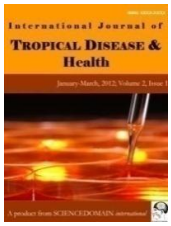

International Journal of TROPICAL DISEASE & Health
7(1): 16-22, 2015, Article no.IJTDH.2015.053
ISSN: 2278–1005

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Nasopharyngyeal Carriage and Antimicrobial Susceptibility of *Streptococcus pneumoniae*, in Children under Five Years at Mbarara Regional Referral Hospital, Uganda

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Authors' contributions

This work was carried out in collaboration between both authors. Author IM did the study design and wrote the protocol and performed the laboratory experiments. Author BJ did the statistical analysis did the overall supervision of the work and wrote the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2015/11595
Editor(s):
(1) Shankar Srinivasan, Department of Health Informatics, University of Medicine & Dentistry of New Jersey, USA.
Reviewers:
(1) Anonymous, Poland.
(2) Anonymous, Brazil.
(3) Anonymous, Turkey.
(4) Anonymous, Poland.
(5) Anonymous, China.
(6) Anonymous, Russia.
(7) Anonymous, Pakistan.
(8) N.S. Abbai, HIV Prevention Research Unit, South Africa.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1008&id=19&aid=7974

Original Research Article

Received 23rd May 2014
Accepted 1st December 2014
Published 31st January 2015

ABSTRACT

Background: Acute respiratory infections (ARI) are a leading cause of childhood morbidity and mortality, causing 25-30% of all deaths in developing countries. Pneumococcal disease is a significant public health problem that usually follows pneumococcal colonization of the nasopharynix. We determined the prevalence of nasopharyngeal carriage of *Streptococcus pneumoniae*, antimicrobial susceptibility patterns and risk factors for nasopharyngeal carriage of *S.*

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pneumoniae among under fives attending Maternal Child Health Clinic at Mbarara Regional Referral Hospital (MRRH).
Methods: We performed a cross-sectional study between August and November 2012. Nasopharyngeal swabs collected from four hundred healthy children were cultured on blood agar and chocolate agar and incubated for 24 hours at 37^0C in carbon dioxide jar. Upon growth the organisms were identified by colonial appearance and standard biochemical tests. Antimicrobial resistance to six antibiotics was performed using Kirby Bauer method on chocolate agar and interpreted according to CLSI guide lines.
Results: The prevalence of *S. pneumoniae* in the cultured samples was reported at 19% (76/400). Of the positive isolates, 75/76 (99%) and 55/76 (77%) were shown to be resistant to cotrimoxazole and tetracycline, respectively. Among the factors assessed for nasopharyngeal carriage of *S. pneumoniae* none was significantly associated with carriage.
Conclusion: Despite the low rate of carriers of *S. pneumoniae*, a remarkable resistance of these isolates to cotrimoxazole and tetracycline was detected.

Keywords: S. pneumoniae; nasopharyngeal carriage; health children; under five years.

1. INTRODUCTION

The nasopharynx of children has resident microbial flora that do not usually harm the child but, in some cases constitute a reservoir of pathogens implicated in respiratory tract infections and invasive diseases [1]. These bacteria carried in the nasopharynx of healthy children reflect the infection causing strains currently circulating in the community, this gives rise to nasopharyngeal colonization by invasive and antibiotic-resistant *Streptococcus pneumoniae* strains [2]. The occurrence of antibiotic resistant *Streptococcus pneumoniae* is a big problem worldwide especially in resource poor developing countries that can only afford inexpensive antibiotics.

The full extent of this antibiotic resistance in developing countries such as Uganda is largely unknown. Data concerning *Streptococcus pneumoniae* carriage rate, risk factors and antibiotic susceptibility patterns is scanty thus complicating empirical therapy.

This study was carried out to determine the nasopharyngeal carriage rate, risk factors and antimicrobial resistant patterns of *Streptococcus pneumoniae* isolated from children under five years attending Maternal Child Health Clinic (MCH) at Mbarara Regional Referral Hospital in South Western Uganda

2. MATERIALS AND METHODS

This was a cross sectional study conducted between August 2012 to November 2012 among apparently health children less than five years at Maternal Child Health of Mbarara Regional Referral Hospital which serves as University Teaching Hospital for Mbarara University of Science and Technology and Referral Hospital with the bed capacity for 500 beds and is located about 300 kilometers from Kampala in South Western region of Uganda. The study participants included 400 children aged between two to sixty months who attended maternal child health services. Consent for participating child was obtained from parents/caregivers using a semi structured consent form.

Convenient sampling procedure was used to recruit up to 400 participants into this study after fulfilling the inclusion criteria. Children were enrolled simultaneously until the desired sample size was accomplished.

2.1 Data Collection

Data was collected using questionnaires that were administered to the parent or care taker of participating child to collect demographic information and socioeconomic status.

2.2 Laboratory Methods and Procedures

Nasopharyngeal specimens were collected by trained health workers. A flexible calcium alginate-tipped sterile swab was used, by inserting into the posterior nasopharynix for a minimum of five seconds before removal, and transported in cold box to the Microbiology laboratory within two hours for processing [3].

Culture and Identification of *S. pneumonia,* Nasopharyngeal specimens were cultured according to standard microbiological procedures. In the laboratory the specimens

were inoculated into 5% chocolate agar with poly-vertex added to enhance growth and blood agar. Cultured plates were incubated for 24-48 hours at 37ºC under 5% Carbon dioxide.

2.3 Phenotypic Identification

After overnight incubations, *S. pneumoniae* colonies appeared small, grayish, mucoid and were surrounded by a greenish zone of α-hemolysis. Further incubation for 24–48 hours the colonies showed centrally depressed ("draughtsman") colonies.

2.4 Gram Stains

This was performed on the colonies suspected to be *S. pneumoniae,* and the gram reactions showed gram positive cocci either in diploid form or Single organisms.

Biochemical identification of *S. pneumonia* was performed using optochin disc 5µg and bile solubility test to differentiate between *S. pneumoniae* and *other viridian streptococci*. The *S.pneumoniae* isolates that were typically susceptible to optochin were also bile soluble, whereas Viridians *streptococci* remained resistant to optochin and bile insoluble [4].

2.5 Antimicrobial Susceptibility

This was performed on Mueller Hinton agar supplemented with 5% Hemoglobin powder using Kirby Bauer disk diffusion method with bacterial suspensions of turbidity equivalent to 0.5 McFarland standard using the following disks (Oxoid, UK) penicillin 10unit, cotrimoxazole 1.25/23.75 µg, tetracycline 30 µg and erythromycin15 µg [5] Penicillin susceptibility was determined using 1 µg Oxacillin [5]. Interpretation for sensitivity or resistance was based on guidelines from the Clinical and Laboratory Standards Institute. Standard strains such as *S. pneumoniae* ATCC 49619 and *S. mitis* ATCC 49456 were included as controls. Isolates were classified as susceptible or resistant to the antibiotics based on the recommendations of the Clinical and Laboratory Standards Institute [5].

2.6 Statistical Analysis

Data was analyzed with STATA 11 software (Stata Corporation, College Station, TX, USA). Paired *t*-tests for continuous data was used to evaluate differences in baseline characteristics, bivariate associations between potential risk factors and Nasopharyngeal colonization were evaluated using logistic regression models. Odds ratios and 95% confidence intervals were used to measure the association between potential factors and nasopharyngeal colonization. A p-value ($P \leq 0.05$) was considered statistically significant in bivariate analyses.

2.7 Ethical Considerations

The study was carried out in accordance with Ethical Guidelines. Ethical clearance was obtained from Faculty Research and Ethic Committee (FREC) and Institutional Review Committee (IRC) of Mbarara University of Science and Technology. Permission was also sought from administration of MRRH. Informed consent obtained from parents/guardian of the child before enrolment into the study. Detailed description about the study was given to the parents/guardians of the children to ensure that they have all information needed to make an informed choice. It was also made clear to parents/guardians that if their children participated in the study it would involve a questionnaire and assurance of confidentiality of any information given as well as test results

3. RESULTS

A total of 400 study participants were recruited into the study, majority being from Mbarara district, Isingiro and some from others districts. The age category ranged from 2months to 60 months, the mean age range was 18months and the majority of the participants recruited were 6 months of age. Of the 400 nasal swabs cultured 76 (19%) grew *S. pneumoniae.*

As shown in Table 1, majority of the participants with high carriage of *S. pneumoniae* came from Mbarara district and their informants were parents as opposed to caretakers. Majority of study participants with high percentage of *S. pneumoniae* carriage included those who didn't seek medical care, those without any history of antibiotic therapy in the last two weeks, none school going children and those aged below 12 months.

From the Table 2, there was no significant risk factor for nasopharyngeal carriage.

As shown in Fig. 1, *S. pneumoniae* isolates were highly resistant toco-trimoxazole with 98.68%, and tetracycline 77.26%, with low resistance to erythromycin 23.7%.

Table 1. The baseline characteristics of the under fives who carried *Streptococcus pneumoniae*

Characteristics	*S. pneumoniae,* n (%) N=76
Informants	
Parent	75(98.68)
Caretaker	01(1.32)
District of participants	
Mbarara	59(77.63)
Isingiro	11(14.47)
Others	06(07.90)
Sex of the participants	
Male	36(47.37)
Female	40(52.63)
Age of the participants (in months)	
02-12	39(51.32)
13-24	20(36.32)
25-60	17(22.36)
Visit to the health service	
Yes	12(15.79)
No	64(84.21)
Antibiotic given in the previous two weeks	
Yes	03(03.95)
No	69(90.79)
Don't know	04(05.26)
Members of house mate treated with antibiotic	
Yes	19(25)
No	57(75)
School going participant	
Yes	08(10.53)
No	68(89.47)
No. of siblings in house hold less than 5 years	
None	12 (15.79)
One child	17 (22.37)
More than one child	47 (61.84)
House types	
Mud/grass	05(06.58)
Mud/iron sheet	24(31.58)
Permanent	47(61.84)
Number of rooms the house has	
On	38(50.00)
Two	28(36.68)
More than two	10(13.16)
No. of the house occupants	
<5years	26(34.21)
or more	50(65.79)
Passive smoking	
Yes	16(21.05)
No	60(78.95)

Table 2. The relationship between the participants characteristics and *S. pneumoniae* carriage status

Variables	Crude OR (95%,CI)	p-values
Age of the participants(in months)		
02-12	1.6(0.856-3.120)	0.540
13-24	0.7(0.423-1.4030	0.393
25-60	1.0	
Antibiotic given		
Yes	1.0	
No	2.3(0.688-7.851)	0.175
Don't know	4.8(0.893-26.174)	0.068

Variables	Crude OR (95%,CI)	p-values
School going child		
Yes	2.0(0.430-9.306)	0.377
No	1.0	
Number of siblings		
One child	1.0	0.393
More than one child	1.3(0.748-2.084)	
Types of house		
Mud/grass	1.3(0.400-4.034)	
Mud/iron sheets	1 .1(0.612-1.824)	0.685
Permanent house	1.0	0.843
Number of rooms		
One room	1.6(0.703-3.440)	0.275
Two rooms	1.2(0.575-2.629)	0.594
More than two rooms	1.0	
House hold smoking		
No	1.0	0.276
Passive smoking	1.4(0.762-2.673)	

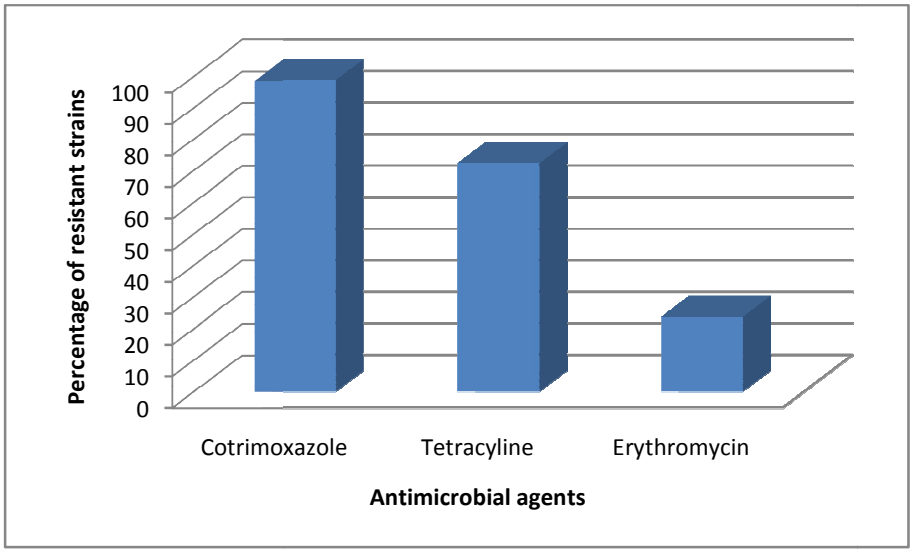

Fig. 1. Antimicrobial susceptibility patternof *S. pneumoniae* isolates

4. DISCUSSION

In this study the nasopharyngeal carriage of *S. pneumonia* was found to be 19.0%. This carriage rate was similar to what has been reported in other studies for example in Michigan, USA, that reported carriage rate of 20%, Asia and Middle East that reported a carriage rate of 22% [6] But our findings also differ from those reported in other African countries such as the Gambia [7] and Kenya [8] which had nasopharyngeal carriage of 87.2% and 55.2% respectively Our study findings also differ from those reported from Mayuge in Eastern Uganda that reported a carriage rate of 62.0% [9]. These differences in our findings and those reported by Joloba et al. [9] could be due to the differences in the study subjects. Whereas this study included only those children that were apparently healthy and came for immunization at the Hospital, the Mayuge study was a study that included children who might be at a greater risk of being carriers.

In most of the studies, rates of nasopharyngeal colonization did not differ significantly between boys and girls: Among Finnish children, the carriage proportions for boys and girls were 48% and 52% respectively [10], in Italian children, it was reported to be 51.4% for males and 49% in females [11] and in Uganda, 56% and 44% for boys and girls respectively [9] This was in agreement with this study in which the

prevalence of nasopharyngeal carriage among male children was 47.4% and 52.6% among females. This finding could therefore indicate that gender does not influence nasopharyngeal carriage of *S. pneumoniae*.

Though several studies have reported a number of risk factors for Nasopharyngeal carriage of *S.pneumoniae* in children under five years [3,9,12,13], none of these seemed to be significant in our study.

In this study, the nasopharyngeal carriage of *S. pneumoniae* among those below 12 months was very high This is in agreement with study conducted in Netherlands [14] and in Kenya [8]. The inverse relationship between nasopharyngeal colonization and age may be related to the increased risk of respiratory infections in infants and young children, since colonization increases significantly during periods of upper respiratory illness.

In this study it was found that most of *S. pneumoniae* strains were resistant to cotrimoxazole (98.68%) and tetracycline (77.26%). The proportion of resistance to cotrimoxazole reported in this study is similar to that reported [9] in Eastern Uganda. Similar Study done in Kiliffi Kenya [8] put resistance to co-trimoxazole as high as 84%. This is not surprising because there is excessive use of co-trimoxazole for prophylaxis by HIV positive individuals. The high levels of resistance to antibiotics among these pneumococci might also be as a result of the unrestricted antibiotic accesses leading to abuse of these drugs.

5. LIMITATION

A key limitation to our study was failure to consider the HIV status and malnutrition status of the study participants, since some few studies reported HIV influence on pneumococcal carriage. We also did not serotype the isolates.

6. CONCLUSION

Though the nasopharyngeal carriage rate of *S. pneumoniae* among under fives at MRRH is low there is a high resistance of these isolates to co-trimoxazole and tetracycline. Due to the high rates of resistance to trimoxazole and tetracycline we recommend that these drugs should not be used for treatment of infections to *S. pneumoniae* infections in children.

ETHICAL APPROVAL

The study was carried out in accordance with Ethical Guidelines. Ethical clearance was obtained from Faculty Research and Ethic Committee (FREC) and Institutional Review Committee (IRC) of Mbarara University of Science and Technology. Permission was also sought from administration of MRRH. Informed consent obtained from parents/guardian of the child before enrolment into the study.

COMPETING INTERESTS

The authors declare that they have no competing interests

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1008&id=19&aid=7974