Risk factors for acquisition of meningococcal carriage in the African meningitis belt

Authors

Laura V Cooper, University of Cambridge, Cambridge, UK

Anna Robson, University of Cambridge, Cambridge, UK

Caroline L Trotter, University of Cambridge, Cambridge, UK

Abraham Aseffa, Armauer Hansen Research Institute, Addis Ababa, Ethiopia

Jean-Marc Collard, Centre de Recherche Médicale et Sanitaire, Niamey, Niger; Bactériologie expérimentale, Institut Pasteur de Madagascar, Antananarivo, Madagascar

Doumagoum Moto Daugla, Centre de Support en Santé Internationale, N'Djamena, Chad

Aldiouma Diallo, Institut de Recherche pour le Développement, Dakar, Senegal

Abraham Hodgson, Navrongo Health Research Centre, Navrongo, Ghana

Jean-François Jusot, Centre de Recherche Médicale et Sanitaire, Niamey, Niger

Babatunji Omotara, Department of Community Medicine, University of Maiduguri, Maiduguri, Nigeria

Samba Sow, Centre pour les Vaccins en Développement, Bamako, Mali

Musa Hassan-King, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Olivier Manigart, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Maria Nascimento, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Arouna Woukeu, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Daniel Chandramohan, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

Ray Borrow, Public Health England Vaccine Evaluation Unit, Manchester, UK

Martin CJ Maiden, University of Oxford, Oxford, UK

Brian Greenwood, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

James M Stuart, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

on behalf of

the MenAfriCar Consortium (*listed at end of manuscript)

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ABSTRACT

Background

Although several studies of risk factors for prevalence of meningococcal carriage in the African meningitis belt have been reported, few have examined risk factors for acquisition. We investigated a range of potential risk factors for acquisition in seven countries of the meningitis belt.

Methods

Households were followed up every two weeks for two months, then monthly for a further four months. Pharyngeal swabs were collected from all available household members at each visit and questionnaires completed. Risks of acquisition over the whole study period and for each visit were analysed by a series of logistic regressions.

Results

Over the course of the study, acquisition was higher in: (i) 5-14 year olds, as compared with those 30 years or older (OR 3.6, 95%CI 1.4-9.9); (ii) smokers (OR 3.6, 95%CI 0.98-13); and (iii) those exposed to wood smoke at home (OR 2.6 95%CI 1.3-5.6). The risk of acquisition from one visit to the next was higher in those reporting a sore throat during the dry season (OR 3.7, 95%CI 2.0-6.7) and lower in those reporting antibiotic use (OR 0.17, 95%CI 0.03-0.56).

Conclusions

Acquisition of meningococcal carriage peaked in school age children. Recent symptoms of sore throat during the dry season, but not during the rainy season, were associated with a

higher risk of acquisition. Upper respiratory tract infections may be an important driver of epidemics in the meningitis belt.

BACKGROUND

Epidemics of meningococcal meningitis occur periodically in the African Meningitis Belt, an area of sub-Saharan Africa stretching from Senegal in the west to Ethiopia in the east.[1] These epidemics are highly seasonal, with the majority of cases occurring during the dry season, predominantly in the first five months of the year.[2] Given that asymptomatic pharyngeal carriage of meningococci is relatively frequent (ranging from 3-30% of the population)[3] and because meningococcal acquisition only occasionally leads to invasive disease, one explanation for this striking seasonality is an increased risk of invasive disease in the dry season, due to mucosal damage from environmental factors such as low absolute humidity and dust.[1,4,5] Another hypothesis suggested by mathematical modelling is that higher rates of meningococcal transmission during the dry season, combined with population immunity, may be sufficient to explain epidemic patterns.[6] Although a review of carriage in the meningitis belt published in 2007 found no evidence to support a seasonal effect on carriage,[3] more recent studies have found a higher prevalence of carriage in the dry season.[7,8]

Studies of carriage prevalence and acquisition will, therefore, lead to a better understanding of the epidemiology of meningococcal meningitis in the African meningitis belt. The African Meningococcal Carriage Consortium (MenAfriCar) undertook 20 cross-sectional carriage surveys in seven African meningitis belt countries from July 2010 to July 2012, involving the collection of over 48,000 pharyngeal swabs. These studies found a higher frequency of carriage in children aged 5-14 years, in the dry season and in rural populations.[7] During these surveys, households with at least one pharyngeal carrier of *N. meningitidis* were recruited for longitudinal studies.[9]

87 Previous longitudinal studies in the meningitis belt have been undertaken mainly at the
88 population level [10–12] and few have investigated the transmission and acquisition of
89 carriage at an individual level.[13,14] The aim of this MenAfriCar study was to investigate
90 a comprehensive set of potential risk factors for the acquisition of carriage of *N.*
91 *meningitidis* across the African meningitis belt.

92 **METHODS**

93 *Household surveys*

94 Households included in this study were recruited during the course of cross-sectional
95 surveys conducted in seven countries in the African meningitis belt (Chad, Ethiopia, Ghana,
96 Mali, Niger, Nigeria, and Senegal) in 2010, 2011, and 2012. Details of the survey methods
97 employed have been published previously.[7] Longitudinal surveys were triggered by the
98 identification of a putative carrier during a cross-sectional survey (Visit 0). This initial
99 identification of carriers relied on conventional microbiology and was later confirmed via
100 molecular methods at the University of Oxford. In some cases, molecular methods did not
101 confirm the presence of meningococci, so 51 of 184 households recruited to the study did
102 not have an index carrier.

103 Within four weeks of the identification of a carrier, all members of the putative carrier's
104 household were invited to take part in further studies (Visit 1). The head of the household
105 was asked about characteristics of the household, including numbers of rooms and
106 bedrooms, sleeping arrangements, location of kitchen and cooking fuel, house construction,
107 drinking water, sanitation, and household assets such as vehicle ownership, livestock, and
108 electrical goods.

A pharyngeal swab sample was obtained from all members of a household who gave their consent and a questionnaire completed which included questions on: smoking; social activities; symptoms of recent respiratory tract infection; socio-economic status and educational level; school attendance; travel history; recent medication including antibiotics; meningitis vaccination; and ethnic group. Carrier households were followed up two-weekly for two months (Visits 2-5) and monthly for a further four months (Visits 5-9). At each follow up visit, each household member was asked for a pharyngeal swab sample and to answer a short follow-up questionnaire on factors that might have changed since the previous visit, such as symptoms of a respiratory tract infection.

Laboratory methods

Pharyngeal swab samples, taken from the posterior pharynx and tonsillar fossa *via* the mouth, were plated directly onto Modified Thayer Martin agar plates in the field, taken to the laboratory within six hours of collection, and processed as previously described.[9] A sample of boiled suspensions of Gram negative oxidase positive bacteria was sent to the University of Oxford for molecular analysis. Amplification and sequencing of the *rplF* gene was used to confirm the presence of, and differentiate between, *Neisseria* species. Confirmed *N. meningitidis* were further characterised by genogroup (including capsule null) and *porA* genosubtype.

Data management

Data were managed using the Teleform system version 10.4.1 (Autonomy, Cambridge UK) with a separate database module linking the main study database with genetic laboratory results from the Oxford PubMLST.org/neisseria database (https://pubmlst.org/neisseria). Data from the longitudinal questionnaires were merged using a common person ID, or

census number, person matching was checked, any duplicate entries were removed, and aberrant values excluded.

Statistical analysis

The genogroup-specific acquisition rates and 95% confidence intervals were calculated as Poisson rates, counting the number of acquisitions occurring in non-index carriers and the time at risk as the days between the first carriage-negative swab and the first positive swab. A series of fixed-effects logistic regressions were used to identify significant risk factors for acquisition. In the first round of regressions, individual risk factors were included in a multivariable logistic regression with the *a priori* variables sex, age group, and country. In the second round, risk factors with $p<0.1$ in round 1 were added to a single model with *a priori* variables. In the third round, risk factors with $p<0.05$ in round 2 were retained in the multivariable model. In the fourth round, all factors dropped in round 3 were added back in to the model one by one and all variables with $p<0.05$ were retained, giving the final models. The study-long and visit-by-visit models were then run with household ID and both household and individual ID as random effects, respectively, to account for clustering, and factors that were no longer significant ($p>=0.05$) were dropped.

Acquisition was assessed over the full study period (study-long) and visit-by-visit. Individuals were defined as positive for study-long acquisition if they had a negative swab (no meningococci isolated) at visits 0 or 1 and a positive swab (any meningococci isolated) at any following visit. Individuals were defined as negative for study-long acquisition if they had a negative swab at visits 0 or 1 and no positive swab at any subsequent visit. Individuals with three or more missed visits in total were excluded, as the possibility of acquisition during this missed period could not be ruled out, and individuals carrying at

visits 0 or 1 were also excluded.

Individuals were defined as positive for visit-by-visit acquisition on a given visit if the individual had a positive swab at the current visit and a negative swab at the previous visit or carried a different strain at the previous visit and the strain was not previously carried during the study. Strains were assessed by genogroup and porA variable regions 1 and 2. Individuals were defined as negative for visit-by-visit acquisition on a given visit if the individual had a negative swab at the previous visit and a negative swab at the current visit. Individuals carrying an identical strain to that obtained at the previous visit and individuals who cleared carriage were excluded from the analysis. Tables S1 and S2 provide the classification of cases for study-long and visit-by-visit acquisition.

We defined the dry season as January to May and the rainy season as June to December. Because we found a significant association between sore throat and season and because previous studies have demonstrated an interaction between meningococcal carriage, upper respiratory tract infection, and season, we also tested for interaction between sore throat and season in our final model and found that the model with an interaction term fitted better than the model with no interaction (Table S4).

Ethics

The study was approved by the ethics committee of the London School of Hygiene and Tropical Medicine and by the relevant ethical authorities in each African centre.[9] The head of the household or another responsible adult gave verbal informed consent for the household to be included in the study. Each individual recruited within that household gave written informed consent; for children under the age of 18 years a parent or guardian gave written consent and children aged over 12 years were additionally asked to give written assent.

RESULTS

Acquisition over course of the study

Overall, 169/861 (20%) of non-index carriers became pharyngeal carriers of a meningococcus at least once over the course of the study. A higher proportion of 5- to 14-year-olds acquired carriage than other age groups, and a higher proportion of participants acquired carriage in Senegal, Niger, Ghana, and Ethiopia relative to Chad and Mali (Table 1). A wide variation in acquisition rates was observed between countries. Genogroup W and capsule-null (*cnl*) meningococci accounted for the majority (83%) of acquisitions. The acquisition rates of genogroup W meningococci was 2.0% per month (95%CI 1.6-2.4) double that of *cnl* meningococci at 1.0% per month (95%CI 0.74-1.4). Genogroups A, C, Y, and other genogroup (i.e. other than A, B, C, W, X, Y, or *cnl*) acquisitions were uncommon, and no genogroup B or X acquisitions were detected.

In the final multivariable model, the highest odds of acquisition were among 5- to 14- year olds, with odds in all age groups under 30 years of age being significantly higher than the reference group of individuals 30 years and older (Table 1). Active smokers had higher odds of acquiring carriage than non-smokers living in households with no smokers, with a lower confidence bound just below 1 (OR 3.57 95%CI 0.98-12.99). Non-smokers living in households with smokers also had elevated odds of acquisition but the difference was not statistically significant. Wood was the ubiquitous cooking fuel, with 96% of participants using this as cooking fuel; 56% of participants had additional wood smoke exposure. Participants with household exposure to wood smoke (independent of using wood as cooking fuel) had higher odds of acquiring carriage than those without (OR 2.60 95%CI 1.26-5.59). Although this trend was not significant in the regression analysis, higher

acquisition rates were observed in households with an indoor kitchen and in households which used wood as the primary cooking fuel than in those who did not.

Visit-specific Acquisition Analysis

Participants who said they had had a sore throat since the previous visit during the dry season were significantly more likely (OR 3.67 95%CI 1.95-6.65) to have acquired carriage in that time period than those who did not have a sore throat in the rainy season (Table 2). Those who reported taking antibiotics since the previous visit were significantly less likely (OR 0.169 95%CI 0.0271-0.564) to have acquired carriage.

DISCUSSION

This longitudinal study found a higher risk of acquisition amongst individuals who reported a sore throat since the previous visit, but only during the dry season. An association between an upper respiratory tract infection and meningococcal carriage has been reported previously.[14] A sore throat could be due to an initial inflammation of the pharynx from meningococcal colonisation or could be caused by a concurrent unrelated infection that predisposes an individual to acquisition.[15] If the latter is true, upper respiratory tract infections in combination with dust and low humidity may be an important driver for the high risk of meningitis epidemics in the dry season. This hypothesis is supported by a recent study indicating an association between upper respiratory tract infection (defined as otitis, severe sore throat, and rhinopharyngitis) and meningitis outbreaks in Burkina Faso.[16] Such upper respiratory tract infections could plausibly increase both the risk of acquisition and the risk of invasion after acquisition.

The 5-14 year-old age group had the highest acquisition rate. The highest prevalence of carriage in cross-sectional MenAfriCar studies and in Burkina Faso in 2009 was similarly highest in 5-14 year olds.[7,17] An overall acquisition rate of 2.4% (95% CI 1.6 to 4.0%) per month was estimated from this same study using a hidden Markov model.[9] There were no significant differences reported by age group, but data were subdivided by control and index households and there was no adjustment for other risk factors.

Additional factors linked to acquisition of meningococci over the course of this study were smoking tobacco and exposure to wood smoke. Smoking, passive exposure to smoke and to smokers has been shown to convey a high risk of carriage and invasive disease in high-income countries.[18–21] Exposure to cigarette smoke has also been linked to the risk of carriage in the meningitis belt.[7,14] The higher risk of acquisition from smoke exposure in this study suggests a direct risk from smoke itself, potentially from interference with mucosal immunity, as exposure to wood smoke was an independent risk factor. Exposure to smoke from wood fires has also been shown as a risk factor for meningococcal meningitis in northern Ghana.[22] Although use of wood as primary cooking fuel was not found to be a significant risk factor, this could be explained by the fact that nearly all study participants relied on wood as primary fuel or that some households used outdoor kitchens, thus moderating the degree of exposure.

Strengths of this study are the multi-centre design across seven countries of the meningitis belt conducted at the same time, including a mix of urban and rural populations with a broad age range, the use of standardised field and laboratory protocols and a large sample size. Measuring acquisition rather than carriage ensures that the risk factors identified in this study are not biased by factors associated with longer carriage duration. A

comprehensive range of risk factors was included, so that important confounding factors are unlikely to have been missed; however, the sampling of carriers and non-carriers was not random and we would expect some misclassification of carriage status from the known low sensitivity of pharyngeal swabbing.

Both the acquisition of meningococci found in this longitudinal study and prevalence of carriage in the MenAfriCar cross-sectional studies varied considerably by country. Although laboratory methods were standardised across centres, differences in laboratory techniques could still have contributed to some of the differences observed. As most meningococcal acquisitions were either genogroup W or capsule-null and outside epidemics, it cannot be assumed that risk factors for acquisition of other genogroups or during epidemics would be the same as that found in this study.[23]

It was surprising that some risk factors such as household crowding that have long been known to raise the risk of carriage and disease [7,13,24,25] were not associated in this study with acquisition. Crowding was measured here by numbers sharing a bedroom or bedmat, and by numbers of people per room in the household. It is possible that crowded living conditions are so prevalent across the meningitis belt countries that any effect of crowding on acquisition is not detectable. A study in rural Gambia did not find any differences in crowding between compounds with and without cases of meningococcal meningitis during an epidemic.[26]

Reported vaccination was clustered in particular time periods and countries corresponding to the introduction of group A conjugate vaccine. Vaccination was not found to be protective against carriage acquisition. However, we would not expect a group A conjugate

vaccine to have a significant impact on carriage in this study as very few group A carriers were detected.

We were not able to draw any conclusions regarding the relationship between carriage acquisition and disease incidence because none of the study sites reported an outbreak of meningitis during the follow-up period.

This study involved multiple countries and examined an exhaustive set of household and individual risk factors for meningococcal acquisition. The importance of identifying these risk factors is that acquisition is a necessary pre-requisite for invasive disease. Acquisition studies also play a potential role in vaccine evaluation. Of particular interest for countries of the African meningitis belt is the finding that symptoms of upper respiratory tract infection are linked to risk of acquisition, but only in the dry season. The evidence is mounting that such infections are an important factor behind the risk of epidemics in the meningitis belt.

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Collard (PI), Ibrahim Dan Dano, Ibrahim Habiboulaye, Bassira Issaka, Jean-François Jusot, Sani Ousmane, Issoufa Rabe.

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Centre pour les Vaccins en Développement, Bamako, Mali: Abdoulaye Berthe, Kanny Diallo, Mahamadou Keita, Uma Onwuchekwa, Samba O Sow (PI), Boubou Tamboura, Awa Traore, Alou Toure.

Centers for Disease Control, Atlanta, USA: Tom Clark, Leonard Mayer.

Department of Community Medicine, University of Maiduguri, Maiduguri, Nigeria: Mary Amodu, Omeiza Beida, Galadima Gadzama, Babatunji Omotara (PI), Zailani Sambo, Shuaibu Yahya.

Faculty of Infectious Disease, London School of Hygiene & Tropical Medicine, London, UK: Daniel Chandramohan, Brian M Greenwood (PI), Musa Hassan-King, Olivier Manigart, Maria Nascimento, James M Stuart, Arouna Woukeu.

Princeton University, USA: Nicole E Basta

Public Health England Vaccine Evaluation Unit, Manchester, UK: Xilian Bai, Ray Borrow, Helen Findlow.

Institut de Recherche pour le Développement, Dakar, Senegal: Serge Alavo, Hubert Bassene, Aldiouma Diallo (PI), Marietou Dieng, Souleymane Doucouré, Jules François Gomis, Assane Ndiaye, Cheikh Sokhna, Jean François Trape.

Navrongo Health Research Centre, Navrongo, Ghana: Bugri Akalifa (deceased), Abudulai Forgor (deceased), Abraham Hodgson (PI), Isaac Osei, Stephen L Quaye, John Williams, Peter Wontuo.

University of Bristol, UK: Thomas Irving.

University of Cambridge, UK; Caroline L Trotter

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Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of the London School of Hygiene and Tropical Medicine and by the relevant ethical authorities in each African centre. The head of the household or another responsible adult gave verbal informed consent for the household to be included in the study. Each individual recruited within that household gave written informed consent; for children under the age of 18 years a parent or guardian gave written consent and children aged over 12 years were additionally asked to give written assent.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and/or analysed during the current study are available in the Unviersity of Cambridge Repository Apollo, [link to be made available upon acceptance of manuscript].

Competing interests

The authors declare that they have no competing interests.

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Author contributions

BMG, JMS, CLT, MN, RB, MCJM, AA, J-MC, J-FJ, DMD, BO, AD, SS, AH, AW designed the study and coordinated the field work. AR, CLT, LVC, BMG, JMS drafted the manuscript. AR, CLT, and LVC analysed data. All authors critically reviewed and approved the manuscript.

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Table 1. *Risk factors for N. meningitidis acquisition over the full study period: single risk factor analysis and multi-variable model. Adjustment was made in both single and multi-variable analysis for age, country, and sex.*

	Single risk factor analysis				Multi-variable model			
Factor	Total	Positive (%)	OR	95% CI	Total	Positive (%)	OR	95% CI
Age								
30 plus					205	11.7	1	
Under 5					91	28.6	3.12	(1.27,8.05)
5-14					108	23.1	3.62	(1.42,9.93)
15-29					161	21.1	2.38	(1.22,4.76)
Country								
Chad					54	5.6	1	
Ethiopia					64	26.6	7.65	(1.81,44.4)
Ghana					74	23	6.77	(1.52,40.1)
Mali					157	5.7	0.532	(0.110,3.22)
Niger					206	28.6	10.0	(2.53,57.3)
Senegal					10	40	13.3	(1.23,159)
Sex								
Female					326	17.5	1	
Male					239	21.8	1.00	(0.585,1.71)
Exposure to wood smoke in house (apart from use in cooking)*								
No	372	20.2	1		261	19.2	1	
Yes	478	19.0	2.74	(1.76,4.32)	304	19.4	2.60	(1.26,5.59)
Tobacco exposure*								
None	234	14.1	1		230	13.5	1	
Passive (secondhand) smoke	312	22.8	1.92	(0.965,3.77)	312	22.8	1.92	(0.823,4.55)
Active smoker	23	30.4	3.75	(1.23,10.8)	23	30.4	3.57	(0.978,13.0)§
Any sore throat reported *								
No	651	17.8	1					
Yes	208	25.5	1.66	(1.09,2.53)				
Any runny nose reported *								
No	184	20.7	1					
Yes	675	19.4	1.57	(0.995,2.51)				
Use gas as primary cooking fuel*								
No	832	20.0	1					
Yes	25	12.0	0.311	(0.0664,1.03)				
Completion of primary school (amongst over 17 years)*								
No	269	18.2	1					
Yes	99	11.1	0.381	(0.170,0.793)				
Household member completed secondary school*								
No	444	22.3	1					
Yes	415	16.9	0.670	(0.455,0.983)				
More than 2 participants per room*								
No	484	14.5	1					
Yes	375	26.4	1.44	(0.996,2.10)				

	Single risk factor analysis				Multi-variable model			
Factor	Total	Positive (%)	OR	95% CI	Total	Positive (%)	OR	95% CI
Attending primary school (ages 5 to 17)								
No	52	25	1					
Yes	254	23.2	0.721	(0.325,1.65)				
Regular social meetings								
None	202	20.3	1					
1-2 per week	68	16.2	0.916	(0.404,1.96)				
3-4 per week	48	8.3	0.531	(0.141,1.61)				
5-7 per week	52	5.8	0.356	(0.0793,1.14)				
Index carrier in household								
No	259	12.0	1					
Yes	600	23.0	1.32	(0.826,2.16)				
Use wood as primary cooking fuel								
No	31	12.9	1					
Yes	828	19.9	1.02	(0.340,3.83)				
Indoor kitchen								
No	660	16.4	1					
Yes	199	30.7	1.28	(0.838,1.94)				

NB Total number of individuals may not sum to 861 in every case because of missing values.

* p-value less than 0.1 in single risk factor analysis.

§p-value less than 0.05.

Table 2. *Risk factors for visit-by-visit N. meningitidis acquisition: single risk factor analysis and multi-variable model. Adjustment was made a priori in both single and multi-variable analysis for age, country, and sex.*

	Single risk factor analysis (plus *a priori*)				Multi-variable model			
Factor	Total	Positive (%)	OR	95% CI	Total	Positive (%)	OR	95% CI
Age								
30 plus					1504	1.8	1	
Under 5					1539	3.4	1.99	(1.22,3.32)
5-14					2129	4.2	2.76	(1.75,4.48)
15-29					1239	3	1.83	(1.08,3.15)
Country								
Chad					990	0.6	1	
Ethiopia					564	4.6	7.54	(2.59,24.5)
Ghana					828	3.5	5.7	(1.96,18.6)
Mali					1574	0.9	1.51	(0.483,5.13)
Niger					2281	5.2	11.5	(4.53,34.5)
Senegal					174	7.5	14.2	(3.6,60.7)
Sex								
Female					3405	2.9	1	
Male					3006	3.6	1.23	(0.907,1.68)
Antibiotic taken*								
No	6592	3.5	1		6150	3.3	1	
Yes	261	0.8	0.197	(0.0323,0.623)	261	0.8	0.169	(0.0271,0.564)
Interaction term*								
No sore throat, rainy	2643	3.3	1		2643	3.3	1	
No sore throat, dry	3481	2.8	0.88	(0.651,1.19)	3481	2.8	0.844	(0.617,1.16)
Sore throat, rainy	123	2.4	0.906	(0.218,2.52)	123	2.4	0.82	(0.192,2.39)
Sore throat, dry	164	11	3.72	(2.09,6.34)	164	11	3.67	(1.95,6.65)
Sore throat*								
No	6566	3.3	1					
Yes	287	7.3	2.64	(1.58,4.19)				
Season								
Rainy: June to December	1944	3.1	1					
Dry: January to May	4467	3.3	1.07	(0.78,1.47)				
Meningitis vaccination								
No	5743	3.7	1					
Yes	1110	2	1.54	(0.899,2.55)				
Attendance at social event								
No	3319	4.4	1					
Yes	3534	2.5	0.851	(0.63,1.14)				
Travel greater than one hour								
No	6055	3.6	1					
Yes	798	2	0.955	(0.538,1.58)				
Cough								
No	5163	3.6	1					
Yes	1690	3	0.955	(0.682,1.31)				
Runny nose								
No	4634	3.8	1					
Yes	2219	2.6	0.961	(0.689,1.32)				

* p-value less than 0.1 in single risk factor analysis.

Table S1. *Case definition for study-long acquisition.*

Classification	Carriage at visits 0 or 1	Carriage at visits 2-9	More than 3 missed visits	Number of individuals
Not acquisition	No	No	No	692
Acquisition	No	Yes	No	169
Excluded	No	No	Yes	231
Excluded	No	Yes	Yes	18
Excluded	Yes	No	No	42
Excluded	Yes	No	Yes	18
Excluded	Yes	Yes	No	159
Excluded	Yes	Yes	Yes	22

Table S2. *Case definition for visit-by-visit acquisition.*

Classification	Carriage at previous visit	Carriage at current visit	Strain previously observed	Number of visit pairs
Not acquisition	No	No	Not applicable	6768
Acquisition	No	Yes	No	226
Acquisition	Yes	Yes	No	47
Excluded	No	Yes	Yes	183
Excluded	Yes	Yes	Yes	366
Excluded	No	No data	Not applicable	1180
Excluded	Yes	No	Not applicable	516
Excluded	Yes	No data	Not applicable	128
Excluded	No data	No	Not applicable	1677
Excluded	No data	Yes	Not applicable	304
Excluded	No data	No data	Not applicable	2115

Table S3. *Odds of sore throat adjusting for age, country, sex and season.*

Factor	Total	Percent reporting sore throat	OR	95% CI
Age				
30 plus	2024	6.6	1	
Under 5	1914	2.4	0.372	(0.261,0.522)
5-14	2806	2.6	0.399	(0.295,0.534)
15-29	1752	7.4	1.13	(0.873,1.45)
Country				
Chad	1038	5.8	1	
Ethiopia	940	7.1	1.27	(0.883,1.83)
Ghana	1135	8.6	1.41	(1.01,1.98)
Mali	1854	1.1	0.174	(0.101,0.287)
Niger	3179	4.3	0.728	(0.532,1.01)
Senegal	350	0		
Sex				
Female	4424	4.3	1	
Male	4072	4.6	1.14	(0.92,1.4)
Season				
Rainy: June to December	3617	4.4	1	
Dry: January to May	4879	4.5	1.27	(1.02,1.57)

Table S4. *Likelihood ratio test comparing visit-by-visit model with and without term of interaction between season and sore throat.*

						Chi-square		
Model	Degrees of freedom	AIC	BIC	Log-likelihood	Deviance	Statistic	Degrees of freedom	p-value
Acquisition ~ Age + Country + Sex + Sore throat + Antibiotic	14	1660	1754	-815.82	1631.6			
Acquisition ~ Age + Country + Sex + Sore throat + Antibiotic + **Season**	15	1662	1763	-815.77	1631.5	0.099	1	0.75
Acquisition ~ Age + Country + Sex + Sore throat + Antibiotic + **Season + Season and sore throat interaction**	16	1656	1764	-811.97	1623.9	7.6	1	0.006

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