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Case Study.

DYNAMICS OF CARRIAGE AND CASES OF MENINGITIS IN KASSENA-NANKANA DISTRICT IN THE UPPER EAST REGION, GHANA.

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Following the major meningitis epidemic that occurred in Kassena- Nankana district in 1997/98, colonization of *meningococci* in both carriage and cases have been studied and followed by mass vaccination with polysaccharide vaccine. Pneumococcal carriage is imperative in mitigating this Public health menace in Kassena-Nankana district since pneumococcal vaccine was readily out of reach. This is a preliminary research which was conducted in Kassena- Nankana district in the upper east region of Ghana examined 300 nasopharyngeal samples within the peak season of the disease (January-May, 2010). Out of this sample 50 % had no growth, 16.67 % carried *Neisseria meningitidis* (W135), 0% for *Streptococcus pneumoniae* while 33.33 % carried other organisms of less importance in this research. *N meningitidis* (W135) in the carriage sample accounted for 15.51 % of the disease out of 303 cases even in the fate 14 % of pneumococcal cases recorded with 69% and 1% for negative cases and other organisms respectively. The absence of *S. pneumoniae* in the nasopharynx yet responsible for a significant disease rate suggested that, the organism had colonized an alternative niche (possibly the nose) while causing the disease. This unraveled knowledge obtained from this research is an attempt to contribute to the dynamics of *N meningitidis* and *S. pneumoniae* in the district by taking into accounts the carriage and case rates of the two within the same study population. Even in the fate of pneumococcal meningitis outbreak, the study also observed the presences of a new hypervirulent meningococcal clone in the nasopharyngeal carriage and cases within this same season.

Keywords: Meningitis , *Streptococcus pneumoniae ,* polysaccharide vaccine.

INTRODUCTION

Meningitis is one of the major diseases endemic in Kasena-Nankana district, it has been battled with by the public health system since it emergence in 1997. Since then, it has stayed and resurfaced as different strains in carriage and cases studies over time. Usually, transmission and carriage are low in open population but increases with close contact and subsequently cause the disease (Stephen *et al*., 2007). Carriage not characterized by any symptom often provides a platform for inhabited causative agent to invade the blood stream and cross the blood-brain barrier to cause the disease under adverse conditions. Symptoms associated with cases include severe headache, fever, stiffness of neck, back muscles (meningismus), photophobia, kernig sign, irritability, vomiting, convulsion and drowsiness among many others (Cheesbrough, 2006).

Successful research on producing meningococcal conjugate vaccine for serogroup A, just like serogroup C conjugate vaccine has prevented serogroup C meningococcal disease in the UK and less number of death was recorded in 2007 (Troller *et al*., 2004). The reduction in morbidity has been associated with the reduction in carriage of sergroup C *meningococci* (Maiden *et al*., 2002; Ramsey *et al*., 2003). Currently, victims pneumococcal meningitis in the Northern Ghana resort to chemoprophylaxis while very little is done to identify the carriage rate and where it has inhabited in the presence of *meningococci*. The unclear periodicity of meningococcal epidemic Africa making prediction difficult in Africa meningitis belt, rather 10-15 cases per 100,000 inhabitants is the epidemic threshold used. in detecting and controlling the disease since public health interventions are difficult to implement and therefore vaccination campaigns are often delayed (WHO 1998, 2000). The reason for the susceptibility of this part of the Africa to major epidemics of meningococcal disease is not fully understood but it appears to be due, at least in part, to the unique climatic features of this

region as epidemics are largely restricted to the hot dry season (Greenwood, 1999).

Following the major meningitis epidemic that occurred in Kassena- Nankana district in 1997/98, colonization of *meningococci* in carriage have been studied and followed by mass vaccination with polysaccharide vaccine. This has reduced incidence of the disease even though sudden disappearance and re-emerging of hypervirulent clones and defied drug in creating immunological memory, immunogenicity on young children, impact on carriage and transmission of the infection (Dellicour and Greenwood, 2007). Research conducted over a period of eight years in northern Ghana have shown the passage of different meningococcal stains through the community over a period several years (Leninkeugel *et al*., 2009). With varying sero groups and serotypes, *Neisseria meningitidis* and *Streptococcus pneumoniae* are the major etiologic agents responsible for the district disease endemicity. Carriage strains are often seen causing the disease in most of the cases during the peak period of the disease even though this was not the case prior to the study. An earlier unpublished routine carriage study indicated that both strains known and vaccinated against had disappeared even though it still had alarming cases reported at various health facilities. This thus could not be reconciled with etiologic strain in cases, the disappearance and the possible sudden emergence of a new strain necessitated this research. Some study subjects in the district who were previously vaccinated with the then available sero group A & C vaccine yet reported at several health facilities with the disease. This kept the knowledge of the district dynamics of carriage and cases of the disease in shredded parts, making prediction even though few attempts have been made in this direction in with less sophisticated or modern techniques. The study considered the carriage and case rate of the two etiologic agents, the type of meningococcal strain in both carriage and cases, and co-colonisation of *S. pneumoniae* and N. *meningitidis* at the swabbed site of the study subject. Thus, this piece of research was carried out to help the unraveling of the knowledge that contributes to dynamics of meningitis case and carriage study in the district.

MATERIALS AND METHODOLOGY

Materials

Tongue depressors, sterile cotton swabs, media (blood agar plates and chocolate agar plates), (state the maker of the media) plastic waste disposal bags, permanent markers, printed data collection forms and labels, storage container, adhesive tape, biochemical reagents, sterile gloves, nose mask among other necessary microbiological laboratory equipments were used in this research.

Methods

The study was conducted in Kassena-Nankana district in the upper east region of Ghana in the Africa meningitis belt and lies in the Guinea savannah woodland with two major seasons; dry (Nov/Dec-Mar/April) and wet season (April/May- Oct/Nov) (Nyarko *et al*., 2002). A sample size of 150 study subjects was randomly sampled and swabbed for the purpose of this study from 37 compounds in a cross- section of the district from January-May, 2010. Ethical issues such as consenting and assenting were carried out before samples were obtained from the subjects; the individual was excluded from the study if he/she had acute illness (in the upper respiratory tract), serious chronic illness, and prior recipients of a meningococcal polysaccharide (within the past 2 years) and conjugate vaccines. The research was carried out from January-May of 2010, a period that is considered the peak because environmental conditions at this time are noted to facilitate the transmission and disease cases.

Nasopharyngeal samples were obtained from informed healthy individuals at dawn (4:00am- 6:00am) who with their unique identification code on BAP and CAP were inoculated immediately in the field while minimizing contamination. The inoculated plates were transported in storage containers to laboratory 2-6 hours for incubation at 35-38 0C in 5 % CO2 enriched atmosphere and the growth were examined for *S. pneumoniae* at intervals of 24, 48 and 72 hours. Plates with small greyish, moist raised central point of the young colonies (become depressed when old and gram positive diplococci colonies with a zone of apha-haemolysis on BAP and CAP (Popouie *et al*., 1996). Differentiating pneumococcal colonies from viridians streptococci that are alike, optochin and bile solubility tests are performed. Also within the study period N. *meningitidis* and *S. pneumoniae* were tested for in cases that were reported at the various hospitals in the district identified using CSF collected from lumbar puncture for commercially available slide agglutination test following to the Manufacturer's instructions.

Latex Agglutination and electrophoresis

Cerebrospinal fluid from suspected cases were collected using lumbar puncture and tested for the presence of N. *meningitidis* (their sero groups) and *S. pneumoniae* using latex agglutination method with a commercially reagents. Cases sampled and examined for the purpose of this research were patient with conditions suspected and diagnosed to be meningitis an experienced Medical practitioner. Electrophoresis was used to identify sero group of the carriage meningococcal after series of preliminary biochemical tests.

Susceptibility to Optochin

Optochin disk containing 5µg ethylhydrocuprine (6mm diameter) was used alongside with BAP, enriched

CO2 environment for incubation period between 18-24 h. Optochin resistant strains were not considered as pneumococci while bile solubility while strains with zone of inhibition greater than 14mm diameter are pneumococci or otherwise confirmed with bile solubility

Bile solubility

Loopful of the test from BAP growth used for McFarland density standard suspension of 0.5ml of sterile saline (divided into two equal part) with 2% deoxycholate (bile salt) in one part under 35-38 0C for two hours. Examine the tube periodically for lysis of cell in the tube with bile salt and the clearing of the tube or loss in turbidity is positive for pneumococci.

RESULTS

Preliminary laboratory examination of the 300 samples showed that 40 % of these growth plates had two or more distinct colonies. During the period of study, the 90 confirmed disease cases caused by meningococcal Neisseria and pneumococcal agent were recorded from various health facilities in the district. In this research, priority of laboratory identification was given to organisms of paramount concern which in one way other is under the broad Gram type of organisms causing the meningococcal carriage (*Neisseria meningitidis* and *Streptococcus pneumoniae*).

Sampled subjects were grouped in to various age classes as represented in table 3.0 with their corresponding carriage rate for the interested Gram types.

Table 1.0 Sample Age-group and carriage level for causative isolates

Age class	NO. OF SUBJECTS	**W 135**	***S. pneumoniae***
2 to 10	35	2	0
11 to 20	35	12	0
21 to 30	39	20	0
31 to 40	20	8	0
41 to 50	30	4	0
51 to 60	18	3	0
61+	23	1	0
Total	**300**	**50**	**0**

Both the carriage and cases within the study period are represented in the tables below.

Table 1.0 Carriage study in the District

Isolate	**Number of Subjects**
N. meningitidis, W135	50
S. pneumoniae	0
Others	100
No growth	150
Total	**300**

Table 2.0 Case study the District

CAUSATIVE AGENTS	**NO_ OF CASES**
S. pneumoniae	43
N. meningitidis	47
Other agents	4
Negative cases	209
TOTAL	**303**

In an investigation in the laboratory confirmed patients with either of the causative agents where nasopharyngeal samples from the 90 confirmed disease cases recorded revealed that, 72.2% of the patients were carrying N. meningitidis (W135) responsible for 16.67% of the cases even though S. pneumoniae caused 14% of the cases instead of the usual N. meningitidis (A).

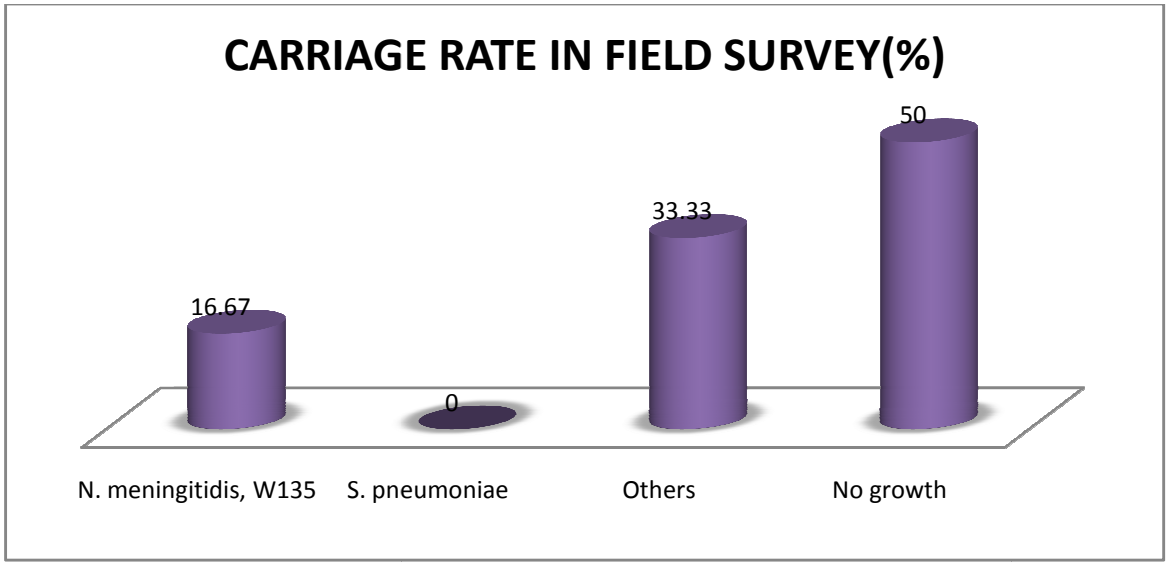

Figure 1.0 Carriage rate in the District

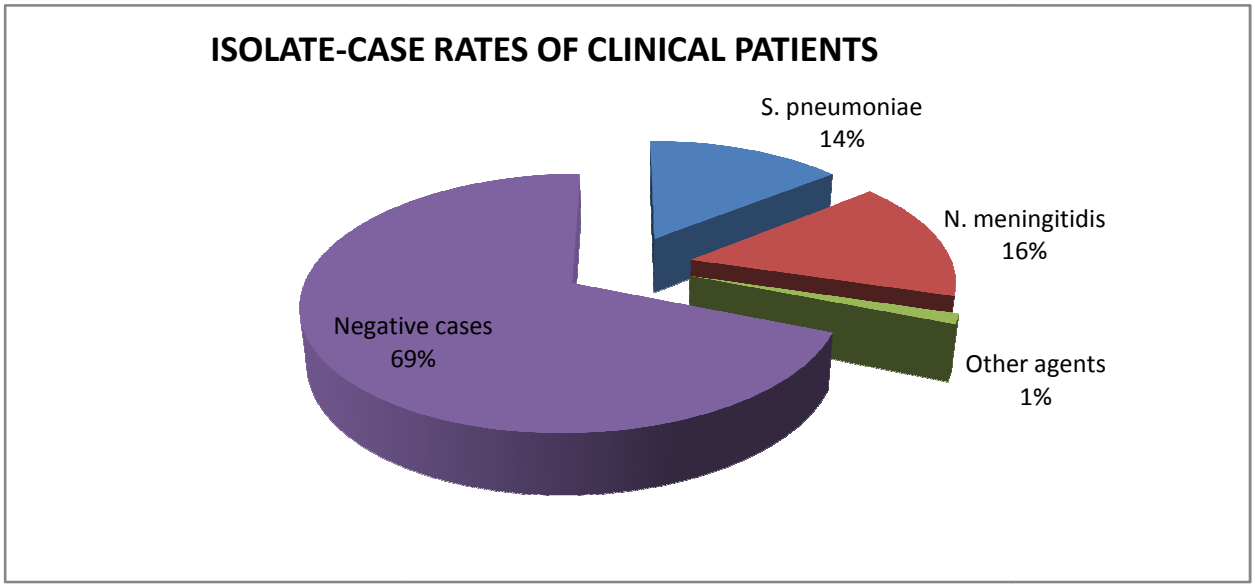

Figure 2.0 Isolate-case rates of clinical patients

DISCUSSION

The alpha-haemolytic colonies produced on BAP and CAP forming 1.33 % of the non-patient samples were found to be viridians streptococci not *S. pneumoniae* after responding negatively to both optochin and bile solubility tests. Viridians streptococci (1.33 % of carriage samples) co-colonized with some other organisms other than Neisseria (these are likely to be micro flora, Cluster Gram positive cocci suspected to be *Staphylococci*). Even though *S. pneumoniae* was absent in the carriage samples (clinical and non-clinical), it accounted for 14.19% of the total disease in the district within the same study period irrespective of other causative agents. This also suggested that even though relatively large number of them was carrying N. meningitids (W135), *S. pneumoniae* had colonized an alternative niche while remarkably surfacing in the cases recorded within the study period. When *S. pneumoniae* co-colonize with nasopharyngeal meningococcal Neisseria, its colonization and growth are inhibited by hydrogen oxide produced by the meningococcal Neisseria in the same niche (Cheesbbrough, 2006).

The 16.67% of the non-clinical samples were carrying meningococcal Neisseria, a replacement of the usual sero group A (N. meningitis sero group W135). The isolate in both carriage and cases was same (N. meningitis sero group W135). The presence of serogroup W135 in the carriage reflected in 15.51% of the total disease cases

examined during the study period. The organism colonizes the nasopharynx and invades the blood into the cerebrospinal cord after the nasopharynx have been traumatized inflicting the disease on the host. This transmission and invasion of a hypervirulent *meningococci* is seriously aided by environmental factors (climatic features of the region) while without the reservation genetic influence of epidemic meningococcal clones (Greenwood, 1999). Serogroup W135 was sourced in both carriage and cases within the same study period which suggested that, serogroup A earlier noted for the disease in the district (Leninkeugel *et al*., 2009) has been replaced by serogroup W135 (a new epidemic clone) may be due to the mass vaccination with N. *meningitidis* serogroup A& C polysaccharide vaccines (Lipsitch, 1999; Grupta and Galvani, 1999). This was the first appearance of serogroup W135 epidemic clone in the district. However, the vaccination of the district populace with serogroup A, C & W135 polysaccharide vaccine was on course after the picking samples from study subjects. A protein conjugate polysaccharide vaccine was yet be tried it is anticipated to produce long term immunity and also reduce carriage even though there was an ongoing mass vaccination with N. meningitides serogroup A,C & W135 polysaccharide vaccine that was used as an immediate public health intervention in the district.

Other organisms of minimal important in this research from both carriage and cases were 33.33% and 1% respectively while respective rates for no growth in non-clinical carriage and negative cases in clinically suspected patients were 50% and 69. In light of the above discussion in the faith of the results of the research, the alarming pneumococcal meningitis in the district that could not be sourced in the nasopharyngeal carriage unlike the meningococcal Neisseria, this is an indication that *S. pneumoniae* had colonized alternative niche which could be the nose while presenting itself in the cases. The hot dry air with dusty particles during dry season traumatizes the epithelial lining of the nose where the organism has access to the blood stream leading to the blood-brain barrier to cause the disease. This research contributes towards the understanding of dynamics of meningococcal disease in district while alerting the public health system of the meningococcal carriage organisms that are likely to cause an outbreak of the disease. It also affirms the carriage age group of the meningococcal disease-causing organisms in the study population is mostly the active young and adolescent or adult age groups, 11-20 and 21-30 years (Cartwright *et al*., 1987; Maiden *et al*., 2002; Ramsey *et al*., 2003; Troller and Greenwood, 2007).

CONCLUSION

S. pneumoniae, the next leading microbial agent aside N. *meningitidis* though not present in carriage yet accounted for 14.19% of the suspected meningococcal disease in Kassena- Nankana district within the study period. The inability to source this alarming pneumococcal case in the nasopharynx suggested that, *S. pneumoniae* is able to alternatively colonize another niche other than in the nasopharynx while causing the disease since the nasopharynx was occupied by inhibitory meningococcal Neisseria. Antibiotics were only administered to those with pneumococcal meningitis in the crucial absence of the vaccine (microbes may develop resistance leading to high level of cases and carriage). The absence of the pneumococcal vaccine even in the state of alarming pneumococcal suggested the public health risk in the study area. The alternative niche (nasal carriage) suggested will be investigated in our next research. This research finding also indicated that, N. *meningitidis* serogroup W135 had just appeared for the first time in carriage and cases replacing N. *meningitidis* serogroup A. The research arrived that, no pneumococcal carriage was found in the nasopharynx in the presence of meningococcal Neisseria that could have possibly causeed the pneumococcal outbreak. Also, mass vaccination with the pneumococcal vaccine was imperative in order to save many lives in the district and the country at large.

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