

**NASOPHARYNGEAL CARRIAGE AND
ANTIMICROBIAL SUSCEPTIBILITY OF
STREPTOCOCCUS PNEUMONIAE, IN
CHILDREN UNDER FIVE YEARS AT MBARARA
REGIONAL REFERRAL HOSPITAL, UGANDA.**

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 nasopharynx. We determined the prevalence of nasopharyngeal carriage of *Streptococcus pneumoniae*, antimicrobial susceptibility patterns and risk factors for nasopharyngeal carriage of *S.pneumoniae* in under fives attending Maternal Child Health Clinic at Mbarara Regional Referral Hospital.

Method: We performed a cross-sectional study between August 2012 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°C 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: Out of the 400 Nasopharyngeal swabs cultured, 76 (19.0%) grew *S. pneumoniae* and among the 76 isolates, 75 (98.68%) were resistant to Trimethoprim-sulfoxide, 55 (77.26%) were resistant to tetracycline. But 2 (97.37%) were susceptible to Cefotaxime. Among the factors assessed for nasopharyngeal carriage of *S.pneumoniae* none was significantly associated with carriage.

Conclusions: Though the nasopharyngeal carriage rate of *S.pneumoniae* was low there is a high resistance of these isolates to co-trimoxazole and tetracycline.

Keywords: *S.pneumoniae*, nasopharyngeal carriage, Health Children, under five years.

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

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. MATERIAL 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 Mbarara Regional Referral Hospital. 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.

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.

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 nasopharynx 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. Pneumoniae* 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 37°C under 5% Carbon dioxide.

Phenotypic identification After overnight incubations, *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.

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

Biochemical identification of *S.pneumoniae* was done by the use of optochin disc 5µg and bile solubility test to differentiate between *S.pneumoniae* and *Streptococcus viridians*. The confirmation was based on optochin sensitivity and bile solubility. The *S. pneumoniae* isolates that were typically susceptible to optochin were also bile soluble, whereas *Streptococci viridans* remained resistant to optochin and bile insoluble [4]

Antimicrobial susceptibility: This was performed 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, trimethoprim-sulfamethoxazole 1.25/23.75µg, tetracycline 30µg, erythromycin 15µg, amoxicillin 10µg, ciprofloxacin 5µg, cefotaxime 30µg [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.viridan* 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].

Statistical analysis

69 Data was analyzed with STATA 11 software (Stata Corporation, College Station, TX, USA). Paired *t*-tests for continuous
70 data was used to evaluate differences in baseline characteristics bivariate associations between potential risk factors and
71 Nasopharyngeal colonization were evaluated using logistic regression models. Odds ratios and 95% confidence intervals
72 were used to measure the association between potential risk factors and nasopharyngeal colonization. A p-value ($P \leq$
73 0.05) was considered statistically significant in bivariate analyses.

74 **Ethical considerations**

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

82
83 **3. RESULTS**

84
85 A total of 400 study participants were recruited into the study, majority being from Mbarara district, Isingiro and some from
86 others districts. The age category ranged from 2months to 60 months, the mean age range was 18months
87 and the majority of the participants recruited were 6 months of age. The 400 nasal swabs cultured 76 (19%) grew
88 *S.pneumoniae* .

89 **Table 2: The baseline characteristics of the under fives who carried *Streptococcus pneumoniae***

Characteristics	<i>S.pneumoniae</i> ,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	
No	03 (03.95)
Don't know	69 (90.79)
	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	
One child	12 (15.79)
More than one child	17 (22.37)
	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	
One	
Two	38 (50.00)
More than two	28 (36.68)
	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)

Most of the participants with high carriage of *S.pneumoniae* came from Mbarara district; their informants were parents as opposed to caretakers. Majority of study participants who didn't seek medical care or having any history of antibiotic therapy in the last two weeks, no school going and age category below 12 months also high percentage of *S. pneumoniae* carriage.

Table 3: 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

School going child			98
			99
Yes		0.377	100
No	2.0 (0.430-9.306)		101
	1.0		102
			103
Number of siblings		0.393	104
One child	1.0		105
More than one child	1.3 (0.748-2.084)		106
			107
Types of house			108
Mud/grass	1.3 (0.400-4.034)	0.685	109
Mud/iron sheets	1.1 (0.612-1.824)	0.843	110
Permanent house	1.0		111
			112
Number of rooms			113
One room	1.6 (0.703-3.440)	0.275	114
Two rooms	1.2 (0.575-2.629)	0.594	115
More than two rooms	1.0		116
			117
House hold smoking		0.276	118
No	1.0		119
Passive smoking	1.4 (0.762-2.673)		120
			121
			122

123 Risk factors for the nasopharyngeal carriage were analyzed using Bi-variate analysis (Table 3), Factors such as no history
124 of antibiotic therapy with (Crude OR 2.3, 95% CI 0.688-7.851, p-value 0.175), Children in age category between 2-12
125 months with (Crude OR1.6 ,95% CI 0.856-3.120 ,p-value 0.540) and Passive smoking with (Crude OR 1.4 ,95% CI
126 0.762-2.763) showed high strength of association, but none of the variables were significantly associated with
127 *S.pneumoniae* carriage P> 0.05.

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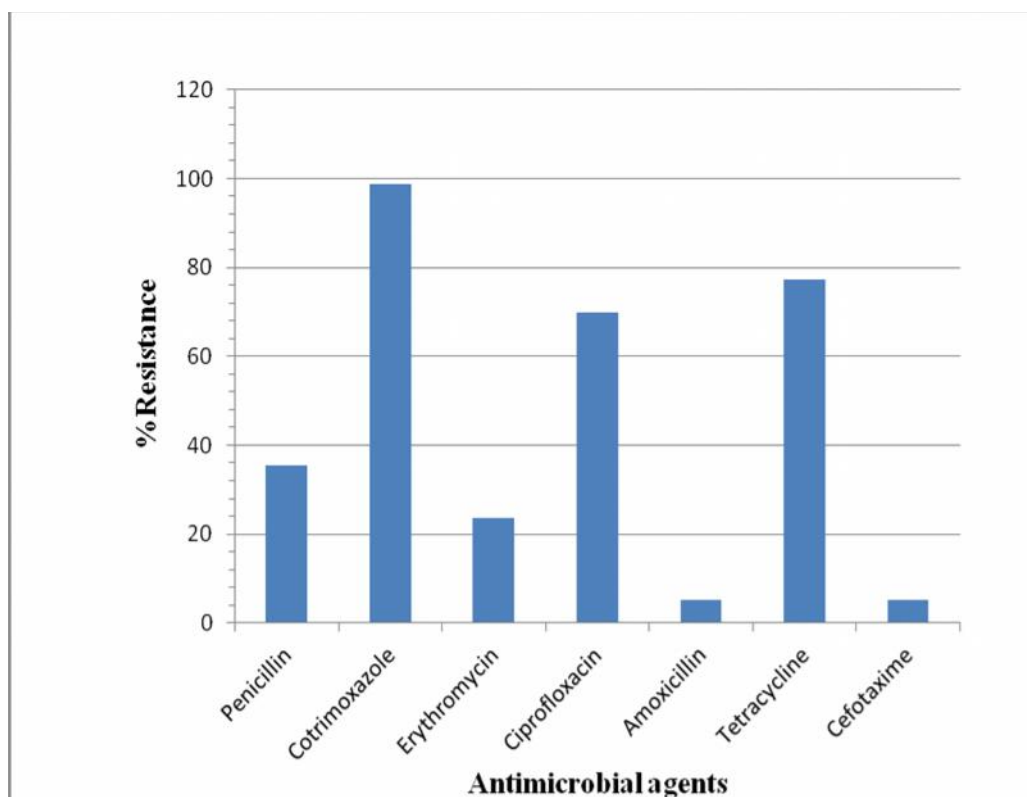


Figure 0.1: Antimicrobial susceptibility pattern of *S. pneumoniae* isolates

As shown in the figure 01 above, *S.pneumoniae* isolates were highly resistant to co-trimoxazole with 98.68%, Tetracycline 77.26% and Ciprofloxacin (69.74%), with low resistance to amoxicillin and cefotaxime.

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 done elsewhere 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%[11] .

These differences in our findings and those reported by Joloba *et al* 2001 could be due to the differences in the study subjects. Whereas this study included only those children that came for immunization at the Hospital that were apparently healthy, the Mayuge study was home based and included children that could be more at risk of nasopharyngeal carriage.

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 [9] in Italian children, it was reported 51.4% for males and 49% in females [10] and in Uganda, 56% and 44% for boys and girls respectively [11] This was in agreement with this study which put the prevalence of male children 36 (47.4%) and 40 (52.6%) respectively this therefore could indicate 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] [11] [12] and [13] none of these seemed to be significant in our study.

The nasopharyngeal carriage of *S.pneumoniae* is high among those below 12 months in this study This is in agreement with study done 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 *S. pneumoniae* was resistant to co-trimoxazole (98.68%), tetracycline (77.26%) and Ciprofloxacin (69.74%). The proportion of resistance to Trimethoprim-Sulphamethoxazole reported in this study is similar to that reported [11] in Eastern Uganda. Similar Study done in Kilifi 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.

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.

4. 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, tetracycline and ciprofloxacin.

COMPETING INTERESTS

The authors declare that they have no competing interests

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.

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