Journal of
Public Health and Epidemiology

Academic
Journals

ABOUT JPHE

The **Journal of Public Health and Epidemiology (JPHE)** is published monthly (one volume per year) by Academic Journals.

Journal of Public Health and Epidemiology (JPHE) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as health observatory, biostatistics, occupational health, behavioral medicine etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JPHE are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jphe@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Public Health and Epidemiology will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Professor Mostafa A. Abolfotouh

Professor of Family & Community Medicine
Head of Medical Team - Biobanking Section.
King Abdullah International Medical Research CEnter, King Saud Bin-Abdulaziz University for Health Sciences, National Guard Health Affairs, Saudi Arabia

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.
Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JPHE to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.
Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni,

1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)
References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section. Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Journal of Public Health and Epidemiology

ARTICLES

Full Length Research Paper

The buruli ulcer morbidity in the amansie West District of Ghana: A myth or a reality?

Ebenezer Owusu-Sekyere

Department of Development Studies, University for Development Studies, Wa, Ghana.

Although there is a lot of literature on the possible causes of Buruli ulcer (BU), no one is sure where the bacterium lives in the environment. It is also a mystery how the mycobacterium enters the human body, although it is clear the bacterium is unable to do so by itself. There is therefore a lot of myth about the disease epidemiology. This research has shown that the myth surrounding the cause of the disease and the origin of the disease pathogen has affected the treatment option sought by patients and the intervention strategies put in place by health expects in the Amansie West Distrct of Ghana. Whereas some patients in the Amansie west district associate the disease with witchcraft and magico-religious beliefs, the study showed that the disease is associated with aquatic environment that have been disturbed either through mining or intense agricultural activity. The aim of this paper is therefore to expand the frontiers of the argument by examining some of the predisposing factors and to identify the spatial pattern in the distribution of BU in the Amansie West District. The paper concludes that despite the myth, the disease causing organism thrives well in arsenic rich aquatic environment. However because of the widely rooted wrong perception, any attempt to manage the disease must first target the myth, in order to manage the reality.

Key words: Buruli ulcer, morbidity, myth, reality, mycobacterium ulcerans.

INTRODUCTION

One mysterious tropical disease whose epidemiology is yet to be unravelled is Buruli ulcer (BU) (Duker et al., 2006). Buruli ulcer, also known as Bairnsdale ulcer is a chronic, indolent, and necrotizing disease of the skin tissue caused by mycobacterium ulcerans (MU). The disease usually begins as a painless nodule or papule and may progress to massive skin ulceration (World Health Organization (WHO), 2012). The large number of cases and the complications associated with the disease as well as its long-term socio-economic impact could have a substantial effect on the national economy (Chauty, 2004). BU is a poorly understood disease that has emerged dramatically since the 1980s. The disease is mostly found in rural areas located near wetlands and slow-moving rivers, especially areas prone to flooding and that are often associated with rapid environmental

change (Merrit et al., 2010). Unlike leprosy and tuberculosis, caused by the organism belonging to the same family as BU, which are characterized by person-to-person transmission, inoculation of mycobacterium ulcerans into the subcutaneous tissues likely occurs through environmental contact, although the mode of transmission is still not entirely clear (Merrit et al., 2010). The agent produces a potent toxin known as mycolactone, which destroys cells in the subcutaneous leading to the development of large skin ulcers (Noeske et al., 2004).

The incubation period, the time between infection with mycobacterium ulcerans and clinical presentation of Buruli ulcer, is usually under three months (Johnson et al., 2004). It affects any part of the body, but predominantly affects the limbs (Asiedu et al., 2000).

E-mail: oseturbo@yahoo.com.

Year	New	Recurrent	Clinical forms		
			Nodules	Ulcer	Others
2008	235	24	36	164	0
2009	177	15	22	125	46
2010 half year	251	5	72	180	47

Source: Ministry of Health, 2012.

About 70% of cases are in people under 15 years of age (Asiedu et al., 2000). The clinical features of Buruli ulcer have been clearly defined by the World Health Organization (Marston et al., 1995). It starts as a localized swelling in the skin that is typically painless and firm. It is referred to as a papule when the skin swelling is less than one centimetre in diameter, and a nodule when it is one to two centimetres in diameter, attached and under the skin (subcutaneous). It is called a plaque when the ulcer has irregular edges and is more than two centimetres in diameter (Amofah et al., 2002). These swellings develop into ulcers. The ulcers typically have undermined edges, which make the real size of the ulcer difficult to estimate visually. The base of the ulcer is filled with dead (necrotic) tissue. The skin adjacent to the lesion, and often an entire limb, may be swollen (Asiedu et al., 2000). Ulcers may remain small and heal without treatment, or may spread rapidly over large areas. Healing is slow, taking an average of four to six months, and often follows a course with shrinking of lesions followed by a further extension. Healing results in scars which are usually depressed and star shaped (Amofah et al., 2002). Death due to Buruli ulcer is rare. The disease may however, result in joint deformities (contractures) from excessive scarring, making movement at joints difficult. Loss of or severe damage to vital organs such as eyes, breast, or genitalia may occur.

Despite the advances in medical sciences, extensive public education and research on the treatment and control of the disease, the World Health Organization reports that incidence of Buruli ulcer disease in Africa has not seen significant reduction over the years. The total Buruli ulcer cases recorded globally in 2012 including that of Ghana was 5,076 with Africa being the worst affected region. Ghana is the second most endemic country with 1,048 Buruli ulcer case after Cote d'Ivoire, with 2,670 Buruli ulcer case (WHO, 2012). This by implication means that Ghana and Cote d'Ivoire contributed 73 percent of the world's incidence. Again, the Ghana Ministry of Health in their 2012 annual report shows the Ashanti Region accounting for over 60% of all cases (Table 1).

Table 1 show that higher number of cases is still being reported yearly with the commonest clinical form being ulcer. This might be due to the fact that patients do report for early treatment, hence the higher number of cases for the ulcerative stage. The report further suggests that the most affected district of the Ashanti Region is the Amansie West, with a prevalence of 151 cases per 100,000 inhabitants. There are uncertainties about the epidemiology of the disease. There are knowledge gaps about where the bacterium lives in the environment and how the mycobacterium enters the human body. Again, the high rate of re-occurrence has fuelled perception among endemic communities that the disease of spiritual origin. But is there a case for superstition in the disease epidemiology? What could be the factors that pre-dispose people to infection? Seeking answers to these and many other lingering questions is the objective of this study. The aim of this paper is therefore to highlight some of the perceived myth surrounding the causes of the disease and also discussing some of the real predisposing factors that affect the transmission and spread of the disease in the Amansie West District of Ghana.

METHODOLOGY

Study setting

The Amansie West District is located in the south-western part of Ashanti Region where the disease was first given public recognition and national documentation in Ghana. The District shares boundaries with the Amansie East District in the west, Atwima Mponua District in the east, Atwima Nwabiagya District in the north and Amansie Central in the South. The Amansie West District falls within latitudes 6° 35 and 6° 51 North and longitudes 1° 40 and 2° 05 West. The District covers an area of about 1,364 sq. km. and forms about 5.4 percent of the total land area of the Ashanti Region. The most endemic towns include Tontokrom Mpatuam, Manso Mem, Manso Atwere, Edubia, Watreso, Abore, Keniago, Essuowin, Ahwerewa and Datano (Amansie West District Assembly, 2004) (Figure 1).

The district is drained by the Oda and Offin rivers in the north with their tributaries such as the Pumpin, Emuuna and Jeni. These rivers have however been polluted by numerous mining activities in the district. A survey carried out by National Buruli Ulcer Programme further detected new foci of BU outside of the previously established endemic communities. The district level prevalence rate is 151 cases per 100,000 inhabitants and this is above the national rate of 22.7 cases per every 100,000 inhabitants (Ministry of Health, 2012). The treatment for Buruli ulcer disease in the study area is provided at the St Martin's hospital at Agroyesum which has specialized Buruli ulcer disease facilities following WHO treatment guidelines. The study area is populated by various segmented identity subgroups of Asantes and settler migrants from other parts of Ghana. These groups rely mainly on subsistence
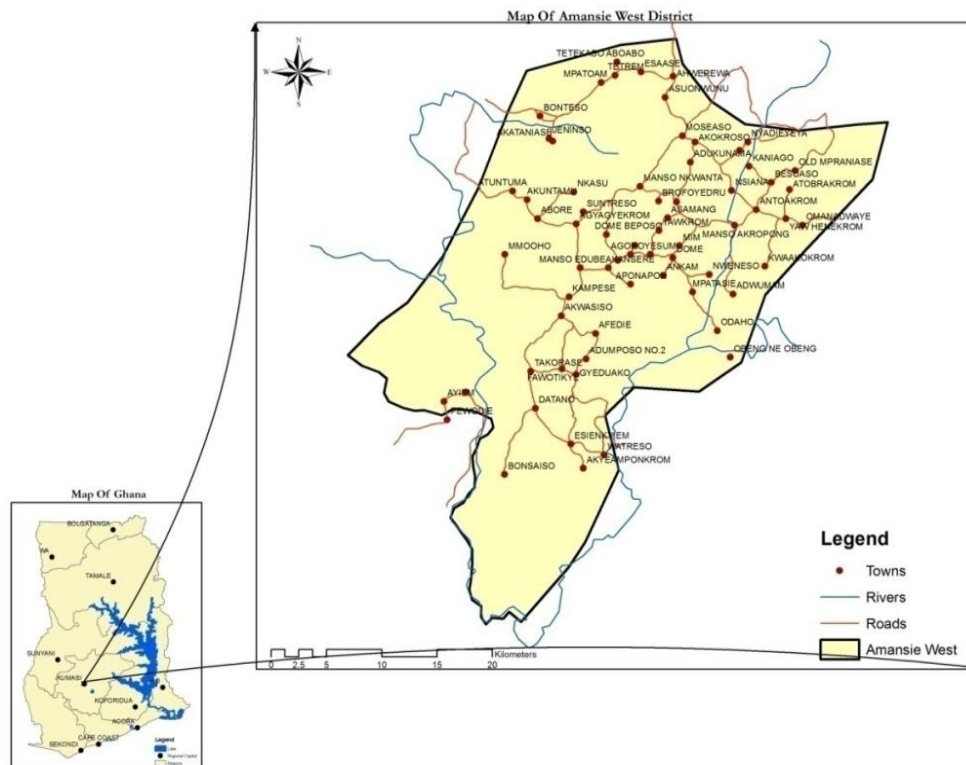
Figure 1. Map of the Amansie West District.

farming and artisanal small scale mining, commonly called 'Galamsey' for sustenance.

Research strategy

The research design consisted of a mixed methods study based on methodological triangulation, combining qualitative data from focused group discussion and quantitative data gathered using a standardized questionnaire. Data for the study was obtained from the three most endemic communities of Kaniago, Edubea and Watreso as well as the St Martin's Hospital where patient's hospital records were well documented, considering the focalized character of BU infection rates. The study population consisted of Buruli ulcer patients who were medically diagnosed with laboratory confirmation for BU and whose disease had reached both ulcerative and non ulcerative stage. Ulcerative and non ulcerative cases were based on clinical findings and confirmed by any two positives of Ziehl-Neelsen (ZN) test for acid fast bacilli, polymerase chain reaction (PCR), and histopathology (Roberts and Hirst, 1999).

Sampling

Sampling was purposive. Following the principle of gradual selection, informants were theoretically selected (in accordance with emerging results/theory) and categorized in relation to relevant criteria (such as gender, age, religion, ethnicity, locality, hospital or traditional treatment for BU, severerity of infection, functional disability, etc.) to allow for maximum variation. In addition, critical cases were continuously selected and analyzed. Snowball sampling (using participants to identify additional cases) was also used in order to increase respondents' confidence in the research team and consequently reduce response bias. In all, 185 participants were selected for the study.

Qualitative data

Qualitative data were gathered during the focus group discussions (FGD). The emphasis on qualitative data collection for the first strand of the study was required, given the exploratory nature of the first phase of research and the sensitive content of research questions related to traditional healing, the acceptability of hospital treatment and the possible mystical origin of the disease. Areas of interest to the researcher were analysis of factors related to patient satisfaction with biomedical treatment (including the doctor-patient relationship, practical and financial implications of hospitalization, the role of caregivers, etc.); on the perceived aetiology of BUD in relation to treatment choice; and on gaining an in-depth understanding of factors directly guiding treatment itineraries. Participant observation was an essential component of the field research as this facilitated in building up confidence with informants and in acquiring an in-depth understanding of more sensitive subjects such as sorcery involvement in the illness and healing processes. The observation of patients' daily activities provided an opportunity for reiterated informal conversations with key respondents, leading to some pivotal insights regarding the doctor-patient relationship, the importance of caregivers and the complexities of the disease's causality

Quantitative data

After the initial qualitative research strand, the study also used topographic map data which captured the geographic location of the study area where Buruli ulcer has been reported. Topographic map of the study area with a scale of 1:500000 obtained from the
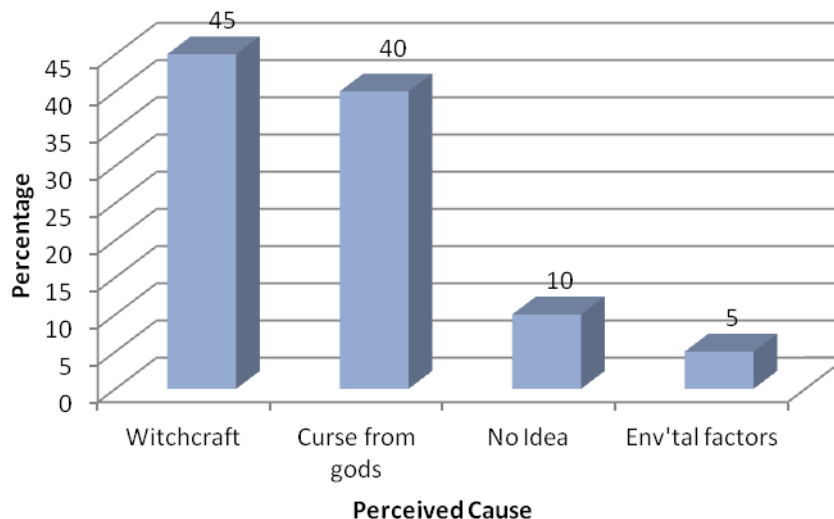
Figure 2. Perception of the causes of Buruli ulcer.

survey department, Accra was digitized using Arc GIS version 9.2. Initially, the map was geo-referenced by defining the X and Y coordinates of corner points of the map into a War Office Coordinate System. The boundary map of the study area was digitized as a polygon and communities as points. Reported cases of BU cases from 1999 to 2011 obtained from Amansie West District Assembly were entered as attribute of the point feature that is the settlements. The purpose was to establish the relationship between the prevalence of the disease and the physical.

RESULTS AND DISCUSSION

Buruli ulcer and superstition

Superstition is a widely held but unjustified belief in supernatural causation leading to certain consequences of an action or event or practice. The limited knowledge of the disease and socio-cultural beliefs and practices strongly influenced the perception of cause and health-seeking behaviours of people affected by BU. The success of any initiative to deal with health problems very much depends on recognition and health seeking behaviour of the people affected as it is often said that perceptions mould people's reactions to the world around them. The individual's health actions therefore need to be considered within the specific socio-cultural belief models which orient local definitions of health and illness (van de Graaf et al., 1999). On the score of this, respondents gave their perception on the causes of the disease (Figure 2).

The question on the possible causes of BU was posed to the infected and members in their household. The study found out that 45% attributed the causes to witch craft, 40% attributed the cause of the disease to curse from the gods of the land. On aggregate, 85% of respondents attributed the disease to supernatural

sources. Among the reasons, the respondents believed that sufferers of the disease might have had an infraction against the social order by breaking the taboos and living contrary to the established norms of the society. In this case, the gods punished them through the disease infection. Secondly, the disease is frequently linked to casting of spells through theft and social misunderstandings among households.

Patients who were less than five years were believed to be paying for the wrongs committed by their fore fathers. 10% were not able to identify the cause of the disease in the community. Only 5% could link the disease to aquatic environment. This revealing trend of perception indicates the low level of awareness on the causes of the disease. It was therefore not surprising that majority of the infected persons did not seek the right medication. This also shows that the numerous public educations to explain the causes of the disease have not achieved their target. This wrong public perception is one of the reasons why many patients do not seek early treatment and only report for medical treatment at the ulcerative stage where it becomes expensive and difficult to treat. It is therefore important that this bad public perception is corrected in order to reduce the rate of spread of the disease in the community.

Risk to health, as an area of study, begun to receive attention in developing countries since the early years 2000 (WHO, 2004a). Until this period, social science theories and empirical data on perceptions of environmental risk focused almost exclusively on factors related to frequently occurring disagreements between the lay population and technical experts. In other words, when assessing the risks, experts placed emphasis on quantitative data, whereas citizens were much more likely to base their opinions on qualitative aspects, such as the nature and origin of the threat to which they are exposed,
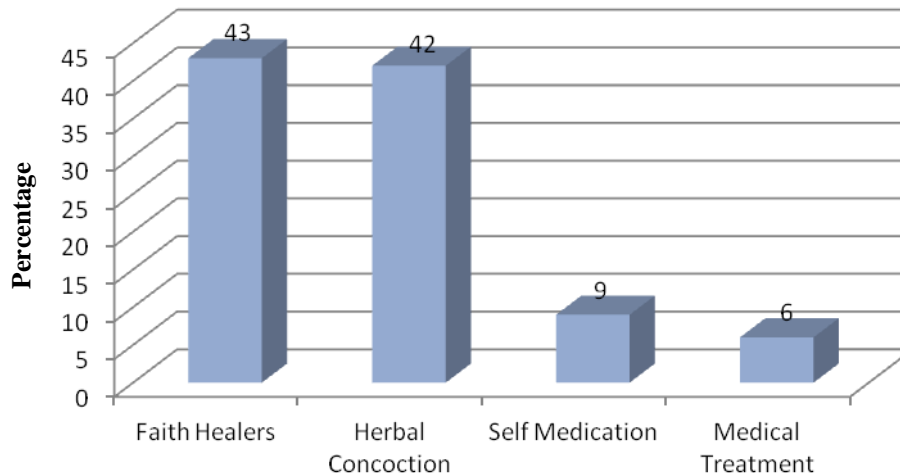
Figure 3. Treatment options sought by respondents.

usually involuntarily (Blake et al., 1995). To this end, a focus group discussion was organized to qualitatively engage the views of the respondents on the causes of the disease. One infected woman had this to say:

"*I do not understand how I got the disease because none of my family members have it even though we live in the same house, drink water from the same source and virtually share everything in common. Because am the only person infected, the family believe that it is the work of the evil spirits*".

This assertion was generally agreed by all. In a related development, a 34 year old male infected person also had this to say:

"*I certainly believed that it is the work of the supernatural forces, my late grandfather told me that there was once a popular fetish priest in this village who wanted to marry my mother but our family members objected. The fetish priest cursed our family; it is believed that my sickness is the result*".

The responses from the focus group discussions (FGD) indicate that there is a poorer understanding of the epidemiology of the disease. This dearth of understanding is a problem as it leads to stigmatization, social exclusion and lack of social integration. In some cases, its impact disproportionately affects girls and women, whose marriage prospects may diminish or who may be left vulnerable. Again, the lack of proper understanding on the cause of BU also influences the treatment option sought by patience.

The research revealed that about 43% of respondents depended on faith healers who were mostly fetish priests or pastors of faith based churches, 42% said they use herbal concoction and 9% could not be specific as they were on self medication. This meant that over 90% of respondents did not seek treatment from biomedical facilities Figure 3. This figure correspond to the over 80% who blamed the cause of the disease to magico-spiritual factors. The implication here is that the perception of cause influenced the treatment sought by the infected. For this reason, it is important that any attempt to encourage early report and treatment should first focus on working on their perception.

The myth surrounding the cause also leads to serious stigmatization as patients are regarded as unclean. Stigma is the reaction of society towards people with certain characteristics (for example, a deformity or an ulcer) which are perceived as abnormal and undesirable; the result is that such people are deprived of the same social inclusion and human right as are enjoyed by others. Fear, devaluation, and social inequality can also be a response to the physical deformities and scars that remain after medical and surgical treatment of the disease. These physical signs visually mark the individual and depart from societal standards of beauty. The disease may be viewed as a sign of a curse, or a punishment for some sin committed. Deformities may also lead many to believe that the person is unable to participate in activities and normal family, educational, and community life. These people may be viewed as a burden to themselves, their families, and their community. In another focus group discussion, one woman had this to say:

"*My husband has divorced me because his family believes that there is witchcraft in my family that is why I am suffering from the diseases*".

A focus group discussion was carried out to find out the extent of stigmatization in the communities under study. A middle aged woman at Tontokrom had this to say:

Figure 4. Map of the estimated risk of BU disease in Amansie West by Ordinary kriging

"I have been ejected from my room .in the family house that I use to occupy because they think my ulcer is as a result of the activities of the witchcraft".
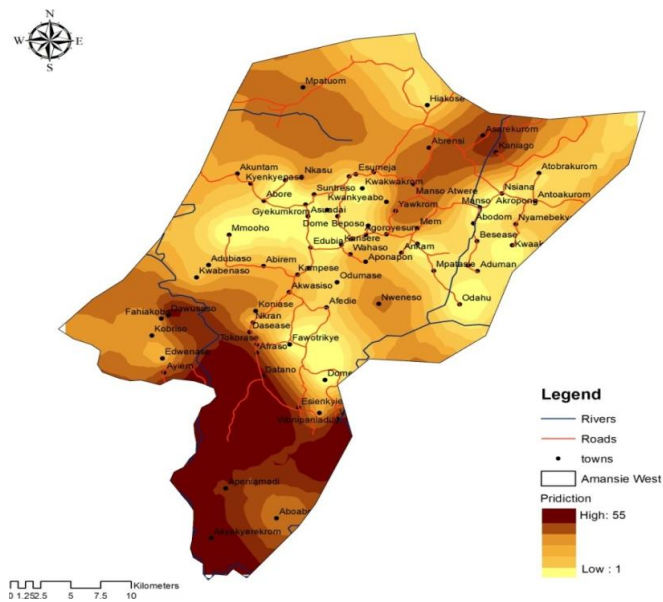
All sorts of bad names are given and in the event, one may voluntarily vacate the community for fear of being stigmatized. This therefore leads to psychological stress in the patients, as they most often feel embarrassed.

The reality

Using basically geospatial analysis, findings from this research indicated that the disease causing organism may be associated with aquatic environment. Geospatial analysis of the study area showed that the risk was higher in the southern portion and especially closer to the confluence of the two major rivers, the Offin and Oda Rivers. This portion is also noted for intensive artisenal mining and agricultural activities. In addition,the area has very poor social infrastructure especially good drinking water and health facilities, thus compounding the problem (Figure 4).

The results of the study indicates that Buruli ulcer endemicity is associ0ted with aquactic environments that have been disturbed either through minig or irrigation. The central portion of the risk map (Figure 4) which is devoid of rivers showed light colours whereas the southern part of the map which had the two major rivers are thick coloured. A lot of authors have reported the association of the disease with an aquatic habitat. Veitch et al. (1997) reported a large outbreak of the disease on Philips Island, Australia and associated the source of

infection to an irrigation which lay in the midst of the cluster of cases. Number of cases been reported from the community reduced after the irrigation site was modified and limited from the public. Scot et al. (2004) noted that cases of Buruli ulcer are associated with tropical wetlands of West and Central Africa, and cases have increased rapidly in these areas since the 1980's, particularly after irrigation and dam construction. Travis (1999) also noted that people living near slow-running waters are more likely to contract the disfiguring disease Buruli ulcer.

Merit et al. (2004) also reported the re-emergence of the disease among people who live/or work close to wetlands, especially slow-flowing (riverine) or stagnant water bodies (marshes, swamps), often created as a result of some form of human environmental disturbances. Bayley (1971) reported cases of the disease along the tributaries of Densu River in the Ga North District of Ghana. James et al. (2003) in Benin also identified three risk areas according to origin of patients reporting at hospitals with Buruli ulcer and noted that most of them were coming from Laguna areas of coastal Benin, marshy inland areas where market crops and rice are cultivated, and river valleys areas. Portaels et al. (1999) reported that re-emergence of the disease in some developing countries may be related to environmental and socio-economic factors like deforestation leading to increased flooding, population expansion without improved agricultural techniques, thus putting more people at a risk of contracting the disease.

Apart from associating aquatic environment with BU infection, it was also observed that high levels of Arsenic (As) concentrations prevail in such environment and this could cause BU occurrence to increase. The eastern and western portion of the Amansie West District, which accounts for most of the BU cases in Ghana, happens to have the highest levels of (As), possibly released into rivers, lakes and ground water by intensive gold mining and agricultural activities. Figure 5 shows the distribution of (As) in the district. The figure indicates that the concentration of (As) corresponds to the two areas drained by the rivers Offin and Oda (portions shown in deep red). These two areas co-incidentally have the highest cases of BU in the district (Figure 5).

This large range of spatial autocorrelation in arsenic concentration model shows a better spatial structure which may be speculated to be related to exposure of arsenic through mining activities into drainage where inhabitants use this water for their everyday activities. Human activities in the Amansie West District have elevated arsenic contamination in the environment (Bell and Broemeling, 2000). High levels of arsenic in drinking water have been detected in most parts of the district, with concentrations frequently exceeding the World Health Organization guideline contaminant level (MCL) of 10 µg/L (Smedley et al., 2002). Some of the human activities that have adversely affected the environment are mining, waste disposal, indiscriminate use of
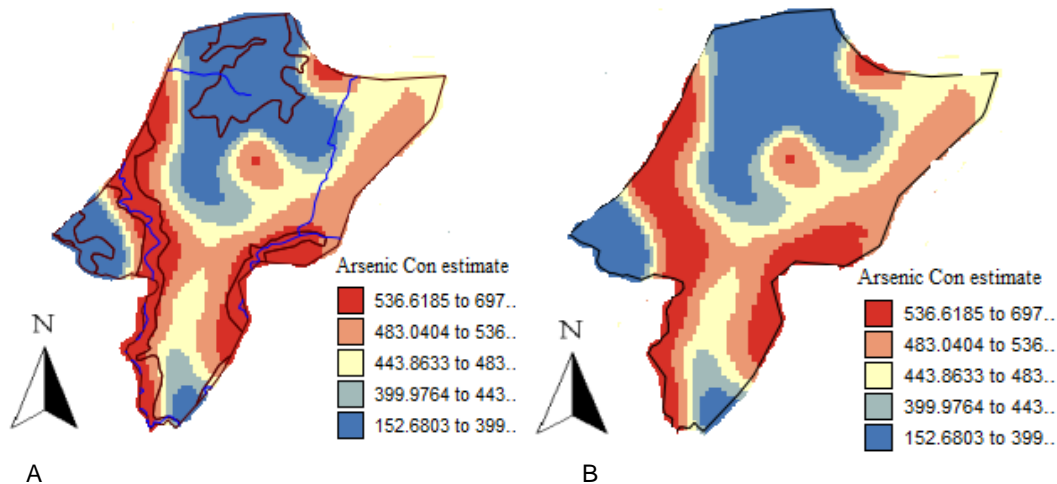
Figure 5. Maps of arsenic concentration on district boundary (A) and Arsenic concentration on the district soil map with the two main rivers; (B) both created by Simple Kriging with local mean.

fertilizers, pesticides, herbicides, manufacturing and chemical spillage.

A similar report was made by Jahan et al. (2002) that in the state of Victoria (Australia), mining of gold had caused an estimated 30,000 tonnes of arsenic to be redistributed to the surface across the landscape through erosion into streams and rivers. Hence, arsenic accumulates in soil, contaminates both surface water and groundwater WHO (2012), is taken up by plants and is then entrenched in mammalian/insectivore food chain (Green et al., 2001).

Arsenic occurs naturally in groundwater from dissolution of arsenic-bearing mineral constituents in underground aquifers, with concentrations typically ranging from < 1 to 1000 μg/L. Elevated levels of arsenic are a cause for concern because arsenic is associated with a number of adverse health outcomes, including several types of cancer, vascular diseases, dermatological ailments, diabetes, respiratory diseases, cognitive decline, and infant mortality (Chen et al., 1995). Mobilization of arsenic from geological formations into groundwater is driven by a host of biogeochemical and hydrologic factors. These factors include sediment mineralogy, well depth, microbial oxidation or reduction of arsenic, competing elemental species for sorption sites, groundwater recharge, groundwater flow path, and presence of fractures in bedrock formations (Smedley et al., 2002).

CONCLUSION

The research has revealed that despite the progress registered in recent years in research and educating the general public on the possible cause of the disease, major gaps still remain unabridged. The reservoirs of mycobacterium ulcerans, its mode of transmission to humans and immune-pathogenesis are still poorly understood. Currently, there is still a wide perception that the disease is cursed by supernatural powers. Again the treatment options discourage patients from reporting early; and no available treatment can prevent recurrence. There is therefore the need for endemic communities to intensify control activities and accelerate research. The priority is to disabuse the minds of patients of the perceived 'mythism' of the disease and to encourage early detection and case-management in the absence of a vaccine. Basic research is needed to understand the biology and epidemiology of the causative agent of this emerging disease. By better understanding the specific water-body systems and how human land-use disturbances affect the ecological interactions associated with Buruli ulcer, scientists will be able to provide the missing epidemiological links necessary to unravel the mysteries of this emerging disease.

REFERENCES

Amansie West District Assembly (2004). Demographic Characteristics and Infrastructural distribution (Ghana: Amansie West District Profile 2004) pp. 12- 26.

Amofah GK, Bonsu F, Tetteh C (2002). Buruli ulcer in Ghana: results of a national case search. Emerg Infect Dis. 8:167–70.

Asiedu K, Scherpbier R, Raviglione M (2000). Buruli Ulcer: Mycobacterium ulcerans Infection. World Health Organisation, Global Buruli Ulcer Initiative, pp. 87–92.

Bayley AC (1971). Buruli Ulcer in Ghana. British Medical J. 2:401-402.

Bell BS, Broemeling LD (2000). A Bayesian analysis for spatial processes with application to disease mapping. Stat. Med. 19:957–974.

Blake BR, Sisodia CS, Mokkui TK (1995). The effects of methyl mercury, tetraethyl lead, sodium arsenite on humoral.

Chauty A (2004). The role of antibiotics in the management of Buruli ulcer at the Pobe Center in Benin, in Proceedings of the 7th WHO Advisory Group Meeting on Buruli Ulcer, WHO, Geneva, Switzerland

Chen CJ, Chuang YC, You SL, Lin TM, Wu HY (1995). A retrospective study on malignant neoplasms of bladder, lung, and liver in a Blackfoot disease endemic area in Taiwan. Br. J. Cancer 53:399-405.

Duker AA, Carranza EJM, Hale M (2006). Spatial dependency of Buruli ulcer prevalence on arsenic-enriched domains in Amansie West District Ghana: implications for arsenic mediation in Mycobacterium ulcerans infection. Int. J. Health Geogr. 3:19.

Green K, Broome L, Heinze D, Johnston S (2001). Long distance transport of arsenic by migrating Bogon Moth from agricultural lowlands to mountain ecosystem. The Victorian Naturalist 118 (4):112-116

James K, Attipou KK, James YE, Blakine M, Tignokpa N, Abete B (2003). "Buruli ulcer in Togo: A hospital study." Sante 13(1):43-7.

Jahan N, Wilson M, Snow ET (2002). Bioaccumulation of arsenic in fish and aquatic food webs in the Victorian goldfields. Proceedings of the Fifth International Conference on Arsenic Exposure and Health Effects, San Diego, CA July 14-18.

Ministry of Health (MOH). (2012). Strategic Objectives and New Paradigm of the Ministry of Health. Ashanti Regional Health Directorate in 2012.

Merrit RW, Walker ED, Wallace JR, Johnson PDR (2010). Ecology and Transmission of Buruli Ulcer Disease: A Systematic Review. PLoS Negl Trop Dis 4(12): e911. doi:10.1371/journal.pntd.0000911.

Marston BJ, Diallo MO, Horsburgh CR, Diomande I, Saki MZ, Kanga J (1995). Emergence of Buruli ulcer disease in the Daloa region of Cote D'ivoire. J. Trop. Med. Am. 52:219-224.

Noeske J, Kuaban C, Rondini S, Sorlin P, Ciaffi L (2004) Buruli Ulcer Disease in Cameroon Rediscovered. Am. J. Trop. Med. Hyg, 70(5):520–526.

Portaels F, Elsen P, Guimaraes-Peres A, Fonteyne P, Meyers WM (1999). Insects in the transmission of Mycobacterium ulcerans infection. The Lancet 353:986.

Roberts and Hirst (1999). Immunomagnetic seperation and PCR for detection of Mycobacterium ulcerans. J. Clin. Microbiol. 35(10):2709-2722

Scot JT, Johnson RC, Aguir J, Debacker M, Kestens L, Gryseels B (2004). Schsitosoma haematobium infection in Buruli ulcer. Emerging Infect. Dis. 3:10.

Smedley PL, Edmunds WM, Pelig-Ba KB (2002). Mobility of arsenic in groundwater in the Obuasi gold-mining area of Ghana: Implications for Health. In: Appleton, J.D.

Travis J (1999). Africa's latest scourge: A flesh-devouring bacterium begins to reveal its secret. Science News 156 (3):40.

Veitch MG (1997) Mycobacterium Ulcerans Infection on temperate Southern Islands Epidemiology of Infection 83:410.

Van der Graaf WTA, Van der Werf TS, Tappero JW, Asiedu K (1999). Mycobacterium ulcerans infection. Lancet 354, 1013–1018.974.

World Health Organization (2004a). Report of the World Health Organization 7th Advisory Group Meeting on Buruli ulcer, 8–11 March 2004, Geneva, Switzerland. Geneva, Switzerland: World Health Organization.

World Health Organization (2012). Buruli ulcer – diagnosis of Mycobacterium ulcerans disease. Geneva, Switzerland: World Health Organization.

Full Length Research Paper

Loss of the *mec*a gene during storage of methicillin-resistant *Staphylococcus aureus* isolates in Northwestern Nigeria

Kumurya, A. S.

Department of Medical Laboratory Science, Faculty of Medicine, Bayero University, P.M.B. 3011, Kano, Nigeria.

Preservation of strains in a microbiology laboratory is of great importance for quality control, teaching, and research. Freezing is a very common method of preservation and storage of microorganisms. The evaluation of new diagnostic or *in vitro* antimicrobial susceptibility tests for methicillin-resistant *Staphylococcus aureus* (MRSA) requires well-defined strain collections. The study was aimed at determining whether loss of *mec*A gene in MRSA is related to the storage method. A total of 1692 non-duplicate *S. aureus* isolates were collected from different human clinical specimens at 8 different health institutions in Northwestern Nigeria from February, 2008 to April, 2010. All the isolates were screened for methicillin resistance using disc diffusion method (DDM), screen agar method (SAM) and latex agglutination techniques (PBP). Thereafter, the isolates were stored in 16% v/v glycerol broth at -80°C. In December, 2011, the isolates were retested by polymerase chain reaction (PCR) which was used to amplify both the *S. aureus* specific sequence gene and *mec*A gene of 100 isolates, with the amplicon size of 107 and 532 bp. The prevalence rate of MRSA on DDM, SAM, and PBP were 26.3, 24.2 and 25.0%, respectively. Surprisingly, the *mec*A gene was lost in 95.0% of 100 MRSA isolates after 2 years of storage at −80°C with the Micro bank system (Pro-lab Diagnostics, Austin, Tex.). This study demonstrates that *mec*A can be lost from MRSA strains stored at −80°C with the Micro bank system. This finding has important implications for the management of strain collections and is of use for all future biobanking projects.

Key words: *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus* (MRSA), *mec*A gene, storage, human, Northwestern Nigeria.

INTRODUCTION

Resistance of staphylococci to methicillin and all β-lactam antibiotics is associated with the low affinity of a penicillin-binding protein, PBP2a, which is not present in susceptible staphylococci. (Hartman and Tomasz, 1984; Pierre et al., 1990; Chambers, 1987, 1997, 1999; Muhammad et al., 2006). This protein is encoded by the *mec*A gene, which is located in the *mec* region and which is DNA of foreign origin (Matsuhashi et al., 1986).

The expression of the *mec*A gene and the resulting production of PBP2a is regulated by proteins encoded by the penicillinase-associated *blaR1–blaI* inducer–repressor system and the corresponding genomic *mec*R1–*mec*I elements (Tesch et al., 1990; Hackbarth and Chambers, 1993; Sharma et al., 1998; Muhammad et al., 2006). Hiramatsu et al. (1992) identified in *Staphylococcus aureus* N315 the *mec*R1–*mec*I regulator element, which is located upstream of the *mec*A gene and is divergently transcribed from *mec*A. The *mec*I gene

E-mail: askumurya.med@buk.edu.ng. Tel: +2347032153561.

codes for a repressor protein and the *mecR1* gene for a β-lactam-sensing transmembrane signalling protein.

Methicillin and oxacillin are, however, not good inducers for this system, often resulting in slow induction of methicillin resistance. Phenotypically susceptible strains, known as pre-methicillin-resistant *S. aureus* (pre-MRSA) and pre-methicillin-resistant coagulase-negative staphylococci (pre-MRCNS), have been discovered, which do not express methicillin resistance, as *mec*A is fully repressed by *mec*I (Hiramatsu, 1995; Weller, 1999). The induction of *mec*A transcription is very slow and might be due to mutations of *mec*I (Weller, 1999).

The distribution of *mec* regulator genes among methicillin-resistant *Staphylococcus* strains from various countries has already been studied by hybridization and sequencing, which showed that the loss or inactivation of the *mec*I gene leads to derepression of *mec*A gene transcription (Suzuki et al., 1993; Shimaoka et al., 1994; Hiramatsu, 1995; Weller, 1999). *In vitro* amplification of DNA by polymerase chain reaction (PCR) is a rapid and sensitive method for the detection of specific DNA sequences and requires fewer cells than DNA hybridization protocols (Tokue et al., 1992; Unal et al., 1992). The method has already been applied for the identification of *mec*A-positive strains directly in clinical specimens or in DNA extracts (Tokue et al., 1992; Ubukata et al., 1992). The present study was aimed at determining whether loss of *mec*A gene in MRSA isolates can be related to the storage method.

MATERIALS AND METHODS

Bacterial isolates

A total of 1692 consecutive non-duplicated *S. aureus* isolates were obtained from clinical samples in 8 health institutions (Microbiology department) across Northwestern Nigeria. The isolates were collected for duration of two years from February, 2008 to April, 2010. The quality control and rejection criteria of specimen were followed (Isenberg, 1998). *S. aureus* ATCC 25923 was used as a reference control organism. All confirmed *S. aureus* isolates were stored in 16% v/v glycerol broth at -80°C.

Disc diffusion method (DDM)

Methicillin disk susceptibility testing was performed according to National Clinical Laboratory Standards (NCCLS, 2003, 2008). Briefly, a bacterial suspension adjusted to 0.5 McFarland was inoculated onto Muller-Hinton agar. Filter paper disks containing 5 µg methicillin and 1 µg oxacillin (Becton Dickinson, Heidelberg, Germany) were placed on the inoculated Muller-Hinton agar using sterile forceps. All plates were incubated in 35°C for 24 h. The diameters of zone of inhibition were recorded.

Screen agar method (SAM)

All isolates were plated on Mueller-Hinton agar with 4% NaCl and 10 mg/l methicillin or 6 mg/l oxacillin. The isolates were inoculated at a final density of 5×10^5 CFU/ml. Methicillin resistance was confirmed by surface growth after incubation at 35°C for 24 h.

Latex agglutination assay (PBP)

Mastalex ^{TM}MRSA kit (Mast diagnostics, UK), a commercially available *S. aureus* agglutination test kit was used for the latex agglutination test. The MRSA screen test is a slide latex agglutination test based on the reaction of latex particles sensitized with monoclonal antibodies against penicillin binding protein 2a (PBP2a or PBP2') of *S. aureus* and PBP2a (a product of *mec*A gene) extracted from tested colonies (Muhammad et al., 2006). The test kit was used according to manufacturer's instruction. Autoagglutination reactions in the negative control were excluded from calculations.

Storage of the isolates

Using sterile swab, the entire growth of an overnight pure culture was sub-cultured in 5 ml of sterile glycerol broth and immediately stored in freezer [Micro bank (Diagnostic pro-lab)] at -80°C. After 24 h the viability of the organism was checked by thawing the suspension at 35°C and inoculated on blood agar plates.

DNA extraction method

Pure culture of *S. aureus* on agar slant was required for molecular analysis of the isolates. Nonviable and mixed cultures were not processed for the molecular analysis. Of the 423 MRSA isolates detected by latex agglutination technique, 100 isolates were randomly selected and used in the molecular analysis with representative from each of the study area. After overnight culture on brain heart infusion (Difco Laboratories) agar plates, one colony of each sample was resuspended in 25 µl of sterile distilled water and the suspension was then placed in a 100°C heat block for 15 min. From this suspension, a 5 µl volume was directly used as a template for PCR amplification (Bignardi et al., 1996; Perez et al., 2001; Anna-Kaarina et al., 2009).

Oligonucleotide primers

The oligonucleotide primers used in this study have been previously described (Martineauf et al., 1998; Meshref et al., 2011) and were obtained from a commercial source (Inqaba Biotechnical Industries (Pty) Ltd., South Africa). The 3-end region of the *S. aureus* specific gene (chromosomal DNA) was amplified using A 30 nucleotide forward primer 5'- AAT CTT TGT CGG TAC ACG ATA TTC ACG - 3' and A30 nucleotide reverse primer, 5'-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3' (which hybridize to 5-34 and (112-83), respectively (Martineauf et al., 1998), while the 3-end region of the *mecA* gene was amplified using A 22nucleotide forward primer 5'-AAA ATC GAT GGT AAA GGT TGG C - 3' and A22 nucleotide reverse primer, 5'- AGT TCT GCA GTA CCG GAT TTG C-3' (which hybridize to sites 1282-1301 and 1814-1793) (Robert Koch institute, 2003) (Table 1). *S. aureus* specific gene and *mecA* gene have the amplicon size of 107 and 532 bp using primers described by Meshref et al. (2011).

*Mec*A gene detection by polymerase chain reaction

PCR assays were all directly performed from the bacterial suspension obtained after the rapid DNA extraction method earlier described. An aliquot of 5 µl of this suspension was added to 95 µl of PCR mixture consisting of 1× reaction buffer [16 mM $(NH_4)_2SO_4$,

Table 1. Methicillin resistance among *Staphylococcus aureus* isolates using conventional methods.

Parameter	Strains isolated Methods used for detection of MRSA		
	DDM	SAM	PBP
Number of isolates (%)			
Methicillin Resistant *S. aureus* (MRSA)	445 (26.3)	415 (24.2)	423 (25.0)
Methicillin Susceptible *S. aureus* (MSSA)	1251 (73.9)	1283 (75.3)	1269 (75.0)

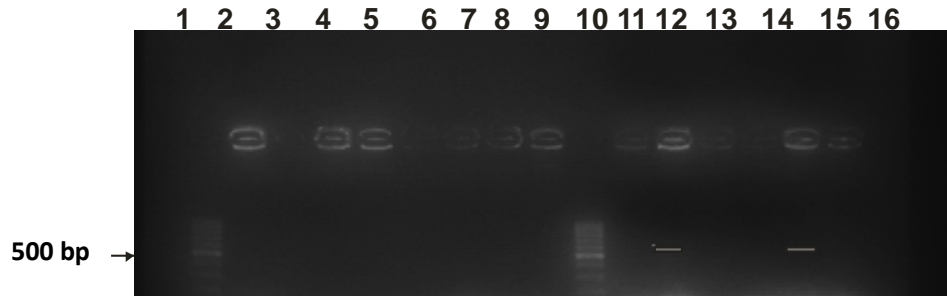$X^2 = 3.14$, df = 4, P = 0.534.

Figure 1. Representative of agarose gel electrophoresis of PCR products. Lanes 12, 14 are positive for *mec*A as indicated by 532 bp PCR product, lane 2 to 9,11,13, 15, are negative for *mec*A, Lane 16: negative control (methicillin susceptible *S. aureus,* ATCC 25923); lane 1,10: molecular weight size marker.

67 mM Tris-HCl (pH 8.8)], a 0.5 mM concentration of each of the four deoxyribonucleoside triphosphates (dATP, dCTP, dGTP, and dTTP) (Inqaba Biotechnical Industries (Pty) Ltd., South Africa), 1.0 µM of each primer, and *mecA* primer, and 1.25 U of The Dream Taq™ Green PCR Master Mix (2×) (Fermentas Life Sciences, supplied by Inqaba Biotechnical Industries (Pty) Ltd., South Africa) is a ready-to-use solution containing Dream Taq™ DNA polymerase, optimized Dream Taq™ Green buffer and 4 mM MgCl₂. For each sample, one reaction was performed with the pair of primers to identify *S. aureus* specific sequence gene and with the *mecA* pairs of primers to detect resistance gene (*mec*A).

In order to reduce the formation of nonspecific extension products, a hot-start PCR protocol was used; the tubes were placed in the thermal cycler when the denaturing temperature was reached. All PCR assays were carried out with a negative control containing all of the reagents without DNA template. DNA amplification was carried out in a Techne PCR system TC-5000 thermocycler (Bibby Scientific Ltd) with the following thermal cycling profile: an initial denaturation step at 94°C for 5 min was followed by 30 cycles of amplification. Each cycle consisted of the following steps; denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s ending with a final extension step at 72°C for 5 min.

After PCR amplification, 5 µl was removed and subjected to agarose gel electrophoresis (1.5% agarose, 1× Tris-borate-EDTA, 100 V, 40 min) to estimate the sizes of the amplification products by comparison with a 100 bp O' GeneRuler™ 100 bp molecular size standard DNA Ladder, ready-to-use designed by Fermentas Life sciences (supplied by Inqaba Biotechnical Industries (Pty) Ltd., South Africa). The gel was stained with ethidium bromide, and the amplicons were visualized using a ultra violet (UV) light box. This protocol, including the rapid DNA extraction method from a single colony and electrophoretic analysis of the amplified products on an agarose gel, was performed in less than 4 h.

Statistical analysis

The statistical package for social sciences (SPSS) for windows version 11.0 was used for statistical analysis and data interpretation. The statistical analysis was done using median, averages, ranges, ± standard deviation, chi square, student' test and Pearson correlation were applied. The p value ≤ 0.05 was considered as "statistically significant".

RESULTS

The prevalence of methicillin resistant among 1692 *S. aureus* isolates in disc diffusion method (DDM) and screen agar method were 26.3 and 24.2%, respectively as shown in Table 1. Four hundred and twenty three *S. aureus* isolates [(were found to be resistant to methicillin by detection of *mec*A product, PBP2a (PBP')] using latex agglutination technique. Ninety five (22.5%) and fifty seven (13.5%) of the 423 MRSA isolates did not grow on oxoid mannitol salt agar (MSA) and mast MSA media without oxacillin, respectively. The difference was statistically significant (P < 0.001).

Out of total 100 isolates of methicillin resistant strains recovered by the conventional techniques, the *mec*A gene was detected from only 5 (5.0%) of the 100 MRSA strains. The size of the amplicon for the mec*A* gene was expected to be 532 base pairs (bp). An amplicon of 532 bp was seen in only 5 of the 100 isolates tested. PCR amplification of *mec*A gene demonstrating amplicon of 532 bp products are given in Figure 1. Table 2 shows the

Table 2. Measurement of specificity and sensitivity of different methods used.

mecA	No. of isolates tested	No. of strains with result indicated					
		PBP		DDM		SAM	
		Pos	Neg	Pos	Neg	Pos	Neg
Positive	5	5	0	5	0	4	0
Negative	95	95	0	95	0	96	0

Pos: Positive; **Neg:** negative.

measurement of specificity and sensitivity of different methods used. Surprisingly, 95 (95.0%) of the 100 isolates did not harbor the *mec*A gene. The *mec*A gene was lost in 95.0% of 100 methicillin-resistant *S. aureus* isolates after 2 years of storage at −80°C with the Micro bank system (Pro-lab Diagnostics, Austin, Tex).

DISCUSSION

The presence of the *mec*A gene is considered the hallmark for identification of MRSA strains and can be difficult to distinguish using phenotypic methods because of the possibility of missing some resistant strains using standard phenotypic susceptibility testing methods due to heterogenous phenomena. This phenomenon was recognized soon after the discovery of MRSA; that in cultures of most strains only a proportion of cells (usually one 10^3 to 10^6) were highly resistant to methicillin while majority expressed resistant level at or near those of susceptible strains such as heterogenecity of resistance characterized of MRSA in contrast to almost all other bacteria (Chambers, 1997; Hiramatsu et al., 2001).

Though *mec*A gene is responsible for phenotypic behaviour of methicillin resistance in this part of the world, it s noteworthy that the loss of *mec*A gene there is methicillin resistant *S. aureus* during storage. This result indicates the acquisition of *mec*A gene is responsible for methicillin resistance; concurring with the previous studies on the role of *mec*A gene in developing high methicillin resistance (Hartman and Tomasz, 1984). In addition to presence of *mec*A gene in MRSA, PBP2a and *ica* gene cluster can also encode resistant in MRSA (Cramton et al., 1999; Memmi et al., 2008). In this study, out of the 100 isolates, 5 (5.0%) of the isolates were confirmed as MRSA by the detection of *mec*A gene. This low level of *mec*A gene detection can be explained due to loss of the *mec*A gene during storage methicillin-resistant *S. aureus* strains at −80°C as studied by Griethuysen et al. (2005) where they found 14.4% MRSA isolates lost *mec*A gene during storage after two years. Loss of the *mec*A gene in such a large percentage of MRSA isolates during storage at −80°C with the Microbank system has been described by Hurlimann et al. (1992) where they described the apparent loss of the *mec*A gene in methicillin-resistant *S. aureus* isolates stored as lyophilized cultures. However, they did not confirm the presence of the *mec*A gene at the time the isolates were stored; therefore, it is not certain that all isolates carried the *mec*A gene to start with (Hurlimann et al., 1992).

Loss of the *mec*A gene has also been observed *in vivo* (Lawrence et al., 1996; Deplano et al., 2000). Katayama et al. (2000) demonstrated that the SCC*mec*, which contains the *mec*A gene, can be integrated to and excised from the *S. aureus* chromosome. However, spontaneous excision of the SCC*mec* did not occur appreciably in the strain that was examined (Katayama et al., 2000). The presence of the *mec*A gene is considered the hallmark for identification of MRSA strains and can be difficult to distinguish using phenotypic methods since heterogenous phenomena may compound the possibility of missing some resistant strains using standard phenotypic susceptibility testing methods. This phenomenon was recognized soon after the discovery of MRSA where cultures of few strains were highly resistant to methicillin while majority expressed resistant level at or near those of susceptible strains such as heterogenecity of resistance which is a characteristic of MRSA in contrast to almost all other bacteria (Hiramatsu, 1995; Chambers, 1997).

Preservation of strains in a microbiology laboratory is of great importance for quality control, teaching, and research (Harbec and Turcotte, 1996). Freezing is a very common method of preservation and storage of microorganisms (Aulet et al., 2001). Studies mainly concentrate on the viability of the microorganisms after a certain storage period. Little attention is given to the influence of storage conditions on characteristics of the stored strain such as antimicrobial susceptibility. The Microbank bacterial preservation system (Pro-lab Diagnostics) is a well-known system for freezer storage of all kinds of microorganisms and is used in laboratories all over the world. One of the issues that remain is whether loss of the *mec*A gene is related to the storage method; this cannot be concluded that the *mec*A gene was lost during storage at −80°C. This study can therefore demonstrate that *mec*A can be lost from MRSA strains stored at −80°C with the Micro bank system. This has important implications for the management of strain collections. Prior to the use of MRSA isolates that have

been previously stored at −80°C in any study, they have to be checked for the presence of the *mec*A gene at that moment in time. Maybe storage of MRSA strains can be improved by altering the storage conditions by, for example, the addition of oxacillin to the cryopreservative. This needs to be evaluated in future studies.

Conclusion

This study demonstrates that *mec*A can be lost from MRSA strains stored at −80°C with the Micro bank system. This finding has important implications for the management of strain collections. On the basis of this finding, attention should also be given to the influence of storage conditions on characteristics of the stored strains such as antimicrobial susceptibility of the microorganisms after a certain storage period.

ACKNOWLEDGMENTS

REFERENCES

Anna-Kaarina J, Sanna Laakso P, Anne sAittakorpi M, Lindfors L, Huopaniemi H, Minna M (2009). Rapid identification of bacterial pathogens using a PCR- and microarray-based assay. BMC Microbiology 9:161.

Aulet de Saab OC, de Castillo MC, de Ruiz Holgado AP, de Nader OM (2001). A comparative study of preservation and storage of Haemophilus influenzae. Mem. Inst. Oswaldo Cruz 96:583-586.

Bignardi GE, Woodford N, Chapman A, Johnson AP, Speller DCE (1996). Detection of the *mec-A* gene and pbenotypic detection of resistance In *Staphylococcus aureus* isolates with border ine or low-level methicillin Resistance. J. Antimicrob. Chemother. 37:53—63.

Chambers HF (1987). Coagulase-negative staphylococci resistant to beta-lactam The study was aimed at determining whether loss of *mec*A gene in methicillin-resistant *Staphylococcus aureus* (MRSA) is related to the storage method.

Deplano A, Tassios PT, Glupczynski Y, Godfroid E, Struelens M (2000). In vivo deletion of the methicillin resistance mec region from the chromosome of Staphylococcus aureus strains. J. Antimicrob. Chemother. 46:617-619.

Griethuysen AV, Loo IV, Belkum AL, Vandenbroucke-Grauls C, Wannet W, Keulen PV, Kluytmans J (2005). Loss of the *mec*A gene during storage of methicillin resistant *Staphylococcus aureus* strains. J. Clin. Microbiol. 43:1361-1365.

Hackbarth CJ, Chambers HF (1993). blaI and blaR1 regulate beta-lactamase and PBP2a production in methicillin-resistant Staphylococcus aureus. Antimicrob. Agents and Chemother. 37:1144–9.

Harbec PS, Turcotte P (1996). Preservation of Neisseria gonorrhoeae at −20°C. J. Clin. Microbiol. 34:1143-1146. Bacteriol. 158:513–516.

Hartman BJ, Tomasz A (1984). Low affinity penicillinbinding protein associated with beta-lactam resistance in *Staphylococcus aureus*. J.

Hiramatsu K (1995). Molecular evolution of MRSA. Microbiol. Immunol. 39:531–543.

Hiramatsu K, Asada K, Suzuki E, Okonogi K, Yokota T (1992). Molecular cloning and nucleotide sequence determination of the regulator region of mecA gene in methicillin-resistant Staphylococcus aureus. *FEBS Letters* 298:133–136.

Hiramatsu K, Cui L, Kuroda M, Ito T (2001). The emergence and evolution of methicillin-resistant *Staphylococcus aureus.* Trends Microbiol. 9:486-493.

Hurlimann-Dalel RL, Ryffel C, Kayser FH, Berger-Bächi B (1992). Survey of the methicillin resistance-associated genes mecA, MecR1-mecI, and femA-femB in clinical isolates of methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 36:2617-2621.

Isenberg HD (1998). Agar screening test to detect oxacillin (methicillin)-resistant *Staphylococcus Spp*. In: Essential procedures for clinical microbiology. ASM Press Washinton DC pp 232-234.

Katayama Y, Ito T, Hiramatsu K (2000). A new class of genetic element, Staphylococcus Cassette Chromosome mec, encodes methicillin resistance in Staphylococcus aureus. Antimicrob. Agents Chemother. 44:1549-1555.

Lawrence C, Cosseron M, Durand P, Costa Y, Leclercq R (1996). Consecutive isolation of homologous strains of methicillin-resistant and methicillin-susceptible Staphylococcus aureus from a hospitalized child. J. Hosp. Infect. 33:49-53.

Martineauf P, Roy PM, Bergeron MG (1998). Species-specific and ubiquitous-DNA based assay for rapid identification of *staphylococcus aureus*. Clin. Microbiol. 36:618-623.

Matsuhashi M, Song MD, Ishino F, Wachi M, Doi M, Inoue M, Ubukata K, Yamashita N, Konno M (1986). Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to beta-lactam antibiotics in Staphylococcus aureus. J. Bacteriol. 167:975–9780.

Meshref AA, Omer MK (2011). Detection of (*mecA*) gene in methicillin resistant *Staphylococcus aureus* (MRSA) at Prince A / Rhman Sidery Hospital, Al- Jouf, Saudi Arabia. J. Med. Genetic Genomics. **3**(3):41–45.

Mohammad R, Mahmood Y, Au F (2006). Comparison of Different Laboratory Methods for Detection of MRSA. Pakistan J. Med. Sci*.* 22(4):442-445.

Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S (1991). Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J. Clin. Microbiol. 29:2240-2244.

Murchan S, Kaufmann ME, Deplano A, de Ryck R, Struelens M, Zinn CE, Fussing V, Salmenlinna S, Vuopio-Varkila J, El Sohl N, Cuny C, Witte W, Tassios PT, Legakis N, van Leeuwen W, van Belkum A, Vindel A, Laconcha I, Garaizar J, Haeggman S, Olsson-Liljequist B, Ransjo U, Coombes G, Cookson B (2003). Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant Staphylococcus aureus: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. J. Clin. Microbiol. 41:1574-1585.

NCCLS (2003)National Committee for Clinical Laboratory Standards, 2003. Performance standard forantimicrobial disk susceptibility tests. Eight edition: Approved standards M2-A8. NCC:S. Wayen P. A. USA.

NCCLS (2008). National Committee for Clinical Laboratory Standards, 2008. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, Wayne, P. A,; NCCLS. 19: M100-S9.

Perez RE, Claverie MF, Villar J, Méndez Á (2001).Multiplex PCR for Simultaneous Identification of *Staphylococcus aureus* and Detection of Methicillin and Mupirocin Resistance. J. Clin. Microbiol. 39(11): 4037-4041.

Pierre J, Williamson R, Bornet M, Gutmann L (1990). Presence of an additional penicillin-binding protein in methicillin-resistant Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, and Staphylococcus simulans with a low affinity for methicillin, cephalothin, and cefamandole. Antimicrob. Agents Chemother. 34:1691–1694.

Sharma VK, Hackbarth CJ, Dickinson TM, Archer GL (1998). Interaction

of native and mutant MecI repressors with sequences that regulate mecA, the gene encoding penicillin binding protein 2a in methicillin-resistant staphylococci. J. Bacteriol. 180:2160–2166.

Shimaoka M, Yoh M, Segawa A, Takarada Y, Yamamoto K, Honda T (1994). Development of enzyme-labeled oligonucleotide probe for detection of mecA gene in methicillin-resistant Staphylococcus aureus. J. Clin. Microbiol. 32:1866–1869.

Suzuki E, Kuwahara-Arai K, Richardson JF, Hiramatsu K (1993). Distribution of mec regulator genes in methicillin-resistant Staphylococcus clinical strains. Antimicrob. Agents Chemother. 37:1219–1226.

Tesch W, Ryffel C, Strässle A, Kayser FH, Berger-Bächi B (1990). Evidence of a novel staphylococcal mec-encoded element (mecR) controlling expression of penicillin-binding protein 2′. Antimicrob. Agents Chemother. 34:1703–1706.

Tokue Y, Shoji S, Satoh K, Watanabe A, Motomiya M (1992). Comparison of a polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 36:6–9.

Ubukata K, Nakagami S, Nitta A, Yamane A, Kawakami S, Sugiura M, Konno M (1992). Rapid detection of the mecA gene in methicillin-resistant staphylococci by enzymatic detection of polymerase chain reaction products. J. Clin. Microbiol. 30:1728–1733.

Unal S, Hoskins J, Flokowitsch JE, Wu CY, Preston DA, Skatrud PL (1992). Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. J. Clin. Microbiol. 30: 1685–1691.

Weller TMA (1999). The distribution of mecA, mecR1 and mecI and sequence analysis of mecI and the mec promoter region in staphylococci expressing resistance to methicillin. J. Antimicrob. Chemother. 43:15–22.

Full Length Research Paper

Prevalence and barriers to the use of insecticide treated nets among pregnant women attending ante-natal clinic at Specialist Hospital Sokoto, Nigeria

Awosan, K. J.[1]*, Ibrahim, M. T. O.[1], Alayande, M. O.[2], Isah, B. A.[1], Yunusa, E.[1] and Mahmud, M. B.[1]

[1]Department of Community Medicine, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.

Malaria is endemic and a major public health problem in Nigeria. In most malaria endemic areas, pregnant women are the main adult group at risk for the severe form of the disease. In the absence of effective vaccine for malaria prevention and development of unacceptable levels of resistance to one drug after another by the malaria parasite, prevention of mosquitoes bite through use of insecticide treated nets (ITNs) remains a very important strategy for malaria control. This study aimed to assess the prevalence and barriers to use of insecticide treated nets among pregnant women in Sokoto. This was a cross sectional descriptive study among 185 randomly selected pregnant women attending antenatal clinic (ANC) at Specialist Hospital Sokoto, in October, 2010. Data collection was done using a set of pretested, semi-structured questionnaires; descriptive statistics was used for analysis. Among the 179 (96.8%) respondents that were aware of ITNs out of the 185 participants, 154 (86.0%) had accurate knowledge of ITNs, 50 (27.6%) use ITNs (but this constitutes 74.6% of the 67 respondents that own an ITN). One hundred and eleven (84.7%) of the 131 respondents who did not sleep under an ITN the night before the survey, gave lack of ITNs as reason for not sleeping under the net. Utilization of ITNs was low despite high knowledge of the commodity among the respondents. Lack of ownership was the major barrier to its utilization. Educational level was statistically significantly associated with ownership and use of ITNs (p < 0.001). Women empowerment (through education and employment) and monitoring of ITNs distribution by the relevant government agencies were suggested as important interventions in improving availability, affordability and use of ITNs.

Key words: Insecticide treated nets, prevalence, barriers, pregnancy.

INTRODUCTION

In most countries of sub-Sahara Africa, malaria is highly endemic and a leading cause of morbidity and mortality (Federal Ministry of Health (FMoH), 2004). Due to repeated exposure to malaria infection (transmitted through the bite of infected female anopheles mosquito), people develop a certain degree of immunity to it during the first decade of life (Brabin, 2000). Despite this immunity, pregnant women, especially primigravidae,

---

*Corresponding author. E-mail: awosankj1@yahoo.com.

have a high susceptibility to *Plasmodium falciparum* infection, manifested by a high prevalence and intensity of parasitaemia (Steketee, 2005).

Every year, at least 30 million pregnancies occur among women in malarious areas of Africa, most of these women reside in areas of relatively stable malaria transmission. Nigeria is the most populous country in Africa, with an estimated population of over 168 million (World Bank, 2012). It therefore has the largest population of persons exposed to malaria infection in sub-Saharan Africa.

In areas of Africa with stable malaria transmission, *P. falciparum* infection during pregnancy is estimated to cause as many as 10,000 maternal deaths each year, 8 to 14% of low birth weight babies, and 3 to 8% of all infant deaths (Guyat and Snow, 2001).

Transmission of malaria disease is very high in Nigeria with an annual incidence of 31,913 per 100,000 population; it is the leading cause of death in children aged under 5 years accounting for 20% of deaths among this group, closely followed by pneumonia and diarrhea diseases that accounted for 17 and 11% of deaths, respectively. Among adults, it is the second leading cause of death with a cause specific mortality rate of 131 deaths per 100,000 population compared to 132 deaths per 100,000 population from human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) (World Health Organization (WHO), 2013). Available record ranks Sokoto State (the study area) among those with very high morbidity and mortality from malaria in Nigeria in 2006 (National Bureau of Statistics (NBS), 2006).

In the absence of effective vaccine for malaria prevention and development of unacceptable levels of resistance to one drug after another by the malaria parasite, coupled with the development of resistance to insecticides by mosquitoes that transmit the disease; prevention of mosquitoes bite through the use of insecticide treated nets remains a very important strategy for malaria control (Lengeler and Snow, 2000; Ter Kuile et al., 2003). Insecticide treated nets (ITNs) have been shown to reduce severe disease and mortality due to malaria in endemic regions and reduce all cause mortality by about 20% (Centre for Disease Control (CDC), 2004).

Current use of malaria preventive measures during pregnancy in Nigeria was found to be low despite the burden of the disease in the country (National Population Commission (NPC) and ICF Macro, 2009). Review on community acceptance of bed nets in other places has shown that various factors influence the use of bed nets, including; cultural, behavioral and demographic factors, ethnicity, accessibility, gender relations and seasonality of malaria (Heggenhougen et al., 2003). This study was conducted to determine the prevalence and barriers to the use of ITNs among pregnant women in Sokoto.

MATERIALS AND METHODS

This was a cross sectional descriptive study carried out in Specialist Hospital, Sokoto State, Nigeria in October, 2010. The hospital is a tertiary healthcare facility located in Sokoto metropolis with a bed capacity of 270. The metropolitan city of Sokoto is the capital of Sokoto State; it lies between longitude 05° 11' to 13° 03' East and latitude 13° 00' to 13° 06' North and covers an area of 60.33 square km. The area has an annual mean rainfall ranging between 500 to 1,300 mm. Dry season starts from October and lasts up to May, and wet season begins in May and last up to October every year.

The study population consisted of pregnant women attending antenatal care (ANC) clinic, in the Obstetrics and Gynaecology Department of the hospital. The department runs the ANC clinic thrice a week, seeing an average of 150 patients per day. Fresh attendees presenting for booking are seen on Wednesdays, while those presenting for revisits are seen on Tuesdays and Thursdays.

The sample size was estimated at 185 based on a prevalence of 13% from a previous national survey (NPC and ICF Macro, 2009), precision level of 5% and an anticipated response rate of 95%. The study subjects were randomly selected by systematic sampling technique; this was done after explaining the objectives of the study to them. One in every four pregnant women presenting for revisits at the ANC clinic of the hospital was recruited over a 4 day period (consecutive Tuesdays and Thursdays in two consecutive weeks) until the required sample size of 185 was obtained. A standardized semi-structured, interviewer-administered questionnaire was developed to obtain the required data. The questionnaire sought information on socio-demographic variables, knowledge of difference between an ordinary net and an insecticide treated nets (ITNs), ownership and source of ITNs, use and barriers to use of ITNs and prevalence of malaria attacks. It was reviewed by senior colleagues in the Department of Community Medicine and Department of Veterinary Parasitology and Entomology of the Usmanu Danfodiyo University, Sokoto. The necessary correction was made based on their inputs to ascertain content validity. The questionnaire was pre-tested in a pilot study among 14 pregnant women presenting for ANC revisit at the maternal and child health unit of Primary Healthcare Centre, Yar- Akija, Sokoto in September, 2010. It was re-administered after a 2 week interval to 10 of the 14 respondents that complied with their follow-up appointment. The instrument shows good internal consistency (Cronbach's alpha = 0.83) and test-retest correlation coefficient was 0.79. Five student midwives assisted in questionnaire administration after pre-training on conduct of survey research, the objectives of the study, selection of study subjects and questionnaire administration. Ethical permission to carry out the study was obtained from the management of the hospital, and informed written consent was also obtained from the study subjects before questionnaire administration.

Data was analyzed using the statistical package for social sciences (SPSS) version 17 computer statistical software package. Frequency distribution tables were constructed; cross tabulations were done to examine relationship between categorical variables. The Chi-square test was used to compare differences between proportions; statistical significance was set at $p < 0.05$.

RESULTS

The study subjects comprised of 185 pregnant women between the ages of 18 and 45 years (Mean = 29.9; standard deviation (SD) = 5.75). Ninety four (50.8%) of

Table 1. Socio-demographic profile of respondents

Socio-demographic profile	Frequency (%)
Age groups (in years)	
18-25	42 (22.7)
26-33	94 (50.8)
34-41	47 (25.4)
42 and above	2 (1.1)
Marital status	
Married	180 (98.4)
Divorced	1 (0.5)
Widowed	2 (1.1)
Tribe	
Hausa/Fulani	140 (77.8)
Igbo	18 (10.1)
Yoruba	20 (11.1)
Other tribes	2 (1.1)
Education	
None	14 (7.6)
Qurranic only	37 (20.0)
Primary	22 (11.9)
Secondary	69 (37.3)
Tertiary	43 (23.2)
Religion	
Christianity	29 (15.7)
Islam	156 (84.3)
Occupation	
Housewife	124 (67.0)
Petty trader	17 (9.2)
Businesswoman	17 (9.2)
Civil servant	22 (11.9)
Professional	3 (1.6)

the 185 respondents were in the 26 to 33 years age group, 180 (98.4%) were married, 140 (77.8%) were Hausa/Fulani, 69 (37.3%) had secondary education, 43 (23.2%) had tertiary education, 156 (84.3%) were Moslems and 124 (67.0%) were housewives (Table 1).

Table 2 shows the distribution of respondents' knowledge of ITNs, ownership and use of ITNs by level of education. One hundred and seventy nine (96.8%) of the 185 respondents have ever heard of ITNs. Among the 179 respondents that were aware of ITNs, 124 (69.3%) reported health workers, 28 (15.6%) reported radio/television, 24 (13.4%) reported friends/relatives, 2

(1.1%) reported newspaper/magazines and 1 (0.6%) reported church/mosque as their source of information on ITNs. One hundred and fifty four (86.0%) of the 179 respondents that were aware of ITNs had accurate knowledge that ITNs differ from ordinary nets by containing chemicals that kill mosquitoes in addition to preventing mosquito bite. There was statistically significant association between educational level of respondents and their knowledge of difference between an ordinary net and an ITN, $x^2 = 120.481$, p < 0.001.

While 126 (68.1%) of the 185 respondents own a bed net (either ordinary or insecticide treated), only 67 (36.2%) own an insecticide treated net. Ownership of ITNs increased statistically significantly from 7.7% among those with no education, to 13.5% among those with qurranic education, 30% among those with primary education, 37.7% among those with secondary education, and 69.1% among those with tertiary education, $x^2 = 31.138$, p < 0.001. Thirty four (50.7%) of the 67 respondents that had ITNs bought them at the market, 17 (25.4%) obtained their nets free at the ANC clinic, 8 (11.9%) bought their nets at the hospital, 5 (7.5%) obtained their nets as donation by Non-Governmental Organizations (NGOs) and 3 (4.5%) obtained their nets as donation by friends/relatives.

One hundred (54.1%) of the 185 respondents slept under a bed-net (either ordinary or an ITN) the night before the survey; only 181 of the 185 participants responded to the question on use of ITN the night before the survey. Fifty (27.6%) of the 181 participants that responded to the question on use of ITNs slept under an ITN the night before the survey (but this constitutes 74.6% of the 67 respondents that own an ITN). Among the 50 respondents that slept under an ITN the night before the survey, 25 (50.0%) had tertiary education, 20 (40.0%) had secondary education, while the remaining 5 (10%) had primary education and below. The proportion of respondents that slept under ITNs rose statistically significantly from 0% among those with no education, to 5.4% among those with qurranic education, 15.0% among those with primary education, 29.0% among those with secondary education, and 59.5% among those with tertiary education, $x^2 = 37.133$, p < 0.001.

One hundred and thirty one of the remaining 135 participants that did not report sleeping under an ITN the night before the survey responded to the question on reasons for not sleeping under it. Of the 131 respondents who gave reasons for not sleeping under an ITN the night before the survey, 111 (84.7%) reported lack of ITNs, 10 (7.6%) and 2 (1.5%) reported excessive heat and seasonality of malaria, respectively as reasons for not sleeping under the net among other reasons (Table 3).

Only 36 (19.5%) of the 185 respondents reported no attack of malaria fever in the past 1 year, 74 (40%) reported one or two attacks of malaria fever, while 75

Table 2. Distribution of respondents' occupation, knowledge of ITNs, ownership and use of ITNs by level of education.

Variable	Educational level				
	None **No. (%)**	**Qurranic only** **No. (%)**	**Primary** **No. (%)**	**Secondary** **No. (%)**	**Tertiary** **No. (%)**
Knowledge of ITN					
Accurate	1 (9.1)	25 (73.5)	16 (71.4)	69 (100.0)	43 (100.0)
Not accurate	10 (90.9)	9 (26.5)	6 (28.6)	0 (0)	0 (0)
N = 179, x^2 = 37.133, p < 0.001					
Own an ITN					
Yes	1 (7.7)	5 (13.5)	6 (30.0)	26 (37.7)	29 (69.1)
No	12 (92.3)	32 (86.5)	14 (70.4)	43 (62.3)	14 (30.9)
N = 181, x^2 = 31.138, p < 0.001					
Slept under an ITN					
Yes	0 (0)	2 (5.4)	3 (15.0)	20 (29.0)	25 (59.5)
No	13 (100.0)	37 (94.6)	17 (85.0)	49 (71.0)	17 (40.5)
N = 181, x^2 = 37.133, p < 0.001					

Table 3. Reasons for not sleeping under an insecticide treated net (ITN)

Reasons for not sleeping under an ITN (N=131)	Frequency (%)
Excessive heat at night	10 (7.6)
Causes suffocation at night	3 (2.3)
Prevent free movement on the bed	5 (3.8)
Do not have ITN	111 (84.7)
Malaria fever is only seasonal	2 (1.5)

Table 4. Distribution of number of malaria attacks in the past 1 year by use of ITN.

No of malaria attacks in the past 1 year	Used an insecticide treated net	
	Yes frequency (%)	**No frequency (%)**
None	13 (26.0)	23 (17.6)
One to two	25 (50.0)	48 (36.7)
Three and above	12 (24.0)	60 (45.8)

x^2 = 12.178, p = 0.016.

(40.5%) reported three or more attacks of malaria fever in the past 1 year. Among the 181 participants that responded to the question on use of ITN, prevalence of three or more attacks of malaria fever in the past 1 year was 45.8% among those that did not use an ITN compared to 24.0% among those that used an ITN, and the difference was statistically significant, x^2 = 12.178, p = 0.016 (Table 4). Educational level (secondary/tertiary education) was the only variable that predicted no attack or only one attack of malaria fever in the past I year (OR = 6.349, p < 0.001, 95% CI = 0.594 to 0.313).

DISCUSSION

High level of awareness (96.8%) and knowledge of the

importance of ITNs in killing mosquitoes and preventing mosquito bites by majority (86.0%) of the respondents was demonstrated in this study. A statistically significant association between educational level of respondents and knowledge of ITNs was also observed (x^2 = 120.481, p < 0.001). This is contrary to the findings in a study in Ogun State, Western Nigeria that reported poor awareness (48.9%) and knowledge (31.2%) of the importance of ITNs among pregnant mothers. However, similar to the findings in this study, it also reported a statistically significant association between educational level and knowledge of ITNs (Runsewe-Abiodun et al., 2012).

A low prevalence (27.6%) of ITNs use was reported by the respondents in this study, despite the high level of awareness and knowledge of the commodity. Contrary to the findings in this study, a study in Owerri, Nigeria reported high awareness and usage of ITNs among pregnant women (Iwu et al., 2010). The major barrier to ITNs use was non-availability as only 36.2% of the respondents in this study own an ITN. However, use of ITNs was high (74.6%) among the respondents that own an ITN. A statistically significant association between educational level and ownership (x^2 = 31.138, p < 0.001) as well as use of ITNs (x^2 = 37.133, p < 0.001) was recorded in this study.

While ITNs ownership (36.2%) in this study was lower than that reported in a study among pregnant women by Belay and Deressa (2008) who reported ITNs ownership of 59.0%, use of ITNs among the respondents that own ITNs in this study (74.6%) was higher than the ITNs usage of 52.1% reported by Deressa et al. (2011). Majority 34 (50.7%) of the respondents that own ITNs in this study bought their nets at the market, 8 (11.9%) bought their nets at the hospital, while 17 (25.4%) got theirs free at the antenatal clinic. This is in contrast to the findings in the study by Njoroge et al. (2009) that reported free/subsidized supply by government as the main source of ITNs by 54.6% of the respondents, 24.6% purchased theirs from the health facility, and none of the respondents bought theirs at the market. This highlights the probability of diversion of the ITNs from the health facilities to the market in the study area.

Conclusion

Utilization of ITNs was low despite high knowledge of the commodity among the respondents. Lack of ownership was the major barrier to its utilization (as utilization of ITN was high among the few that have it). Educational level was a major determinant of ownership and utilization of ITN; this could be due to its strong association with occupation and by extension purchasing power (as most

of the respondents bought their ITNs at the market). Women empowerment through education and employment should be promoted to enable them access and use the commodity. Distribution of ITNs should be monitored by the relevant government agencies to make the commodity available in the health facilities and prevent illegal diversion.

REFERENCES

Belay M, Deressa W (2008). Use of insecticide treated nets by pregnant women and associated factors in a predominantly rural population in northern Ethiopia. Trop. Med. Int. Health 13(10):1303-1313.
Brabin BJ (2000). An analysis of malaria in pregnancy in Africa. Bull. World Health Org. 61:1005-1016.
CDC (2004). Malaria during pregnancy. Department of Health and Human Services, US Centers for Disease Control and Prevention. Atlanta.
Deressa W, Fentie G, Girma S, Reithinger R (2011). Ownership and use of insecticide treated nets in Oromia and Amhara regional states of Ethiopia two years after a nationwide campaign. Trop. Med. Int. Hlth. 16(12):1552–1561.
FMoH (2004). Annual Report of Malaria Control Programme in Nigeria. Federal Ministry of Health. Abuja.
Guyatt HL, Snow RW (2001). Malaria in pregnancy as an indirect cause of infant mortality in sub-saharan Africa. Trans. Royal Soc. Trop. Med. Hyg. 95:569-576.
Heggenhougen HK, Hackenthal V, Vivek P (2003). Bednets usage and its acceptance at the local level. "In" The behaviour and social aspect of malaria control: an introduction and annotated biography. Geneva: Special Programme for Research and training in Tropical Diseases (TDR). World Health Org. 3(1):97-106.
Iwu RU, Ijioma BC, Egeruoh AS, Awurum IN, Ohalete CN (2010). Awareness and use of insecticide treated net among pregnant women attending antenatal clinic at Federal Medical Centre and General Hospital Owerri, Imo State. Report and Opinion, 2(12). Available at: http://www.sciencepub.net/report
Lengeler C, Snow RW (2000). From efficacy to effectiveness: insecticides treated bed nets in Africa. Bull. World Health Org. 74:325-332.
NBS (2006). Annual abstracts of statistics. National Bureau of Statistics, Abuja.
Njoroge FK, Kumani VN, Ongore D, Akwale WS (2009). Use of insecticide treated bednets among pregnant women in Kilifi District, Kenya. East Afr. Med. J. 86(7):314-22.
NPC, ICF Macro (2009). Nigeria Demographic and Health Survey 2008. Abuja, Nigeria: National Population Commission and ICF Macro.
Runsewe – Abiodun T, Iyabo IA, Christy SA (2012). Awareness and knowledge about Insecticide Treated Nets (ITNs) amongst pregnant mothers in Ogun State, Western – Nigeria: a descriptive cross sectional study. Edu. Res. J. 2(5):138–145.
Steketee RW (2005). Recent findings in perinatal malaria. Bull. Int. Paed. Assoc. 20:418-33.
Ter Kuile FO, Terlouw DJ, Phillips-Howard PA, Hawley WA, Friedman JF, Kariuki SK, Shi YP, Kolczak MS, Lal AA, Vulule JM, Nahlen BL (2003). Reduction of malaria during pregnancy by permethrin-treated bed nets in an area of intense perennial malaria transmission in western Kenya. Am. J. Trop. Med. Hyg. 68(4):50-60.
World Bank (2012). Nigeria, Country at a glance. The World Bank Group. Available at: http://www.worldbank.org/en/country/nigeria
WHO (2013). World Health Statistics 2013. World Health Organization, Geneva, Switzerland. Available at: http://www.who.int/gho/publications/world_health.

Short Communication

Prevalence of motorcycle helmet use in Sri Lanka: An observational study

Samath D. Dharmaratne[1,2,3], Achala Upendra Jayatilleke[3,4]*, Asanka N. Abeyrathna[1], Inoka D. M. Mabharana[1] and K. G. B. Kumbukgolle[1]

[1]Department of Community Medicine, Faculty of Medicine, University of Peradeniya, Peradeniya, 20400 Sri Lanka.
[2]Institute for Health Metrics and Evaluation, Department of Global Health, School of Public Health, University of Washington, USA.
[3]Institute of Violence and Injury Prevention, 25 1/2 -9B, Tower Building, Station Road, Colombo 04, 00400 Sri Lanka.
[4]Postgraduate Institute of Medicine, University of Colombo, 160, Prof. Nandadasa Kodagoda Mawatha, Colombo 07, 00700 Sri Lanka.

In Sri Lanka, helmet use is mandatory by law. This study was conducted to determine the prevalence of motorcycle helmet use in Sri Lanka. An observational study was conducted in four locations: two locations entering and leaving the city of Kandy, a rural area in the Kandy district, and the high way (A1) leading to Colombo from Kandy. All the motorcyclists that passed the observation locations were observed. Of all the 1254 motorcycle users, 1140 subjects used helmets (90.1%), as follows: 863 'riders' (97.7%), 275 'pillion riders' (second passengers) (84.1%), two 'third passengers' (5.4%) and none of the 'fourth passengers'(0.0%). Out of 106 observed child passengers, only 25 (23.5%) wore helmets. The significant finding of this study was that majority of adult motorcycle users used helmets while majority of children did not. Motorcycle helmet laws need to be strictly implemented against non-use of helmets by children in Sri Lanka. A further qualitative study is needed to examine the reasons for non-use of helmets among children.

Key words: Helmet, motor cycles, motor vehicle crashes, Sri Lanka, vulnerable road users.

INTRODUCTION

Vulnerable road users (pedestrians, motorcyclists and cyclists) make up almost three quarters of road traffic deaths in the South East Asia region (WHO, 2008). In Sri Lanka, 51% of the total vehicles involved in road traffic collisions (RTCs) are motorcycles (WHO, 2009). The number and the percentage of motorcycles, out of the total vehicle population, have increased from 834,586 (48%) in the year 2000 to 1,422,140 (56%) by the year 2005, which is a 70% increase (Department of Motor Traffic, 2012). Consequent to this increase, Sri Lanka is facing the problem of rapidly rising motorized two wheeler crashes. It is estimated that 35% of fatal RTCs in

Colombo, in 2002 occurred from motorized two-wheeler crashes (Sri Lanka Police, 2012).

A large proportion of deaths and severe injuries due to motorcycle crashes results from injury to the head (WHO, 2006; Servadei et al., 2003; Nixon et al., 1987; Norvel and Cummings, 2002). Usage of standard helmets is an effective way of preventing head injuries (WHO, 2009). It has been revealed that motorized two-wheeler users without wearing helmets are three times more likely to sustain head injuries than those who are wearing helmets (Norvel and Cummings, 2002). The motorcycle helmet use reduces the likelihood of crash fatality by 40% and is

Table 1. Distribution of helmet use.

Helmet use	Rider (%)	Pillion rider (%)	Third passenger (%)	Forth passenger (%)	Total
Helmet used	863 (97.7)	275 (84.1)	2 (5.4)	0 (0.0)	1140 (100.0)
Without helmets	20 (2.3)	52 (15.9)	35 (94.6)	7 (100.0)	114 (100.0)
Total	883 (100.0)	327 (100.0)	37 (100.0)	7 (100.0)	1254 (100.0)

Table 2. Distribution of helmet use among pillion riders.

User	Sex	Helmet (+)	Helmet (-)	Total
Adults	Male	112 (96.5)	4 (3.5)	116 (100)
	Female	141 (94.0)	9 (6.0)	150 (100)
Children	-	42 (68.9)	19 (31.1)	61 (100)

Table 3. Distribution of helmet use by area.

Helmet use	Urban	Rural	A1 High way	Total
Helmet+	334(88.6)	46 (80.7)	760 (92.7)	1140 (90.9)
Helmet-	43 (11.4)	11 (19.3)	60 (7.3)	114 (9.1)
Total	377 (100.0)	57 (100.0)	820 (100.0)	1254 (100.0)

70% effective in preventing severe head injuries (Liu et al., 2008).

Mandatory helmet laws reduce head injuries among motorcyclists significantly (Pitaktong et al., 2004). Motorcycle helmet wearing rates can be increased up to 90% when helmet laws are enforced effectively (Kraus, 1995). In Sri Lanka, both the drivers and the passengers of motorized two-wheelers are required by law to wear helmets except children in school uniform. A WHO survey has placed Sri Lanka at point seven in a 0 to 10 scale of mandatory helmet law enforcement (WHO, 2009). However, to date, there is no information on the prevalence of helmet wearing among motorcycle users in Sri Lanka. Therefore, the aim of the present study was to determine the prevalence of motorcycle helmet use in Kandy, Sri Lanka.

MATERIALS AND METHODS

Study design

This is an observational study conducted in Kandy, Sri Lanka in March, 2009. This study was conducted in three locations; Kandy city, a rural area in the Kandy district and the high way that connects Kandy to country capital, Colombo (A1 high way). Ethical approval for the study was obtained from the Ethical Review Committee, Faculty of Medicine, University of Peradeniya.

Data collection

In March 2009, all the motorcycles entering and leaving the city of

Kandy were observed in two locations for an hour from 9 March (Monday) to 13 March (Friday). The two locations were: the main entrance to the Kandy city and a randomly selected rural area in Kandy (Katugastota). All the motorcycles were observed entering and leaving that area for an hour during the five days of the week mentioned previously. Then, we travelled 110 km along the main highway from Kandy to Colombo (A1 highway), which was a 4 h journey, and observed all the motorcyclists who travelled on A1 for the use of helmets. This journey covered the seven main cities other than Colombo and Kandy.

All the authors except the second author were involved in data collection. The data on helmet use of the motorcycle users (rider, pillion rider and other passengers) were collected along with their age (whether adult or child) and sex on a structured data sheet by the first author. Those who were apparently less than 12 years were categorized as children and whether the helmets met the minimum apparent standards was also ascertained. Data was entered into a Microsoft Excel data sheet and was analyzed using Statistical Package for Social Sciences (SPSS) statistical software.

RESULTS

In this study, 883 motorcycles and 1,254 users were observed. Out of the 1,254 users, 1,140 (90.9%) used helmets. Except for 6 (0.5%), all the others used standard helmets. Out of the 883 riders, 863 (97.7%) used helmets. Table 1 depicts the helmet wearing rates among the motorcycle riders and passengers.

Of the 883 observed motorcycles, 74 (8.6%) carried a third passenger and 14 (1.6%) carried a fourth passenger as well. All the 3rd and the 4th passengers were children. Among all the 1,254 passengers, 106 (8.5%) were children; of them, only 25 (23.5%) were wearing a helmet at the time of observation. Table 2 shows the helmet wearing rates of motorcycle passengers. Out of 883 riders, only 2 (0.2%) were females. Those two female riders were wearing a helmet at the time of observation.

Table 3 shows helmet use among motorcyclists according to the area. The helmet use was 88.6, 80.8, and 92.7% in Kandy city (urban area), rural area, and the highway, respectively. Helmet use was more prevalent when the users were on the highways.

DISCUSSION

Majority of the riders (97.7%) used helmets, which is very close to helmet wearing rates of United States of America which is 99% (Kraus et al., 1995). Several other high-income, low- and middle-income countries have achieved

helmet wearing rates over 90% upon enforcement of helmet laws (WHO, 2006).

Most significant finding of this study was that, 76.5% of the children were not using helmets indicating the unprotected nature of child passengers as compared to adults. An important reason for low helmet use among children may be because they were exempted from the mandatory helmet law when they are in school uniform (WHO, 2009). This is not scientifically acceptable and the policy makers need to amend the mandatory helmet law including children of all ages and children in school uniforms. More exploratory studies are needed to examine the reasons for low child helmet wearing rates.

The prevalence of helmet use in the rural area was lower than the urban area and on the A1 high way, which could be due to the absence of traffic police officers in rural areas (Fernando, 2012). Police stations in rural areas might be under staffed and having traffic police officers frequently on the road might not be practically possible. Policy makers should consider placing more traffic police officers on the rural roads to improve helmet use on rural roads.

It is a disturbing incidental finding that 8.6% of motorcycles were carrying a third passenger and 1.4% carrying a fourth passenger. Also, the usage of helmet was low when it comes to other riders e.g. pillion rider, 3rd and 4th passengers. In fact, none of the 4th passengers wore helmets at the time of observation. Although, only two people are permitted to ride on a motorcycle, police does not enforce this law in Sri Lanka especially if the third or the fourth passenger is a child. This might place the 3rd and the 4th passengers at an additional risk. Hence, police need to be advised and sensitized on this issue and requested to strictly enforce the mandatory helmet use law and the laws on number of passengers to travel on motorcycles.

A recently published multicenter study revealed that the widespread use of non-standard helmets in low- and middle-income countries may limit the potential gain of the motorcycle helmet use programs (Ackaah et al., 2012). The present study suggests that most helmet users in Sri Lanka apparently use standard helmets. Though, that is an encouraging finding, one limitation of this study is that only the helmet was observed, and did not examine them. Hence, this finding needs to be confirmed by future research that should physically examine the helmets to see whether they meet the required standards.

Another limitation of the study is that the age of child riders by observation were assessed. Hence, our estimations of the age of those children might not be accurate in some instances. Similarly, because this was an observational study, the sex of the motorcycle users could be inaccurate in some instances (WHO, 2006).

However, the strength of this study was its possible generalizability to Sri Lanka. This study was conducted in Kandy, Sri Lanka. Kandy is one of the 24 districts in Sri Lanka and has several similar characteristics and road conditions to the other 23 districts. Also, the A1 highway that runs through the four districts was covered. Hence, our findings might be generalized to Sri Lanka.
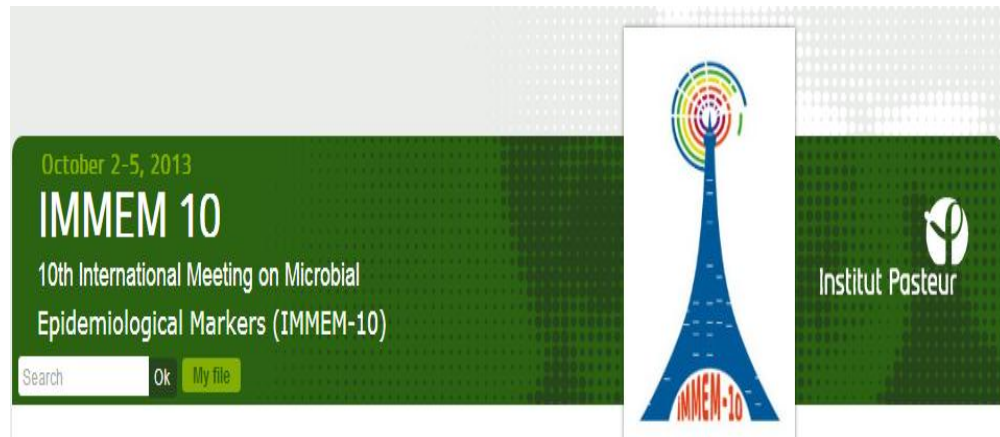
In conclusion, the prevalence of helmet use by children motor cycle riders in Sri Lanka is not satisfactory. Hence, the policy makers should amend the mandatory helmet law including children of all ages and children in school uniforms. Police should strictly enforce helmet laws for all the people riding motorcycle, including the pillion riders irrespective of their age. Third and fourth passengers riding on motorcycles should be stopped. Future studies are required to examine in detail helmet usage in Sri Lanka. In order to produce more reliable results, further qualitative studies on helmet use in Sri Lanka were recommended. These studies need to examine the ages of children who ride motorcycles, the reason for non-use of helmets and the physical quality of the used helmets.

REFERENCES

Ackaah W, Afukaar F, Agyemang W, Thuy Anh T, Hejar AR, Abdul G, Gururaj G, Elisa HS, Martha H, Hyder AA, Inclán-Valadez C, Kulanthayan S, Norton R, Odero W, Owoaje ET, Peden M, Rajam K, Abdul Razzak J, Oluwafunmilola Sangowawa A, Shah J, Le Tuan P, Umar Rs R, Thi Van Anh N, Van der Putten M, Vajanapoom N, Vichit-Vadakan N, Yellappan K, Yu J (2012). The use of non-standard motorcycle helmets in low- and middle-income countries: a multicenter study. Inj. Prev. 0:1-6. Doi:10.1136/injuryprev-2012-040348.

Department of Motor Traffic Sri Lanka (2012). Total vehicle population 2000-2006 [online]. Available at: http://www.motortraffic.gov.lk/statistics.php. Accessed January 10.

Fernando M (2012). Traffic laws alone can't prevent fatal accidents. Sunday Observer. April 8 http://www.sundayobserver.lk/2012/04/08/sec04.asp. Accessed August 25, 2012.

Kraus JF, Peek C, Williams A (1995). Compliance with the 1992 California motorcycle helmet use law. Am. J. Public Health 85:96-99.

Liu BC, Ivers R, Norton R, Boufous S, Blows S, Lo SK (2008). Helmets for preventing injury in motorcycle riders. Cochrane Database of Systematic Rev. Issue 1. Art. No.: CD004333. DOI: 10.1002/14651858.CD004333.pub3.

Nixon J, Clacher R, Pearn, Corcoran (1987). Bicycle accidents in childhood. BMJ. 294:1267-1269.

Norvel DC, Cummings P (2002). Association of Helmet use with death in motorcycle crashes: A matched –pair cohort study.156:483-487.

Pitaktong U, Manopaiboon C, Kilmarx PH, Jeeyapant S, Jenkins R, Tappero J, Uthaivoravit W, van Griensven F (2004). Motorcycle helmet use and related risk behaviors among adolescents and young adults in Northern Thailand. Southeast Asian J. Trop. Med. Public Health. 35:232-241.

Servadei F, Begliomini C, Gardini E, Giustini M, Taggi F, Kraus J (2003). Effect of Italy's motorcycle helmet law on traumatic brain injuries. Inj. Prev. 9:257-260.

Sri Lanka Police Service (2012). Statistics on road traffic accident reported [online]. Available at: http://www.police.lk /divisions/trafficnews.asp. Accessed January 10, 2012.

World Health Organization (2008). Global status report on road safety – Time for action. Geneva, WHO.

World Health Organization (2006). Helmets: A road safety manual for decision makers and practitioners. Geneva, WHO.

World Health Organization (2009). Regional Office for South-East Asia. Regional report on status of road safety: the South-East Asia Region. India, WHO.

UPCOMING CONFERENCES

10th International Meeting on Microbial Epidemiological Markers (IMMEM-10), Paris, France, 2 Oct 2013

7th International Conference on Health Informatics, Angers, France, 3 Mar 2014

Conferences and Advert

January 2014

International Conference on Biological, Health and Environmental Sciences, London, UK, 19 Jan 2014

March 2014

7th International Conference on Health Informatics, Angers, France, 3 Mar 2014

International Conference on Developmental Origins of Adiposity and Long-Term Health, Munich, Germany, 13 Mar 2014

April 2014

Conference on Environmental Pollution and Public Health, Shanghai, China, 12 Apr 2014

Journal of
Public Health and Epidemiology

Related Journals Published by Academic Journals

Journal of Diabetes and Endocrinology
Journal of Medical Genetics and Genomics
Journal of Medical Laboratory and Diagnosis
Journal of Physiology and Pathophysiology
Medical Practice and Reviews
Research in Pharmaceutical Biotechnology