RESEARCH ARTICLE

Streptococcus pneumoniae serotype specific anti-microbial susceptibility profiles among PCV-10 vaccinated and unvaccinated children attending Gertrude's Children's Hospital: a cross-sectional study [version 1; peer review: 2 approved with reservations]

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Abstract

Background: The spread of antimicrobial resistance threatens effective control and treatment of pneumococcal disease worldwide. In Kenya, an estimated one in every five children dies from pneumococcal disease every year. Of these, $\geq 50\%$ are attributable to antibiotic resistance. Consequently, the WHO has recommended that continuous regional surveillance be done to detect early resistance to available antibiotics and make necessary changes. We therefore investigated antimicrobial susceptibility patterns of *Streptococcus pneumoniae* among PCV-10 vaccinated and unvaccinated children ≤ 5 years old at Gertrude's Children's Hospital.

Methods: A 0.5 McFarland standard of freshly subcultured organisms were inoculated on Mueller–Hinton plates with 5% sheep blood agar. A standard disk dispenser was used to dispense various antibiotic disks on the Mueller–Hinton agar plate. Incubation was done overnight (20–24 hours) at 37°C in 5% CO₂ and clearance zones read using a Vanier caliber. Antimicrobials tested included vancomycin (30µg, ≥ 17 mm); erythromycin (15µg, ≥ 21 mm); clindamycin (2µg, ≥ 19 mm); oxacillin (1µg, ≥ 19 mm) and ceftriaxone (1µg, ≥ 30 mm).

Results: Thirty nine (92.86%) *Streptococcus pneumoniae* isolates were susceptible to erythromycin; 39 (92.86%) were susceptible to vancomycin; eight (19.86%) *Streptococcus pneumoniae* isolates were susceptible to oxacillin, while 34 (80.95%) were non-susceptible; 40 (95.24%) isolates were susceptible to clindamycin; and 24 (57.86%) isolates were susceptible to ceftriaxone, while 18 (42.86%) were non-

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susceptible. Children who attended daycare centers exhibited a four-fold significant risk of being resistant to ceftriaxone. All antibiotics studied were effective against *Streptococcus pneumoniae* except oxacillin and ceftriaxone, which exhibited high levels of non-susceptibility. Attendance of daycare centers, consumption of antibiotics two weeks prior to collection of sample and subject age were shown to be associated with an increased risk of *Streptococcus pneumoniae* being resistant to penicillins and ceftriaxone.

Conclusions: The law guiding use of antibiotics in Kenya should be meritoriously enforced to curb abuse of the available antibiotics.

Keywords

Streptococcus pneumoniae, Antimicrobial Resistance, Optochin, Kirby Bauer Test

This article is included in the **Pathogens** gateway.

This article is included in the **Antimicrobial Resistance** collection.

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Introduction

Globally, there is a growing concern over the burden of antimicrobial resistance (AMR) because it is presently estimated to account for more than 700,000 deaths per year worldwide (WHO, 2014). Hypothetically, studies have reported that if no appropriate measures are taken to arrest this unfortunate trend, AMR may cost up to 10 million lives and around US \$100 trillion per year by 2050 (de Kraker *et al.*, 2016). What is more appalling is that, contrary to some other health trepidations, AMR is a problem that concerns every country regardless of its level of income and development as resistant pathogens do not respect borders and jurisdictions (Cole, 2016).

Diverse forms of pneumococcal disease are rated among the highest vaccine preventable etiologies of child morbidity and mortality globally (Lowth, 2015). This is true despite availability of effective vaccines and antibiotic agents; it is therefore a matter of grave concern that *Streptococcus pneumoniae* is developing resistance to available antimicrobial agents. Paucity of quality and verifiable data on the pneumococci antimicrobial susceptibility profiles is a precursor for inadequate treatment guidelines, especially for countries that are still largely developing (Assamala, 2014). The gap in public health capacity is also an issue, given the changing resistance machineries and the emergence of multidrug-resistant *Streptococcus pneumoniae* that can only be detected through systematic screening in quality assured microbiology laboratories (O'Neill, 2016).

Overtime, there has been unswerving universal increase in antibiotic resistance by *Streptococcus pneumoniae* isolates, a trend that has made treatment options more challenging (Henriques-Normark & Tuomanen, 2013). The bacterium's polysaccharide capsule is a major virulence factor (Mitchell & Mitchell, 2010). The bacterial serotype is determined on the basis of the capsule's antigenic features, which have also been expansively correlated with AMR patterns. Although *Streptococcus pneumoniae* antimicrobial resistance is a wide-reaching concern, low and middle income countries (LMICs) are vulnerable to exceptional difficulties because substitute and/or effective therapeutic choices are either unattainable or too expensive for them to afford (Reardon, 2014). Experiential prescription of antibiotics for treatment of various forms of pneumococcal disease without data on antimicrobial susceptibility profiles is a very common practice, especially in Africa (Feldman & Anderson, 2016). Purchase of antibiotics over the counter and failing to comply with rules of antibiotic consumption and/or use significantly augments occurrence of AMR in Kenya (Donkor & Badoe, 2014). Unregulated and illicit hawking of major antibiotics at bus-stops by unlicensed persons and back-street manufacturing of counterfeit antimicrobial agents contribute majorly to pneumococci AMR (Laxminarayan *et al.*, 2013).

The end result of 'abuse' of antimicrobial agents meant for treatment of *Streptococcus pneumoniae* infections is apparent; the pneumococcus has connivingly developed resistance to novel antibiotic agents (Ventola, 2015). Since the rates of *Streptococcus pneumoniae* AMR vary on the basis of geographic location, time, age, and site of infection, AMR surveillance must

be incessant and guidelines be derived from local AMR data (Feldman & Anderson, 2016). As AMR impinges on the quality of patient care, concerted efforts must be made to understand the best way to circumvent it. To treat various forms of pneumococcal disease, antibiotics are the most commonly prescribed drugs in hospitals. Judicious use of antimicrobial agents is therefore one of the major avenues that can be employed to arrest the worrying trend of AMR.

An audit of *Streptococcus pneumoniae* antibiotic susceptibility patterns is imperative in the execution of a lucid and pragmatic antibiotic strategy. We therefore sought to analyze antimicrobial resistance profiles to commonly used antibiotics meant for treatment of pneumococcal disease among children ≤ 5 years in Nairobi County.

Methods

This was a descriptive cross-sectional study. *Streptococcus pneumoniae* anti-microbial susceptibility patterns among PCV-10 vaccinated and unvaccinated children below five years of age were measured between May 2017 to February 2018.

Ethical statement

Ethical approval for this study was given by the Kenyatta University Ethics Review Committee {KU/ERC/APPROVAL/VOL-UME.1 (12)} and permission to carry out the study obtained from the National Commission of Science Technology and Innovation (NACOSTI/P/17/65428/15801). Gertrude's Children's Hospital (GCH) research committee issued permission for the study to be conducted at the GCH outpatient clinic (GCH/ERB/VOLMMXVII/121). Biological mothers and/or legal guardians to eligible study subjects were approached to sign a written informed consent to allow their children participate in the study; this was done after reading and having the study explained to them. Participation in the study was completely on voluntary terms.

Study location

This study was conducted among children attending GCH, Nairobi County. GCH is the leading stand-alone hospital in East and Central Africa specializing in pediatric care. The hospital is accredited by the Joint Commission International (JCA). *Streptococcus pneumoniae* laboratory isolation, disk diffusion for AMR profiling and isolate stocking was done at the GCH main laboratory, while *Streptococcus pneumoniae* serotyping was done at KEMRI Wellcome Trust, Kilifi.

Study population

To take part in this study, biological mothers and/or legal guardians were approached at the GCH outpatient clinic when they brought their children for medical attention. For inclusion in this study, the subject was required to: be at most five years of age; be a resident of either Nairobi or Kiambu Counties; have been vaccinated with either three doses of PCV-10 or none (any child who had received less than three doses was considered ineligible); not be consuming any antibiotics at the time of the study; be clinically diagnosed by a physician at the site as having some form of pneumococcal disease; and the

parent or guardian needed to voluntarily sign an informed consent form. Subjects who had known HIV/AIDS infection or had any immunosuppressive conditions were excluded from the study.

Sample size determination

To determine the minimum sample size, the Fisher's formula (1998) was used, with a prevalence rate of 16% (Tuti *et al.*, 2017) being the current occurrence rate of pneumococcal disease in Kenya.

$$n = \frac{z^2 \hat{p}(1 - \hat{p})}{m^2}$$

Where: n = desired minimal sample size; z = standard normal deviation = 1.96 (from the tailed normal table); $\hat{p}$ = prevalence rate; and m = desired degree of accuracy at 95% confidence level = 0.05. $n = 1.96^2 \times 0.16 (1-0.16) / 0.05^2 = 206$; therefore, $n = 206$.

Sociodemographic characteristics

A standardized questionnaire was used to collect data on the subjects' sociodemographic features. The characteristics assessed included whether the child attended daycare or not, exposure to both cigarette and cooking smoke, breastfeeding frequency during the first six months after birth (classified as: none, meaning the child was never breastfed at all; moderate, meaning the child partially breastfed and partially given other food; and exclusive, meaning the child was entirely dependent on breast-milk), child's age and PCV-10 immunization status.

Isolation and identification of *Streptococcus pneumoniae*

Nasopharyngeal swabs collected using flocked cotton swabs (Copan) were the specimen of choice for this study. All swabs were initially suspended in Amies Media and transported to the laboratory within three hours of collection. Each swab was inoculated onto a selective gentamicin blood agar (GBA) enriched with 5% sheep blood. The plates were incubated at 37°C in 5% CO₂ and examined at 16–24 hours and then again at 40–48 hours for growth of *Streptococcus pneumoniae*.

Isolates were identified as *Streptococcus pneumoniae* on the basis of colony morphology (mucoid, draughtsman's appearance, α -haemolysis), susceptibility to optochin (ethyl hydrocupreine hydrochloride) and, where necessary, solubility in bile salts (desoxycholate salts). The isolation of a single colony indicated nasopharyngeal carriage.

Optochin sensitivity test

All colonies on GBA plates demonstrating *Streptococcus pneumoniae* characteristics were evenly inoculated on plain blood agar (BA) plates. This was done using a sterile inoculating loop. An optochin disk was aseptically placed on the preparation using a sterile antibiotic dispenser and incubated for 24–48 hours at 37°C in 5% CO₂ atmosphere. After 24–48 hours, a clearance zone of ≥ 14 mm around the optochin disk was a confirmation that the organism was *Streptococcus pneumoniae*. Plates with clearance zones < 14 mm but ≥ 6 mm were further subjected to a bile

solubility test. Bile soluble stocks confirmed that the organism was *Streptococcus pneumoniae*.

Bile solubility test

Using a sterile cotton inoculating swab, *Streptococcus pneumoniae* colonies from the blood agar plate were added to 1.0 ml of 0.85% saline solution to achieve 1.0 McFarland standard turbidity. The cell suspension was divided into two equal volumes of 0.5 ml/tube. Then, 0.5 ml of 2% sodium desoxycholate solution (bile salts) were added to one tube and 0.5 ml of 0.85% saline solution to another tube. The two tubes were gently mixed and incubated for up to two hours at 37°C in 5% CO₂. The tubes were vortexed while observing for any clearance of turbidity within 10 minutes and subsequently checked until two hours later. Any clearance in the tube containing bile salts and not in the saline tube confirmed that the organism was *Streptococcus pneumoniae*.

Quellung reaction

Capsular serotyping was done using the Quellung reaction test according to (Habib *et al.*, 2014). Frozen vials containing *Streptococcus pneumoniae* stocks stored at -70°C were thawed at room temperature for about 30 minutes. Next, 10 μ l of the stored *Streptococcus pneumoniae* cells were suspended in 50 μ l PBS and gently vortexed. Subsequently, 10 μ l of the suspended cells were added on to a glass slide and mixed with 10 μ l pooled antisera (Statens Serum Institute, cat. No.16744). The glass slide was swirled gently while observing for any agglutination reaction until a positive reaction was observed with various pooled antisera. The process was repeated with individual groups under various antisera pools. After that, 10 μ l of the suspended cells in PBS were added to a glass slide and mixed with various *Streptococcus pneumoniae* serotype-specific antisera included in the antisera pools that gave a positive reaction. This was done until a positive reaction with the particular serotype specific antisera was observed.

Those serotypes that did not belong to any pool were typed directly until a positive agglutination reaction was observed. The cells/PBS/serotype-specific antisera mixture on the glass slide were covered with a cover slip and observed under a phase contrast microscope with a $\times 400$ objective lens with oil emulsion.

Disk diffusion method

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guideline (Hegstad *et al.*, 2014). A 0.5 McFarland standard of freshly subcultured pneumococci organisms was inoculated on a 150 mm Mueller–Hinton plate with 5 % sheep blood (MH-BA). A standard disk dispenser was used to dispense the various antibiotic disks on the MH-BA agar (CLSI, 2004). Incubation was done overnight (20–24 hours) at 35°C in 5% CO₂ and various clearance zones read with the aid of a vernier caliber. Antimicrobial agents tested included vancomycin (30 μ g, ≥ 17 mm), erythromycin (15 μ g, ≥ 21 mm), clindamycin (2 μ g, ≥ 19 mm), oxacillin (1 μ g, ≥ 19 mm) and ceftriaxone (1 μ g, ≥ 30 mm). Because of ambiguity caused by poorly resolved concentration gradient around disks

containing cephalosporins, this method may not give accurate results for ceftriaxone.

Statistical analysis

Data were entered, cleaned and analyzed on SPSS version 22. Descriptive statistics were used to summarize socio-demographic and antibiotic susceptibility patterns of *Streptococcus pneumoniae* isolates.

Adjusted logistic regression was used to establish the strength of relationship between selected risk factors and susceptibility patterns of the selected antibiotic agents studied.

Results

A total of 206 subjects were recruited to participate in this study. It was found that 98% ($n=202$) of the subjects' mothers/guardians were non-smokers as compared to 2% ($n=4$) who were smokers. About 96% ($n=197$) of the subjects did not share a household with an alcoholic, while 4% ($n=9$) did. Regarding housing, 55% ($n=113$) of the subjects lived in single rooms, 20% ($n=42$) lived in one-bedroom houses, 22% ($n=46$) lived in two-bedroom houses, while 2% ($n=5$) lived three or more bed roomed houses. It was found that 46% ($n=94$), 30% ($n=62$), 18% ($n=38$), 4% ($n=9$) and 1.5% ($n=3$) of the subjects cooked using gas, stove, charcoal, firewood and electricity, respectively. In addition, 75% ($n=154$) of the subjects' households disposed of their waste in the public sewer system, 23% ($n=47$) disposed of their waste in private septic tanks and 2% ($n=5$) disposed of their waste using other undisclosed ways. The majority, 90% ($n=185$) of the subjects lived in households that used electricity as their source of light, while 5% ($n=11$) used traditional lamps, 2% ($n=5$) used candles for lighting, 1% ($n=3$) used solar energy and 0.49% ($n=1$) used lanterns. Within the two weeks preceding their visit to the clinic, 54% ($n=112$) of the subjects had consumed antibiotics, while 46% ($n=94$) had not. Daycare centers were not attended by 81% ($n=167$) of the subjects as compared to 19% ($n=39$) who did attend. Approximately 30% ($n=61$) of the subjects shared their households with three people other than their parents/guardians, 25% ($n=52$) shared with two other people, 18% ($n=37$) shared with one other person, 16% ($n=33$) shared with four other people, 6% ($n=12$) shared with five other people and 5% ($n=10$) shared with more than five other people. Lastly, 65% ($n=133$) of the subjects were exclusively breastfed, 35% ($n=72$) were moderately breastfed, while 0.49% ($n=1$) had never been breastfed (Table 1).

From the 206 subjects recruited, a total of 42 pneumococci were isolated. Out of the 42 total *Streptococcus pneumoniae* isolates, 39 (92.86%) were susceptible to erythromycin and vancomycin, eight (19.86%) were susceptible to oxacillin, 40 (95.24%) were susceptible to clindamycin and 24 (57.86%) were susceptible to ceftriaxone (Table 2). All the eight (19.05%) isolates of serotype 28F were susceptible to erythromycin, vancomycin and clindamycin; one isolate (2.38%) was susceptible to oxacillin and three (7.14%) were susceptible to ceftriaxone. All the five (11.9%) isolates of serotype 6A were susceptible to erythromycin; four (9.52%) were susceptible to vancomycin, one (2.38%) was susceptible to oxacillin, four (9.52%) were

susceptible to clindamycin and two (4.76%) were susceptible to ceftriaxone.

Isolates from serotypes 13, 15B, 21, 35B, 35F, 39, 48 and untypeable were susceptible to erythromycin, vancomycin, clindamycin and ceftriaxone but were non-susceptible to oxacillin, except serotype 21 and untypeable. Isolates from serotypes 3, 23A, 23B, 20, 11A, 17F, 19B and 7C were susceptible to erythromycin, vancomycin and clindamycin. Serotypes 3, 23A and 11A were resistant to erythromycin and serotypes 11A and 7C were resistant to vancomycin. Serotypes 3, 23A, 23B, 20, 17F, 19B and 7C were non-susceptible to oxacillin. Serotype 17F was resistant to clindamycin, while serotypes 3, 23B, 11A, 17F and 7C were resistant to ceftriaxone (Table 3).

Compared with males, *Streptococcus pneumoniae* isolated from female subjects were more likely to be resistant to erythromycin (OR 1.94; CI 0.23, 16.10) but odds of being resistant to vancomycin, oxacillin, clindamycin and ceftriaxone decreased when the subject was female: OR 0.14 (95% CI 0.01, 2.81), OR 0.51 (95% CI 0.11, 2.26), OR 0.51 (95% CI 0.11, 2.26), OR 0.2 (95% CI 0.01, 4.43) and OR 0.56 (95% CI 0.17, 1.86), respectively. The odds of being resistant to erythromycin, vancomycin, oxacillin, clindamycin and ceftriaxone decreased when the subject was aged 13–24 months, 25–36 months and 37–48 months old, but the odds increased when the subject was aged 49–60 months old. The odds of being resistant to erythromycin, vancomycin, oxacillin and clindamycin increased when the subjects had consumed antibiotics two weeks prior to the study. However, the odds of being resistant to ceftriaxone decreased. The odds of being resistant to the tested range of antimicrobial agents decreased when the subjects had had none, moderate and exclusive breastfeeding types with the exception of oxacillin whose odds of resistance increased when the subjects had had moderate breastfeeding. Odds of being resistant to the tested antimicrobial agents decreased when the subjects had received a full dose of PCV-10, except for vancomycin and ceftriaxone whose odds of resistance increased when the subjects had received a full dose of PCV-10. The odds of being resistant to the tested antimicrobial agents significantly increased when the subjects had been attending daycare centers (Table 4).

Discussion

Antimicrobial resistance is a major global public health hazard in the waiting lounge. Infections that are resistant to antibiotics are often hard to treat, the cost of managing them is much higher and they generally pose more stress on families' finances. Its envisaged that by 2050, general global mortality due to antimicrobial resistance will be at 10 million people annually (de Kraker *et al.*, 2016); *Streptococcus pneumoniae* is likely to be one of the major culprits, the need for its earlier containment has never been more urgent.

The WHO (2014) recommends the use of penicillins as the first line of antibiotic therapy for treatment of pneumococcal disease and; clindamycin, erythromycin, vancomycin and cephalosporins as alternative antibiotic interventions. In the current study, most (93%, $n=39$) of the *Streptococcus pneumoniae* isolates were

Table 1. Socio-economic Demographic Characteristics of PCV-10 Vaccinated and Unvaccinated Children Attending Gertrude's Childrens Hospital.

Characteristic	Description	<i>n</i>	%
Education level of mother			
	Primary School	61	29.61
	Secondary School	88	42.72
	Tertiary College	38	18.45
	University	19	9.22
Mother's smoking status			
	Smoker	4	1.94
	Non-smoker	202	98.06
Alcoholic in the house			
	Yes	9	4.37
	No	197	95.63
Size of the house of residence			
	Single room	113	54.85
	One bedroom	42	20.39
	Two bedrooms	46	22.33
	Three bedrooms	5	2.43
Cooking fuel			
	Firewood	9	4.37
	Stove	62	30.1
	Charcoal	38	18.45
	Gas	94	45.63
	Electricity	3	1.5
Waste disposal method			
	Public sewerage system	154	74.76
	Private septic tank	47	22.82
	Other	5	2.43
Source of light			
	Candle	5	2.43
	Traditional lamp	11	5.34
	Lantern	1	0.49
	Solar	3	1.46
	Electricity	185	89.81
	Missing	1	0.49
Recent consumption of antibiotics (two weeks prior to study)			
	Yes	112	54.37
	No	94	45.63
Attendance at day care center			
	Yes	39	18.93
	No	167	81.07

Characteristic	Description	n	%
No. of household occupants			
	One	37	17.96
	Two	52	25.24
	Three	61	29.61
	Four	33	16.02
	Five	12	5.83
	≥ Five	10	4.85
Breast feeding type			
	None	1	0.49
	Moderate	72	34.95
	Exclusive	133	64.56
Child's weight at birth			
	1.1-2.0 kgs	5	2.43
	2.1-3.0 kgs	6	2.91
	3.1-4.0 kgs	37	17.96
	4.1-5.0 kgs	158	76.7
Child immunization			
	Yes	106	51.46
	No	100	48.54

n, total number of subjects per category from a total of 206; %, percentage of subjects per category.

Table 2. Susceptibility of *Streptococcus pneumoniae* isolates to various antimicrobial agents among PCV10 Vaccinated and Unvaccinated Attending Gertrude's Childrens Hospital.

Antimicrobial agent	n=42	Percent (%)
Erythromycin		
Sensitive	39	92.86
Resistant	3	7.14
Vancomycin		
Sensitive	39	92.86
Resistant	3	7.14
Oxacillin		
Sensitive	8	19.05
Resistant	34	80.95
Clindamycin		
Sensitive	40	95.24
Resistant	2	4.76
Ceftriaxone		
Sensitive	24	57.86
Resistant	18	42.86

The table above summarises the susceptibility of *Streptococcus pneumoniae* to various antibiotic agents at different concentrations. Vancomycin (30µg, ≥17mm); erythromycin (15µg, ≥21mm); clindamycin (2µg, ≥19mm); oxacillin (1µg, ≥19mm) and ceftriaxone (1µg, ≥30mm) (CLSI, 2017).

sensitive to erythromycin and vancomycin. Erythromycin and vancomycin act by inhibiting protein synthesis and development of the cell wall in the target bacteria (Tenson *et al.*, 2003). *Streptococcus pneumoniae* tolerance to erythromycin and vancomycin can be attributed to the previous irregular exposure to the drug agents (Tenson *et al.*, 1997).

However, in the current study, the number of erythromycin and vancomycin tolerant *Streptococcus pneumoniae* is almost medically insignificant as compared to previous studies, which reported that rates of nasopharyngeal *Streptococcus pneumoniae* susceptibility to erythromycin and vancomycin were even higher among healthy preschool children in Vilnius (90.4 and 98 % in 2006, respectively) (Heelan *et al.*, 2004).

About 19% (n=8) of the *Streptococcus pneumoniae* isolates were susceptible to oxacillin. Being an antibiotic in the penicillin group of drugs, it is expected that it will be one of the most prescribed and irregularly used on the local market. The current results are similar to those observed among non-invasive *Streptococcus pneumoniae* strains in pediatric populations in Poland and Russia where only 29.5% to 29.2% and 16.7% to 19.3% of the isolates in these respective countries were susceptible to penicillins over a period of two consecutive years (Albrich *et al.*, 2004; Mayanskiy *et al.*, 2014) before routine PCV vaccination. What is common in both scenarios is that with poorly enforced National Antibiotic Use Plans (NAUP), the general population is predisposed to purchase of antibiotics over the counter without valid a physician's prescription, effectively exposing the general populace to the dangers of resistant

Table 3. *Streptococcus pneumoniae* serotype susceptibilities to various antimicrobial agents among PCV-10 Vaccinated and Unvaccinated Children Attending Gertrude's Childrens Hospital.

Serotype	Erythromycin, n (%)		Vancomycin, n (%)		Oxacillin, n (%)		Clindamycin, n (%)		Ceftriaxone, n (%)	
	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
28F	0	8 (19.05)	0	8 (19.05)	7 (16.67)	1 (2.38)	0	8 (19.05)	5 (11.9)	3 (7.14)
6A	0	5 (11.90)	1 (2.38)	4 (9.52)	4 (9.52)	1 (2.38)	1 (2.38)	4 (9.52)	3 (7.14)	2 (4.76)
3	1 (2.38)	3 (7.14)	0	4 (9.52)	4 (9.52)	0	0	4 (9.52)	2 (4.76)	2 (4.76)
23A	1 (2.38)	2 (4.76)	0	3 (7.14)	3 (7.14)	0	0	3 (7.14)	0	3 (7.14)
23B	0	3 (7.14)	0	3 (7.14)	2 (4.76)	1 (2.38)	0	3 (7.14)	2 (4.76)	1 (2.38)
20	0	3 (7.14)	0	3 (7.14)	2 (4.76)	1 (2.38)	0	3 (7.14)	0	3 (7.14)
11A	1 (2.38)	1 (2.38)	1 (2.38)	1 (2.38)	2 (4.76)	0	0	2 (4.76)	1 (2.38)	1 (2.38)
17F	0	2 (4.76)	0	2 (4.76)	1 (2.38)	1 (2.38)	1 (2.38)	1 (2.38)	2 (4.76)	0
19B	0	2 (4.76)	0	2 (4.76)	1 (2.38)	1 (2.38)	0	2 (4.76)	1 (2.38)	1 (2.38)
7C	0	2 (4.76)	1 (2.38)	1 (2.38)	2 (4.76)	0	0	2 (4.76)	1 (2.38)	1 (2.38)
13	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
15B	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
21	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)
35B	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
35F	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
39	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
48	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)
Untypeable	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	1 (2.38)	0

The table above shows *Streptococcus pneumoniae* serotypes susceptibility profiles to selected antimicrobial agents. The antimicrobial agents are vancomycin (30µg, ≥17mm), erythromycin (15µg, ≥21mm), clindamycin (2µg, ≥19mm), oxacillin (1µg, ≥19mm) and ceftriaxone (1µg, ≥30mm).

pneumococcal infections. This, coupled up with failure to consume the correct regimen, results in antibiotic selection pressure, which consequently brings forth resistant strains that are then horizontally spread across the population (Neu, 1992). This would explain the unexpected high resistance levels of *Streptococcus pneumoniae* serotypes to oxacillin. These results are consistent with those of Bronzwaer *et al.* (2002), who reported increased (53%, $n=115$) antibiotic failure, especially to different derivatives of penicillins.

High levels of sensitivity and tolerance to clindamycin and ceftriaxone agree with findings by scientists at the St. Jude Children's Research Hospital, who demonstrated that clindamycin and azithromycin, which kill bacteria by inhibiting protein synthesis, are more effective than a standard first-line treatment with the beta-lactam antibiotic ampicillin, which causes the bacteria to lyse, or burst (Sutcliffe *et al.*, 1996).

Clindamycin is especially recommended for *Streptococcus pneumoniae* treatment because of its spectrum of activity includes group A streptococci (Puopolo *et al.*, 2007). Usually, it is reserved for cases of *Streptococcus pneumoniae* with high resistance to penicillins and/or cases of subjects being allergic to first line antibiotic therapies (Karlström *et al.*, 2009). The

high level of ceftriaxone tolerant *Streptococcus pneumoniae* (CT-*Streptococcus pneumoniae*) observed in this study is concerning. This is because broad spectrum cephalosporins like ceftriaxone are recommended for invasive pneumococcal disease in cases where other first line antibiotics have failed (Molyneux *et al.*, 2011). Resistance would only be expected in cases where the subjects had been inappropriately exposed to other cephalosporins as the first line of therapy (Von Mollendorf *et al.*, 2014). Resistance to penicillins has also been reported to have a nexus with resistance to cephalosporins as they both target the beta-lactam ring of the bacteria (Drawz & Bonomo, 2010). Irregular and non-prescribed purchase of antibiotics at local pharmacies and shops may be the other culprits behind these unfortunate findings.

Pneumococcal resistance to ceftriaxone was found to be high (up to 66–67%) in Estonia (Tadesse *et al.*, 2017) and Romania, while the rates were lower in Norway (4.6%) (Steens *et al.*, 2015), the Netherlands (12.9%) (Voets *et al.*, 2012) and the Czech Republic (15.7 %) (Zemlickova *et al.*, 2010). *Streptococcus pneumoniae* has over 90 serotypes belonging to different groups according to their capsular polysaccharides and the degree of cross-reactivity (Hathaway *et al.*, 2012). The various *Streptococcus pneumoniae* serotypes behave differently when exposed

Table 4. Effect of Selected Risk Factors on *Streptococcus Pneumoniae* Susceptibility to Various Antimicrobial Agents among PCV-10 Vaccinated and Unvaccinated Children Attending Getrudes Children's Hospital

Risk factor	Erythromycin		Vancomycin		Oxacillin		Clindamycin		Ceftriaxone	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Gender										
Male	1		1		1		1		1	
Female	1.94 (0.23, 16.10)	0.541	0.14 (0.01, 2.81)	0.196	0.51 (0.11, 2.26)	0.372	0.2 (0.01, 4.43)	0.308	0.56 (0.17, 1.86)	0.341
Age (months)										
6–12	1		1		1		1		1	
13–24	0.34 (0.01, 7.98)	0.504	0.34 (0.01, 7.98)	0.504	0.25 (0.03, 2.32)	0.224	2.07 (0.18, 23.36)	0.557	0.3 (0.05, 1.74)	0.181
25–36	0.76 (0.09, 6.61)	0.801	0.76 (0.09, 6.61)	0.801	0.26 (0.03, 2.09)	0.207	0.41 (0.02, 11.05)	0.598	0.58 (0.13, 2.49)	0.463
37–48	0.64 (0.03, 16.05)	0.789	0.64 (0.03, 16.05)	0.789	0.23 (0.02, 2.87)	0.251	1.15 (0.04, 33.33)	0.936	0.34 (0.04, 2.87)	0.321
49–60	1.16 (0.04, 32.08)	0.930	1.16 (0.04, 32.08)	0.93	0.1 (0.01, 1.62)	0.104	2.07 (0.06, 66.3)	0.682	0.79 (0.07, 9.22)	0.85
Recent use of antibiotics (two weeks)										
No	1		1		1		1		1	
Yes	1.30 (0.16, 10.78)	0.810	1.3 (0.16, 10.78)	0.81	0.8 (0.18, 3.58)	0.771	4.11 (0.19, 91.08)	0.371	0.89 (0.27, 2.98)	0.856
Breast feeding type										
None	1		1		1		1		1	
Moderate	0.09 (0.00, 6.45)	0.270	0.78 (0.03, 23.53)	0.885	1.93 (0.06, 62.17)	0.71	0.29 (0.01, 10.76)	0.502	0.16 (0.01, 4.41)	0.28
Exclusive	0.47 (0.02, 13.89)	0.660	0.06 (0, 4.13)	0.192	1 (0.04, 27.7)	1	0.18 (0.01, 6.72)	0.356		
PCV-10 immunization										
No	1		1		1		1		1	
Yes	0.52 (0.06, 4.29)	0.541	1.59 (0.19, 13.17)	0.67	0.64 (0.14, 2.84)	0.553	0.16 (0.01, 3.64)	0.253	1.24 (0.37, 4.09)	0.729
Day care attendance										
No	1		1		1		1		1	
Yes	1.69 (0.20, 14.37)	0.633	5.35 (0.62, 45.99)	0.126	2.14 (0.32, 14.27)	0.431	2.9 (0.27, 31.05)	0.378	4.97 (1.17, 21.14)	0.03

Odds ratio (OR)=1: exposure does not affect odds of outcome; OR>1: exposure associated with higher odds of outcome; OR<1: exposure associated with lower rates of outcome. Confidence intervals (CI) are used to estimate the precision of the OR. The higher the CI the lower the precision of the OR and the lower the CI the higher the precision of the OR. P-value is a measure of the significance level of the exposure.

to different antibiotic agents (Reinert, 2009). In this study, seven serotype 28F strains out of eight were tolerant to oxacillin, while only three were sensitive to ceftriaxone. Oxacillin, which is a penicillin, is normally given as a first line of treatment for the various forms of pneumococcal disease infections and ceftriaxone is a broad spectrum antibiotic normally given after penicillin has failed (File, 2006). The mode of resistance to the two antimicrobial agents is shared to some extent as both involve the use of a four atom beta-lactam ring (Kong *et al.*, 2010). As such, resistance in one is likely to influence resistance in another. Inappropriate exposure to one or both of these agents prior to the collection of the study samples may explain the high resistance levels in both.

Most strains of serotypes 6A, 3, 23A, 23B, 20, 11A and 17 were oxacillin and ceftriaxone tolerant but highly susceptible to erythromycin, vancomycin and clindamycin. These results agree with findings from a previous study by Liñares *et al.* (2010), who demonstrated *Streptococcus pneumoniae* serotype susceptibility patterns to different antibiotics and intimated that serotype 28F and 6A are susceptible to erythromycin, vancomycin and clindamycin but highly resistant to penicillin and ceftriaxone. Serotypes 19A, 7C, 13, 15B, 21, 35B, 35F, 39, 48, and the untypeable *Streptococcus pneumoniae* were highly intolerant to erythromycin, clindamycin, vancomycin, oxacillin and ceftriaxone. While most *Streptococcus pneumoniae* serotypes in this study were susceptible to majority of the antimicrobials studied, resistance to oxacillin and ceftriaxone was clinically significant and largely worrying. A similar study examined antibiotic susceptibility of non-invasive *Streptococcus pneumoniae* in children aged less than six years with signs of an acute respiratory tract infection in Moscow, Russia. Non-susceptibility to penicillin, erythromycin and MDR was found in 28%, 26% and 22% of pneumococcal strains, respectively (Mayanskiy *et al.*, 2014).

The slightly lower rates of *Streptococcus pneumoniae* resistance may be due to the enrolment of both vaccinated unvaccinated children and differences in the enactment and implementation of the antibiotic use/consumption policies. Although none of the risk factors had a significant influence on the *Streptococcus pneumoniae* nasopharyngeal carriage, odds demonstrated that age, gender, recent consumption of antibiotics by subjects, attendance at daycare centers, breast-feeding type and vaccination status had a bearing on the effectiveness of antibiotics. For instance, being female and young increased the subject's susceptibility to erythromycin tolerance, while decreasing

susceptibility to vancomycin, clindamycin, ceftriaxone and oxacillin. Children exposed to antibiotics within the two weeks prior to sample collection and PCV-10 unvaccinated children had increased odds of being resistant to all the antibiotics studied. Breastfeeding type, attendance at day-care centers and PCV-10 vaccination status play a major role in antibiotic susceptibility according to the findings of this study.

With the exception of oxacillin, the odds of *Streptococcus pneumoniae* being resistant to clindamycin, vancomycin, erythromycin, ceftriaxone decreased when subjects had been moderately or exclusively breastfed. According to Lynch & Zhanel (2009), due to the likelihood of horizontal transfer of *Streptococcus pneumoniae* resistant genes within a community, subjects who attended daycare centers and had not received PCV-10 immunization had increased chances of being resistant to all the sampled antibiotics. These results are consistent with findings from Lynch & Zhanel (2010) who postulated that age, PCV7 vaccination, attendance at day care or school, previous respiratory infection and non-susceptibility to penicillin were significantly or insignificantly associated with antibiotic resistance. While some of the results may not have strong research significance, they pose significant clinical concerns.

Data availability

This project contains the following underlying data:

Name of Repository: Harvard dataverse

Data Name: Streptococcus Pneumoniae Serotype Epidemiology, Antibiotic Susceptibility Profiles and Associated Risk Factors among Children Attending Getrudes Children's Hospital
DOI: <https://doi.org/10.7910/DVN/VDMKS2> (Walekhwa, 2019)

Extended data

This project contains the following extended data:
Questionnaire

Acknowledgements

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Version 1

Reviewer Report 10 August 2020

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Izabela Korona-Glowniak

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The manuscript deals with an interesting topic in the field of pneumococcal resistance epidemiology. The link between antibiotic use and the emergence of bacterial resistance has been clearly established and many studies have shown the increase in antibiotic resistance of *S. pneumoniae*. Due to geographical diversity of *S. pneumoniae* resistance, which depends on the local antimicrobial policy, epidemiological studies in each geographical region should be determined and analyzed separately.

The topic is interesting and worthy of note, yet the manuscript have to be improved.

1. Abstract: Methods paragraph is too detailed.
2. Method: In my opinion, the methods - especially related to diagnostic tests - are described too extensively.
3. Results: The authors should avoid duplicating the results in tables and in the text. The first paragraph of the Results is unnecessary. All data are shown in Table.
4. The last sentence of the Result " The odds of being resistant to the tested antimicrobial agents significantly increased when the subjects had been attending daycare centers" is an over interpretation of the statistical analysis. All but one tests showed insignificant differences between the analyzed groups.
5. The term odds in description of the statistical analysis results sounds awkward. The authors should rewrite the text using other terms, for example risk.
6. Discussion: The first paragraph of the Discussion is a repetition of information given in the Introduction.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: microbiology, antibiotic resistance, microbiological diagnostics,

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 05 November 2019

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Bartholomew N. Ondigo

Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

Abstract

- Provide N, number of children sampled.
- Clearance zones read in what units of measurements?
- Results – This need to be tailored as per the objective of the manuscript: patterns of *Streptococcus pneumoniae* among PCV-10 vaccinated and unvaccinated children ≤ 5 years old at Gertrude's Children's Hospital. However, the results are written focusing on day care centres.
- Key words – italicise scientific names.

Introduction

- Paragraph 1- Repetition of globally and worldwide in the same sentence. The focus of this paragraph need to be antimicrobial resistance.
- Paragraph 2 - Rewrite to introduce *Streptococcus pneumonia* as the causative agent of pneumococcal disease.
- Paragraph 3 – Should focus briefly on the mechanisms of antimicrobial resistance.
- Paragraph 4 – The problem and uniqueness of the study.

Methods

- This was a descriptive cross-sectional study conducted among children attending Gertrude's Children's Hospital (GCH) in Nairobi.
- Ethical statement: This was obtained and approved from "not given...". This statement need to be rewritten to reflect: Written consent was sought from each child guardian/parent with each consent form indicating clearly the study purpose, any anticipated consequences of the research, the anticipated uses of the data, possible benefits of the study and possible harm, confidentiality of the data and the option to withdraw their children participation at any given time.
- Study location: Provide reference for Joint Commission International (JCA).
- SPSS version 22 – source, city, state?

Results

- Confirm if the 4 kids were smokers, and if smokers what was the criteria used.
- Consume antibiotics does not sound medically – better word to use is take antibiotics.
- Have headings for the several parts of the results focusing on:
 - Socio-Demographic characteristics – a brief summary (Table 1).
 - Antimicrobial susceptibility patterns of *Streptococcus pneumoniae* among PCV-10 vaccinated children ≤ 5 years (N=?) by selected antibiotic agents. Table 3 need to be split to show vaccinated children, then describe the results in text.
 - Antimicrobial susceptibility patterns of *Streptococcus pneumoniae* among PCV-10 unvaccinated children ≤ 5 years (N=?) by selected antibiotic agents. Table 3 need to be split to show unvaccinated children, then describe the results in text.
 - Risk factors and susceptibility patterns of vaccinated children by selected antibiotic agents.
 - Risk factors and susceptibility patterns of unvaccinated children by selected antibiotic agents.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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