Evaluation of Pneumococcal Urinary Antigen Test in University Teaching Hospitals of Rwanda

A thesis submitted to the School of Graduate Studies in fulfillment of the requirements for the award of a Masters of Medicine Degree
By
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Kigali, February 2016
I, Pierre Laurent Lussungu, hereby declare that the work submitted in this thesis is a result of my own study except where otherwise acknowledged. This thesis has not been submitted for another degree award in this or any other University or institute.

Sign: _______________________________       Date _______________________________

**Supervisor:** Prof. DEVON Hall

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Dr Pierrot Lundimu Tugirimana

Dr Cyprien Ntirenganya,

Sign…………………………………………………………………………………………………………………

Date:


**Declaration narrative**

I declare that the following thesis is my own research work and does not contain any material published before. I am submitting it for award of the degree of master in medicine in internal medicine at university of RWANDA. Till now, It was not submitted before for degree or examination at any other university.

Dr LUSSUNGU Laurent Pierre

February, 2016
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Finally, I thank the School of Medicine and the Ministry of Health for the technical support during my training.
LIST OF ACRONYMS AND ABBREVIATIONS

BAL : bronchoalveolar lavage

CAP : community acquired pneumonia

CHUK : centre hospital universitaire de KIGALI

CHUB : centre hospital universitaire de BUTARE.

IDSA : infectious disease society of America

NS : non significant

SPUAT : streptococcal pneumonia urine antigen test

UAT : urine antigen test
Dedication

This goes to my beloved parents, Brothers, I will always remember you. I will always strive for your recognition.
1. INTRODUCTION

1.1 BACKGROUND
Pneumonia is an infection of the lower respiratory tract that is usually accompanied by cough, fever, malaise, and chest x-ray abnormalities. Pneumonia is a common illness affecting approximately 450 millions of people a year and occurs in all parts of the world (1–3). There is evidence that pneumococcal carriage rate and pneumonia is more common in tropical countries with fewer resources (18).

The disease is classified as community acquired pneumonia (CAP), healthcare associated or hospital-acquired according to how and where it was contracted (3). The infectious diseases society of America (IDSA) defines CAP as an acute infection the pulmonary parenchyma frequently associated with at least two symptoms of the active infection occurring in individuals who have not been hospitalized or resided in a long-term care for 14 days before the onset of the symptoms.

CAP is a common disease, representing the most frequent cause of the hospital admission and the mortality of the infection origin in developed countries (2, 4). The streptococcus pneumonia is the leading cause of CAP and is responsible for 30-40 % of the CAP. The etiology can be established following a routine diagnostic work up when reliable laboratory, bronchoscopic and x-ray facilities are available.

1.2 STATEMENT OF THE PROBLEM
Streptococcus pneumonia is suspected to cause an important proportion of the community acquired pneumonia whose etiology cannot be detected with the conventional tests (2). This is even more of a possibility in low resource countries where reliable diagnostic studies are not available. In Rwanda, it is unusual for blood and sputum specimens to be submitted for diagnosis of pneumonia and bronchoscopic procedures are available only in teaching hospitals.
Reviewing microbiological logs, it is extremely rare to have positive blood or sputum cultures for pneumococcus. The use of a pneumococcal urine antigen to detect the presence of pneumococcus will do the following:

1. Provide evidence of the prevalence of pneumococcus in the community.
2. Compare the detection of pneumococcus from urinary antigen with the culture of sputum and blood culture. A wide variation of results will encourage laboratories to improve the use of sputum gram stains, sputum cultures and blood cultures for detection of pneumococcus.
3. Give clinicians an opportunity to evaluate patients with positive urinary antigens to identify the clinical presentation of pneumococcal pneumonia.
4. If the pneumonia is clinically compatible with pneumococcal pneumonia, more targeted antibiotics can be used for treatment and once patients are afebrile, earlier discharge would be considered.

The streptococcal pneumonia is responsible of 30-40% of CAP (2,5,6). The streptococcal CAP may be under diagnosed and that may be responsible of for at least one of three episodes of the CAP without etiological diagnosis (4,7).

Data on the real etiologies of community acquired pneumonia is essential to decision makers for the development of the national health policies that will help for prevention and the control of the disease. Medically certified information on streptococcal pneumonia and its antibiogram in order to allow physicians to take advance of them for the good provide of health to our population is essential(8)

1.3 JUSTIFICATION OF THE STUDY

In our settings, there are a number of obstacles to establish the pneumococcal etiology with conventional diagnostic methods in patients presenting with CAP symptoms. The isolation of streptococcal pneumonia from blood or pleural fluid, the gold standard test, is rarely positive compared to places where adequate laboratory facilities are available. Even when proficient laboratories are available, only 15-30 % of cases are culture positive(3,9). Moreover, the diagnosis based on sputum culture is controversial due to both nasopharyngeal carriage of pneumococcus in healthy individuals and inadequate sputum specimen(1,9).
In addition, 30 % of patients with CAP have been treated with antibiotics before admission, which may decrease the sensitivity of the conventional methods(11). This is more likely in Rwanda where antibiotics may be started at the village health centers or the regional hospitals before transfer to the teaching hospitals.

Invasive specimens such as obtained by broncho-alveolar lavage [BAL], bronchoscopically retrieved protected specimen, or transthoracic needles aspiration are generally considered to be the reliable respiratory samples for determining the etiology of pneumonia(10,12). These more high technology and invasive procedures are rarely available for the diagnosis of pneumonia. The isolation of the pneumococci from these specimens is considered as proof of pneumococcal origin. However, invasive techniques are not routinely used because they require specialist training and they may have side effects(13).

CAD presents a challenge to the physician and therefore its treatment is often empirical since the diagnosis of CAP still relies on clinical and on microbiological results which are consisting of blood culture and culture of an appropriate sputum sample, including gram stain (14,15). That why many cases of CAP are of unknown etiology; the sensitivity of the blood remaining around 10-15 % and that of sputum culture being between 10 and 30%(19).

Even when the physician becomes more aggressive using invasive sampling techniques such as broncho-alveolar lavage or trans-thoracic aspiration, 40% of CAP remains of unknown etiology [17]. In addition to all those difficulties related to etiological diagnosis, numbers of patients presented with suspected pneumococcal pneumonia had a use of prior antibiotics therapy that reduced the sensitivity and specify of standard and conventional methods of its diagnosis. Given these limitations, the need for new rapid and specific tests for the streptococcus pneumonia deems to be important as pneumococcal pneumonia is considered to be the main cause of cultivable bacteria. In a area of increasing prevalence of antimicrobial resistance, quick and unequivocal identification of the etiology of pneumonia would considerably improve the patient management.
The evaluation of a rapid urinary antigen test (UAT) for the detection of streptococcal pneumonia in adults suffering from CAP would help the physician in our settings. The use of a simple UAT may improve the diagnosis with the advantage to an easy availability of urine specimens in the situations where sputum may not be able to be produced; the yield of sputum culture for streptococcus rapidly decreases with initiation of antibiotics. The use of the urinary antigen test in high resource countries has been somewhat controversial because it compares more favorably to the sensitivity of laboratory culture results. Countries with low resources and inadequate laboratory facilities may be a place where this test may be much more helpful in the diagnosis and treatment of pneumococcal disease.

1.4 HYPOTHESIS
The pneumococcal urinary antigen test is a more useful diagnostic test in countries where laboratory cultures are not reliable?

1.5 OBJECTIVES:
✓ Compare the positivity rate of binaxnow urinary antigen to sputum and blood cultures tests.
✓ Develop clinical abilities of health care providers to suspect pneumococcal pneumonia based on clinical presentation.
✓ Determine whether antibiotics changes are made based on clinical correlation with positive antigen tests.
✓ Compare length of stay of pneumonias with positive and negative pneumococcal antigen tests.
2. METHODS

2.1 Study design
We will conduct a prospective study.

2.2 Sample size
There is scantiness in the prevalence of community acquired pneumonia in tropical countries in particular in Africa. But using a neighbor country prevalence in CAP that was 19%, we are estimating to have a sample size of 225 at a significance level of p<0.05

2.3 Study area and population
The study will be conducted at BUTARE university teaching hospital and KIGALI university teaching hospital in internal medicine departments for a period of 6months.

For the purpose at increasing the number of samples and reducing prior antibiotic treatment issue, some peripheral hospital to BUTARE hospital and KIGALI hospital will be provided in pneumococcal urine antigen tests and blood culture medium. KABUTARE , GISAGARA, KIBAGABAGA, RWAMAGANA and MASAKA District hospital are on the list.

2.4 Inclusion criteria
All patients aged of 21 years old and above , admitted in internal medicine wards with clinical features consisting with a acute lower respiratory tract infection including two or more of the following symptoms: Productive cough, Pleuretic chest pain, Dyspnea, Pulmonary consolidation on physical examination

And one or more systemic symptoms including temperature >37,8;sweating , altered mental status , aching and pain , headache, coryza, and sore throat.

These patients will be enrolled at the accident and emergency department when they present.
2.5 Exclusion criteria

- Patients hospitalized within previous 5 days
- The pneumonia is distal to bronchial obstruction like cancer
- Patients with suspected broncho-aspiration or patients who will not complete the follow up at least 4 weeks
- Patients with TBC or who have an emerging alternative diagnosis (pulmonary edema, pulmonary or septic emboli, or any malignancy,) or those whose with immunosuppression du to neutropenia < 500 not attributable to current pneumonia.
- Patients with chemotherapy agents during the last 6 months prior to admission or steroids, azathioprine, for the last 3 months prior to admission.

Data collected will include antibiotics taken before admission, days of symptoms and evolution of illness, clinical signs, radiological features and treatment with antibiotics.

2.6 Laboratory tests

- Sputum specimens will be obtained at admission for gram stain (presence of > 25 polymorph nuclear leucocytes and < 10 squamous epithelial cells at x 10 magnification).
- Two sets of blood cultures will be taken within 6h after admission
- Urine samples will be collected soon after admission to hospital and will be processed as soon as possible. If it is not possible to perform the urine test immediately the urine will be frozen at – 20°C, being immediately thawed before test. The detection of the pneumococcal antigen will be performed.

2.7 Etiological diagnosis.

The etiological diagnosis will classified as certain or probable. Certain when it complies with one of the following criteria:
- positive sputum for stain
- Positive blood sample,
- Positive S. pneumonia antigen detection in urine and is clinically consistent with pneumococcal pneumonia.

Probable when it complies with the following criteria:
- Presence of a clinical compatible picture when negative testing.
- Presence of a predominant type of microorganisms after gram staining and/or isolation of a predominant type of respiratory pathogen in the culture and or the detection of pneumococcal antigen and type B H. influenza in sputum sample.

2.8 Radiological studies
A chest radiography will be performed at the time of admission. Any findings consisting in pulmonary infiltrate either segmental either lobar without prior lesions will be considered as pneumonia.

2.9 Data entry and Statistical analysis
Data collected will be recorded making use of Microsoft Excel 2013. All other statistical analyses will performed using STATA for Mac, Version 13 (StataCorp LP, Texas USA).

Participant descriptive characteristics will be summarized as: mean± standard deviations for continuous variables, and as number or percentages for categorical variables. The significance level of p<0.05 will be used.

2.10 Ethics considerations
This study will be approved by the research and ethical committee of the school of medicine of the University of RWANDA.

A statement of the research purpose in Kinyarwanda will be communicated to eligible patients. They will be given an opportunity to ask questions about the study and to review the informed consent form. Participation will be voluntary. If they agreed to participate, written (when possible) or verbal consent documented by signature of a literate witness and thumbprint will be collected. The participants will have the right to withdraw the consent and exit the study at any time without any repercussion.

The collected data sheets will be kept in a locked room and the database will be password protected. The research project will be reviewed by CHUK and CHUB ethical committees.
3. RESULTS

3.1 Univariate analysis of variables

Table 1: Distribution of socio demographic characteristics of respondents

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>104 (46.64%)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>119 (53.36%)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>33 (12%)</td>
</tr>
<tr>
<td>Provenance</td>
<td></td>
</tr>
<tr>
<td>Kigali City</td>
<td>99 (44.39%)</td>
</tr>
<tr>
<td>East</td>
<td>19 (8.52%)</td>
</tr>
<tr>
<td>North</td>
<td>4 (1.79%)</td>
</tr>
<tr>
<td>South</td>
<td>85 (38.12%)</td>
</tr>
<tr>
<td>West</td>
<td>16 (7.17%)</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>55 (25.23%)</td>
</tr>
<tr>
<td>Primary</td>
<td>93 (42.66%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>55 (25.23%)</td>
</tr>
<tr>
<td>University</td>
<td>15 (6.88%)</td>
</tr>
<tr>
<td>Profession</td>
<td></td>
</tr>
<tr>
<td>Jobless</td>
<td>56 (26.42%)</td>
</tr>
<tr>
<td>Peasant farmer</td>
<td>62 (29.25%)</td>
</tr>
<tr>
<td>Private sector</td>
<td>31 (14.62%)</td>
</tr>
<tr>
<td>Public agent</td>
<td>34 (16.04%)</td>
</tr>
<tr>
<td>Retired</td>
<td>7 (3.30%)</td>
</tr>
<tr>
<td>Student</td>
<td>22 (10.38%)</td>
</tr>
<tr>
<td>Deaths, n (%)</td>
<td>14 (6.45%)</td>
</tr>
<tr>
<td>Prior antibiotic use, n (%)</td>
<td>107 (47.98%)</td>
</tr>
<tr>
<td>Microbiologic testing obtained, n (%)</td>
<td></td>
</tr>
<tr>
<td>Pneumococcal urinary antigen test</td>
<td>221 (100%)</td>
</tr>
<tr>
<td>Blood culture</td>
<td>94 (100%)</td>
</tr>
<tr>
<td>Sputum Gram stain and culture</td>
<td>122 (100%)</td>
</tr>
<tr>
<td>Co morbidities, n (%)</td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>24 (11.16%)</td>
</tr>
<tr>
<td>Excessive ethanol</td>
<td>63 (28.77%)</td>
</tr>
<tr>
<td>Asthma</td>
<td>13 (5.88%)</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>2 (0.91%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10 (4.52%)</td>
</tr>
</tbody>
</table>

Distribution of socio-demographic variables presented in table 1 show that 53.36% of respondents were female and 46.64% were male with a current age mean of 33 years old and the
standard deviation is around 12 years, means that more patients are concentrated at age 21 and 45 below and above the mean.

More respondents were located in Kigali city and southern province with a percentage of 44.39% and 38.12% respectively.

42.66% have a primary education, 25.23% have a secondary education, 25.23% have no education and 6.88% have a university education.

The percentage of deaths was not so high (6.45%) and the prior antibiotic use was 47.98%. The percentages of patients who were currently smoking and with an excessive ethanol use are respectively 11.16% and 28.77%. The table shows also that the percentage of patients with asthma; immunosuppression and diabetes were respectively 5.88%; 0.91% and 4.52%.
3.2 Bivariate analysis of variables
Bivariate analysis was used to determine the empirical relationship between 2 variables; binaxnow urinary antigen and blood cultures tests

Table 2: Association analysis between urinary antigen test and blood cultures test

<table>
<thead>
<tr>
<th>STREPTOCOC</th>
<th>CAL</th>
<th>PNEUMONIA</th>
<th>URINARY</th>
<th>ANTIGEN</th>
<th>BLOOD CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST</td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>-----------</td>
<td>--------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>24</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>38.46</td>
<td>61.54</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>71.43</td>
<td>32.88</td>
<td>41.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>49</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.91</td>
<td>89.09</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28.57</td>
<td>67.12</td>
<td>58.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>73</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.34</td>
<td>77.66</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pearson chi2 (1) = 9.9847 Pr = 0.002
According to urinary antigen test, 41.49% are positive tested and 58.51% are negative tested. And the percentage of blood culture positively and negatively tested are 22.34% and 77.66% respectively. By using chi square test to determine the relationship between urinary antigen test and blood culture test, there is enough evidence that blood culture is associated with urinary antigen test with p value<0.05

Table 2.1. sensitivity and specificity of the test

<table>
<thead>
<tr>
<th>STREPTOCOCCAL PNEUMONIA URINARY ANTIGEN TEST</th>
<th>BLOOD CULTURE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>73</td>
</tr>
</tbody>
</table>

the sensitivity of the SPUAT is 71% and the specificity about 67%
Table 3: Association analysis between urinary antigen test and sputum for gram stain and culture

<table>
<thead>
<tr>
<th>STREPTOCOC</th>
<th>CAL</th>
<th>PNEUMONIA</th>
<th>URINARY</th>
<th>SPUTUM FOR GRAM STAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTIGEN</td>
<td>AND CULTURE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEST</td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>41</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.45</td>
<td>74.55</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70.00</td>
<td>41.00</td>
<td>45.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>59</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.23</td>
<td>90.77</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.00</td>
<td>59.00</td>
<td>54.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>100</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.67</td>
<td>83.33</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Pearson chi² (1) = 5.6459 Pr = 0.017

The table above shows that the percentage of positive and negative urinary antigen test are respectively 45.83% and 54.17% and the percentage of positive and negative sputum for gram stain and culture are respectively 16.67% and 83.33%. By using chi square test to determine the relationship between urinary antigen test and sputum for gram stain and culture, there is relationship with p-value <0.05.
Determine whether antibiotics changes are made based on clinical correlation with positive antigen tests

Table 4: Association between urinary antigen test and antibiotics changes

<table>
<thead>
<tr>
<th>ANTIGEN</th>
<th>STREPTOCOC</th>
<th>CAL</th>
<th>PNEUMONIA</th>
<th>URINARY</th>
<th>antibiotic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST</td>
<td>Prior</td>
<td>No prior</td>
<td>Unavailable data</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
<td>----------</td>
<td>-------------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td>60</td>
<td>42</td>
<td>6</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55.56</td>
<td>38.89</td>
<td>5.56</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56.07</td>
<td>40.78</td>
<td>54.55</td>
<td>48.87</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>47</td>
<td>61</td>
<td>5</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41.59</td>
<td>53.98</td>
<td>4.42</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43.93</td>
<td>59.22</td>
<td>45.45</td>
<td>51.13</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>107</td>
<td>103</td>
<td>11</td>
<td>221</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.42</td>
<td>46.61</td>
<td>4.98</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Pearson $\chi^2 (2) = 5.0647 \text{Pr} = 0.079$

The table above shows that the percentage of prior antibiotic, no prior antibiotic and unavailable data are respectively 48.42%, 46.61% and 4.98% and the percentage of positive and negative urinary antigen test are respectively 48.87% and 51.13%. By using chi square test to determine the relationship between the 2 variables there is no correlation between the two variables, with p. value<0.05.
Table 5: Compare length of stay of pneumonias with positive and negative pneumococcal antigen tests.

<table>
<thead>
<tr>
<th>STREPTOCOC</th>
<th>CAL</th>
<th>PNEUMONIA</th>
<th>URINARY</th>
<th>LENGTH IN THE HOSPITAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTIGEN</td>
<td></td>
<td></td>
<td></td>
<td>TEST</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>Negative</td>
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</table>

Pearson chi² (2) = 50.8922, Pr = 0.000

The table above shows that the percentage of patients staying in hospital less than 1 week, between 1 and 3 weeks and more than 3 weeks are respectively 19.46%, 39.37% and 41.18%. And the percentage of positive and negative urinary antigen test are respectively 48.87% and 51.13%. By using chi square test to determine the relationship between the 2 variables there is
association between length in hospital and positive and negative urinary antigen test, With p. value<0.05.

**Table 6: Diagnostic test leading to the confirmation of definite or probable pneumococcal pneumonia**

<table>
<thead>
<tr>
<th>Diagnostic test positive</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture only</td>
<td>21</td>
<td>12.28</td>
</tr>
<tr>
<td>Sputum culture only</td>
<td>22</td>
<td>12.87</td>
</tr>
<tr>
<td>UAT(^+) only</td>
<td>108</td>
<td>63.16</td>
</tr>
<tr>
<td>Blood culture + Sputum</td>
<td>6</td>
<td>3.51</td>
</tr>
<tr>
<td>UAT(^+) + Sputum</td>
<td>14</td>
<td>8.19</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>171</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

The table above shows that the diagnostic test leading to the confirmation of definite or probable pneumococcal pneumonia, only UAT\(^+\) represent with high of 63.16% for the diagnostic test positive.
Table 7: Differences between patients with positive and negative test

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UAT positive</th>
<th>UAT negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.048</td>
</tr>
<tr>
<td>Male (%)</td>
<td>41.75</td>
<td>58.25</td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>55.08</td>
<td>44.92</td>
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</tr>
<tr>
<td>Mean age</td>
<td>48.86</td>
<td>51.13</td>
<td>0.019</td>
</tr>
<tr>
<td>Death (%)</td>
<td>64.29</td>
<td>35.71</td>
<td>0.277 (NS)</td>
</tr>
<tr>
<td>Asthma</td>
<td>38.46</td>
<td>61.54</td>
<td>0.460 (NS)</td>
</tr>
<tr>
<td>Immunosuppression (%)</td>
<td>100</td>
<td>0</td>
<td>0.146 (NS)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>29.17</td>
<td>70.83</td>
<td>0.058 (NS)</td>
</tr>
<tr>
<td>Ethanol use (%)</td>
<td>60.66</td>
<td>39.34</td>
<td>0.045</td>
</tr>
<tr>
<td>Prior antibiotics (%)</td>
<td>56.07</td>
<td>43.93</td>
<td>0.079 (NS)</td>
</tr>
</tbody>
</table>

Abbreviation: *NS: not significant

The table above shows that sex, age and ethanol use are statistically significant associated with positive and negative urinary antigen test; (p value=0.048, 0.019 and 0.045 respectively).
4. DISCUSSIONS

4.1 Prevalence

Our study demonstrated a high prevalence of positive pneumococcal urinary antigen test in Rwanda patients hospitalized with community acquired pneumonia. The s. pneumonia antigen was detected in 84% in non concentrated urine samples. The streptococcus pneumonia was detected in 17% patients with definitive pneumococcal pneumonia.

From patients with a diagnosis of probable pneumococcal pneumonia, urine antigen was detected in 36 of 52 of urine samples.

These data agree with previously published studies and showed the potential help of UAT in the diagnostic evaluation of patients with CAP.

In contrast to this high rate of UAT positivity, large prospective studies in Europe and Australia demonstrated low rate of positive UAT (19,23,26). Also a large Spanish retrospective analysis including over four hundred thousands hospitalized CAP patients over 5 years showed pneumococcus to be the cause in 17% (27).

This difference between this low rate of UAT positivity and our study may be explained by the declining incidence of pneumococcal infection in Europe due to a good vaccinal protection against streptococcus as well as efficacy antibiotics use. Still the explanation above given may not explain the entire scenario in this disparity. Therefore this is partially explained because it still unclear and ambiguous at this stage (16).

This prevalence of positive SPUAT in our study can reflect both real daily practice and local pneumonia etiology. Unfortunately, we could not confirm this high rate by combining it with other conventional diagnostic methods in all positive SPUAT patients. This rate is interesting to note: the rate with positive UAT patients and definitive etiology of CAP is comparable to the study of CHARLES et Al (26).
However, it will be tendacious to set up appropriate conclusions and epidemiology considerations when considering the rate, the prevalence and sensitivity of pneumococcal infection from this study, given it was about a test evaluation. This study has also similarities with previous studies: a high rate of SPUAT positivity was found by SORDER et Al in their study. In a total of 471 patients with CAP, 43 % patients were exclusively detected in urine antigen test (19).

4.2 Blood culture and spuat

With blood culture as the reference, positive UAT patients were 41,49 % and 58,51% for negative UAT patients. While only blood culture positivity tested were 22,34% and negatively UAT patients. We should take in account several issues that may be taken in considerations when we are analyzing these results of high positivity of UAT findings.

The relevance of microbial culture is still unclear. Only 22,34 % patients have positive cultures. The findings which have been reported elsewhere (15), obviously showed the limitations of the diagnostic yield of the cultures for etiological diagnosis of CAP.

In addition to this, 48,4% positive UAT patients have already received a antibiotic before they presented to the hospital at the time of samples collection.

In contrast to the culture tests, the results of urine antigen test are not affected by a prior antibiotic use or administration. This because the administration of any antibiotic before can be considered as a fact that can increase the rate of negative cultures and this not only for streptococcus species but also for others species (19,20).

Therefore the positivity of UAT with no cultural evidence leads to confusion. Is it a real true positive or just a cross reactivity results to other streptococcus species? It will remain therefore unclear in our study if whether the positive UAT patients were real true or false negative testing since the cultural yield, the reference method had a low rate of sensitivity.
The rate may also be taken with caution, because UAT testing can be positive in some adults with previous streptococcal carriage with no pneumonia. UAT testing may be found positive in healthy patients who had been colonized with S. pneumonia.

UAT may be found positive in healthy people who had been colonized with S. pneumonia (18). Unknown etiology or probable etiology with a positive SPUAT is a big problem. Considering the several limitations of cultural evidence and all related difficulties, we can raise the assumption that most of these patients with undetected pneumococcal pneumonia or probable or indefinite etiology may use an additional diagnostic testing for more etiological yield and should be considered for probable pneumococcal in origin when positive UAT. Because, it might be a true pneumococcal infection with no blood culture evidence (12,13,21).

Yet, the pneumococcal carriage should be raised also when we consider this high sensitivity rate and low rate of cultural methods. Although MARCOS et Al (19) in his study, found that the pneumococcal carriage in adults was not really very important so that it will impact with SPUAT positivity. But STRALINE et Al (20) revealed in contrast to this hypothesis that 1 of five carriers had weakly positive results in adults.

However, in our study, it had showed that 47% patients did not react to the SPUAT while they had a culture positive (16,2%) table1 . considering the fact that accuracy of UAT positivity might be affected by multiple cross reactivity to other microorganisms whether easily by the urine contamination by the skin flora , these findings in our study strongly suggested that a negative UAT results will be of great importance in clinical practice when we want to rule out pneumococcal infection in pneumonic patient at admission(21,13,19).

Also , the relative high rate of positive culture while SPUAT is negative showed the potential role of other pathogens(22)
4.3 Sputat and sputum for gram stain and culture

This is the first study done in RWANDA to evaluate and establish the SPUAT in pneumonic patients in admission as a diagnostic tool.

Sputum samples were available in 53.3%, see the table 3.

Not a surprise, the sputum evidence for the diagnosis of CAP had a low rate of positivity.

Only 14 patients had positive sputum for S. pneumonia while 6 patients for other microbes. Given that MARCOS et al (19) found a high rate of sputum positivity (68%) in his study, we can raise the assumption that many of our sputum sampling were not adequately taken. MARCOS et al used also other additional respiratory tools to increase the yield that were not available to us. Tracheo-bronchial aspirations, broncho-alveolar lavage or pleural puncture were used in their study. The latter diagnostic tools were of big help to adequate samples. In their study streptococcal pneumonia was detected from respiratory samples in 50% while in ours, only 6% were recovered with S. pneumonia. Good quality sputum is very very difficult in our settings.

4.4 Sputat and antibiotic changes

In our study, 48.2% of patents had a prior antibiotic use while 46.6% of patients did not. Among this, 56% patients were negative SPUAT patients and 46% of patients had no prior antibiotics use. The use of antibiotic in daily practice in Rwanda is common. These data agrees with the findings of MARCOS and al whodetected 38% of patients who had also prior antibiotics use.

4.5 Sputat and length of hospitalization

ROSENBAUM R et al (21) from his study in 2015 suggested that the mean length of most pneumonic patients admitted in general ward was between one week. Our findings disagree in a way and don’t support the ROSENBAUM results. In our study, most of positive UAT patients spent more than one week in admission: 56% patients spent more than 3 weeks compared to 1.3% that went less than a week. This suggested the possible cross reactivity with the UAT testings, possibilities of incidence of penicillines resistances and also existing co infections. All theses are high risk factors for lengthening of hospital stay.
4.6. Study limitations

The present results are much associated with the particular population of the admitted patients in Rwanda and probably can not be generalized or extended to the general pneumonic patients. In our study, there are many limitations. Fistly, it was a multi center study in nature and this impact that we could not follow appropriately all admitted patients to records exactly all the findings given we used our colleagues.

Secondly, some patients with good clinical scenario fitting with pneumonia were not included just because we were not there the time of admission or diagnostic material was not available. The lack of microbiological material to confirm our diagnosis was a big challenge of this study and this may unavoidably underestimate either the prevalence or the specificity and sensitivity of the test.

On the other hand, the sputum was not always easy to get. Yet gotten, it was not always of a good criteria. Also not all blood cultures were taken with no prior antibiotics.

Thirdly, the lack of both sputum and blood cultures samplings raised the issues of specificity and sensitivity accuracy because SPUAT positivity could not be coupled every time the others. That why, the proportions of SPUAT positivity is so high when compared to standard test.

That why also, our results showed a low diagnostic usefulness of SPUAT in the diagnosis of CAP.
CONCLUSIONS

This is the first study on streptococcal pneumonia done in RWANDA by using a urine antigen test. Lacking of available traditional methods was one the challenge we faced and we could only establish an etiological diagnosis in few population of our study.

It is commonly a big challenge to establish a microbiological diagnosis of community-acquired pneumonia. Our study SPUAT sensitivity and specificity is low; number of factors may explain this findings. However this test has high rate sensitivity and specificity as a method for diagnosis of pneumococcal community acquired pneumonia.

Given the fact that it is not influenced by prior antibiotic therapy use at the time of admission as well as it is both rapid and easy to use, this test may be used to increase the diagnostic yield with the conventional methods for the diagnosis of community acquired pneumonia.

With our results, we recommend this test as a complementary tool to the conventional ones to increase our yield of diagnosis. Nonetheless, numbers of cautions should be taken for its optimal use and practitioners should combine adequately both clinical scenario and all available diagnostic tools for accurate management of patients.

The rate of etiological diagnosis is low. We may increase this with improvement of laboratory services of district hospitals.
REFERENCES


APPENDICES

1. BUDGET
2. CONSENT FORM
3. QUESTIONNAIRE
4. WORK PLAN

1. Budget
Most of our expenses will be supported by our own funds because we are conducting this study is for our MMED dissertation. We do not have a funder till now.

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>COSTS</th>
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<tr>
<td>communications</td>
<td>250,000frws</td>
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<tr>
<td>Printings and photocopy of papers for questionnaires</td>
<td>100,000frws</td>
</tr>
<tr>
<td>Perdiem for four medical officers at district hospitals and six laboratory technicians (3000 per participants for doctors and 1000 frws for technicians)</td>
<td>1,500,000frws for doctors, 500,000frws for technicians</td>
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<tr>
<td>transportations</td>
<td>15,000frs/week for 6 months: 360,000frws</td>
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<tr>
<td>Statistics analysis and PI perdiem</td>
<td>1,000,000frws</td>
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<tr>
<td></td>
<td>3,710,000frws</td>
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</table>
2. Consent form

Study: Evaluation of pneumococcal urinary antigen test in university teaching hospitals in Rwanda.

My name is Dr LUSSUNGU Laurent Pierre Boileau. We are conducting a study in which we would like to assess the utility and the impact of a rapid antigen test detection of streptococcus pneumonia as the cause the pneumonia in CHUB and CHUK.

Community acquired pneumonia is one of the common causes of consultations to our hospitals and having an etiological diagnosis will significantly help in the management of this disease; that is why we are intending to perform this testing to increase our diagnostic tools.

Our purpose in this study is the clinical impact in patients with CAP using this test in order to improve the lives of patients by providing new data to clinicians.

Procedures

We will need to know about your presenting symptoms, your past medical history and your family history. Measurements of blood pressure, temperature, heart rate, ... will be taken.

We will also perform some laboratory and imaging studies like:

- samples of blood for FBC, blood culture, blood smear for malaria, sputum for gram stain and AFB
- Samples for renal function test, liver function test,
- Chest X-RAY will be done
- You will give urine for streptococcal pneumonia urinary antigen test detection immediately after being included in the study.

Your records will be handled as confidently as possible. All records will be coded and kept confidentially so that only myself and your doctor will have access.
3. Benefits

The information we will get from this study will help in the better management of patients with community acquired pneumonia in our hospital as well as in general population.

If you have understood and are willing to take part in this study, then kindly sign below. You have the right to decide to participate or to withdraw at any point in this study without jeopardy to your medical care.

A close attendant may give us information and allow us to do investigations with respect to family approval. He may also sign for you if you not well enough to sign in person.

I ................................................................. wish to take part in this study
Date ........................................................... signature

Or I (closer attendant) ............................................................... accept [name of the patient] ............................................................... to take part in this study, signed .................................

Date .................................................................
INFORMED CONSENT FORM / IBISOBANURO KU BUSHAKASHATSI

Bwana/Madame

Dushimishijwe no kubashishikarizabunobushakashashatsikubijyanyen’indwaraz’igufuzikunzekubaakarandekandizikiba sirabenshimuri aka kareredutuyemo. Iyindaray’umusongaikündaguterwan’agakokoko mu bwokobwa streptococcuspneumoniaieigateraindwarakomeyekandiabantubagapfaaribenshi.

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Muzatangainkaligupima streptococcal pneumonia.
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Kwinjira mu bushakashatsi.umukono
Itariki………………………………………………………………………..
QUESTIONNAIRE

1. Sociodemographic characteristics

   Age......................... Sex  ♂ Male ☐ Female ☐

   Provenance : Kigali city ☐ Est. ☐ West ☐ South ☐ North ☐

   Level of education : none ☐ primary ☐ secondary ☐ university ☐

   Profession : peasant farmer ☐ student ☐ jobless ☐ private sector ☐

   Public agent ☐ retired

2. comorbidities

   current smoking : yes ☐ no ☐ stopped ☐

   ethanol use yes ☐ no ☐

   if yes, how many bottles/per day.................................

   asthma yes ☐ no ☐

   COPD yes ☐ no ☐

   Immunosuppression yes ☐ no ☐

   Diabetes yes ☐ no ☐

   Renal failure yes ☐ no ☐

   Hypertension yes ☐ no ☐

3. antibiotic treatment

   - Prior Antibiotic treatment yes ☐ no ☐
   - No prior antibiotic yes ☐ no ☐
   - Unavailable data yes ☐ no ☐
4. microbiological testing

- Streptococcal pneumonia urinary antigen test: positive\(\checkmark\) negative\(\checkmark\)
- Blood culture positive\(\checkmark\) negative\(\checkmark\) if positive which microbes
- Sputum for gram stain and culture positive\(\checkmark\) negative\(\checkmark\)

5. imaging study

- Unilobar lesion yes
- Bilateral lesion yes
- Parapneumonic effusion yes

6. length in the hospital

- Less than 1 week..............yes
- Between 1 week and 3..............yes
- More than 3 weeks..................yes

7. outcome

Death yes no

8. Final diagnosis

- Streptococcal pneumonia yes
- Other bacterial pneumonia yes
- Unknown etiology yes
## WORK PLAN

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<th>march</th>
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