Antibiotic resistance of *Streptococcus pneumonia* serotypes in Kisii, Kenya

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Research article

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Abstract

Background: Pneumonia remains the leading cause of death in children under five worldwide, accounting for about 1.6 million deaths a year. Streptococcus pneumoniae has proved to be the most competent bacterial pathogen causing pneumonia infections among the infants and the elderly, leading to great morbidity and mortality. Currently there are over 94 different serotypes of Streptococcus pneumoniae, and quite a number have emerged as drug-resistant strains which introduce a twist to the current treatment strategies. Detailed information of current antibiotic susceptibilities, resistance and serotype distribution will be important in designing new strategies for treatment and management of pneumococcal infections.

Methods: A total of 309 S. pneumoniae isolates were collected from patients attending the Kisii Teaching and Referral Hospital between 2017 and 2018. Nasopharyngeal samples collected and serotyped using PCR. The samples were cultured and their sensitivity to drugs tested using various concentrations of; Oxacilin, Erythromycin, Tetracycline, Ofloxacin, Trimethoprim/sulfamethoxazole and Chloramphenicol.

Results: Out of the 309 samples collected, 235 were positive for S. pneumonia, although 10 of the samples had incomplete data. A total of 41 S. pneumonia serotypes isolated from the 309 samples with the most prevalent serotypes being; Non-typable (11%), 15B (7.6%), 19F (7.0%), 19A (6.7%), 23B (5.8%), 23F (5.3%), 6A (4.4%). All the serotypes were highly resistant to Oxacilin, and trimethoprim/sulfamethoxazole, while showing high sensitivity to Erythromycin, Ofloxacin and chloramphenicol. The rate of sensitivity to Tetracycline was 75% with the other 25% showing intermediate sensitivity.

Conclusions: The data collected showed a high rate of S. pneumonia (76%) consisting of various serotypes for various ages. The various serotypes isolated showed a marked resistance routinely used antibiotics for patient samples of various ages. The isolated serotypes also consisted of serotypes 19A, 6A, and 5B that are lacking in the pneumococcal vaccine (PCV 10). This data will provide relevant information for a better approach to both clinical treatment, management and prevention of infections with S. pneumonia.

Background

Streptococcus pneumoniae is the leading cause of pneumoniae, an infectious disease that causes up to 156 million new episodes each year worldwide, of which 151 million episodes are in the developing world. Pneumoniae has been categorized as the largest infectious cause of death among children and elderly worldwide. Pneumoniae killed 920 136 children under the age of 5 in 2015, accounting for 16% of all deaths of children under five years old. A great burden of the disease is in the Africa that accounts for more than half of the world’s deaths. In the sub-Saharan Africa, up to 26% of children below age of five die of pneumoniae, with Kenya ranked 18 with 125,000 deaths due to pneumoniae annually (mortality rank of 39).
There are over 94 different serotypes of pneumococcus that have been isolated, with only a few being invasive. The various serotypes have been seen to show variations based on the geographical location and age of the patient, with the children between 0-23 months having the highest risk\(^5,6\). Treatment of pneumoniae is by use of various antibiotics, though various serotypes have been seen to show resistance to specific antibiotics.

This study aimed to document the various serotypes of *P. pneumococcus* in Kisii, Kenya and their sensitivity and susceptibility to various antibiotics commonly used in the treatment of Pneumoniae.

**Methods**

The study was done at the Kisii teaching and referral hospital, which is the major referral hospital in Kisii County, also serving Nyamira county, Migori and other neighboring counties. A total of 309 *S. pneumoniae* isolates were collected from children attending the Hospital between 2017 and 2018. The sample population comprised of children who has received pneumococcal conjugate vaccine (PCV-10) but presented with clinical signs and symptoms of streptococcus pneumonia. Nasopharyngeal (NP) samples were collected using NP swab and inoculated on Blood Agar media then incubated at 37 degrees celcious with 5 -10% Co2 for 24-48 hrs to determine the growth of *S P* (procedure developed by Yukon Hospital) Drug sensitivity was done using nutrient agar whereas identification of SP was done by gram stain morphology before being serotyped using multiplex PCR\(^7\). The samples were then tested for sensitivity to various Tantibiotics using a standard minimum inhibitory concentration according to CLSI (2017). The antibiotics used in the study included; Oxacilin, Erythromycin, Tetracycline, Ofloxacin, Trimethoprim/sulfamethoxazole and Chloramphenical (the commonly used antibiotics in the area of study during the study period).

**Results**

**Streptococcus pneumoniae carriage rate**

Nasopharyngeal swabs for *S. pneumoniae* isolated were 309, of which 226 samples tested positive for various serotypes of *S. pneumoniae* and the rest (83 cases) tested negative. The positive samples comprised of 110 (49%) samples from boys and 116 (51%) samples from girls the positive samples represented a carriage rate of 73%, though there was no significant difference in the carriage rates between the various genders. The study showed that various ages of children were affected differently with ages between 2-4.9 years seeming to be most affected while the infections seemed to be low for children below 2years and as the children got closer to 5 years (Figure 1).

**Serotype distribution**
Serotyping was done using polymer chain reaction (PCR), 41 serotypes were isolated, with the following being more prevalent among the typable serotypes; st15B (7.5%), st19F (7.1%), and st19A (6.6%) (Figure 2)

**Drug sensitivity**

The isolates were tested with various antibiotics using CLSI standard inhibitory concentration (MIC, 2017). The isolates were classified as resistant, intermediate or sensitive. A summary of the antimicrobial sensitivity of the antibiotics is shown in below (table 1).

**Table 1: Anti-microbial susceptibility of *Streptococcus pneumoniae* isolates.**

| Drugs                  | Resistant | Intermediate | Sensitive |
|------------------------|-----------|--------------|-----------|
|                        | Frequency | Percentage   | Frequency | Percentage | Frequency | Percentage |
| Oxacilin               | 225       | 99.6%        | 1         | 0.4%       | 0         | 0%         |
| Tetracycline           | 0         | 0%           | 56        | 25%        | 170       | 75%        |
| trimethoprim/sulfamethoxazole | 226       | 100%         | 0         | 0%         | 0         | 0%         |
| Erythromycin           | 0         | 0%           | 0         | 0%         | 226       | 100%       |
| Chloramphenicol        | 0         | 0%           | 0         | 0%         | 226       | 100%       |
| Ofloxacin              | 0         | 0%           | 0         | 0%         | 226       | 100%       |

All the serotypes were highly resistant to Oxacilin, and trimethoprim/sulfamethoxazole, while showing high sensitivity to Ofloxacin and Chloramphenical. The rate of sensitivity to Erythromycin was high though 7 isolates of st11A showed resistance. Tetracycline showed 75% sensitivity with the other 25% showing intermediate sensitivity. The intermediate sensitivity by tetracycline was across all the serotypes.

**Discussion**

**Streptococcus pneumoniae carriage rate**
The study showed the carriage rate of S. pneumoniae among the study population was high (at 73%) than what has been reported in other studies including a study at Thika, Kenya (17%)\textsuperscript{8}, Kilifi Kenya (53% during dry period and 62% during rainy season)\textsuperscript{9}. The carriage rate is also higher than 24% which has been recorded for Kenya\textsuperscript{10}. The carriage rate was however lower than that reported in Nairobi, Kenya (Kibera), where 90% of the children were colonized and Tanzania with a carriage rate of 86% for children below 5 years\textsuperscript{11,12}. Based on the study by Abdullahi et al (2008) the distribution and carriage rate may be influenced by climate and geographical location of the carrier. In this respect kisii being majorly a highland standing about 1700m above sea level, it could be conducive for colonization of most pneumococcal serotypes. Other predisposing factors to a higher carriage may include weight gain, infection status of caregivers and HIV status\textsuperscript{11,13}.

**Serotype distribution**

The most prevalent serotypes among the typable serotypes were st15B, st19F, st19A, 23B, 23F, and 6A, this agrees with other studies done in kilifi and thika and Kibera, (all in Kenya). In both cases serotypes 19F, 23F and 6A featured as most common invasive serotypes, however the three studies did not feature as a major invasive serotype as did in the current study\textsuperscript{8,9,12}. The pneumococcal vaccine (PCV 10) used in Kenya against pneumococcal, has 10 serotypes; 1, 4, 5, 6B, 7F, 9V, 14, 8C, 19F and 23F. The vaccine however lacks important serotypes that has been seen to be invasive in Kenya including 6A, 19A, 23B and 15B, this means that it may not be very effective especially in the study region hence requires a review of the serotype components. A large number of serotypes (about 11%) were untypable, but showed different sensitivities to different antibiotics, since they dint fall to any specific category, makes it hard to ascertain their treatment and management, a matter that needs further studies. Most African countries tend to have similar invasive serotypes, whereas countries in other continents tend to differ. In Pakistan, serotype 18 has been seen to be the most invasive serotype\textsuperscript{13}, whereas China has the following as the most prevalent serotypes 19F, 19A, 15, 6B, 6A, and 17\textsuperscript{15}. In Spain, the most frequent serotypes were 3, 8 and 12F among strains causing invasive pneumococcal disease. The most prevalent serotypes in Trinidad and Tobago were 19F, 6B, 23F, 3, 19A, 6A, 14 and 9V. Though serotypes in countries out of Africa tended to be varied, all seemed to miss out serotype 1 and 5 especially for developed world, though it has been isolated in Europe and some American countries \textsuperscript{16,17}.

**Drug sensitivity**

Antimicrobial susceptibility of S. pneumonae to different antimicrobial agents are shown in Table 1. All the serotypes were highly resistant to Oxacilin, and trimethoprim/sulfamethoxazole, while showing high sensitivity to Erythromycin (96.9%), Ofloxacain (100%) and Chloramphenical (100%). The rate of sensitivity to Tetracycline was 75% with the other 25% showing intermediate sensitivity. Both sensitivity and intermediate sensitivity to tetracycline was across all the serotypes.
The data collected is in agreement with a number of studies done, though the percentages of susceptibility differs. Studies in Tanzania suggest Sensitivity to erythromycin to be 58.33%, with resistance of 41.67%\textsuperscript{12}. Another study done in thika revealed the following, 100% sensitivity to tetracycline, 96% sensitivity to erythromycin with 4% resistance, 98% sensitivity to chloramphenicol with a 2% resistance\textsuperscript{8}. A similar study in Nairobi shows a 32% resistance to Tetracycline\textsuperscript{18}. A study at Kibera showed tetracycline non-susceptibility in 19.2%, non-susceptibility for chloramphenicol at (1.9%) and non-susceptibility to erythromycin at 0.9%.

Other studies have also indicated low resistance to chloramphenicol\textsuperscript{19,20}. Resistance of the antibiotics have been suggested to be due to mutations in chromosomal genes or plasmids transferred from resistance bacteria or due to cost, with cheap antibiotics easily abused\textsuperscript{8}. In the current study, the approximate vaccine coverage of PCV10 (10%) and PCV13 (33.6%) which is lower than coverage recorded for Tanzania (37% and 49% for PCV 10 and PCV13 respectively).

Conclusions

The common invasive serotypes in Kisii are 15B, 19F, 19A, 23B, 23F, and 6A, although there many nontypable serotypes and serotype combinants. There is a marked resistance to routinely used antibiotics for various ages and various serotype. The isolated serotypes also consisted of serotypes 19A, 6A, and 5B that are lacking in the pneumococcal vaccine (PCV 10). This data will provide relevant information for a better approach to both clinical treatment, management and prevention of infections with S. pneumoniae.

However further research needs to be carried out to establish the possibility of high levels of oxacillin resistance and high levels of macrolide susceptibility.

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Declarations

Ethics approval and consent to participate

Study protocols were reviewed and approved by the Ethical Committee of Kisii Teaching and Referral Hospital. All participants provided written informed consent prior to study commencement. For participants younger than 18 years of age, written informed consent was obtained from each participant’s parents or legal guardian.

Consent for publication

All participants in the study signed a written consent for publication of the data while maintaining confidentiality.

Competing interests

The authors declare that they have no competing interests

Availability of data and materials

Data and materials are available on request from the corresponding author.

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The authors didn’t receive any funding in the study.

Authors contributions

Vincent Obino Orucho designed, performed sampling, data collection, data analysis and participated in manuscript preparation. Cyrus Orucho Ochoi did the research planning, data collection, data analysis and preparation of the manuscript. Maureeen Kerubo Nyangau, participated in data analysis, discussion of the results and development of the manuscript. All the
authors read and approved the final manuscript.

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**Figures**

![Bar Chart](image)

**Figure 1**

Distribution of S. pneumonae infection among various ages
Figure 2

Frequency of the isolated S. pneumoniae serotypes