catarrhalis* is an important cause of exacerbations of chronic obstructive pulmonary disease in adults [17]. However, in our study it cannot be inferred that this organism was a cause

of pneumonia because of limited access to clinical information. The study also revealed that *P. aeruginosa* was the second most isolated potential pathogen but the percentage was higher than in other studies such as those conducted in Iran, Nigeria and Ethiopia [14,18,19]. A prevalence of 3-10% for Gram negative bacilli has been reported elsewhere [20] in contrast to our study where a disproportionately high percentage were isolated including *P. aeruginosa*. The higher incidence seen in our study may represent the pattern of local flora, as some of the Gram-negative bacilli, such as *P. aeruginosa* and *K. pneumoniae*, were also endemic in other parts of Africa. More research is needed to investigate these patterns of infection that may have implications on the diagnosis and management of pneumonia in adult patients. Furthermore, a change toward Gram negative bacteria and opportunistic organisms may occur with increasing age and the severity of the concomitant medical illness as reported elsewhere [21].

Improved detection of many bacterial and viral pathogens associated with pneumonia has been observed with the use of PCR [22]. An added advantage of the PCR technique is its ability to detect microorganisms after initiation of antibiotic treatment [23]. Recent studies have shown that application of both PCR and culture methods has a higher and wider microbial yield [8]. This study utilised PCR as a tool for identifying organisms from the sputum samples detecting a significant number of potential pathogens. Our prevalence of 71% is similar to findings from other studies, although results vary considerably from 39% to 76% [8,25-28]. This variation is attributed to differences in the distribution of pneumonia in adults in different geographic regions as reported elsewhere [29].

Cytomegalovirus was detected as the most common single organism using PCR. The high incidence of CMV cannot be inferred as the definitive cause of pneumonia as CMV can be detected as a latent virus in lymphocytes and salivary glands. Consequently, CMV is often detected in the sputum of patients with HIV/AIDS representing active viral shedding without necessarily causing clinical disease. In our study, it was not possible to establish a relationship between CMV infection and HIV status as HIV testing was not done. *Klebsiella pneumoniae* was found as the second most frequent potential pathogen. Other potential pathogens detected included *H. influenzae* non-type B, *S. aureus*, *S. pneumoniae*, *H. influenzae* type B, rhinovirus, *M. catarrhalis*, *Pneumocystis jirovecii*, Respiratory Syncytial Virus (RSV) A/B, parainfluenza virus, adenovirus, Human metapneumoviruses A/B, Human bocavirus, Human coronavirus 63, influenza type B, parainfluenza type 1, parainfluenza type 3, parainfluenza type 4, *Salmonella species*, *Mycoplasma pneumoniae*, influenza virus type B, Human coronavirus 63 and parainfluenza type 2. This is an important finding as it suggests that novel empirical antimicrobial treatment can be considered. In contrast, *S. pneumoniae* was most frequently detected followed by influenza, rhinovirus, respiratory syncytial virus, parainfluenza virus, enterovirus, metapneumovirus and adenoviruses in Norway [8]. A similar study done in Netherlands identified *S. pneumoniae* as the most common organism, followed by *Coxiella burnetii* and influenza A virus [29]. Other reports have shown that viral infection in patients with pneumonia varies from 4% to 39% [30]. In the present study, respiratory viruses accounted for 52.9% of tested sputum samples. The higher rates of viral pathogens that were found as a single or combined agent can be attributed to the novel laboratory tests that were used and possible context specific differences that may influence disease distribution.

Our study established multiple isolates in 30% of the samples analysed by PCR. This is consistent with assertions that the incidence of mixed infections does not usually exceed 30% as has been reported elsewhere [31]. Mixed isolates have also been reported in various studies conducted elsewhere [27,32,33]. *K. pneumoniae* and *H. influenzae* non type B were the most frequently found coinfections in multiple infected patients. Similar findings were observed in other studies conducted in Nigeria and Ethiopia [14,15]. The identification of mixed infections is very important for treatment strategies and to avoid a misleading impression of clinically resistant strains. Establishing a microbiological diagnosis for pneumonia from samples with mixed isolates is challenging as no single pathogen can be said to be the cause of the infection. Interactions between different pathogens *in vivo* and their contribution to infection are yet to be elucidated [33]. Multiple concurrent infections might interfere with the pulmonary cleansing function thus helping to establish a conducive atmosphere for the development of pneumonia. It is not well understood whether a viral infection alone causes pneumonia or acts in conjunction with other respiratory pathogens, and a number of investigators postulate that a viral infection usually precedes a secondary bacterial infection [32,34,35]. Several studies have shown that viral infection is an important activator of secondary bacterial infection with prior viral infection impairing mucosal barriers in the respiratory system and making the host susceptible to bacterial infection [36,37].

Comparison of bacterial PCR and culture results revealed differences, with PCR yielding more positives than culture, which confirmed the increased sensitivity of PCR reported by others [8,38,39]. A high percentage of negative sputum cultures may be due to fastidious bacteria, viral agents, and previous antibiotic therapy for which the method is not sensitive to. A number of stud-

ies have revealed that during antibiotic treatment, sputum specimens show no growth on culture for bacteria in contrast to the PCR technique that remains positive [30,40,41]. Besides, conventional culture methods cannot detect viral infection. In this study, sputum culture detected only one case of *S. pneumoniae* and *H. influenzae*, illustrating low sensitivity for these organisms [42]. The high diagnostic yield of aetiological agents of pneumonia that was revealed with the application of PCR technique demonstrates that causative agents can be established in the majority of pneumonia patients if PCR technique is employed in routine practice.

Conclusion

By combining conventional diagnostic methods with real-time PCR techniques for both common bacteria and a number of respiratory viral agents, a higher microbial yield was obtained. Cytomegalovirus and *Moraxella catarrhalis* were the leading organisms detected using PCR and culture methods, respectively. Mixed infections were frequent, with *K. pneumoniae* and *H. influenzae* non-type B being the most common organisms. To our knowledge, this is the first study describing the distribution of pneumonia associated pathogens from sputum in adults in Zambia. Potential viral infections should be given more attention in adult pneumonia cases as this could have implications for patient management.

Ethical Approval

Approval for this study was granted by the University of Zambia Biomedical Research and Ethics Committee (UNZABREC) based at University of Zambia Ridgeway campus. Permission to use patients' data, sputum specimens and bacterial isolates was obtained from UTH Management. Results of Culture and PCR diagnosis were relayed back to the attending physician for management of patients.

Consent for Publication

Not applicable.

Availability of Data and Materials

Data generated in this study will not be made available so as to protect the patients' confidentiality.

Competing Interests

The authors declare that they have no competing interests.

Funding

Financial support to buy reagents used for the study was sourced from the Government of the Republic of Zambia through the Ministry of Health. The UTH Bacteriology laboratory provided some reagents during culture and identification of microorganisms.

Authors' Contributions

Conceived and designed the experiments: JM, GK, JCM, CLM. Performed the experiments: JM, SM Analyzed the data: VD. Wrote, read and approved the manuscript: JM, GK, VD, JM, CL, TK.

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