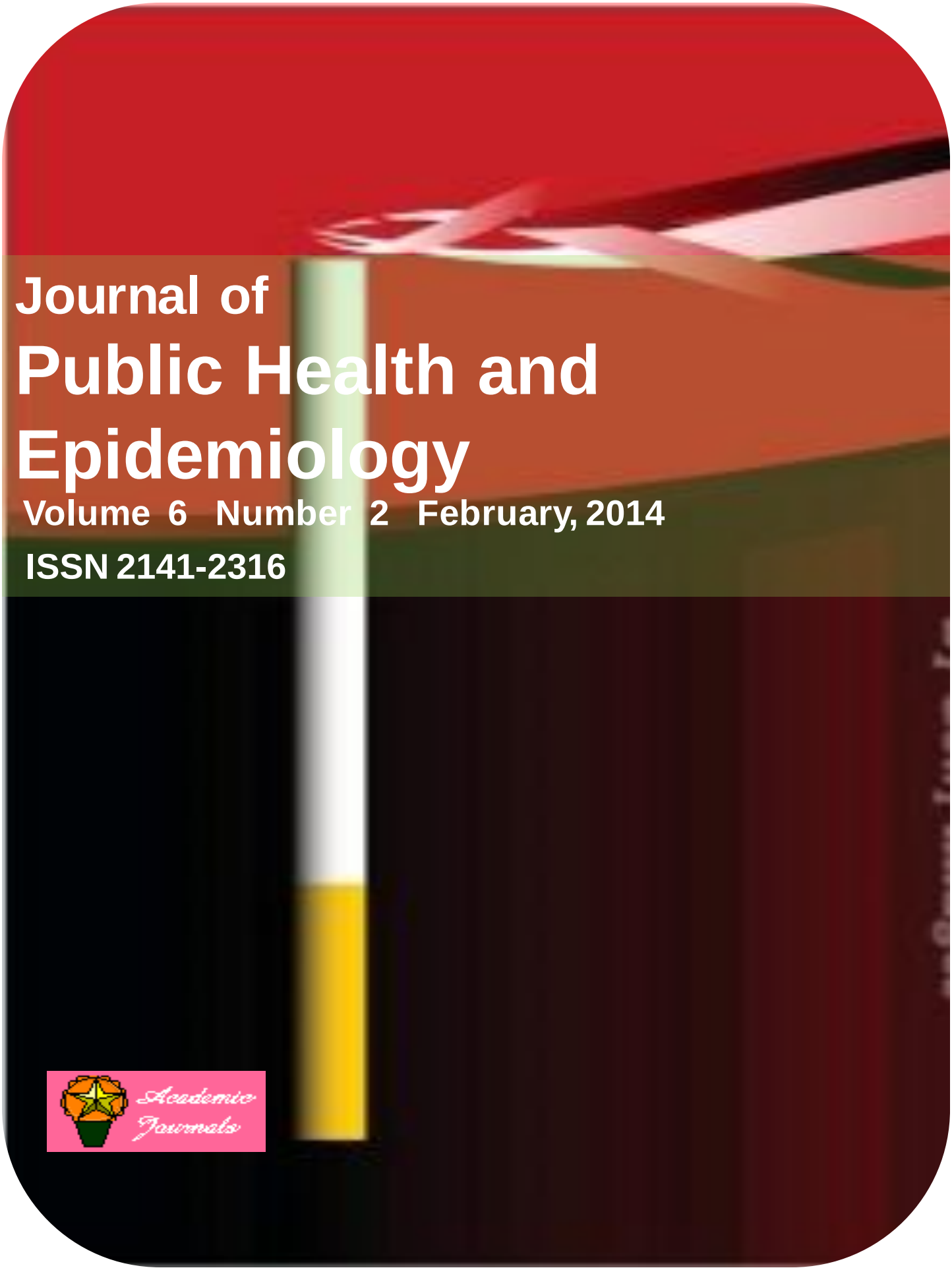

Journal of Public Health and Epidemiology

Volume 6 Number 2 February, 2014

ISSN 2141-2316

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

Editorial Board

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.

Fees and Charges: Authors are required to pay a $650 handling fee. Publication of an article in the Journal of Public Health and Epidemiology is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Journal of Public Health and Epidemiology

Table of Content: Volume 6 Number 2 February 2014

ARTICLES

Research Articles

academicJournals

Vol. 6(2), pp. 70-75, February 2014
DOI: 10.5897/JPHE2013.0606
ISSN 2006-9723 ©2014 Academic Journals
http://www.academicjournals.org/JPHE

Journal of Public Health and Epidemiology

Full Length Research Paper

Absence of vancomycin-resistance genes in *Staphylococcus aureus* isolated from potable water

Hussein H. Abulreesh

Department of Biology, Faculty of Applied Science, Umm Al-Qura University,
P. O. Box 7388, Makkah 21955, Saudi Arabia.

Accepted 20 January, 2014

The aim of this study was to investigate the carriage of vancomycin-resistance genes by *Staphylococcus aureus* found in drinking water supplies. A total of 100 samples (potable water and faucet swabs) were analyzed for the presence of *S. aureus* and their carriage of vancomycin-resistance genes. Mannitol salt agar was used for the isolation of staphylococci, and confirmation of *S. aureus* was carried out by means of Gram staining technique, growth on blood agar, production of catalase and coagulase. Antibiotic susceptibility testing was performed by disk diffusion method, and polymerase chain reaction (PCR) was used to investigate the presence of vancomycin-resistance genes (*vanA*, *vanB*, *vanC*, *vanS*, *vanY*). Staphylococci were recovered from 25% of water samples (n = 75), of which seven samples were positive for *S. aureus*, on the other hand 76% (n = 25) of the faucet swabs yielded typical staphylococcal colonies, with 53% being positive for *S. aureus*. The disk diffusion method showed that all *S. aureus* resistance to penicillin G, and about 66.6% (n = 51 isolates) were resistance to oxacillin. Of all 51 *S. aureus* isolates, only 14% were resistant to vancomycin by disk diffusion method, however, the DNA extracted from all confirmed *S. aureus* did not yield any PCR products with all the primers used for detecting vancomycin-resistance genes. Vancomycin-resistant *S. aureus* remains rare. The disk diffusion method may give false-resistance with vancomycin, therefore, caution is required with the investigation and interpretation of vancomycin susceptibility testing by agar diffusion method.

Key words: *Staphylococcus aureus*, vancomycin-resistance genes, water, biofilm.

INTRODUCTION

Staphylococcus aureus remains an important pathogen that is associated with various hospital-and community-acquired infections, and has been generally regarded as a public health problem (Casey et al., 2007). *S. aureus* is recognized as an important agent of food poisoning. This form of infection results after the ingestion of one or more performed staphylococcal enterotoxins (SEs) on food that has been previously contaminated with the bacteria (Seo and Bohach, 2007).

Multidrug-resistant *S. aureus* is wide spread in the environment and has been recovered from foodstuffs, nasal mucosa and skin of healthy humans, clinical cases, food-producing animals, food-catering and aquatic environments (Acco et al., 2003; Normanno et al., 2007; Kluytmans, 2010; Abulreeh and Organji, 2011), this may highlight the potential public health problems associated with the ubiquity of antibiotic-resistant *S. aureus* in the environment.

Staphylococci in potable water may be regarded as a natural flora or one of the genera that are commonly found in water supplies known as heterotrophic plate count (HPC) bacteria (Allen et al., 2004). Despite the suggestion that it is not possible to establish health-based standards for the presence of HPC bacteria in

E-mail: hhabulreesh@uqu.edu.sa. Tel: +966-555519597.

Table 1. Properties and sequences of the primers for vancomycin-resistance genes.

Primer pair	Sequence	Annealing temperature (°C)	Product size
vanA/*vanA*1	5'-ATGAATAGAATAAAAGTTGCAATAC 5'-CCCCTTTAACGCTAATACGAT	62	1029
vanB/*vanB*1	5'-CCCGAATTTCAAATGATTGAAAA 5'-CGCCATCCTCCTGCAAAA	59	457
vanC/*vanC*1	5'-GCTGAAATATGAAGTAATGACCA 5'-CGGCATGGTGTTGATTTCGTT	58	811
vanS/*vanS*1	5'-AACGACTATTCCAAACTAGAAC 5-GCTGGAAGCTCTACCCTAAA	60	1094
vanY/ *vanY*1	5'-ACTTAGGTTATGACTACGTTAAT 5'-CCTCCTTGAATTAGTATGTGTT	55	866

drinking water, the incidence of high concentration of *S. aureus* in water intended for human consumption may represent potential health hazards, especially if these strains possess determinants of antibiotic resistance and are able to produce enterotoxins (Percival et al., 2004). Pavlov et al. (2004) found that *S. aureus* isolated from drinking water were the most virulent and resistant to multi-antibiotics among all of the HPC bacteria recovered from water supplies in South Africa. Furthermore, water contaminated with *S. aureus* was reported to cause food poisoning when used to cool boiled eggs (Anon., 1972).

Vancomycin (glycopeptides) has become an approved and highly recommended antibiotic worldwide for the treatment of *S. aureus* infections, particularly methicillin-resistant *S. aureus* (MRSA) (Sotozono et al., 2013). Although vancomycin-resistant *S. aureus* is rare, the emergence of strains with decreased susceptibility and/or vancomycin intermediate resistance has been reported (Stienkraus et al., 2007; Gould, 2008; Shang-Yi et al., 2012).

The aim of the present study was to investigate the incidence of *S. aureus* and their carriage of vancomycin-resistance genes in potable water intended for human consumption in Makkah, Saudi Arabia.

METHODOLOGY

Sampling

A total of 100 samples were examined in this study for the presence of vancomycin-resistant *S. aureus*. These samples consisted of potable tap water from households (50 samples), potable water from households main reservoir or tank (25 samples) and households faucets swabs (25 samples). All water samples were collected into sterile polypropylene bottles. Faucets were internally swabbed using sterile cotton swabs. All samples were packed in ice and kept in darkness during transport; bacteriological assays were begun within six hours on the same day as sampling.

Isolation and identification of *Staphylococcus aureus*

S. aureus was recovered from potable water samples by means of membrane filtration technique. A volume of 100 ml of water from each sample was filtered onto a 0.45 µm membrane filter. The membrane filter was placed onto mannitol salt agar (Oxoid, Basingstoke, UK), and incubated at 34°C for 48 h. Each of the faucets swab was streaked onto mannitol salt agar (Oxoid) and incubation was at 34°C for 48 h (Abulreesh and Organji, 2011). Typical staphylococcal colonies on mannitol salt agar (colonies surrounded by yellow zones) were subcultured onto Blood agar plates (Saudi Prepared Media Laboratory, Riyadh, Saudi Arabia). The identification of the colonies was carried out using the following tests: Gram staining, observation of type of haemolysis on blood agar plates, production of catalase and coagulase using the BBL staphyslide latex test (Becton, Dickinson and Company, Maryland, USA). The *S. aureus* NCTC 12989 was used as a control strain.

Antibiotic susceptibility testing

Antibiotic susceptibilities were tested by the disk diffusion method according to the guidelines of the British Society for Antimicrobial Chemotherapy (BASC, 2010) using Mueller-Hinton agar (Oxoid) (Abulreesh and Organji, 2011). Six commercial antimicrobial disks (Mast Diagnostics, Bootle, UK) were used: Erythromycin (60 µg ml^{-1}), Rifampicin (15 µg ml^{-1}), Penicillin G (2 IU), Kanamycin (1000 µg ml^{-1}), Vancomycin (5 µg ml^{-1}) and Oxacillin (1 µg ml^{-1}) (Bio-Rad, Hercules, USA). *S. aureus* NCTC 12989 used a control to ensure the accuracy of testing.

Detection of vancomycin-resistance genes by PCR

PCR was performed on all confirmed *S. aureus* isolates to detect vancomycin-resistance genes. DNA was extracted by suspending a loop of *S. aureus* colony in a 100 µl sterile, pure water and boiling for 5 min. The suspension was then centrifuged for 5 min at 1260 *g*, and 10 µl of the supernatant were used as target DNA. Primers *vanA*, *vanB*, *vanC*, *vanS* and *vanY* (Bioneer, Alameda, USA) were used to detect vancomycin-resistance genes (Miele et al., 1995), the sequence of these primers and the expected product size is listed in Table 1. The PCR reaction mixture contained 10 mM Tris-

Table 2. Isolation of *Staphylococcus aureus* from potable water and faucets swabs.

Sample type: Source	No. of samples tested	No. with typical staphylococcal colonies (%)	No. with confirmed *S. aureus*	% of samples with *S. aureus*
Potable water:				
Household faucets	50	15 (30)	6	40
Reservoir/tank	25	4 (16)	1	25
Faucet swabs	25	19 (76)	10	53
Total	100	38	17	44.7

Table 3. Antibiotic susceptibility of *Staphylococcus aureus* isolated from potable water by disk diffusion method.

Parameter	R	IR	S	NCTC 12989†
	N (%)	N (%)	N (%)	
OX	16 (76.2)	0.0 (0.0)	5 (23.8)	S
VA	2 (9.5)	0.0 (0.0)	19 (90.5)	S
KA	0.0 (0.0)	0.0 (0.0)	21 (100)	S
PG	21 (100)	0.0 (0.0)	0.0 (0.0)	R
RP	0.0 (0.0)	0.0 (0.0)	21 (100)	S
E	0.0 (0.0)	0.0 (0.0)	21 (100)	S

R: Resistant, IR: Intermediate resistance, S: sensitive, OX: Oxacillin, VA: Vancomycin, KA: Kanamycin, PG: Penicillin G, RP: Rifampicin, E: Erythromycin, N: number of isolates tested, †: Control strain (*Staphylococcus aureus* NCTC 12989)

HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.15 mM (each of) dNTP, 1.2 U of Taq polymerase (ABgene, Surry, UK). The final reaction volume 0.05 ml contained 50 pmol of each primer and 500 ng of *S. aureus* DNA. The PCR program consisted of initial denaturation step at 94°C for 3 min; this was followed by denaturation at 94°C for 30 s, primers annealing at appropriate temperature (Table 1) for 2 min, and DNA extension at 72°C for 2 min. After the last cycle, the reaction was terminated by incubation at 72°C for 6 min and the products were stored at 4°C. PCR products (5.0 µl) were analysed by 1% agarose gel (Bioline, London, UK) electrophoresis and made visible by ethidium bromide (0.5 mg ml^{-1}) staining and UV transillumination (Miele et al., 1995).

RESULTS

Isolation of *Staphylococcus aureus* from potable water and faucet swabs

In potable water, staphylococci were detected in 30% of the samples from household faucets, and in 16% of the samples from household reservoirs/tanks. High recovery rate of staphylococci was observed in household faucet swabs (76%) (Table 2). *S. aureus* was present in six water samples out of 15 samples taken from household faucets (40%) and one water sample out of 4 samples taken from household reservoirs/tanks (25%). In faucet swabs, *S. aureus* was detected in 53% of the samples (10 out of 19) that yielded typical staphylococci colonies (Table 2).

Antibiotic susceptibility testing by disk diffusion

Antibiotic susceptibility patterns of *S. aureus* isolates from potable water and faucet swabs are shown in Tables 3 and 4, respectively. All *S. aureus* isolates from water and from faucet swabs were resistant to penicillin G, but sensitive to kanamycin and rifampicin (Tables 3 and 4). Resistance to oxacillin was exhibited by 76.2% and 60% of the *S. aureus* recovered from potable water (Table 3) and faucet swabs, respectively (Table 4). Small number of *S. aureus* recovered from water (9.5%, n = 2.0) exhibited resistance to vancomycin (Table 3), while 16.6% (n = 5.0) of the isolates derived from faucet swabs were resistant to vancomycin (Table 4). No isolates from water were resistant to erythromycin, however, two isolates from faucet swabs showed resistance and intermediate resistance, respectively to erythromycin (Table 4).

Detection of vancomycin-resistance genes by PCR

No vancomycin-resistance genes were detected in all 51 *S. aureus* isolates recovered from potable water and faucet swabs.

DISCUSSION

Staphylococci are ubiquitous with widespread distribution in the environment, and their presence in aquatic

Table 4. Antibiotic susceptibilities of *Staphylococcus aureus* isolated from faucet swabs by disk diffusion method.

Parameter	R	IR	S	NCTC 12989†
	N (%)	N (%)	N (%)	
OX	18 (60)	0.0 (0.0)	12 (40)	S
VA	5 (16.6)	0.0 (0.0)	25 (83.3)	S
KA	0.0 (0.0)	0.0 (0.0)	30 (100)	S
PG	30 (100)	0.0 (0.0)	0.0 (0.0)	R
RP	0.0 (0.0)	0.0 (0.0)	30 (100)	S
E	1.0 (3.3)	1.0 (3.3)	28 (93.33)	S

R: Resistant, IR: Intermediate resistance, S: sensitive, OX: Oxacillin, VA: Vancomycin, KA: Kanamycin, PG: Penicillin G, RP: Rifampicin, E: Erythromycin, N: number of isolates tested, †: Control strain (*Staphylococcus aureus* NCTC 12989).

environments is well established (Percival et al., 2004). In the present study, 46% of water samples collectively yielded staphylococci, with 65% of these isolates confirmed as *S. aureus* (Table 2). Similar and higher percentages of *S. aureus* occurrence in water were reported worldwide (Harakeh et al., 2006; Faria et al., 2009), including Makkah, Saudi Arabia (Mihdhdir, 2009; Abulreesh and organji, 2011). Although *S. aureus* can be found among the genera that normally exist in potable water as HPC bacteria (Allen et al., 2004), there are many reasons for potential concern when *S. aureus* are present in drinking water supplies. Common food preparation practices such as washing boiled potatoes, pasta, shelfish, and cooling of boiled eggs could possibly leave these food items contaminated with *S. aureus*. If these food items used for preparation of salads are left at room temperature, or improperly refrigerated, the possibility of staphylococcal food intoxication certainly exists if these *S. aureus* contaminants were toxigenic.

Staphylococci are well-known for their ability to produce biofilm formation on different surfaces such as water line pipes in dental clinics (Lancellotti et al., 2007) and on stainless steel pipes milk processing plants (Michu et al., 2011), this feature is considered as one of the main virulence factors of nosocomial staphylococcal-related infections (Piette and Verschraegen, 2009). In this study, 76% of the faucet swabs samples (n = 25) yielded typical staphylococci, of which 53% were confirmed as *S. aureus* (Table 2). Abulreesh and Organji (2011) reported higher recovery rate of staphylococci from faucet swabs and faucet filter swabs. The presence of *S. aureus* in biofilm formation within drinking water distribution system may, in part, cause aesthetic and hygienic problems as staphylococci within the biofilm consortia can inherit resistance to disinfectants, and their long term persistence, together with other HPC bacteria, can deteriorate the overall microbiological quality of potable water. Further potential waterborne pathogens may take refuge within the biofilm formation and survive for longer periods, with the possibility of acquiring resistance to antibiotics due to transferrable resistance genes (Lee and Kim, 2003; Parsek and Singh, 2003; Zhu et al., 2008).

Resistance to antimicrobial agents is a major public health concern as resistant bacteria can disseminate in the environment with possible transmission to human through contaminated food and water. In the current study, all *S. aureus* that were recovered from water (Table 3) and faucet swabs (Table 4) exhibited resistance to penicillin G, while resistance to oxacillin was observed in 76.2% and 60% of the isolates from water and faucet swabs, respectively. *S. aureus* is well-known for its remarkable resistance to β-lactam agents. Thus, methicillin-resistant *S. aureus* (MRSA) is currently the most commonly identified antibiotic-resistant pathogen worldwide (Ippolito et al., 2010), and their presence in drinking water and biofilm is widely reported (Harakeh et al., 2006; Lancellotti et al., 2007; Abulreesh and Organji, 2011).

Vancomycin has been successfully used for the last 50 years for the treatment of staphylococcal infections, particularly the cases involving MRSA. However its reliability has been questioned by the emergence of *S. aureus* strains that exhibit complete or intermediate resistance (Holmes et al., 2012). Various published accounts, using disk diffusion method reported the incidence of vancomycin-resistant strains in clinical (Mehdinejad et al., 2008), food (Ateba et al., 2010) and water and biofilm (Lancellotti et al., 2007; Abulreesh and Organji, 2011) specimens.

In the current study, few isolates from water (9.5%, n = 2.0) and from faucet swabs (16.6%, n = 5.0) exhibited resistance to vancomycin by disk diffusion method (Tables 3 and 4). In the United States, the Clinical and Laboratory Standard Institute (CLSI) only approve methods that determine minimum inhibitory concentrations (MIC) values for the detection of vancomycin-resistance in staphylococci due to its accuracy; this is also standardized in other countries such as Japan and European Union (Srinivasan et al., 2002). Therefore, the use of disk diffusion method for investigating resistance to vancomycin in staphylococci may not be appropriate

and possibly yield false-resistance patterns. This was the case in the current study, when all *S. aureus* isolates yielded no PCR products with all primers used, suggesting the absence of vancomycin-resistance genes despite five isolates exhibited resistance when tested by disk diffusion technique. Thus, caution is required when investigating and interpreting vancomycin-resistance in *S. aureus*.

The first isolation of *S. aureus* with *vanA* was reported in Michigan Hospital in 2002 (Anon., 2002), subsequently, several other strains were reported (Chang et al., 2003; Anon., 2004; Zhu et al., 2008), however all these isolates were of clinical origins. Despite the sporadic occurrence of *S. aureus* that carry vancomycin-resistance genes, it is generally accepted that these strains are rare (Srinivasan et al., 2002; Holmes et al., 2012; Askari et al., 2013; Culos et al., 2013). The DNA isolated from all 16 isolates reported in this study did not give any PCR products with the primers *vanA*, *vanB*, *vanC*, *vanS* and *vanY*. This result further confirms the rarity of vancomycin-resistant *S. aureus* in general and in the environment in particular.

REFERENCES

Abulreesh HH, Organji SR (2011). The prevalence of multidrug-resistant staphylococci in food and the environment of Makkah, Saudi Arabia. Res. J. Microbiol. 6:510-523.

Acco M, Ferreira FS, Henriques JAP, Tondo EC (2003). Identification of multiple strains of *Staphylococcus aureus* colonizing nasal mucosa of food handlers. Food Microbiol. 20:489-493.

Allen MJ, Edberg SC, Reasonar DJ (2004). Heterotrophic plate count bacteria – what is their significance in drinking water. Int. J. Food Microbiol. 92:265-274.

Anon (1972). Staphylococcal food poisoning, Milwaukee, Wisconsin. Morbid. Mort. Week Rep. 21:422-423.

Anon (2002). *Staphylococcus aureus* resistant to vancomycin- United States 2002. Morbid. Mort. Week Rep. 251:565-567.

Anon (2004). Brief report: vancomycin-resistant *Staphylococcus aureus*-New York 2004. Morbid. Mort. Week Rep. 53:322-323.

Askari E, Tabatabai SM, Arianpoor A, Nasab MN (2013). *VanA*-positive vancomycin-resistant *Staphylococcus aureus*: systematic search and review of reported cases. Infect. Dis. Clin. Pract. 21:91-93.

Ateba CN, Mbewe M, Moneoang MS, Bezuidenhout CC (2010). Antibiotic resistant *Staphylococcus aureus* isolated from milk in the Mafikeng area, North West province, South Africa. South Afr. J. Sci. 106:1-6.

BSAC (2010). Methods for antimicrobial susceptibility testing. Version 9.1. March 2010. British Society for Antimicrobial Chemotherapy, Birmingham, UK.

Casey AL, Lambert PA, Elliot TSJ (2007). Staphylococci. Int. J. Antimicrob. Agents 29:S23-S32.

Chang S, Sievert DM, Hageman JC, Boulton ML, Tenover FC, Downes FP, Shah S, Rudrik JT, Pupp GR, Brown WJ, Cardo D, Fridkin SK (2003). Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. New Eng. J. Med. 348:1342-1347.

Culos KA, Cannon JP, Grim SA (2013). Alternative agents to vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Am. J. Therap. 20:200-212.

Faria C, Vaz-Moreira I, Serapicos E, Nunes OC, Manaia CM (2009). Antibiotic resistance in coagulase negative staphylococci isolated from wastewater and drinking water. Sci. Total Environ. 407:3867-3882.

Gould IM (2008). Clinical relevance of increasing glycopeptides MICs against *Staphylococcus aureus*. Int. J. Antimicrob. Agents 31:1-9.

Harakeh S, Yassin H, Hajjar S, El-Fadel M (2006). Isolates of *Staphylococcus aureus* and *saprophyticus* resistant to antimicrobials isolated from the Lebanese aquatic environments. Mar. Poll. Bull. 52:912-919.

Holmes NE, Johnson PDR, Howden BP (2012). Relationship between vancomycin-resistant *Staphylococcus aureus*, vancomycin-intermediate *S. aureus*, high vancomycin MIC, and outcome in serious *S. aureus* infections. J. Clin. Microbiol. 50:2548-2552.

Ippolito G, Leone S, Lauria FN, Nicastri E, Wenzel RP (2010). Methicillin-resistant *Staphylococcus aureus*: the superbug. Int. J. Infect. Dis. 14:S7-S11.

Kluytmans JAJW (2010). Methicillin-resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency? Clin. Microbiol. Infect. 16:11-15.

Lancellotti M, de Oliveira MP, de Ávila FA (2007). Research on *Staphylococcus spp* in biofilm formation in water pipes and sensibility to antibiotics. Braz. J. Oral Sci. 6:1283-1288.

Lee DG, Kim SJ (2003). Bacterial species in biofilm cultivated from the end of the Seoul water distribution system. J. Appl. Microbiol. 95:317-324.

Mehdinejad M, Sheikh AF, Jolodar A (2008). Study of methicillin resistance in *Staphylococcus aureus* and species of coagulase negative staphylococci isolated from various clinical specimens. Pak. J. Med. Sci. 24:719-724.

Michu E, Cervinkova D, Babak V, Kyrova K, Jaglic Z (2011). Biofilm formation on stainless steel by *Staphylococcus epidermidis* in milk and influence of glucose and sodium chloride on the development of ica-mediated biofilm. Int. Dairy J. 21:179-184.

Miele A, Bandera M, Goldstien BH (1995). Use of primers selective for vancomycin resistance genes to determine van genotype in enterococci and to study gene organization in *VanA* isolates. Antimicrob. Agents Chem. 39:1772-1778.

Mihdhidr AA (2009). Evaluation of bacteriological and sanitary quality of drinking water stations and water tankers in Makkah Al-Mokarama. Pak. J. Biol. Sci. 12:401-405.

Normanno G, La Salandra G, Dambrosio A, Quaglia NC, Corrente M, Parisi A, Santagada G, Firinu A, Crisetti E, Celano GV (2007). Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. Int. J. Food Microbiol. 115:290-296.

Parsek M, Singh P (2003). Bacterial biofilms: An emerging link to disease pathogensis. Ann. Rev. Microbiol. 57:677-701.

Pavlov CM, de Wet E, Grabow WOK, Ehlers MM (2004). Potentially pathogenic features of heterotrophic plate count bacteria isolated from treated and untreated drinking water. Int. J. Food Microbiol. 92:275-287.

Percival SL, Chalmers RM, Embrey M, Hunter PR, Sellwood J, Wyn-Jones P (2004). Microbiology of Waterborne Diseases. Elsevier Academic Press, San Diego, USA.

Piette A, Verschraegen G (2009). Role of coagulase-negative staphylococci in human disease. Vet. Microbiol. 134:45-54.

Seo KS, Bohac GA (2007). *Staphylococcus aureus*. In: Doyle M. P. and Beuchat L. R. ed. Food Microbiology: Fundamentals and Frontiers 3rd Ed. ASM Press, Washington D.C. USA. 493-518.

Shang-Yi L, Tun-Chieh C, Feng-Jui C, Yen-Hsu C, Yee-In L, Kristopher-Siu L, Po-Liang L (2012). Molecular epidemiology and clinical characteristics of hetero-resistant vancomycin intermediate *Staphylococcus aureus* bacteremia in Taiwan medical center. J. Microbiol. Immunol. Infect. 45:435-441.

Srinivasan A, Dick JD, Perl TM (2002). Vancomycin resistance in staphylococci. Clin. Microbiol. Rev. 15:430-438.

Sotozono C, Fukuda M, Ohishi M, Yano K, Origasa H, Saiki Y, Shimomura Y, Kinoshita S (2013). Vancomycin ophthalmic ointment 1% for methicillin-resistant *Staphylococcus aureus* or methicillin-resistant *Staphylococcus epidermidis* infections: a case series. BMJ Open 13:1-6.

Stienkraus G, White R, Friedrich L (2007). Vancomycin MIC creep in non-vancomycin-internediate *Staphylococcus aureus* (VISA), vancomycin-susciptible clinical methicillin-resistant *Staphylococcus aureus* (MRSA) blood isolates from 2001-05. J. Antimicrob Chemo. 60:788-794.

Zhu W, Clark NC, McDougal LK, Hageman J, McDonald LC, Patel JB (2008). Vancomycin-resistant *Staphylococcus aureus* isolates associated with Inc18-like *vanA* plasmids in Michigan. Antimicrob. Agents Chem. 52:452-457.

academicJournals

Vol. 6(2), pp. 76-84, February 2014
DOI: 10.5897/JPHE2013.0592
ISSN 2006-9723 ©2014 Academic Journals
http://www.academicjournals.org/JPHE

Journal of Public Health and Epidemiology

Full Length Research Paper

Assessment of long-lasting insecticidal net coverage, use and physical integrity one year after universal distribution campaign in Plateau department in South-East Benin

Filémon T. Tokponnon[1, 2,,5,6]*, Yolande Sissinto[3], Aurore Hounto Ogouyémi[3], Arthur Sovi[5,6], Virgile Gnanguenon[5,6], Adicath Adéola Adéothy[3], Abel Wapo[3], Bruno Aholoukpe[1,2], Patrick Makoutodé[3], Alioun Adéchoubou[1], Ossè Razaki[5,6], Raphael N'guessan[7], Dina Gbénou[4], Mariam Oke[1,2], Dorothée Kinde Gazard[2], Achille Massougbodji[3] and Martin C. Akogbéto[5]

[1]National Malaria Control Program, Cotonou, Benin.
[2]Ministry of Health, Benin.
[3]Faculté des Sciences de la Santé de l'Université d'Abomey Calavi, Benin.
[4]World Health Organisation, Benin.
[5]Faculte des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin.
[6]Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin.
[7]London School of Hygiene and Tropical Medicine, Kepel Street, London, UK.

Accepted 20 January, 2014

Long-lasting insecticidal nets (LLINs) have proved to be an important tool for the malaria control and other vector-borne diseases. Benin, by its National Malaria Control Program, conducted in July, 2011 a universal distribution campaign where approximately 5 million of nets were distributed. But after this mass-distribution, questions arise: Do people effectively use or not use the mosquito nets freely distributed? To clarify these questions, this study was conducted on LLINs coverage, use and physical condition in Benin one year after their distribution. The households were randomly selected from 32 clusters. Data on bed net ownership and usage, physical condition of the nets, other characters and issues related to sourcing were asked of all targets to assess the origins of LLIN found at the household level. Of the total surveyed, 88.96% had at least one LLIN. 87.19% of these nets come from the last campaign, 9.1% were from pregnant women voucher clinic delivery systems and 3.20% were purchased full price. 84% of the nets were reportedly used the night preceding the survey. Around 89% of the total of LLINs observed was in good condition. The universal distribution campaign in Benin has significantly increased LLIN coverage and ownership policy in the community.

Key words: Long-lasting insecticidal nets (LLINs), coverage, use, physical integrity, Benin.

INTRODUCTION

Long-lasting insecticidal nets (LLINs) have proved to be an important tool for the control of malaria and other

*Corresponding author. E-mail: filemont@yahoo.fr. Tel: (229) 97272631. Fax: (229) 21333930.

vector-borne diseases (World Health Organisation (WHO), 2005). Several studies in malaria endemic countries have shown the usefulness of LLINs in reducing man-vector contact from malaria (Greenwood et al., 2005; Lengeler, 2004; Eisele et al., 2006; Thwing et al., 2008). It is a technology based on the slow release of pyrethroid insecticides, rendering it wash-resistant and extending insecticide residual effectiveness to at least three years without the need of re-treatment. This is why the recent objective of roll back malaria (RBM) focuses duplication of efforts including maintaining universal coverage of nets to achieve a 75% reduction compared to 2000, the number of reported malaria cases in endemic countries in the African region, 2015 (OMS, 2011; AFR/RC50/12 – WHO, 2012) .

Benin, by its National Malaria Control Program, supported since 2000 the initiative RBM and conducted in July, 2011 a universal distribution campaign where approximately 5 million of nets were distributed. This distribution is intended to significantly increase the national coverage of insecticide-treated nets. Before the distribution, the proportion of households owning at least one net at national level was estimated at 52% (PNLP-AFRICARE BENIN-CRS BENIN, 2010). After the distribution of July, 2011, this proportion was around 86.4% (PNLP, 2011). In the net level analysis, factors independently associated in both surveys with reduced likelihood that a net would be used were: increasing net age, increasing damage of nets, increasing household net density (nets/person). Some studies have shown that several factors are associated with LLIN ownership and effective use (Graves et al., 2011). Factors associated with ownership were assumed to be: cultural beliefs and practices mechanisms of LLIN distribution and distance to LLIN suppliers, rumours about LLINs and social support and pressure (Wiseman et al., 2007; Baume and Marin, 2007). But factors associated with net used were reported to be: perceived benefits and disadvantages of nets, trust in health workers providing health education and LLINs, knowledge of appropriate net use/care practices, and net-hanging skills, household size and composition, the number of children under five years of age and use of other vector control measures (Toé et al., 2009; Edelu et al., 2010). Other studies conducted in Benin showed that several mosquito nets after 12 month of use had already holes (Gnanguenon, personnal communication). From these observations, questions arise: Do people effectively use the mosquito nets freely distributed? Or do people not use LLINs to their insufficient number or their poor physical condition? To clarify these questions, we conducted a study on insecticide-treated nets coverage, use and physical condition in plateau department in southern Benin one year after the distribution. This study aimed to provide useful data on the effectiveness of the distribution campaign.

METHODOLOGY

Study area

Plateau is a county of Benin in West Africa. The selection of this county was based on its geographic accessibility, the high use of mosquito nets by children under 5. Entomological surveys conducted in the plateau have shown that there are both high and low pyrethroids resistance areas (Yadouleton et al., 2010). According to the general report of the distribution campaign, 85.5% of the households received a LLIN with an average of 2.7 LLINs/Household. Ketou, Pobe, Adja-Ouere, Ifangni and Sakete are the top 5 cities that compose it. But our study was focused on only four districts (Ketou, Pobe, Ifangni and Sakete). Ifangni district is located at 2° 43' 14"E and 6° 38' 56"N; its area is 242 km² representing 7.28% of Plateau territory. Sakete district is located at 2° 39' 7"E and 6° 46' 3"N; covering an area of 432 km², it represents 13.29% of plateau territory. Ketou is located is at 2° 36' 4"E and 7° 27' 21"N; it has an area of 1775 km², representing 54.38% of Plateau county's area. Pobè district is located at 2° 41' 51"E and 7° 5' 12"N; it has an area of 400 km², representing 11% of the county's area. 32 rural villages were selected through all four districts (Figure 1).

Study design

Larvae prospection's for insecticides susceptibility tests on *Anopheles gambiae,* main malaria vector in Benin have been conducted in many villages of the targeted districts. This baseline study on the resistance of malaria vectors to deltamethrin in the department of Plateau helped to make the choice of sentinel villages where various activities were held in our work. A total of 32 clusters were selected including 17 clusters at Ifangni, 6 at Sakété, 2 at Pobè and 8 at Kétou. Each cluster (village) was composed of several hamlets and comprised a minimum of 100 Children under five years old. Household cross-sectional surveys were undertaken in each cluster in May and August, 2012, conducting during high malaria transmission period. The survey covered the targeted groups in different villages. The choice of the targeted population has followed the standards set in the collection of basic data on morbidity and mortality due to malaria in the monitoring/evaluation RBM/RBM in Benin in 2004 (Kinde-Gazard et al., 2004). The targeted persons by cluster were: 30 mothers of children or care for children less than 5 years to evaluate fever or malaria during the last two weeks, 25 Pregnant women in the third trimester of pregnancy and women who gave birth in the last 6 months to assess malaria prevention, 30 householders for the availability and use of LLINs.

The households were randomly selected from each cluster. Data on bed net ownership and usage, physical condition of the nets, demographics of household members, other characters and issues related to sourcing were asked of all targets to assess the origins of LLIN found at the household level. Data were gathered using an adapted version of the standard Malaria Indicator Survey (RBM, 2013). Specific questions relating to the Universal Campaign Coverage process were asked to the householder and to the mothers of children or care for children less than 5 years. The physical condition of the nets was estimated using one of the two indicators recommended by WHO: the proportion of LLINs with any hole(s) (WHO, 2011). The main hole category in the LLINs was recorded as follows:

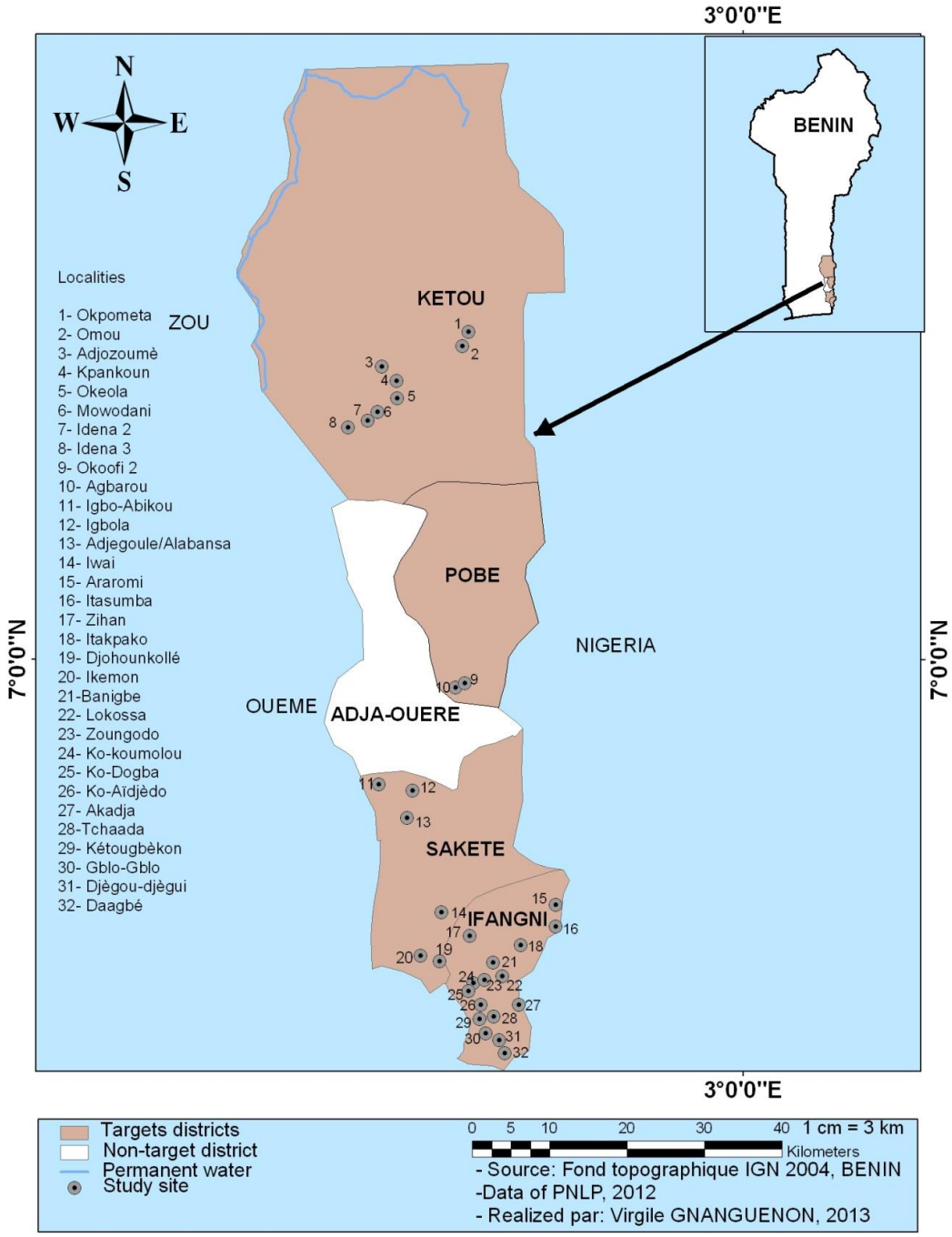

Figure 1. Map showing the study villages in Plateau department (Benin).

T1: holes size < thumb (0.5 to 2 cm);
T2: holes size > thumb < fist (2 to 10 cm);
T3: holes size > fist < head (10 to 25 cm);
T4: holes size > head (> 25 cm).

Statistical analysis

Interviews were conducted using questions. At the end of the survey, data were recorded with Epi-Info and data were transferred

Table 1. Background characteristics of the households surveyed.

Characteristic	Frequency (%)
Sex of the heads of households (n = 960)	
Male	386 (40.20)
Female	574 (59.80)
Educational level of the heads of households	
Illiterate	655 (68.23)
Elementary	188 (19.58)
Post elementary	117 (12.19)
Household size (n = 4688)	
Children under 5 years	1339 (28.56)
Pregnants women	800 (17.06)
Person over 5 years	2549 (54.37)

into SPSS 16.0 software. The investigators had cleaned and analysed the data using the same software program. Household ownership of LLIN was calculated as a proportion of households having at least one LLIN among the total surveyed households. LLIN use was estimated as the proportion of households using at least one LLIN in the LLIN owning households. Once the LLIN ownership and use were determined; the data were filtered into a separate file of LLIN owned households. Then, households using LLINs were compared to those who did not use any to identify the factors associated with LLINs non-use. Barriers of LLIN use were determined by using enter method multivariate logistic regression model. Data on the physical integrity of LLINs and those relating to the origins of LLINs were extracted. The various holes observed were divided into four and frequencies were estimated to assess the general condition of operation of LLINs (WHO, 2011; Kilian, 2012).

Ethics approval

This study was planned and approved by the Ministry of Health. The protocol was also reviewed and approved by National Ethics Committee for Health Research at the Ministry of Health. A briefing note indicating the objectives of the study, the advantages and disadvantages was given to the respondents in order to obtain consent. Confidentiality was respected and questionnaires were anonymous.

RESULTS

Study households characteristics

960 households and 4688 people were included in the survey and the response rate was 100% (Table 1). 59.80% of the heads of households interviewed were females versus 40.20% of males. 68.23% of the heads of households were illiterate, 19.58% have elementary school degree and 12.19% have an educational level over elementary school degree. Of the 4688 people included (Table 1), 1339 (28.56%) were under five years old; 800 (17.06%) were reported to be pregnant women and 2549 (54.37%) were people over five years old (without pregnant women).

LLINs ownership

Of the total surveyed households, 88.96% had at least one LLIN while 11.04% did not have any type of mosquito nets. The majority of households that owned LLIN had either one or two LLINs irrespective of their household size. 28.22% (25.47 to 31.16) had one LLIN, 31.85% (29.00 to 34.89) had two LLINs, 16.76% (14.54 to 19.27) had three LLINs and 12.18% (10.27 to 14.41) had four LLINs. The number of ITNs did not vary significantly between cluster (Table 2) and the average LLIN ownership among LLIN owned household was 1.82 (Table 2). 87.19% (84.76 to 89.27) of these nets come from the National distribution campaign of 2011, 9.1% (7.79 to 11.78) were from pregnant women voucher clinic delivery systems and 3.20% (2.21 to 4.61) were purchased full price (Figure 2).

Factors associated with ITN ownership

The sex of the heads of households (male and female) as well as their education level were not associated with ITN ownership ($p > 0.05$). Also, the composition of the household size in children under five, pregnant women and persons over five years old was not associated with ITN ownership ($p > 0.05$) (Table 3).

ITNs utilization

Of the total of 1746 reported LLINs, 42.96% (n = 750) LLINs were observed by the surveyors. 84% of the nets were reportedly used the night preceding the survey. The

Table 2. LLINs ownership by cluster.

Parameter	Cluster	LLINs	No. of households	Mean	CI 95%
Ketou	Okpometa	52	30	1.73	[1.40-2.05]
	Omou	69	30	2.3	[2.03-2.56]
	Adjozounme	82	30	2.73	[2.54-2.92]
	Kpankoun	59	30	1.97	[1.69-2.25]
	Oke Ola	38	30	1.27	[1.02-1.52]
	Mowodani	74	30	2.47	[2.24-2.70]
	Idena2	45	30	1.5	[1.11-1.88]
	Idena3	49	30	1.63	[1.24-2.01]
Pobe	Okoffi 2	73	30	2.43	[2.20-2.75]
	Agbarou	44	30	1.47	[1.11-1.82]
Sakete	Igboabikou	39	30	1.3	[1.04-1.55]
	Igbola	54	30	1.8	[1.44-2.15]
	Adjegounle/Alabansa	48	30	1.6	[1.22-1.97]
	Iwai	61	30	2.03	[1.68-2.37]
	Ikemon	52	30	1.73	[1.51-1.94]
	Djohounkolle	53	30	1.77	[1.51-2.02]
Ifangni	Akadja	69	30	2.3	[2.08-2.51]
	Araromi	40	30	1.33	[0.92-1.73]
	Banigbe	48	30	1.6	[1.28-1.91]
	Daagbe	49	30	1.63	[1.23-2.02]
	Djegou Djedji	65	30	2.17	[1.93-2.40]
	Gblo Gblo	23	30	0.77	[0.43-1.10]
	Ita Kpako	59	30	1.97	[1.62-2.32]
	Itassumba	56	30	1.87	[1.54-2.19]
	Ketougbekon	47	30	1.57	[1.24-1.90]
	Ko Dogba	56	30	1.87	[1.54-2.19]
	Ko-Aîdjedo	70	30	2.33	[2.12-2.54]
	Kokoumolou	51	30	1.7	[1.42-1.97]
	Lokossa	42	30	1.4	[1.00-1.79]
	Tchaada	64	30	2.13	[1.85-2.40]
	Zian	70	30	2.33	[1.98-2.67]
	Zougoudo	45	30	1.5	[1.09-1.90]
	Total	1746	960	1.82	[1.76-1.87]

CI = confidence interval.

proportion of nets in use varies from 47 to 100%, but not significantly different from one cluster to another (Table 4). It was only at Araromi that the level of net use was significantly low compared to other clusters. Among the target groups, 76.4% (73.46 to 79.34) of pregnant women really used LLINs, 82.88% (80.87 to 84.91) children under 5 years used nets while 69.78% (68.22 to 71.34) people over 5 years were reported as using LLINs (Figure 3). On the 807 LLINs observed, around 16% was found with hole(s) (Table 5). No significant difference was observed between categories of holes (T1 to T4) and while comparing the physical condition of the nets by the level of instruction of their owners ($p < 0.05$).

DISCUSSION

ITNs coverage was increased by the universal distribution

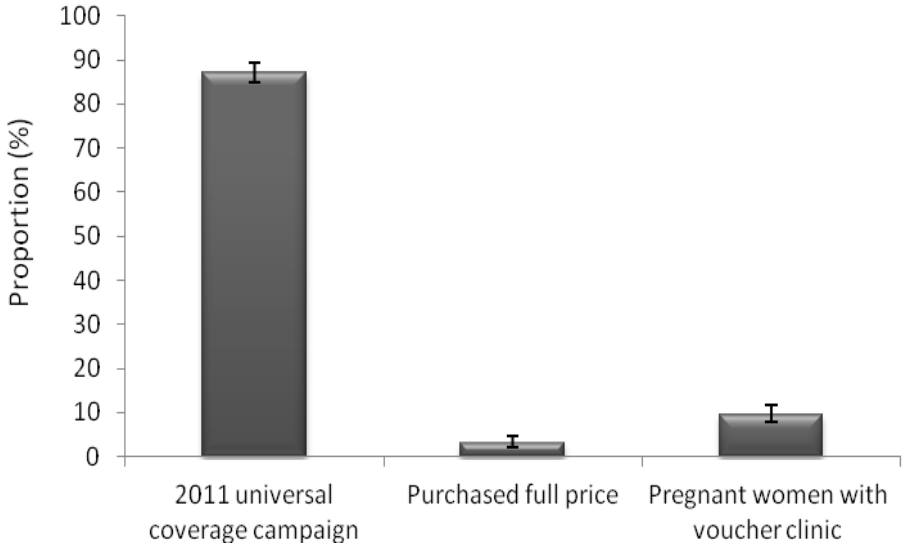

Figure 2. Source of ITN ownership.

Table 3. Multivariate regression on ITN ownership.

Factors	% ITN ownership (n)	CI = 95%	p-value
Heads of households	88.96 (854)		
Sex of the heads of households			
Male	43.85 (421)	[40.72-46.99]	0.680
Female	45.10 (433)	[41.96-48.25]	
Educational level of the heads of households			
Illiterate	60.31 (579)	[57.22-63.41]	
Elementary	17.5 (168)	[15.10-19.90]	0.332
Post elementary	11.15 (107)	[9.16-13.14]	

n = number CI = Confidence Interval.

distribution campaign with a proportion of 88.96% of households that owned at least one ITN. This proportion is higher when compared to the preceding distribution campaign evaluation (Tokponnon et al., 2013), and the average number of ITNs per household was around 2. Mosquito nets (LLINs) that were from the distribution campaign were significantly higher (87%) comparatively to those that were purchased full price (3%) or received from pregnant women antenatal consultation (9%). In our study, the average number of LLINs per household visited was 1.82 considering the 960 households. The average household size was 4.88 people visited. Thus we can say that about two nets are available for 5 people at the household level. However, the objective of this campaign is to increase the distribution level of 56% coverage (PNLP, 2012) at least 80% and have a LLIN for two people in the general population.

In the protocol of the LLIN distribution campaign of July, 2011, households of two people were covered with two LLINs and this rule has been well respected in rural areas in which the distribution has been fewer problems (Tokponnon et al., 2013). Our observations were consistent with the same requirements a year after the campaign. A disadvantage of the distribution was that 11% of households did not receive LLINs (PNLP, 2012). There were no significant differences between the availability of LLINs in different villages when considering the proportion of households with at least two LLINs. This

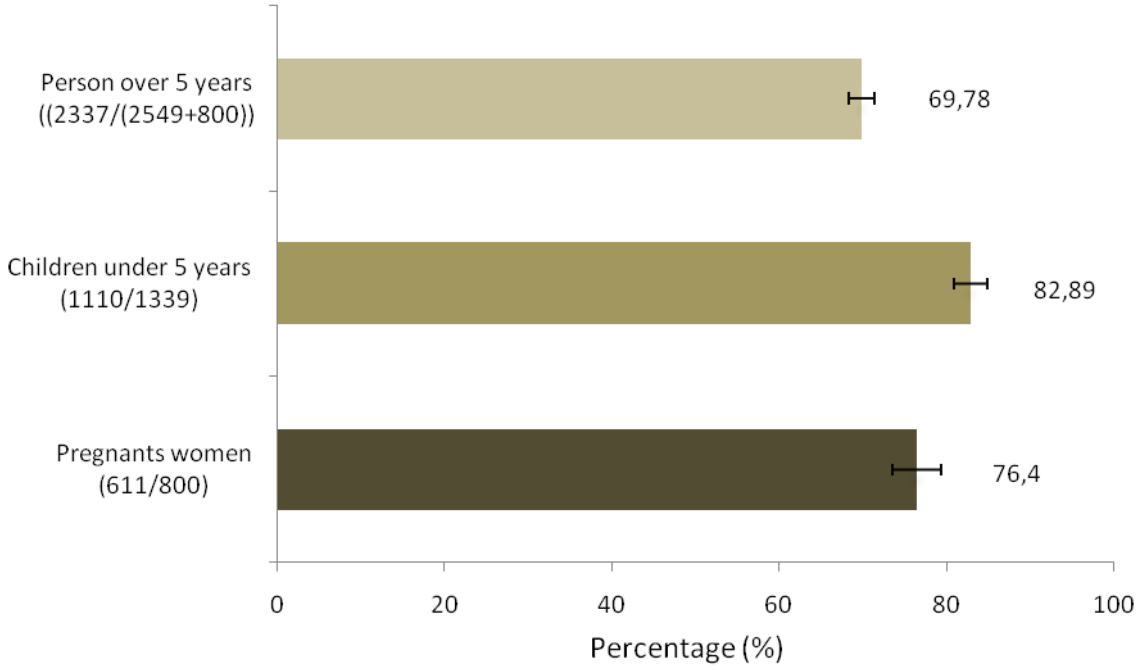

Figure 3. Net usages in target and non target group.

Table 4. Multivariate regression on ITN use.

Districts	Clusters	LLIN utilization			Proportion M+ (%)	OR	p
		M-	M+	Total			
Ketou	Okpometa	7	23	30	77	3.75	0.0194
	Omou	1	29	30	97	33.14	0.0012
	Adjozounme	0	30	30	100	69.41	0.0039
	Kpankoun	3	27	30	90	10.26	0.001
	Oke Ola	7	23	30	77	3.75	0.0194
	Mowodani	9	21	30	70	2.66	0.0698
	Idena 2	10	20	30	67	2.28	0.1208
	Idena 3	8	22	30	73	3.14	0.0379
Pobe	Okoffi 2	4	26	30	87	7.42	0.002
	Agbarou	13	17	30	57	1.49	0.4391
Sakete	Igboabikou	3	27	30	90	10.26	0.001
	Igbola	4	26	30	87	7.42	0.002
	Adjegounle/Alabansa	11	19	30	63	1.97	0.1967
	Iwai	4	26	30	87	7.42	0.002
	Ikemon	1	29	30	97	33.14	0.0012
	Djohounkolle	5	25	30	83	5.71	0.0044
Ifangni	Akadja	6	24	30	80	4.5714	0.0094
	Araromi	16	14	30	47	1	-
	Banigbe	6	24	30	80	4.5714	0.0094
	Daagbe	9	21	30	70	2.66	0.0698

Table 4. Contd.

Djegou - Djegi	6	24	30	80	4.5714	0.0094
Gblo-Gblo	14	16	30	53	1.3	0.6058
Ita - Kpako	0	30	30	100	69.41	0.0039
Itassumba	2	28	30	93	16	0.0007
Ketougbekon	11	19	30	63	1.97	0.1967
Ko Dogba	9	21	30	70	2.66	0.0698
Ko Aïdjedo	0	30	30	100	69.41	0.0039
Kokoumolou	9	21	30	70	2.66	0.0698
Lokossa	10	20	30	67	2.28	0.1208
Tchaada	9	21	30	70	2.66	0.0698
Zian	2	28	30	93	16	0.0007
Zougoudo	9	21	30	70	2.66	0.0698
Total	208	752	960	78.3		

M+: household who's everybody use LLIN the days before survey. M-: household who's not everybody use LLIN the days before survey. OR: odd ratio; Significant at $p < 0.05$ level.

Table 5. Physical condition of LLINs.

Level of instruction	LLINs without hole	LLINs with holes				Total LLINs with hole(s)	% LLINs with holes
		T1	T2	T3	T4		
Illiteracy	542	28	52	15	11	106	16,36
Primary school	163	10	12	3	3	28	14,66
Post primary school	102	7	3	4	1	15	12,82
Total	807	45	67	22	15	149	15,58

proportion was higher compared to those with three or four due to the fact that LLIN distribution campaign has limited the number of nets distributed for two people and a maximum of 8 LLINs for large households at the same size.

This observation is a good performance and the coverage attained was similar to what has been achieved in other countries (Tanzania, Nigeria and Togo) (West et al., 2012; Stevens et al., 2013). Continuous distribution of LLINs to pregnant women and children under five is an important way to increase nets coverage and replace torn nets (RBM, 2011). An additional continuous distribution system, via NGOs for example, can also be planned for other households. 78% of the net observed were reported to be used the previous night, and the proportion of nets use did not vary significantly between clusters, suggesting that awareness for net use was a success. But full net used by target group was not achieved.

76.4% of pregnants women were using ITNs while only 82.88% of children under five were using ITNs. This observation suggests that net usage could be improved in target group.

The high ITNs usage could be a consequence of the increased availability of ITNs at the household level due to the universal distribution campaign. This observation has already been noticed in Tanzania by West and colleagues (West et al., 2012). The level of ITN use could also be influenced by high temperature and mosquito density (Graves et al., 2011). But comparatively to other studies, ITN usage observed in target group in this study was higher than those observed in Tanzania and Nigeria (Ye et al., 2012; West et al., 2012). ITN ownership and use seems to reflect the general coverage and ITNs ownership in the whole population. The proportion of LLINs from the distribution campaign found in good condition was significantly higher than those observed in serviceable condition or torn out.

Conclusion

The universal distribution campaign in Benin has significantly increased ITN coverage and ownership policy in the community. Non target persons and target

persons (pregnant women and children under five) had similar level in ITN ownership and usage. The level of ITN ownership and usage were also similar between villages. This is an important indicator of universal coverage goal. But additional effort must be done to fully achieve universal coverage goal, and routine distribution must be used to maintain ITN coverage.

ACKNOWLEDGEMENTS

We are grateful to the WHO and Bill & Melinda Gates Foundation which supported financially this study. The authors would also like to thank the populations of 32 clusters, the team of health system in the Plateau department, and the investigators for their collaboration.

REFERENCES

AFR/RC50/12 - WHO (2012). Regional Office for Africa [http://www.afro.who.int/fr/downloads/doc_details/1106-afrrc5012.html?tmpl=component].

Baume CA, Marin MC (2007). Intra-household mosquito net use in Ethiopia, Ghana, Mali, Nigeria, Senegal, and Zambia: are nets being used? Who in the household uses them? Am. J. Trop. Med. Hyg. 77:963–971.

Edelu B, Ikefuna A, Emodi J, Adimora G (2010). Awareness and use of insecticide-treated bed nets among children attending outpatient clinic at UNTH, Enugu - the need for an effective mobilization process. Afr. Health Sci. 10:117–119.

Eisele TP, Macintyre K, Yukich J, Ghebremeskel T (2006). Interpreting household survey data intended to measure insecticide-treated bednet coverage: results from two surveys in Eritrea. Malar. J. 5:36.

Graves PM, Ngondi JM, Hwang J, Getachew A, Gebre T, Mosher AW, Patterson AE, Shargie EB, Tadesse Z, Wolkon A, Reithinger R, Emerson PM, Richards FO (2011). Factors associated with mosquito net use by individuals in households owning nets in Ethiopia. Malar. J. 10:354.

Greenwood BM, Bojang K, Whitty CJM, Targett GAT (2005). Malaria. Lancet 365:1487–1498.

Kilian A (2012). Textile Testing Methods for Nets.

Kinde-Gazard D, Gbenou D, Tohon S, Da Silva C, Nahum A, Quenum A, Houndigande E, Houndekon R, Ekoue S, Massougbodji A (2004). Indicateurs de suivi et d'évaluation en 2001 de l'initiative Faire reculer le paludisme au Bénin. Bull. Soc. Pathol. Exot. 97:349–352.

Lengeler C (2004). Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst. Rev. CD000363.

OMS (2011). Rapport 2011 sur le paludisme dans le monde [http://www.who.int/malaria/world_malaria_report_2011/fr/]

PNLP-AFRICARE BENIN-CRS BENINRS (2010). Evaluation Finale Du Projet D'appui à La Lutte Contre Le Paludisme. Cotonou, 42p.

PNLP (2011). Evaluation post campagne de la distribution gratuite de MIILD en 2011 au Bénin. Cotonou, 58p.

PNLP (2012). Campagne de Distribution Gratuite Des Moustiquaires Imprégnées à Longue Durée D'action Aux Ménages Du Bénin. Cotonou: Ministère de la Santé.11p.

RBM (2011). Continuous LLIN Distributions: A Guide to Concepts and Planning/ K4Health [http://www.k4health.org/toolkits/continuous-distribution-malaria/continuous-llin-distributions-guide-concepts-and-planning].

RBM (2013). Malaria Indicator Surveys - Access to Reports, MIS Datasets, Survey Information [http://www.malariasurveys.org/surveys.cfm].

Stevens ER, Aldridge A, Degbey Y, Pignandi A, Dorkenoo MA, Hugelen-Padin J (2013). Evaluation of the 2011 long-lasting, insecticide-treated net distribution for universal coverage in Togo. Malar. J. 12:162.

Thwing J, Hochberg N, Vanden Eng J, Issifi S, Eliades MJ, Minkoulou E, Wolkon A, Gado H, Ibrahim O, Newman RD, Lama M (2008). Insecticide-treated net ownership and usage in Niger after a nationwide integrated campaign. Trop. Med. Int. Health 13:827–834.

Toé LP, Skovmand O, Dabiré KR, Diabaté A, Diallo Y, Guiguemdé TR, Doannio JM, Akogbeto M, Baldet T, Gruénais M-E (2009). Decreased motivation in the use of insecticide-treated nets in a malaria endemic area in Burkina Faso. Malar. J. 8:175.

Tokponnon F, Aholoukpe B, Denon E, Gnanguenon V, Bokossa A, N'guessan R, Oke M, Kinde-Gazard D, Akogbeto M (2013). Evaluation of the coverage and effective use rate of Long-Lasting Insecticidal Nets after Nation-wide scale up of their distribution in Benin. Parasit. Vectors., 6:265.

West PA, Protopopoff N, Rowland MW, Kirby MJ, Oxborough RM, Mosha FW, Malima R, Kleinschmidt I (2012). Evaluation of a national universal coverage campaign of long-lasting insecticidal nets in a rural district in North-west Tanzania. Malar. J. 11:273.

WHO (2005). World Malaria Report 2005. [http://www.who.int/malaria/publications/atoz/9241593199/en/]

WHO (2011). Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions. [http://apps.who.int/iris/handle/10665/44610]

Wiseman V, Scott A, McElroy B, Conteh L, Stevens W (2007). Determinants of bed net use in the Gambia: implications for malaria control. Am. J. Trop. Med. Hyg. 76:830–836.

Yadouleton AW, Padonou G, Asidi A, Moiroux N, Bio-Banganna S, Corbel V, N'guessan R, Gbenou D, Yacoubou I, Gazard K, Akogbeto MC (2010). Insecticide resistance status in *Anopheles gambiae* in Southern Benin. Malar. J. 9:83.

Ye Y, Patton E, Kilian A, Dovey S, Eckert E (2012). Can universal insecticide-treated net campaigns achieve equity in coverage and use? the case of northern Nigeria. Malar. J. 11:32.

academicJournals

Vol. 6(2), pp. 85-91, February 2014
DOI: 10.5897/JPHE2013.0535
ISSN 2006-9723 ©2014 Academic Journals
http://www.academicjournals.org/JPHE

Journal of Public Health and Epidemiology

Full Length Research Paper

Epidemiological studies of *Fasciola gigantica* in cattle in Zaria, Nigeria using coprology and serology

A. A. Aliyu[1], I. A. Ajogi[1], O. J. Ajanusi[2] and R. C. Reuben[1]

[1]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[2]Department of Veterinary Parasitology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Accepted 13 January, 2014

Fasciolosis is an important helminth disease of livestock and other ruminants. A cross sectional study to determine the prevalence of *Fasciola gigantica* in cattle was carried out in 9 randomly selected farms and 1 slaughter house between February and May, 2012. Faecal and blood samples were collected from 186 cattle in the farms and 200 cattle at slaughter. The faecal samples were analysed using the formol-ether sedimentation technique and the blood by Indirect ELISA kit (Bio-X-Diagnostic, ID VET Jemelle-Belgium) to detect *F. gigantica* eggs and antibodies to *F. gigantica* antigens, respectively. Of the 200 faecal samples collected at slaughter, 39 (19.5%) had *F. gigantica* eggs, as compared to 27 (14.5%) positives out of the 186 samples collected from the farms, giving an overall prevalence rate of 66 (17.1%). There was no significant difference (P>0.05) between prevalence of infection of cattle sampled in the farms and slaughter house. 23 (11.5%) of the sera prepared from the 200 blood samples obtained at slaughter had antibodies to *Fasciola hepatica* antigens, as against 5 (2.6%) for sera from 186 blood samples collected in the farms, giving an overall seroprevalence of 28 (7.3%). There was significant difference (P<0.05) between infection at slaughter and on farms. Out of the 200 cattle from slaughter, 20 (10.0%) had *F. gigantica* eggs and also were seropositive for *F. hepatica* antigens, and of the 186 cattle from farms, only 5 (2.7%) had *Fasciola* eggs and were also seropositive for *F. hepatica* antigens. Both at slaughter and on farms, infection was more prevalent in females than in males. The overall prevalence for females using coprology and ELISA were 19.3 (41/212) and 7.5% (16/212), respectively. The respective values for males were 13.7 (24/174) and 6.89% (12/174). However, the difference in the prevalence of females and males obtained was not statistically significant (P>0.05). No statistical difference was observed in breed prevalence. This study has established *F. gigantica* prevalence of 17.1 and 7.3% by coprological and serological examinations of faeces and blood of cattle in Zaria. It is recommended that cattle should be dewormed regularly and further serological screening be embarked in other local government areas of Kaduna State, so as to know the current status of *F. gigantica* infection in cattle.

Key words: Cattle, fasciolosis, prevalence, farms, slaughter house.

INTRODUCTION

Fasciolosis also known as fascioliasis, distomatosis and liver rot, is an important helminth disease caused by trematode species, *Fasciola hepatica* (the common liver fluke) and *Fasciola gigantica*. The disease belongs to the plant-borne trematode infection and the definitive host rangeisverybroadandincludesmanyherbivorousmammals

*Corresponding author. E-mail: kalomovaah@yahoo.com or reubenrine@yahoo.com.

and humans (Mas-Coma et al., 2005). *F. hepatica* has a worldwide distribution due to its capacity to infect many different species and the ability of the intermediate snail host to adapt to wide range of ecological niches (Garcia et al., 2007).

F. hepatica infects more than 300 million cattle and 250 million sheep worldwide and together with *F. gigantica*, causes significant economic losses to global agriculture; estimated at more than US$3 billion annually through lost productivity, such as a reduction of milk and meat yields (Mas-Coma, 1997).

Fasciolosis is a disease of public health and economic importance (Ashrafi et al., 2006). It causes serious disease of cattle, sheep, goats, buffalo and other ruminants. In cattle, the disease is debilitating, decreasing production of milk and result in losses due to condemned livers when the animals are slaughtered (Vassilev and Jooster, 1991). It can also lead to chronic low-grade anaemia and emaciated carcasses at slaughter.

In any country of the world, the goal of cattle production is to make money or provide some advantages such as a cheap source of labour and animal protein for the populace (Dixon et al., 2001). They also play an important role in the social and cultural life of most of the communities in the northern part of Nigeria (Tewe, 1997). However, the productivity of cattle has been limited by parasitic infections including fasciolosis (Keyyu et al., 2005) and this threatens the food security of the population and its social balance.

Apart from its veterinary importance throughout the world, fasciolosis is now recognized as an important emerging zoonotic disease of humans. Prior to 1992, the total number of reported human cases of fasciolosis was estimated to be less than 3000. More recent figures suggest that between 2.4 and 17 million people are currently infected, with a further 91.1 million living at risk of infection (Keiser and Utzinger, 2005). Drinking untreated water may be a source of infection due to the presence of free-floating metacercarial cysts. Vegetables washed in contaminated water may also become a source of infection (Taira et al., 1997; Mas-Coma et al., 2005). Although reported incidence and prevalence of the disease varies widely from country to country, prevalence rate in developed countries can reach up to 77%, but ranges from 30 to 90% in cattle in tropical countries (Spithill et al., 1999).

In Nigeria, the first incidence of fasciolosis was reported by Burke (1939) when about 3000 goats died of the disease in the then Borno province. In a South-Western State of Nigeria, a gross total liver loss of 8.292 kg was observed with about 75% loss of value in 29.952 kg of partially condemned livers in a single abattoir over a three-year period (World Bank, 2006). Estimating that each of the 36 states and the Federal Capital Territory will record similar losses in at least one abattoir per state, this will translate to huge loss of resources (US$ 5,762,010) for the country. These enormous losses are especially important for a low-income food-deficient country (LIFDC) like Nigeria (World Bank, 2006).

In Zaria, Kaduna State, cattle population was reported to be 1,144,000 (KDSG, 2008) and about 99% of these cattle were being managed/reared under semi-intensive, extensive or pastoral system. Most herdsmen graze their herds along the river banks during the prolonged dry season when the upland pasture is poor in quantity and quality. These animals are thus exposed to high risks of *Fasciola* infection (Olumide and Mpoko, 2001).

Most prevalence studies in Zaria and other parts of the country have been based mainly on abattoir records, with few on faecal examination (Ademola, 2003; Kamani et al., 2007). A serological method like enzyme-linked immunosorbent assay (ELISA) which detects all stages of the infection will be needed to have a more reliable figure on prevalence of the infection. This study on prevalence will therefore provide information on the seroprevalence and the current status of *F. gigantica* at slaughter and on farms in Zaria, and also to determine association between *F. gigantica* infection and age, sex and breed of cattle in Zaria.

MATERIALS AND METHODS

Study area

The study area is Zaria, a major city in Kaduna State in Northern Nigeria located within latitudes 11°7, 11°12 N and longitudes 07°41 E. It has an estimated population of 547,000 and a growth rate of 3.5% per annum. Zaria is characterized by a tropical climate, a monthly mean temperature ranging from 13.8 to 36.7°C and an annual rainfall of 1092.8 mm. It is approximated that about 40 to 75% of its working population derive their principal means of livelihood from agriculture (ABU, 2000). Agricultural activity in Zaria can be divided into two types: rain-fed (from May to October) and irrigation farming in the dry season (from November to April).

Sampling procedure

Samples were collected from cattle in 7 Fulani herds and 2 farms located in Basawa, Dogarawa, Dakachi, Hanwa, Jaja, Tukur-tukur and Zango, an institutional farm (belonging to Ahmadu Bello University and located in Samaru, a private farm (located in Zaria city) as well as in slaughtered cattle). Animals to be sampled were selected based on the simple random (without replacement) method (Fatimah, 2003). At least 20% of the animals in the herd were sampled. Slaughter cattle from Zaria abattoir (Zango) were also selected based on systematic random technique, the first forty cattle slaughtered were sampled (Fatimah, 2003).

Visits were made to the farms and nomadic herds between March and May, 2012, while visits to the abattoir took place once a week (7:00 to 10:00 am), the period when animals are slaughtered in the abattoir between February and April, 2012.

Sample collection and handling

From each animal sampled, blood and faecal samples were collected. The estimated age by dentition (Pace and Wakeman, 2003), sex and breed were recorded. On farms, each animal was properly restrained and 10 ml of whole blood was drawn from the jugular vein using a 10 ml syringe and 18G " 1.5" needle. Blood was

Table 1. Prevalence of *F. gigantica* at slaughter and on farms in Zaria by coprology.

Site	No. examined	No. Positive (%)
Abattoir	200	39 (19.5)
Farms	186	27 (14.5)
Total	386	66 (17.1)

χ^2= 1.689, P value=0.1938.

then transferred into clean, plain, labeled 10 ml bottles and placed in a receptacle allowing for clotting. Serum was then carefully extracted using sterile Pasteur pipettes and deposited in clean 5 ml serum vials which were properly labeled and stored at -25°C in a deep freezer, for subsequent serological analysis. About 4 g of faecal samples were collected from farm and abattoir prior to slaughter using a polythene bag worn over the fingers. All the samples were properly labeled and transported to the Parasitic Zoonosis Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for examination.

Enzyme-linked immunosorbent assay (ELISA)

The serum samples were analyzed using indirect ELISA Kit obtained from Bio-X-Diagnostics (Jemelle, Belgium) according to manufacturer's instructions. The format of the ELISA plates was such that alternate columns (1, 3, 5, 7, 9, and 11) were coated with *F. hepatica* antigen and the even columns (2, 4, 6, 8, and 12) contained only the monoclonal antibody. This is a genuine negative control to differentiate specific anti-*F. hepatica* antibodies from non-specific ones.

The test blood sera were diluted 1:100 in the dilution buffer and 100 µl was applied to a coated and uncoated well respecting the following pattern. Positive serum: wells Al and A_2, sample 1: wells B1 and B2, sample 2: wells C1 and C2 and the plate incubated at 21°C for 1 h.

After the incubation period, plates were washed and 100 µl of conjugate, a peroxidase-labeled anti-bovine lgG1 monoclonal antibody, was added to each well and incubated at room temperature for 1 h and was washed again. 100 µl of substrate chromogen tetramethylbenzidine (TMB) was added to each well and incubated for 10 min at 21°C protected from the light and uncovered. 50 µl of the stop solution (1 M phosphoric acid) was added and the plate read with an ELISA plate reader at 450 nm according to manufacturer's instruction. The test was considered valid if the ratio of the mean optical density (OD) values of the positive and negative controls (OD Pc and OD) was greater than 1.26, following the manufacturer's instruction.

Coprological analysis of sample

Detection of eggs was performed using the formol-ether sedimentation technique as described by Arora and Brij, (2010). Four grams of fecal sample was thoroughly mixed in 10 mm of water and strained through two layers of gauze in a funnel. The filtrate was centrifuged at 2,000 rpm for 2 min. The supernatant was discarded and the sediment resuspended in 10 mm of physiological saline. It was again centrifuged and the supernatant was discarded. The sediment was resuspended in 7 mm of formol saline, after which 3 ml of ether was added. The tube was closed with a stopper and shaken vigorously. The stopper was removed and the tube centrifuged at 2,000 rpm for 2 min. Four layers became visible: the top layer of ether, a second layer of plugs of debris, a third layer of formalin and a fourth layer of sediment. The plug of debris was detached from the side of the tube with the aid of a glass rod and the liquid was discarded leaving a small amount of formal saline for resuspending the sediment. A little was transferred to a clean glass slide at a time, covered with a cover slip and examined under the microscope at ×10 magnification to view the eggs. The procedure was repeated until the whole sediment was examined.

Statistical analysis of results

Data collected were reduced to contingency tables and Statistical Package for Social Science (SPSS), version 17.0 (SPSS Chicago Inc) was used to determine Chi-square or Fisher's exact test where appropriate. P values less than 0.05 (P<0.05) were considered to be statistically significant.

RESULTS

Prevalence of *F. gigantica* infection in Zaria by coprology

During the study period, a total of 386 faecal samples were collected, made up of samples collected from 200 individual cattle at slaughter and 186 individual cattle on farms. 39 (19.5%) of the cattle sampled at slaughter and 27 (14.5%) of those sampled in the farms had *F. gigantica* eggs in their faeces with overall prevalence rate of 17.1% (Table 1). Prevalence of *F. gigantica* was thus higher in cattle at slaughter than in cattle on farms, although this was not statistically significant (χ^2=1.689, df=1, P=0.1928).

Of 106 female cattle sampled in the abattoir, 25 (23.6%) had *F. gigantica* eggs, as compared to 14 of the 94 males (14.8%) that were sampled. Of the 106 female cattle sampled in the farms, 16 (15.1%) had *F. gigantica* eggs; while 10 of the 80 (12.5%) sampled males had *F. gigantica* eggs (Table 2). There was no association between sex and infection for cattle sampled at slaughter and on farms (χ^2=2.397, df=1; p=0.1215; χ^2=0.2552, df=1, P=0.6134).

Out of the 173 local breeds sampled in the farms, 27 (15.6%) had *F. gigantica* eggs; while no egg was detected in the samples collected from the 13 foreign breeds (Table 3). Of the 56 young cattle (2 years and below) that were sampled in the farms, 13 (23.2%) had *F. gigantica* eggs in their faeces. Of the 130 adult cattle (>2 years) sampled, 14 (10.8%) had *F. gigantica* eggs (Table 4). There was statistically significant association between the age of cattle and infection (χ^2=4.885; df=1; P=0.0271).

Seroprevalence of *F. gigantica* in Zaria

A total of 23 (11.5%) of the sera collected at slaughter and 5 (2.7%) of those collected from the farms were seropositive for *F. gigantica* infection; giving an overall

Table 2. Sex-specific prevalence of *F. gigantica* at slaughter and on farms in Zaria by coprology.

Sex	Abattoir		Farms	
	No. examined	No. positive (%)	No. examined	No. positive (%)
Male	94	14 (14.9)	80	10 (12.5)
Female	106	25 (23.6)	106	16 (15.1)
Total	200	39 (19.5)	186	26 (13.9)

χ^2= 2.397, P value=0.1215; χ^2= 0.2552, P value=0.16134.

Table 3. Breed-specific prevalence of *F. gigantica* on farms in Zaria by coprology.

Cattle breed	No. examined	No. infected (%)
Local breed	173	27 (15.6)
Foreign breed	13	0 (0)
Total	186	27 (14.5)

Table 4. Age-specific prevalence of *F. gigantica* on farms in Zaria by coprology.

Age	No. examined	No. positive (%)
Young cattle (2 years and below)	56	13 (23.2)
Adult cattle (above 2 years)	130	14 (10.8)
Total	186	27 (14.5)

χ^2= 4.885, P value=0.0271.

seroprevalence rate of 7.3%. Seroprevalence was higher in cattle at slaughter than in cattle on farms. There was statistically significant difference (χ^2=11.12; df=1, P=0.0009) (Table 5).

Of the 106 female cattle sampled in the abattoir, 13 (12.3%) were seropositive for infection; while 10 of the 94 sampled males (10.6%) were seropositive. Of the 106 female cattle sampled in the farms, 3 (2.8%) were seropositive for infection; while 2 of the 80 males (2.5%) were seropositive. There was no significance difference (χ^2=0.1294, df=1, P=0.7191, P=1.0000) (Table 6). Out of the 173 local breeds of cattle examined in the farms, 5 (2.9%) were seropositive, while none of the foreign breeds were infected, giving an overall seroprevalence rate of 2.7% (Table 7).

Of the 56 young cattle (2 years and below) that were sampled in the farms, 3 (5.4%) were seropositive for infection, while 2 (1.5%) of the 130 adult cattle (age above 2 years) were seropositive, giving a seroprevalence rate of 5 (2.7%). Infection rate did not differ significantly (P=0.1616, df=1) between young cattle and adult cattle (Table 8).

Overall prevalence of *F. gigantica* by coprology and serology in Zaria.

The overall prevalence obtained by coprology (17.1%) was higher than the 7.3% obtained using ELISA (Table 9). It was statistically significant (χ^2=11.12, df=1, P=0.000029).

DISCUSSION

Fasciolosis and other helminthes infections have been reported in Northern Nigeria to be wide spread (Elkanah et al., 2006). Studies on the prevalence of fasciolosis due to *F. gigantica* have been carried out in different parts of Nigeria (Ademola, 2003; Kamani et al., 2007). There is however, a general paucity of information regarding seroprevalence of *F. gigantica* at slaughter and on farms in and around Zaria, Nigeria. This study therefore provides current status on the prevalence of *F. gigantica* at slaughter and on farms in the area.

In this study, coprology gave a higher prevalence than ELISA which is a far more sensitive technique. The likely reason for this unusual result is that the antigen and monoclonal antibody used in the ELISA were those of *F. hepatica*. Although they can also detect antibodies to *F. gigantica*, the level of detection will be much lower, since the two species might have marked dissimilarities in their antigenic epitopes. This is in tandem with the findings of Meshgi et al. (2008) in Iran where they found differences in the excretory and somatic antigen of *F. hepatica* and *F. gigantica*.

The prevalence of *F. gigantica* as determined by coprology and serology was higher at slaughter than on

Table 5. Seroprevalence of *Fasciola gigantica* in cattle at slaughter and on farms in Zaria.

Location	No. examined	No. positive (%)
Abattoir	200	23 (11.5)
Farms	186	5 (2.7)
Total	386	28 (7.3)

χ^2= 11.12, P value=0.0009.

Table 6. Sex-specific seroprevalence of *F. gigantica* at slaughter and on farms in Zaria.

Sex	Abattoir		Farms	
	No. examined	No. positive	No. examined	No. positive
Male	94	10 (10.6)	80	2 (2.5)
Female	106	13 (12.3)	106	3 (2.8)
Total	200	23 (11.5)	186	5 (2.7)

χ^2=0.1294, P value=0.7191; P value=1.0000.

Table 7. Breed-specific seroprevalence of *Fasciola gigantica* on farms in Zaria.

Cattle breed	No. examined	No. positive (%)
Local breed	173	5 (2.9)
Foreign	13	0
Total	186	5 (2.9)

Table 8. Age–specific seroprevalence of *Fasciola gigantica* on farms in Zaria.

Age	No. examined	No. positive (%)
Young cattle (2 years and below)	56	3 (5.4)
Adult cattle (above 2 years)	130	2 (1.5)
Total	186	5 (2.7)

P value=0.7191.

Table 9. Comparison of infection detection by coprology and ELISA.

Technique	No. examined	No. positive (%)
Sedimentation	386	66
ELISA	386	28
Total	772	94

χ^2=17.47 P value=0.000029.

farms in Zaria. This could be attributed to the period during which sampling took place, which was the dry season. During this period of the year, the upland pasture is poor both in quantity and quality, as a result of which herdsmen graze their herds along the river banks, where the pasture may be contaminated with metacercariae (the infective stage of *F. gigantica*) which encyst from cercariae released from infected digenea snails which are abundant at the river banks during the dry season. Since most of the slaughtered animals were from the field, a higher prevalence rate may be expected as the on-farm cattle are less exposed to infection. In addition, the on-farm cattle are treated routinely with anthelmintics. Some of the farms sampled in this study for instance, the private and the institutional farms do not allow their cattle to drink water outside, thus reducing their chances of exposure to the infective parasitic stages.

In this study, female cattle had a higher prevalence rate compared to their male counterparts. This trend agrees with the results of studies in Egypt (Dhar et al., 1988; Fatima and Chislti 2008) and Nigeria (Ulayi et al., 2007). Studies as Schillhorn Van Veen (1997), Soulsby (1982)

and Ibrahim et al. (2001) have suggested that there is hormone-controlled relaxation of immunity in female animals during pregnancy and lactation, which increases their susceptibility to infection. Since coprology and the type of ELISA (antibody-detecting) employed in this study cannot differentiate between a new, recent and old infection, the female animals in this study might have contracted the infection during pregnancy and lactation, hence, the observed higher prevalence rate. This higher prevalence in females than males could also be attributed to the fact that more females were sampled than the males.

This study revealed that the local breed had high infection than the foreign breeds on farms. This result is in agreement with the findings of Ulayi et al. (2007). The higher prevalence obtained for the local breed could be due to the fact that cattle of this breed is the most predominant in the study area and very often, the extensive system of management under which they are reared, coupled with the dwindling grazing lands owing to increased food crops farming, compels them to graze in areas that could be heavily infested with the intermediate hosts of the liver fluke in the late dry season when there is acute shortage of feed.

In this study, the prevalence of *F. gigantica* was higher in the young cattle (<2 years old) than adult cattle (>2 years old). The reason for this could be the development of acquired immunity in the older animals which results in resistance, as opined by earlier investigators (Phiri et al., 2005).

The prevalence of 17.1% obtained in this study is much lower than the 71.1% reported by Fabiyi and Adeleye (1982) on the Jos Plateau; the 65.4% reported by Schillhorn Van Veen (1980) and 52.1% reported by Olusegun-Joseph et al. (2000) in Zaria. This could suggest that herdsmen and herd owners are now much aware and seek for anthelmintic intervention. On the other hand, the prevalence rate obtained in this study was higher than the 10.51 and 10.0% reported, respectively by Ekwunife and Eneanya (2006) and Ngwu et al. (2004) both in South Eastern Nigeria. This may be because animals in the South Eastern region of Nigeria are not subjected to dry season fadama grazing since the upland pasture is available all year round. Also, since most of the animals slaughtered in the region are from the northern part of Nigeria, it is not unlikely that only very healthy cattle are transported down South for sale and slaughtered.

Conclusion

This study has, through coprological and serological means, established the current prevalence (17.1 and 7.3%) of *F. gigantica* at slaughter and on farms in Zaria, Kaduna State. Coprology gave a higher prevalence than the more sensitive ELISA technique. Hence, proper meat inspection at abattoirs and public health enlightenment on the disease should be intensified.

REFERENCES

Ademola I (2003). Meteorological changes and their influence on the epidemiology of fasciolosis in food animals slaughtered in Oyo State. Nig. Vet. J. 24:35-38

Arora DR, Brij BA (2010). Medical Parasitology. 3rd edition, CBS Publishers and Distributors Pvt Ltd, pp. 158-159.

Ashrafi K, Valero MA, Panova M, Periago MV, Massoud J, Mas-Coma S (2006). Phenotypic analysis of adults of *Fasciola hepatica, Fasciola gigantica* and intermediate forms for the endemic region of Gilan, Iran. Parasitol. Int. 55:249-260

Burke J (1939). Case report: Growing South African Nguni goat's kids on the annals of Borno eastern Nigeria. Nigeria National Resource. Accessed from www.gooogle.com on 16 /03 /2012

Dhar DN, Sharma DK, Raina L (1988). Fasciolosis in animals-Kashmir Valley. J. Vet. Parasitol. 2:31-35.

Dixon J, Gulliver A, Gibbon D (2001). Farming systems and poverty: Improving farmers livelihoods in a changing world. Rome and Washington D.C: FAO and World Bank.

Ekwunife CA, Eneanya CI (2006). *Fasciola gigantica* in Onitsha and environs. Ani. Res. Int. 3(2):448-450

Elkanah SO, Oneyeke JOA, Anyanwu GI (2006). Prevalence and clinical significance of *Bancroftian filariasis* in Lau L.G.A, Taraba State. 30th Annual Conference of the Nigeria Society of Parasitology 22nd-25th November 2006, Jos. 94:18

Fabiyi JP, Adeleye GA (1982). Bovine fasciolosis on the Jos Plateau, north Nigeria with particular reference to economic importance: Bul. Anim. Health Prod. Afr. 30:41-43.

Fatima MA, Chislti M Z (2008). Report of *Fasciola gigantica* Cobboid, Parasitic trematode in ruminant. Proceedings of 2nd Jammu Kashmir Science Conference. 489-494

Garcia HH, Moro PL, Schantz PM (2007). Zoonotic helminth infections of humans: echinococcosis, cysticercosis and fasciolosis. Infect. Dis. 20:489-494.

Ibrahim MA, Patrick VK, Alhaji AM (2001). An analysis of the effect of socio–economic status of farmers and season variation on the marketing of pregnant does for meat in Borno State. Agro-Satellite 1:2-12.

Kaduna State Government (KDSG). (2008). Kaduna State Achievement In: Data on Estimated Annual Animal population and Fish Production Investment Opportunities in Kaduna State. pp. 16-18

Kamani J, Yidawi JP, Buba S, Dahiru JJ, Bukar MDY (2007). Seasonal incidence of ovine fasciolosis in Maiduguri, Borno State, Nigeria. An abattoir Survey. In: Proceedings of the 44th Annual congress of the Nig. Vet. Med. Assoc. 27:222-225.

Keiser J, Utzinger J (2005). Emerging food borne trematodiasis. Emerg. Inf. Dis. 11:1507-1514.

Keyyu ID, Monrad J, Kyvsgard NL, Kassuku AA (2005). Epidemiology of *Fasciola gigantica and Amphistomes* in cattle on traditional, small-scale dairy and large-scale dairy farms in the Southern highlands of Tanzania. Trop. Ani. Health Prod. 37:303-314

Mas-Coma S, Barques MD, Valero MA (2005). "Fasciolosis and other plant–borne trematode zoonosis". Int. J. Parasitol. 35 (11):1255-78.

Mas-Coma S (1997). Secondary reservoir role of domestic animals other than sheep and cattle in fasciolosis transmission in northern Bolivian, Altiplano. Res. Rev. Parasitol. 57:39-46.

Meshgi B, Eslami A, Hemmatzadeh F (2008). Detection of somatic and excretory/secretory antigens of *Fasciola hepatica* and *Fasciola gigantica* using SDS-PAGE. Ir. J. Vet. Res. 9:22

Ngwu GI, Ohaegbula ABO, Okafor FC (2004). Prevalence of *Fasciola gigantica*, *Cysticercus bovis* and some other disease conditions of cattle slaughtered in Nsukka urban abattoir. Anim. Res. Int. 1(1):7-11.

Olumide O, Mpoko B (2001). Post Harvest Technologies in Nigeria's livestock Industry; challenges and capacities. A presentation at the GFAR-GI PHT workshop, Estebe, Uganda, pp. 17-21.

Olusegun-Joseph TS, Doherty VF, Edeghagba BO (2000). Prevalence of fasciolosis in cattle slaughtered at Zaria abattoir. Accessible from www.jenvscs.com on 15/07/2012

Pace JE, Wakeman DC (2003). Determining the age of cattle by their teeth. Institute of Food and Agricultural Sciences, University of Florida, Gainesville, 32611

Phiri AM, Phiri IK, Sikasunge CS, Moirad I (2005). Prevalence of fasciolosis in Zambian cattle observed at selected abattoirs with emphasis on age, sex and origin. J. Vet. Med. 52:414-416.

Schillhorn Van Veen TW (1997). Sense or nonsense? Traditional methods of animal parasitic disease control. *Vet. Parasitol.* 71:177-194

Soulsby FJL (1982). Helminths, Arthropoda and Protozoa of domestic animals. 7th edition, Bailliere and Tindall, pp 44-51

Spithil TW, Smooker PM, Copeman DB (1999). *Fasciola gigantica:* epidemiology, control, immunity and molecular biology. *Journal Parasitology*, Wallingford, Oxon. UK, CABI Publication, pp 465-525

Taira N, Yoshifuji H, Boray JC (1997). Zoonotic potential of infection with *Fasciola* spp by consumption of freshly prepaid raw liver containing immature flukes. J. Parasitol. (2)77:775 – 779.

Tewe OO (1997). Sustainability and Development: Paradigms from Nigerian's livestock industry: Inaugural lecture series. University of Ibadan Press, Ibadan, pp 4

Ulayi BM, Umaru-Sule B, Adamu S (2007). Prevalence of *Dicrocoelium hospes* and *Fasciola gigantica* in cattle at slaughter in Zaria. Nig. J. Ani. Vet. Adv. 6(9):1112-1115.

Vassilev GD, Jooste R (1991). Production losses and control of fasciolosis in cattle in Zimbabwe. Zimb. Vet. J. 22:45-56

World Bank (2006). World Bank list of economic (on line) July 2006. Accessible from www.iscb.org on 20/03/2012

academicJournals

Vol. 6(2), pp. 92-100, February 2014
DOI: 10.5897/JPHE2013.0594
ISSN 2006-9723 ©2014 Academic Journals
http://www.academicjournals.org/JPHE

Journal of Public Health and Epidemiology

Full Length Research Paper

Risk factors of osteoporosis among adults in Ethiopia, the case of Tigrai region: A case control study

Alemayehu B. Kahsay[1]*, Fikre E. Gashe[2], Zenebe G. Debessai[3], Abebe Tegegne[4] and Tsigabu-Bezabih[5]

[1]Department of Nursing, College of Health Sciences, Mekelle University, Ethiopia.
[2]School of Public Health, College of Health Sciences, Addis Ababa University, Ethiopia.
[3]Department of Economics, College of Business and Economics, Mekelle University, Ethiopia.
[4]Department of Medicine, College of Health Sciences, Mekelle University, Ethiopia.
[5]Department of medicine, College of Health Sciences, Mekelle University, Ethiopia.

Accepted 20 January, 2013

Osteoporosis is one of the most common public health problems affecting adults and elderlies in developing countries. This study aims to examine the potential risk factors of osteoporosis among adults in Tigrai, Northern Ethiopia. This is a case-control study. Cases and controls were assigned by two radiologists after radiographic examination of the wrist. An interviewer administered questionnaire was used and information on demographic characteristics and potential risk factors were collected. Data was processed and analyzed using statistical package for social sciences (SPSS) version 19. Binary logistic regression was used to control confounders. A total of 130 osteoporotic cases and 266 controls participated in the study. The mean ± standard deviation (SD) age of cases and controls were 60.9 ± 10.1 and 46.9 ± 8.7 years, respectively. The multivariate analysis adjusted for age and sex showed that rural residents were 1.93 times more likely to develop osteoporosis than the urban inhabitants with an adjusted odds ratio (AOR) 1.93 (95% CI, 1.11, 3.36). The strongest association was also found when the work of the respondent involves decreased physical activity with AOR 3.53 (95% CI, 1.98 and 6.30). Furthermore, milk consumption greater than four times a week and smoking showed a significant association with the AOR 0.33 (95% CI, 0.19 and 0.58), and AOR 0.17 (95% CI, 0.05 and 0.58), respectively. Residing in the rural setting and smoking were positively associated with osteoporosis. In contrast, milk intake greater than four times a week, and when work involves vigorous exercise, appeared to be associated with a reduced risk of osteoporosis. Therefore, the findings from the study suggest the need for changes of lifestyle that includes encouraging adults to stop smoking, engage in vigorous physical activities aging and adequate dietary intake including milk. Strategies to identify and manage osteoporosis in the primary health care setting need to be established.

Key words: Case control, osteoporosis, risk factors, bone mineral density, DR-F digital radiography, Ethiopia.

INTRODUCTION

The patient profile in health institutions all over the developing world is changing. Non-communicable diseases (NCDs) have already established themselves as the predominant cause of disease and death in many middle-income countries including Ethiopia (WHO 2010).

Osteoporosis is one of the non-communicable diseases defined as a skeletal disorder characterized by low bone density and micro-architectural deterioration of bony tissue

*Corresponding author. E-mail: alemayehub35@gmail.com.

(Conference, 1991). It is one of the most common public health problems in adults and older people worldwide (Ross 1996). It is a common silent disease affecting both women and men over the age of 50 years. A significant number of clinical symptoms associated with osteoporosis become evident only after the occurrence of hip, vertebral, or wrist fractures. These fractures lead to many problems such as mortality, morbidity, and economic problems to individuals and the society (Bonjour 2001).

Previous studies have suggested that osteoporosis is a condition that can be prevented and treated if diagnosed early and accurately (Devine 1997). Unfortunately, it is often un-diagnosed until a fracture occurs; making the number of people who are screened for the disease very low. The correction of lifestyle factors including cigarette smoking and excessive intake of alcohol in preventing osteoporosis is a significant strategy that may be less costly when compared with medical and pharmacological interventions (Sharkey et al., 2000). Low bone mineral density in the adults can result from either low peak bone mass or accelerated bone loss, or a combination of the two. Nutritional factors play a role in both the attainment of peak bone mass and in the rate of age-related bone loss. Adequate calcium intake particularly milk and regular weight-bearing exercises are recommended for maintaining bone health and preventing excessive bone mass loss with advancing age (Eastell and Lambert 2002).

The exact prevalence of osteoporosis in developing countries is not well documented. However, according to the extrapolation of the prevalence of osteoporosis from developed countries and regions it is estimated that there are over 7.3 million cases of osteoporosis in Ethiopia (Census 2004). Despite this fact there has been a great interest in studying osteoporosis in developing countries. Currently, there has been no published study that examined the risk factors of osteoporosis in Ethiopian men and women. Hence, we undertook this study to determine the potential risk factors of osteoporosis in the Ethiopian context.

METHODOLOGY

Study design

This is a hospital based, case control study with descriptive and analytic elements.

Study setting

The study was conducted in Tigrai region, Ethiopia, with urban inhabitants making 19.5% of the population. The region is predominantly Tigrai-Tigrinya people at 96.6% of the population, and 95.6% of the population are followers of the Ethiopian Orthodox. Public zonal hospitals are 12 in number and many other health centers are providing health services to the community. Currently, there is one specialized training hospital which provides advanced services to the community where the study is conducted and the digital x-ray machine is planted (CSA 2007). The study was conducted from May, 2012 up to October, 2012 on apparently healthy men and women aged 40 and above (the recommended age strata) who visited the regional referral and training hospital for any type of care where the Digital X-ray machine is situated were the source population.

Sample size

The sample size was calculated using two proportion formula for unmatched case control design. Assuming the proportion of cases with exposure rate of 27.51% (main exposure variable age people aged 50 and above) from studies conducted elsewhere (Sharma et al., 2006) and the odds of exposure (OR = 2.15) higher than controls, with 95% confidence interval and 80% power with case to control ratio of 1 to 2, a total of 133 cases and 266 controls were required.

Sampling technique

First, all relatively healthy adult men and women aged 40 and above who came to the referral hospital outpatient department during the data collection period were purposely approached to participate in the study. Second, radiographic examination of the wrist was conducted consecutively on those who volunteered to participate until the required sample size was obtained, for cases and controls, respectively. Pregnant women, chronically debilitated patients (with any known or unknown diseases), those with known diseases that affect bone metabolism (diabetes, hypertension, cancer, kidney diseases, etc.), and severely deformed patients (Kyphosis, anomalies of the anterior arm, etc.) were excluded from the study.

Ascertainment of cases and controls

Cases were osteoporotic patients that were screened by two senior radiologists after undergoing a digital X-ray scanning onto their non-dominant wrist, while the controls were all participants without osteoporosis that were identified as osteoporosis free after undergoing the same procedure. Each X-ray result was visually inspected from the computer and read by the two radiologists independently; cases and controls were assigned when both agree. The X-ray results were discarded when they were considered to have inappropriate or poor quality by the two radiographers. X-ray results on which the radiologists did not agree were excluded from the analysis in this study. The percentage of agreement was 97.6%.

Data collection method

Data were collected using a pre-tested structured interviewer-administered World Health Organization (WHO) steps questionnaire for the identification of chronic disease risk factors (Strong and Bonita, 2002). Information on selected socio-demographic characteristics and lifestyle behaviors including physical activity, and anthropometric measurements of weight and height was obtained. Weight was measured with participants standing without shoes and wearing light clothing. Height was measured with participants standing upright and the head in the Frankfurt plane position. Height was recorded to the nearest 0.5 cm, and weight was recorded to the nearest 100 g. Body-mass index (BMI) was calculated as weight in kilograms over height in meters squared [weight (kg) / (height (m))^2]. Each participant was asked to provide information on current smoking habits and alcohol intake in average numbers of drinks per day at present. Coffee, milk intake, fruit and vegetable consumption

were obtained. Medical history including personal and family history of fracture and previous use of steroids (predinsolone) were also acquired.

In this study, excess alcohol consumption is defined as an intake of any liquor (one bottle of beer, a glass of wine, spirits, fermented cider, Tella or Tej (Local beer)) more than 2 drinks a day. Consumption of coffee greater than 2 cups (70 ml) a day was also considered as excess. Furthermore, drinking less than five glasses of milk per week (Heidi et al., 2003) and consuming fruits (orange, banana, etc.) and vegetables (Cabbage, spinach etc.) less than 5 times a week was considered as not adequate. In addition, when the work of the participant involves vigorous-intensity activity that causes large increases in breathing or heart rate like (carrying or lifting heavy loads, digging or construction work etc.) for at least 10 min continuously was considered as sufficient (WHO, 2011). The cutoff points are set after calculating the mean score of the continuous variables.

Data analysis

Data were entered and analyzed using statistical package for social sciences (SPSS) statistical software Version 19. Descriptive summary measures were used to depict levels of exposure in cases and controls. The associations between the exposure and outcome variables were determined using χ^2 statistics and backward stepwise binary logistic regression analysis to control confounders. Independent variables which have a $P < 0.3$ to the dependent variable in bivariate regression models were exported to a multiple regression model for sex and age adjustment. The major assumptions of logistic regression analysis (absence of multicollinearity and interaction among independent variables) were checked to be satisfied. The fitness of logistic regression models was assessed using the Hosmer-Lemeshow statistic and pseudo R squared values (Cox & Snell and Nagelkerke R squares). Crude odds ratios (COR) and adjusted odds ratios (AOR) along with their 95% confidence intervals (CI) were used to measure the degree of association and to test its statistical significance.

Ethical considerations

The study was conducted in line with national and international ethical recommendations to conduct biomedical research involving human subjects. Ethical clearance was secured from institutional review board of College of Health Sciences Addis Ababa University and an approval and letter of collaboration were solicited from Tigrai Regional Health Bureau. Informed written consent was taken from the study subjects after the objective of the study were briefed. Radiation safety procedures were in place. Osteoporosis related education was given to all study subjects.

RESULTS

Background information of the study subjects

A total of 396 participants- 130 osteoporosis cases and 266 healthy controls participated in the study. Among which majority 83 (63.8%) of the cases and 135 (50.8%) of the controls were females, 75 (57.7%) and 134 (50.4%) of the controls were rural dwellers. Sixty seven (51.5%) of the cases and majority (89.5%) of controls were in the age group of 40 to 50 years. Furthermore, 23 (17.7%) of the cases and 39 (14.7%) of the controls had a family history of fracture after a minor bump or fall. There was a significant difference in age and gender of the participants with the p-value of 0.001 and 0.01, respectively (Table 1).

Lifestyle characteristics of the study subjects

Among the lifestyle variables the number of current smokers was few in both the cases and the controls. Alcohol intake ≤ 2 drinks per day was observed in 87 (66.9%) of the cases and in the majority (72.2%) of the controls. In addition, this study revealed that 101 (77.7%) of the cases and 204 (76.7%) controls consume coffee ≤ 2 cups a day. It was also observed that 85 (65.4%) of the cases and 90 (33.8%) of the control drink ≤ 4 cups of milk per week. Furthermore, 86 (66.2%) and 29 (22.3%) of the cases and 194 (72.9%) and 75 (28.2%) of the controls consume fruits and vegetables ≤ 4 times a week, respectively. The mean (SD) BMI of the cases and controls was almost similar. In addition, a significant difference was found in the weight of the study participants with a p-value of 0.004 (Table 2).

Bivariate and multivariate analysis of the study subjects

The multivariate analysis adjusted for age and sex showed that rural residents were 1.93 times more likely to develop osteoporosis than the urban inhabitants with an adjusted odds ratio (AOR) 1.93 (95% CI, 1.11, 3.36). The strongest association was also found when the work of the respondent involves decreased physical activity with AOR 3.53 (95% CI, 1.98, 6.30). Furthermore, milk consumption greater than four times a week and smoking showed a significant association with the AOR 0.33 (95% CI, 0.19, 0.58), and AOR 0.17 (95% CI, 0.05, 0.58), respectively. Whereas, alcohol intake, coffee intake, fruit and vegetable consumption, steroid intake, BMI, occupation, education, monthly income, personal and family history of fractures were not associated with osteoporosis (Table 3).

DISCUSSION

We used unmatched case control study which is the first of its kind to identify risk factors of osteoporosis in the Ethiopian context. This study confirms some previously identified risk factors of osteoporosis such as consumption of milk, physical activity, current smoking status and residing in the rural. Normally we could expect that a

Table 1. Socio-demographic and fracture history related variables of the respondents by their osteoporosis status, Tigrai, Northern Ethiopia, 2012 (n=396).

Characteristics (n=396)	Osteoporosis status		P-value
	Cases n (%)	Controls n (%)	
Age	Mean (SD) =60. 9 (10.1) Median =58 IQR=15. 25	Mean (SD) =46. 9 (8.7) Median =45 IQR=10. 25	0.001
Sex			
Male	47 (36.2)	131 (49.2)	0.01
Female	83 (63.8)	135 (50.8)	
Residence			
Urban	55(42.3)	132 (49.6)	0.17
Rural	75(57.7)	134 (50.4)	
Ethnicity			
Tigrian	122 (93.8)	248 (93.2)	0.79
Non Tigrian	8 (6.2)	18 (6.8)	
Marital status			
Currently married	120 (92.3)	252 (94.7)	0.21
Never married /Separated/ Divorced	10 (7.7)	14(5.3)	
Educational status			
No formal education	44 (33.8)	70 (26.3)	0.09
Primary education	55 (42.3)	111 (41.7)	
Secondary education	17 (13.1)	50 (18.8)	
College/ University	14 (10.8)	35 (13.2)	
Occupational status			
Employed (Government, NGO, private)	99 (76.2)	181 (68)	0.001
Others (Traders, Farmers, retired)	31 (23.8)	85 (32)	
Monthly income*			
< 1000	75 (57.7)	117 (44.0)	0.14
1001-2000	29 (22.3)	81 (30.5)	
2001-3000	14 (10.8)	33 (12.4)	
>3000	12 (9.2)	35 (13.1)	
History of parents' fracture			
Yes	23 (17.7)	39 (14.7)	0.44
No	107 (82.3)	227 (85.3)	
Personal history fracture			
Yes	17 (13.1)	32 (12.0)	0.76
No	113 (86.9)	234 (88.0)	
Body Mass Index (BMI)			
≤18.5	8 (6.3)	20 (7.6)	0.66
>18.5	118 (93.7)	244 (92.4)	

*ETB = Ethiopian Birr.

Table 2. Lifestyle, anthropometric, nutrition and drug related variables of the respondents by their osteoporosis status, Tigrai, Northern Ethiopia, 2012 (n=396).

Characteristics (n=396)	Osteoporosis status	
	Cases n (%)	Controls n (%)
Smoking status (Current)		
Yes	7 (5.4)	8 (3.0)
No	123 (94.6)	258 (97.0)
Alcohol frequency		
≤2 drinks a day	87 (66.9)	192 (72.2)
>2 drinks a day	43 (33.1)	74 (27.8)
Coffee intake		
≤ 2cups a day	101 (33.1)	204 (66.9)
>2 cups a day	29 (31.9)	62 (68.1)
Milk intake		
≤ 4 Cups a week	85 (65.4)	90 (33.8)
> 4 Cups a week	45 (34.6)	176 (66.2)
Fruit intake		
≤ 4 Times a week	86 (66.2)	194 (72.9)
> 4 Times a week	44 (33.8)	72 (27.1)
Vegetable intake		
≤ 4 Times a week	29 (22.3)	75 (28.2)
> 4 Times a week	101 (77.7)	191 (71.8)
Used steroids for three months		
Yes	11 (8.5)	19 (7.1)
No	119 (91.5)	247 (92.9)
Work involves vigorous exercise		
Yes	42 (32.3)	149 (56.0)
No	88 (67.7)	117 (44.0)

sedentary lifestyle which is more common in the urban population than is in the rural contributes to the development of osteoporosis, but, in this study rural residents were 1.93 times more likely to develop osteoporosis AOR 1.93 (95% CI: 1.11, 3.37), which might be related to the high prevalence of malnutrition in rural Tigrai.

In this study, there was no significant difference between the social and demographic variables- education, monthly income, and family history of fracture and osteoporosis. This was discordant with findings in England, Turkey, Sweden, and Italy which revealed that osteoporosis was associated with poor education, family history of fracture, and low monthly income (Hui et al., 1988; Cankurtaran and Yavuz 2005; Olszynski et al., 2004; Biino et al., 2011).

Several studies in the USA reported positive associations between earlier reported milk consumption or calcium intake and BMD, but several other studies did not find this association. The milk's main selling point is calcium, and milk-drinking is touted for building strong bones in children and preventing osteoporosis in older per sons (Heidi et al., 2003; Katherine 2003; Nguyen et al., 2000). Our study indicated that milk consumption up to four times a week has a protective effect of osteoporosis with the AOR 0.33 (95% CI: 0.19, 0.57) and this result is consistent with many other studies from India, UK, Iran, and USA (Heidi et al., 2003; Nguyen et al., 2000; Ruchira et al., 2010; Lunt et al., 2001).

Evidence from the UK, USA, Australia, and India showed that exercise may help build and maintain bone density at any age. Studies have shown bone density increase by doing regular resistance exercises, such

Table 3. Multivariate analysis (final model) of the risk factors of osteoporosis among adults in Tigrai, Northern Ethiopia, December, 2012, (n= 396).

Risk factors (n=396)	COR (95%, CI)	AOR (95%, CI)
Residence		
Urban	1	1
Rural	1.34 (0.88, 2.05)	1.93 (1.11, 3.36)*
Educational status		
No formal education	1	1
Primary education	0.79(0.48,1.30)	1.62 (0.82,3.20)
Secondary education	0.54 (0.28, 1.05)	1.38 (0.57,3.31)
College/ University	0.64(0.31, 1.32)	1.24 (0.47,3.27)
Occupational status		
Employed (Government, NGO, private)	1	1
Others (Traders, Farmers, retired)	7.50 (4.56, 12.20)*	1.26 (0.64,2.50)
Monthly income*		
< 1000	1	1
1001-2000	0.56 (0.33, 0.93)*	0.94 (0.48,1.82)
2001-3000	0.66 (0.33, 1.32)	1.69 (0.73, 3.90)
>3000	0.54 (0.26, 1.09)	1.78 (0.73, 4.35)
History of parents' fracture		
Yes	1	1
No	0.79 (0.46, 1.40)	0.68 (0.34,1.36)
Personal history of fracture		
Yes	1	1
No	0.90 (0.49,1.71)	0.85 (0.39, 1.83)
Used steroids for three months		
Yes	1	1
No	0.83 (0.38, 1.81)	0.66 (0.27,1.64)
Body Mass Index (BMI)		
≤18.5	1	1
>18.5	1.21 (0.52, 2.83)	1.83 (0.65,5.21)
Smoking status (Current)		
Yes	1	1
No	0.55 (0.19, 1.54)	0.17 (0.05, 0.58)*
Alcohol frequency		
≤2 drinks a day	1	1
>2 drinks a day	1.28 (0.82,2.01)	1.47 (0.83, 2.59)
Coffee intake		
≤ 2cups a day	1	1
>2 cups a day	0.95 (0.57,1.56)	1.13 (0.60,2.11)
Milk intake		
≤ 4 Times a week	1	1

Table 3. Contd.

> 4 Times a week	0.27 (0.17, 0.42)*	0.33 (0.19, 0.58)*
Fruit intake		
≤ 4 Times a week	1	1
> 4 Times a week	1.38 (0.88, 2.17)	1.14 (0.64,2.03)
Vegetable intake		
≤ 4 Times a week	1	1
> 4 Times a week	1.37 (0.84, 2.24)	1.54 (0.82, 2.89)
Work involves vigorous intensity activity		
Yes	1	1
No	2.67 (1.72, 4.14)*	3.53 (1.98, 6.30)*

All variables adjusted for age and sex, COR= Crude odds ratio (Unadjusted odds ratio), AOR = Adjusted odds ratio, *****Significant association.

as lifting weights two or three times a week. This type of weight-bearing exercise appears to stimulate bone formation and retain calcium in the bones that are bearing the load. The force of muscles pulling against bones stimulates this bone-building process. So any exercise that places force on a bone will strengthen that bone (Jha et al., 2010; Todd and Robinson 2003; Mozaffer et al., 2008; Forwood and Larsen 2000). The result of our study also supports these evidences; when the work of the participants does not include vigorous intensive exercise they are at risk of developing osteoporosis with an adjusted odds of 3.53 (95% CI: 1.98, 6.29) compared to their counterparts.

Literatures from UK, Australia, USA and Europe also indicated that cigarette smoking is a risk factor for the development of osteoporosis; the reason is that nicotine and toxins in cigarettes affect bone health from many angles. Cigarette smoke generates huge amounts of free radicals molecules that attack and overwhelm the body's natural defenses. The result is a chain-reaction of damage throughout the body, including cells, organs, and hormones involved in keeping bones healthy (Law and Hackshaw 1997; Nguyen et al., 1994; Lunt et al., 2001; Daniel 1976; Williams et al., 2005.). Though the number of smokers is small, our study also revealed that non-smokers are 0.17 times less likely to develop osteoporosis than the smokers with the AOR 0.17 (95% CI: 0.05, 0.58).

Moderate alcohol consumption is not harmful to bone health, but heavy drinking is a health risk for many reasons, including the effects on bones. Researches from Austria and Europe showed that chronic heavy alcohol use, especially during adolescence and young adult years by interfering calcium absorption, can dramatically affect bone health and increase the risk of osteoporosis later in life (Malik et al., 2009; De Vernejoul et al., 1983). However, this study observed alcohol intake had no significant association with the development of osteoporosis; this may be attributed to the fact that many people hesitate to tell the truth about the frequency and amount of alcohol intake due to high religiosity in the study area. Furthermore, it may be due to lack of statistical power of the study.

A study in USA implied that caffeine increases urinary calcium output and has been implicated as a risk factor for osteoporosis that leads to hip fracture (Douglas 1990), while some other researches in Sri Lanka, California, and Minnesota indicate that the effect of caffeine on bone mineral density is because of the interaction between cigarette and alcohol abuse (Glynn et al., 1995; Heaney 2002; Cooper et al., 1992). This study however revealed no significant association between caffeine intake and osteoporosis. Studies from the USA and Korea confirm that a high BMI (above 30 kg/m^2) has a protective effect for both men and women, in contrast low BMI less than 19 kg/m^2 can lead to osteoporosis (Wardlaw 1996; Barrera et al., 2004; Kenny et al., 2000; Shin et al., 2004). While our study failed to show the association between BMI and osteoporosis. A similar reason can be given that the difference in mean body weight and mean height of the cases and the controls was almost similar.

A study in the USA has linked higher intakes of fruits and vegetables with better bone health. Though it is not clear why fruits and vegetables promote healthy bones, many scientists believe that fruits and vegetables contain certain nutrients such as calcium, magnesium, potassium, vitamin C, vitamin K or a combination of these vitamins that are beneficial for bones. Some studies

indicate that the higher fruit intake was found to be significantly associated with higher BMD in both sexes. High vegetable consumption, however, did not positively impact BMD Zalloua et al., 2007. Further UK and USA studies also indicate consumption of more fresh fruits and vegetables is unlikely to be detrimental to bone health and may be beneficial (Prentice 2004; Vatanparast et al., 2005). However, the current study did not show an association between fruit and vegetable consumption and osteoporosis, under reporting of dietary intake by the participants might explain why this difference was not apparent in the study.

Corticosteroids have several adverse effects on bone metabolism. Direct inhibition of osteoblast function, direct enhancement of bone resorption, inhibition of gastrointestinal calcium absorption, increase in urinary calcium loss, and inhibition of gonadal hormones mainly affect the trabecular bone. In our study, corticosteroid intake did not show a significant association, this could be because of the problem of reporting the exact type of medication (Walsh et al., 2002; Sinigaglia et al., 2000; IP et al., 1994) Studies from UK and USA confirmed that family and personal history of osteoporosis or osteoporotic fracture is a risk factor for osteoporosis (Kanis et al., 2004; Ralston and de Crombrugghe, 2006), while our study found a lack of association between family and personal history of fracture among the cases and controls. The reason for the lack of association is not clear.

LIMITATIONS OF THE STUDY

Though the study is the first of its kind in the region the results have to be interpreted within the context of potential limitations. First, the population is composed of volunteers not randomly selected who do not ensure representation of the general population; therefore, this may introduce selection bias. Second, because we excluded individuals with diseases deemed to interfere with bone metabolism, the risk factors of osteoporosis reported here could be an underestimate of the risk factors in the general population. Third, we have used mean scores for milk and fruit and vegetable consumption for comparison with other literatures; this should have to be interpreted cautiously. Fourth, we did not use the gold standard diagnostic DEXA to assign cases and controls that might lead to inflate the false negative rate. However, this research did not intend to establish the prevalence of osteoporosis but to identify associations with low bone mass (osteoporosis). On the other hand, our study is the first to report risk factors of osteoporosis in men and women in Ethiopia, while many of the published studies in the literature come from American, European and Asian countries.

Conclusion

Residing in the rural setting and smoking was positively associated with osteoporosis. In contrast, milk intake greater than four times a week, and when work involves vigorous exercise appeared to be associated with a reduced risk of osteoporosis. Therefore, the findings from the study suggest the need for changes of lifestyle that includes encouraging adults to stop smoking, engage in vigorous physical activities for active aging and adequate dietary intake including milk. Strategies to identify and manage osteoporosis in the primary health care setting to reach the rural people need to be established. Large community-based studies using the gold standard bone density scanner for a better knowledge of the risk factors of osteoporosis for early prevention and treatment is needed.

ACKNOWLEDGEMENTS

We express our gratitude to the study participants, data collectors and supervisors for their commitment throughout the study.

REFERENCES

Barrera G, Bunout D, Gattás V, de la Maza MP, Leiva L, Hirsch S (2004). A high body mass index protects against femoral neck osteoporosis in healthy elderly subjects. Nutrition 20(9):769-771.

Biino G, Casula L, de Terlizzi F, Adamo M, Vaccargiu S, Francavilla M, Loi D, Casti A, Atzori M, Pirastu M (2011). Epidemiology of osteoporosis in an isolated Sardinian population by using quantitative ultrasound.". Am. J. Epidemiol. 174 (4):432-9.

Bonjour JP, Chevalley T, Ammann P, Slosman D, Rizzoli R (2001). Gain in bone mineral mass in prepubertal girls 3.5 years after discontinuation of calcium supplementation: A follow-up study. Lancet. 358:1208-1212.

Cankurtaran M, Yavuz B B (2005). General characteristics, clinical features and related factors of osteoporosis in a group of elderly Turkish men." Aging Clin. Exp. Res. 17(2):108-15.

Census Bureau US (2004). Statistics by Country for Osteoporosis. International Data Base.

Conference, CD (1991). Diagnosis, prophylaxis and treatment of Osteoporosis. . Am. J. Med. 90: p. 107-10.

Cooper C, Atkinson EJ, Wahner HW, O'Fallon WM, Riggs BL, Judd HL, Melton LJ 3rd (1992). Is caffeine consumption a risk factor for osteoporosis? J. Bone Miner Res. 7(4):465-71.

CSA (2006). The Census by the Central Statistical Agency of Ethiopia. 2007.

Daniel HW (1976) Osteoporosis of the Slender SmokerVertebral Compression Fractures and Loss of Metacarpal Cortex in Relation to Postmenopausal Cigarette Smoking and Lack of Obesity. Arch. Intern. Med. 136(3):298-304.

de Vernejoul MC, Bielakoff J, Herve M, Gueris J, Hott M, Modrowski D, Kuntz D, Miravet L, Ryckewaert A (1983). Evidence for defective osteoblastic function. A role of alcohol and tobacco consumption in osteoporosis in middle-aged men. Clin. Orthop. Relat. Res. 179:107-115.

Devine A, Dick IM, Heal SJ, Criddle RA, Prince RL (1997). A 4-year follow-up study of the effects of calcium supplementation on bone density in elderly postmenopausal women. Osteoporos. Int. 7:23-28.

Eastell R, Lambert H (2002).Strategies for skeletal health in the elderly. Proc. Nutr. Soc. 61(2):173-80.
Forwood MR, Larsen JA (2000). Exercise recommendations for osteoporosis 'A position statement of the Australian and New Zealand Bone and Mineral Society. Aust. Family Physician 29(8):761-764.
Glynn NW, Meilahn EN, Charron M, Anderson SJ, Kuller LH, Cauley JA (1995). Determinants of bone mineral density in older men. J. Bone Miner. Res. 10(11):1769-77
Heaney RP (2002). Effects of caffeine on bone and the calcium economy. Food Chem. Toxicol. 40(9):1263-1270.
Hingorjo MR, Syed S, Qureshi MA (2008). Role of exercise in osteoporosis prevention - Current concepts. J. Pak. Med. Assoc. 58(2): 78-81.
Hui SL, Slemenda CW, Johnston CC (1988). Age and bone mass as predictors of fracture in a prospective study. J. Clin. Invest. 81 (6):1804-1809.
Ip M, Lam K, Yam L, Kung A, Ng M (1994).Decreased bone mineral density in premenopausal asthma patients receiving long-term inhaled steroids. Chest 105(6):1722-1727.
Kalkwarf HJ, Khoury JC, Lanphear BP (2003). Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. Am. J. Clin. Nutr. 77(1):65.
Kanis JA, Johansson H, Oden A, Johnell O, De Laet C, Eisman JA, McCloskey EV, Mellstrom D, Melton LJ 3rd, Pols HA, Reeve J, Silman AJ, Tenenhouse A (2004). A family history of fracture and fracture risk: a meta-analysis. Bone 35(5):1029-37.
Katherine LT (2003). Does milk intake in childhood protect against later osteoporosis? Am. J. Clin. Nutr. 77: p. 10-1.
Kenny AM, Prestwood KM, Marcello KM, Raisz LG (2000)."Determinants of bone density in healthy older men with low testosterone levels." J. Gerontol. A Biol. Sci. Med. Sci. 55(9):492-7
Kiel DP, Felson DT, Hannan MT, Anderson JJ, Wilson PW (1990). Caffeine and the risk of hip fracture: the Framingham study. Am. J. Epidemiol. 132(4):675-684.
Law MR, Hackshaw AK (1997). A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect. BMJ 315:841-6.
Lunt M, Masaryk P, Scheidt-Nave C, Nijs J, Poor G, Pols H, Falch JA, Hammermeister G, Reid DM, Benevolenskaya L, Weber K, Cannata J, O'Neill TW, Felsenberg D, Silman AJ, Reeve J (2001). The effects of lifestyle, dietary dairy intake and diabetes on bone density and vertebral deformity prevalence: the EVOS study. Osteoporos. Int. 12 (8):688-98.
Lunt M, Masaryk P, Scheidt-Nave C, Nijs J, Poor G, Pols H, Falch JA, Hammermeister G, Reid DM, Benevolenskaya L, Weber K, Cannata J, O'Neill TW, Felsenberg D, Silman AJ, Reeve J (2001).The effects of lifestyle, dietary dairy intake and diabetes on bone density and vertebral deformity prevalence: the EVOS study.". Osteoporos. Int. 12(8):688-98.
Malik P, Gasser RW, Kemmler G, Moncayo R, Finkenstedt G, Kurz M, Fleischhacker WW (2009). Low bone mineral density and impaired bone metabolism in young alcoholic patients without liver cirrhosis: a cross-sectional study. Alcohol. Clin. Exp. Res. 33(2):375-81.
Nguyen TV, Center JR, Eisman JA (2000). Osteoporosis in elderly men and women: effects of dietary calcium, physical activity, and body mass index. J. Bone Miner Res. 15(2):322-31.
Nguyen TV, Kelly PJ, Sambrook PN, Gilbert C, Pocock NA, Eisman JA (1994). Lifestyle factors and bone density in the elderly: Implications for osteoporosis prevention. J. Bone Miner. Res. 9(9):1339-1346.
Olszynski WP, Shawn Davison K, Adachi JD, Brown JP, Cummings SR, Hanley DA, Harris SP, Hodsman AB, Kendler D, McClung MR, Miller PD, Yuen CK (2004). Osteoporosis in men: epidemiology, diagnosis, prevention, and treatment. Clin. Ther. 26(1):15-28.
Prentice A (2004). Diet, Nutrition and the prevention of osteoporosis; MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge, UK. Public Health Nutr. 7(1A):227-243.
Ralston SH, de Crombrugghe B (2006). Genetic regulation of bone mass and susceptibility to osteoporosis. Genes Dev. 20(18):2492-2506.
Ross PD (1996). Prediction of fracture risk. II: Other risk factors. Am. J. Med. Sci. 312(6):260-269.
Ruchira MJ, Ambrish M, Nidhi M, Edward MB (2010). Pilot case-control investigation of risk factors for hip fractures in the urban Indian population". BMC Musculoskelet Disord. 11: p. 49.
Sharkey N A, Willian NL, Guerin JB (2000). The role of exercise in the prevention and treatment of osteoporosis and osteoarthritis. Nurs. Clin. North Am. 35(1):209-21.
Sharma S, Tandon VR, Mahajan A, Kour A, Kumar D (2006) Primary screening of osteoporosis and osteopnea in urban women from Jammu using calcaneal QUS. Ind. J. Med. Sci. 60(5):183-189.
Shin A, Choi JY, Chung HW, Park SK, Shin CS, Choi YH, Cho SI, Kim DS, Kim DI, Lee KM, Lee KH, Yoo KY, Kang D (2004). "Prevalence and risk factors of the distal radius and calcaneus bone mineral density in Korean population." Osteoporos. Int. 15(8):639-44.
Sinigaglia L, Nervetti A, Mela Q, Bianchi G, Del Puente A, Di Munno O, Frediani B, Cantatore F, Pellerito R, Bartolone S, La Montagna G, Adami S (2000). A multicenter cross sectional study on bone mineral density in rheumatoid arthritis. Italian Study Group on Bone Mass in Rheumatoid Arthritis. J. Rheumatol. 27(11):2582-2589.
Strong K, Bonita R (2003). The SuRF Report 1. Surveillance of Risk Factors related to Noncommunicable Diseases: Current status of global data. Geneva: World Health Organization.
Todd JA, Robinson RJ (2003). Osteoporosis and exercise. Postgrad Med. J. 79(932):320-323.
Vatanparast H, Baxter-Jones A, Faulkner RA, Bailey DA, Whiting SJ (2005) Positive effects of vegetable and fruit consumption and calcium intake on bone mineral accrual in boys during growth from childhood to adolescence the University of Saskatchewan, Pediatric Bone Mineral Accrual Study. Am. J. Clin. Nutr. 82(3):700 - 6.
Walsh LJ, Lewis SA, Wong CA, Cooper S, Oborne J, Cawte SA, Harrison T, Green DJ, Pringle M, Hubbard R, Tattersfield AE (2002). The Impact of Oral Corticosteroid Use on Bone Mineral Density and Vertebral Fracture. Am. J. Respir. Crit. Care Med. 166(5):691-5.
Wardlaw GM (1996). Putting body weight and osteoporosis into perspective.Am. Clin. Nutr. 63: p. 433S-6S.
WHO (2010). Package of essential non-communicable diseases (PEN) interventions for primary health care in low-resource settings'. World Health Organization Report, Geneva.
WHO (2011). The WHO STEPwise approach to noncommunicable disease risk factor surveillance (STEPS).
Williams F, Cherkas L, Spector T, MacGregor A (2005). The effect of moderate alcohol consumption on bone mineral density: a study of female twins. Ann. Rheum Dis. 64: p. 309-310.
Zalloua PA, Hsu YH, Terwedow H, Zang T, Wu D, Tang G, Li Z, Hong X, Azar ST, Wang B, Bouxsein ML, Brain J, Cummings SR, Rosen CJ, Xu X (2007). Impact of seafood and fruit consumption on bone mineral density." Maturitas 56(1):1-11.

academicJournals

Vol. 6(2), pp. 101-110, February 2014
DOI: 10.5897/JPHE2013.0613
ISSN 2006-9723 ©2014 Academic Journals
http://www.academicjournals.org/JPHE

Journal of Public Health and Epidemiology

Full Length Research Paper

Patient satisfaction with outpatient health services in Hawassa University Teaching Hospital, Southern Ethiopia

Anteneh Asefa[1]*, Andargachew Kassa[2] and Muluken Dessalegn[3]

[1]School of Public and Environmental Health, Hawassa University, Hawassa, Ethiopia.
[2]School of Nursing and Midwifery, Hawassa University, Hawassa, Ethiopia.
[3]Addis Ababa Science and Technology University, Addis Ababa, Ethiopia.

Accepted 20 January, 2014

The level of patients' satisfaction is one among the mechanisms used in assessing the quality of health care services. This cross sectional study was conducted in Hawassa University Teaching Hospital to assess level of satisfaction of patients with outpatient health services and factors associated with it. Multiple logistic regression was used to assess the relationship between patients' satisfaction and possible predictors. Four-fifth (80.1%) of patients reported to be satisfied with the hospital's outpatient services. Respondents who claimed to have had a long stay in the hospital were found to be more satisfied than those who claimed to have had a very long stay (adjusted odds ratio (AOR) = 4.54, 95% CI: 2.38, 8.65). Furthermore, there was negative association between patients' satisfaction and not getting required services in the hospital (AOR = 0.78, 95% CI: 0.41, 0.96), lack of privacy (AOR = 0.52, 95% CI: 0.27, 0.78), and absence of good dialogue with outpatient service providers (AOR = 0.28, 95% CI: 0.12, 0.41). Health managers and service providers should devise innovative ways to reduce waiting time, have good dialogue with patients, and maintain privacy of patients in order to improve the level of satisfaction of patients.

Key words: Patient, outpatient, satisfaction, hospital.

INTRODUCTION

Growing demand for health care, rising costs, constrained resources, and evidence of variations in clinical practice have increased interest in measuring and improving the quality of health care in many countries of the world (Campbell et al., 2000). Avedis Donabedian described quality in three dimensions (structures, processes, and outcomes) which are important for the determination of clients' satisfaction, which is by far an indicator of quality of service (USA Institute of Medicine, 2001). Therefore, the level of patients' satisfaction is one among the mechanisms used in assessing the quality of health care services (Aldebasi and Ahmed, 2011) and addressing patients' expectations was found to be associated with high client satisfaction and better health outcomes (Ruiz et al., 2007; McKinley et al., 2002; Williams et al., 1995). But, patients' perceptions about health care systems seem to have been largely ignored by health care managers in developing countries (Yildlz

*Corresponding author. E-mail: antex98@yahoo.com. Tel: +251 911 754 684. Fax: +251 462 208 755.

and Erdogmus, 2004) and clinicians lack both awareness and adequate training to address patients' expectations (Rozenblum et al., 2011). It is inherently problematic to link outcome and patient satisfaction. One reason is that a particular course of treatment may take weeks or even months to resolve into "cure," whereas satisfaction surveys are mostly conducted immediately after treatment service use or discharge.

According to the current health tier system of Ethiopia, hospitals in the country are categorized into three groups. The first categories of hospitals are primary hospitals which serve a catchment population of 60,000 to 100,000 each. General hospitals which serve 1 to 1.5 million people constitute the second categories. Hospitals like Hawassa University Teaching hospital constitute the third category (specialized hospitals) which serve 3.5 to 5 million people each (Ethiopia Health Sector Development Program IV, 2010).

The aim of this study is to assess the level of patients' satisfaction with outpatient health services and factors associated with it in Hawassa University Teaching Hospital. Hence, this study can contribute to the delivery of evidence based information which can be used by stakeholders aiming at improving quality of hospital services in Ethiopia and other low and middle income countries.

MATERIALS AND METHODS

Study setting

The methods and materials and references part are typed in small fonts. Would you reconsider these parts and make the size of fonts similar. Hawassa University Teaching Hospital is found in the Southern Nations Nationalities and People's Region State (SNNPRS), Ethiopia. The teaching hospital is located in Hawassa city, the capital city of SNNPRS, Ethiopia which is 273 km far from Addis Ababa. The teaching hospital is established and started its activities in 2005, serving a catchment population of 5 million. The hospital was selected purposively since it is the largest hospital in the region which provides a range of services in its outpatient and inpatient units.

Study design and population

A cross-sectional study which deployed interviewer administered questionnaires was carried out to assess the level of patients' satisfaction with outpatient services. Stable patients who received care from four main outpatient clinics (medical, surgical, obstetrics/gynecology, and pediatrics) in June, 2011 were the study subjects of the study. For pediatric age group patients, adult caregivers who accompanied them were used as respondents.

Sample size and sampling

A single population proportion sample size determination formula was used with the following assumption: Proportion of patients satisfied with hospital care services to be 54%, according to a study done in Eastern Ethiopia (Abdosh, 2006), margin of error of 0.047, non response rate of 10%, and the desired confidence level of 95%. Thus the final sample size calculated was 475. Allocation of samples into outpatient departments was done proportionately. The proportionate allocation was done by considering the average number of patient flow of the four departments in the same month of the preceding year (June, 2010) and the month prior to the actual data collection period (April, 2011). A systematic random sampling method which used patients' registration book as a sampling frame was employed to select respondents.

Study variables

Dependent variable

Patients' satisfaction was the independent variable of this study. Patients' satisfaction was assessed using a Likert scale to identify the level of satisfaction respondents claimed. The options were: very satisfied, satisfied, neutral, dissatisfied, and very dissatisfied. In this study patients who have reported to be satisfied and very satisfied were considered as satisfied whereas those who reported to be neutral, dissatisfied and very dissatisfied were considered as unsatisfied.

Independent variables

In this study, socio-demographic characteristics (age, religion, marital status, level of education, occupation, estimated monthly income), outpatient service related characteristics (length of stay in the hospital, amount of payment paid, comfortableness of waiting areas, existence of good dialogue with service providers, availability of required medical services and drugs, presence of privacy in the clinics, and favorability of conditions to ask questions) were the independent variables.

Data collection

Exit interviews of patients were conducted in four confidential rooms using a structured and pre-tested questionnaire. Data were collected by literate and trained data collectors who were not health professionals to minimize the risk of information bias.

Statistical analysis

Data were entered using EPI INFO software version 3.5.1 and analyzed using statistical package for social sciences (SPSS) 18. Both descriptive and analytical statistical procedures were employed. Univariate, bivariate and multivariate logistic regressions with odds ratios along with the 95% confidence intervals were used to ascertain the association between covariates and the dependent variable. The Cornfield approximation was used for calculating the 95% confidence intervals (CI) for the odds ratio (OR). A logistic regression was carried out to determine the adjusted effect of each factor on patients' satisfaction. Variables with more than two categories were entered in the model in the form of two "indicator" contrasts comparing each category to the first group as a reference. A backward stepwise procedure based on the likelihood ratio was used to select the variables included in the final model. The significance for variable removal and entry was set to 0.10 and 0.05, respectively. The Hosmer and Lomeshow test was used to check the goodness-of-fit of the model. Odds ratios and 95% confidence intervals were derived from each variable coefficient in

the final model. The significance of each coefficient was tested by the Wald test, and statistical significance was considered at $p < 0.05$.

Ethical considerations

Ethical clearance was obtained from the Institutional Review Board of College of Medicine and Health Sciences, Hawassa University. Oral informed consent was secured from all respondents.

RESULTS

Socio-demographic characteristics of respondents

A total of 452 study participants were included in the study, this makes the response rate of the study 95.2%. Majority (56.6%) of the respondents were from Hawassa city and the remaining (43.4%) were from outside Hawassa city. Concerning religion of respondents, 39.2 and 36.1% of the respondents were Protestant Christians and Orthodox Christians, respectively. With regards to educational status of respondents, 13.5% of them were not able to read and write and 31.9% have attended college level and/or more level of education. More than three-fifth (62.8%) of the respondents were married whereas nearly one-fourth (23.9%) of the respondents were students by occupation followed by government employees (17.5%) and farmers (17%). The self reported median (IQR) monthly income of the respondents was 500 (200 to 1000) Ethiopian Birr, and 45.5% of them estimated their average monthly income to be less than 650 Ethiopian Birr (Table 1).

Means of transport used and estimated time taken to arrive at the hospital

Nearly one-third (66.4%) of respondents used public transport such as bus, taxi, and three wheel vehicles to come to the hospital. As to how the patients visited the hospital, 57.5% of them visited the hospital by their personal decision and 29.6% of respondents came after referral from another health institution. As the time taken to arrive at the hospital is concerned, 73% of the respondents took one hour or less to arrive at the hospital (Table 2).

Departments visited, and resources spent (time and money) in the hospital by respondents

Among the total respondents, 35.2 and 31.9% visited the medical and surgical outpatient departments of the hospital, respectively. The remaining 17.3 and 15.7% of respondents visited the gynecologic/obstetrics and pediatrics departments, respectively. Respondents were asked to estimate the amount of time they spent to use various hospital services to determine the total waiting time. Nearly one-third (32.7%) of patients waited for more than 90 min to enter to the outpatient departments after they have gone through the registration process. The estimated median (IQR) time respondents claimed to have spent waiting to enter the outpatient departments was calculated to be 60 (25 to 120) min. Among the total respondents, 50.5% had a length of stay of more than two hours in the hospital, and the estimated median (IQR) length of stay in the hospital was found to be 125 (60 to 219) min (Table 3). Based on the respondents' rating of the length of stay they had to receive care, the length of stay was reported to be very long by more than one-fourth (26.3%) of respondents. On the other side, 37.4, 21.7 and 13.9% of the respondents reported the length of stay in the hospital to be long, fair and short, respectively (Table 3). As shown in Table 3, among the total respondents, 369 (83.2%) have paid for services and the median (IQR) amount of money paid was 67 (42.3 to 111) Birr. This payment may be for registration, drug, treatment procedures, laboratory investigation or any of the combinations. As to the patients' perception towards the amount of payment requested by the hospital for the aforementioned activities/services, 44.7 and 1.6% of the respondents rated the payment to be fair and very expensive, respectively.

Friendliness of outpatient services and availability of advised medical services or drugs in the hospital

Nearly 86% of respondents reported the outpatient department they visited to be convenient to ask questions. Furthermore, the setup in which outpatient services were provided was claimed to maintain the privacy of patients by 81% the respondents and 92.7% of respondents declared to have had a good dialogue with outpatient service providers (Table 4). A scale (very politely, politely, neither politely nor impolitely, impolitely, and very impolitely) was used to assess the degree of politeness/impoliteness of outpatient service providers who served the respondents. Thus, 15.7 and 69.7% of respondents described outpatient service providers as very polite and polite during service provision. The remaining 10.8, 3.1 and 0.7% of respondents described outpatient service providers as neither polite nor impolite, impolite, and very impolite, respectively. Among the total patients interviewed, 71.2% reported to have got all ordered services from the hospital (laboratory tests, diagnostic services and drugs). Exactly 92.5 and 87.6% of the respondents wish the hospital for their future visit and would like to recommend the hospital to their friend

Table 1. Socio-demographic characteristics of outpatient service users, Hawassa University Teaching Hospital, Hawassa, 2011.

Variables	Frequency (%)
Place of residence	
Hawassa city	256 (56.6)
Out of Hawassa city≤50 km	74 (16.4)
Out of Hawassa city > 50km	122 (27.0)
Total	452 (100.0)
Religion	
Protestant	177 (39.2)
Orthodox	163 (36.1)
Muslim	90 (19.9)
Catholic	7 (1.5)
Others	15 (3.3)
Total	452 (100.0)
Marital status	
Married	284 (62.8)
Single	154 (34.1)
Widowed	8 (1.77)
Divorced	6 (1.33)
Total	452 (100.0)
Educational status	
Not able to read and write	61 (13.5)
Able to read and/or write	14 (3.1)
Grade 1 – 6	82 (18.1)
Grade 7 – 12	151 (33.4)
College and above	144 (31.9)
Total	452 (100.0)
Occupation	
Housewife	74 (16.4)
Merchant	49 (10.8)
Government employee	79 (17.5)
Private employee	36 (8.0)
Student	108 (23.9)
Farmer	77 (17.0)
Others	29 (6.6)
Total	452 (100.0)
Estimated average monthly income (n=367)	
< 650 birr	167 (45.5)
≥ 650 birr	200 (54.5)
Total	367 (100.0)
Median (IQR)	500 (200 - 1000)

Table 2. Pre-outpatient service use characteristics of respondents, Hawassa University Teaching Hospital, Hawassa, 2011.

Variables	Frequency (%)
Mode of transport used to arrive at the hospital	
Walking	32 (7.1)
Private vehicle	117 (25.9)
Public transport	300 (66.4)
Others	3 (0.7)
Total	452 (100.0)
How respondents visited the hospital	
Came after referral	134 (29.6)
Came upon recommendation from friend or relative	47 (10.4)
Came due to emergency	9 (2.0)
Came upon personal decision	260 (57.5)
Others	2 (0.4)
Total	452 (100.0)
Time taken to arrive at the hospital (in minutes)	
≤ 60	330 (73.0)
60-120	43 (9.5)
121-240	29 (6.4)
>240	50 (11.1)
Total	452 (100.0)

or relative, respectively (Table 4).

Patients' satisfaction with outpatient services

Among the total respondents, 80.1% were found to be satisfied (those who reported to be very satisfied and satisfied) with the outpatient services of the hospital whereas the remaining 19.9% were dissatisfied. Level of satisfaction by fully exhausted scales showed that 11.7, 68.4, 2.4, 12.2, and 5.3% of respondents reported to be very satisfied, satisfied, neutral, dissatisfied and very dissatisfied, respectively (Table 4). Binary and multiple logistic regressions were performed to identify factors associated with patients' satisfaction using different covariates. As depicted in Table 5, respondents who claimed to have had a long stay in the hospital were found to be more satisfied than those who claimed to have had a very long stay (AOR = 4.54, 95% CI: 2.38, 8.65). On the other hand, respondents who did not get all the required items/services from the hospital were less satisfied than their counterparts (AOR = 0.78, 95% CI: 0.41, 0.96). Besides, respondents who did not report the presence of adequate privacy in the clinic they visited were less likely satisfied with the outpatient service they received (AOR = 0.52, 95% CI: 0.27, 0.78). Absence of good dialogue with outpatient service providers was also found to be negatively associated with respondents' satisfaction (AOR = 0.28, 95% CI: 0.12, 0.41). Sociodemographic factors, perceived comfortableness of the hospital's waiting area, and cost of services were not found to affect the satisfaction status of respondents (Table 5).

DISCUSSION

The results of this study showed that 80.1% of patients were satisfied with the outpatient health service they received. Patients' satisfaction was associated with length of stay to receive care, presence of good dialogue with service providers, maintenance of privacy during care, the favorability of situations to ask questions, and availability of required services. Most of these variables were also found to be determinants of patient satisfaction in studies carried out in Thailand (Net et al., 2007) and Tanzania (Muhondwa et al., 2008). But sociodemographic characteristics were not found to be associated with patients' satisfaction. This finding is in agreement with the finding of a study carried out at Calabar Teaching

Table 3. Outpatient departments visited and resources spent by respondents, Hawassa University Teaching Hospital. Hawassa, July 2011.

Variables	Frequency (%)
Outpatient department visited	
Internal medicine	159 (35.2)
Surgery	144 (31.9)
Gynecology/obstetrics	78 (17.3)
Pediatrics	71 (15.7)
Total	452 (100.0)
Time taken to get entered into outpatient clinics (in minutes)†	
< 15	61 (13.5)
15-30	111 (24.6)
31-60	118 (26.1)
61-90	14 (3.1)
> 90	148 (32.7)
Total	452 (100.0)
Median (IQR)	60 (25-120)
Length of stay in the hospital for outpatient service use (in minutes)	
< 60	107 (23.7)
61-120	117 (25.9)
121-180	78 (17.3)
> 180	150 (33.2)
Total	452 (100.0)
Median (IQR)	125 (60-219)
Respondents' rating of length of stay in the hospital	
Very long	119 (26.3)
Long	169 (37.4)
Fair	98 (21.7)
Short	63 (13.9)
Very short	3 (0.7)
Total	452 (100.0)
Total amount of money paid for services (in Ethiopian birr) (n=376)	
< 20	45 (12.0)
20-70	149 (39.6)
> 70	182 (48.4)
Total	376 (100.0)
Median (IQR)	67 (42.3–111)
Respondents' rating of the amount of money paid for services in the hospital (n=376)	
Very cheap	20 (5.3%)
Cheap	149 (39.6%)
Fair	168 (44.7%)
Expensive	33 (8.8%)
Very expensive	6 (1.6%)
Total	376 (100.0)

†Non paying patients and fee waived services

Table 4. Outpatient service characteristics and perceptions of respondents, Hawassa University Teaching Hospital. Hawassa, July 2011.

Variables	Frequency (%)
The environment was convenient to ask questions	
Yes	388 (85.8)
No	60 (13.3)
Don't know/no response	4(0.9)
Total	452 (100.0)
Patient's privacy was maintained in the outpatient department	
Yes	368(81.4)
No	84(18.6)
Total	452 (100.0)
Had good dialogue with outpatient service provider	
Yes	419(92.7)
No	25 (5.5)
Don't know/no response	8(1.8)
Total	452 (100.0)
Politeness of outpatient service providers	
Very polite	71 (15.7)
Polite	315 (69.7)
Neither polite nor impolite	49 (10.8)
Impolite	14 (3.1)
Very impolite	3 (0.7)
Total	452 (100.0)
Have got all ordered diagnostic or laboratory tests and/or drugs from the hospital	
Yes	322(71.2)
No	130(28.8)
Total	452 (100.0)
Wish the hospital for future visit	
Yes	418(92.5)
No	34(7.5)
Total	452 (100.0)
Would like to recommend this hospital for a friend or relative	
Yes	396(87.6)
No	56(12.4)
Total	452 (100.0)
Satisfaction with outpatient services	
Very satisfied	53 (11.7)
Satisfied	309 (68.4)
Neutral	11 (2.4)
Dissatisfied	55 (12.2)
Very dissatisfied	24 (5.3)
Total	452 (100.0)

Table 5. Factors affecting respondents' satisfaction with outpatient services, Hawassa University Teaching Hospital. Hawassa, July 2011.

Explanatory characteristics	Satisfaction status		COR (95% CI)*	AOR (95% CI)*†
	Satisfied	Unsatisfied		
Respondents' rating of the amount of money they paid for services				
Very cheap	17	3	1.00	
Cheap	124	25	0.86 (0.24, 3.21)	0.51 (0.11, 2.49)
Fair	134	34	0.69 (0.19, 2.51)	0.47 (0.10, 2.26)
Expensive	21	12	0.31 (0.08, 1.28)	0.35 (0.06, 1.96)
Very expensive	4	2	0.35 (0.04, 2.87)	0.52 (0.03, 8.10)
Respondents' rating of length of stay in the hospital				
Very long	66	53	1.00	
Long	143	26	4.42 (2.54, 7.68)**	4.54 (2.38, 8.65)**
Fair	91	7	10.44 (4.46, 24.4)**	11.09 (3.87, 31.77)**
Short	59	4	11.85 (4.04, 34.71)**	36.07 (4.55, 285.98)**
Very short	3	0	-	-
Patients' perception of the waiting area				
Very comfortable	7	1	1.00	
Comfortable	311	66	0.67 (0.08, 5.56)	2.91 (0.25, 34.30)
Uncomfortable	37	17	0.31 (0.04, 2.73)	1.76 (0.13, 23.31)
Very uncomfortable	2	5	0.06 (0.01, 0.82)	0.28 (0.01, 6.17)
Not applicable	5	1	0.71 (0.04, 14.33)	1.97 (0.07, 52.97)
Had good dialogue with outpatient service provider				
Yes	349	70	1.00	
No	11	14	0.16 (0.07, 0.36)	0.28 (0.12, 0.41)**
Don't know	2	6	0.07 (0.01, 0.34)	0.12 (0.05, 0.39)**
Have got all ordered services or drugs from the hospital				
Yes	266	56	1.00	
No	96	34	0.59 (0.37, 0.97)	0.78 (0.41,0.6)**
There was adequate privacy				
Yes	309	59	1.00	
No	53	31	(0.19, 0.55)	0.52 (0.27, 0.78)**
There was convenient environment to ask question/s				
Yes	324	64	1.00	
No	38	26	0.29 (0.16, 0.51)	0.38 (0.17, 0.83)**

*p value<0.05**statistically significant, †controlled for age, marital status, educational status, occupation, average monthly income, residential place

Hospital in Nigeria (Udonwa and Ogbonna, 2012).

The level of patient satisfaction in this study is in agreement with a study conducted in six regions of Ethiopia (Bekele et al., 2008), and higher when compared with other studies conducted in Eastern Ethiopia (Abdosh, 2006), Jimma (Oljira and Gebreselassie, 2011),

and Addis Ababa (Tateke et al., 2012), which revealed level of patient satisfaction to be 54, 57.1 and 65.9%, respectively. A systematic review made on measurement of patient satisfaction with health care showed personal contact and interview methods usually result in higher recorded satisfaction than other methods (Crow et al., 2002). Hence, similar condition might have happened and inflate proportion of satisfied patients in this study.

Moreover, a high proportion of satisfied patients in this study may not imply that the services rendered in the outpatient departments were of high quality. This is because patient satisfaction cannot show the real treatment outcome, which is another indicator of quality of health services (Yildlz and Erdogmus, 2004). Besides, patients who did not claim the existence of a convenient environment to ask questions and patients who did not have a good dialogue with outpatient health service providers were less satisfied. A study carried out in health centers in central Ethiopia also revealed that good dialogue and non-verbal communications to be predictors of high degree of patients' satisfaction (Birhanu et al., 2010). This is also supported by a study conducted in United Arab Emirates public hospitals which identified perceived welcoming approach of service providers as a significant determinant of patient satisfaction (Masood et al., 2008).

Perceived long length of stay in the hospital negatively affected patients' satisfaction in this study. This is in agreement with other studies conducted in outpatient setups in Ethiopia (Abdosh, 2006; Tateke et al., 2012; Oljira and Gebreselassie, 2010) and studies conducted in inpatient setups in Vietnam (Thi et al., 2002). However, studies of mental health services have found the opposite to be true and those seem to be areas more likely conditioned by a long stay (Rosenheck et al., 1997). Mostly patients do not want to get partial service or transfer somewhere else unless there is special personal need (Mirzaei et al., 2013). The same is true in this study where patients who did not get all required services or drugs from the hospital were less satisfied than their counterparts (AOR = 0.78, 95% CI: 0.41, 0.96). This study assessed patient experiences in the hospital, which definitely affects patient satisfaction. But a study done in Norway showed it is not only patient-reported experiences which affect patient satisfaction, but also the fulfillment of expectations, which is distinct in its scope (Bjertnaes et al., 2011).

High proportion of self-referred patients in this study is due to the fact that the hospital is not operating as a full blown referral hospital and it provides outpatient services to patients coming without any referral slip. Besides, the hospital is providing fee-waived outpatient health services for students coming from government owned higher institutions. These conditions may bias the patients' evaluation of satisfaction with services rendered. Thus, this study is limited in measuring all possible factors which can affect patients' satisfaction with outpatient health services and we recommend future studies to address this gap and identify all possible predictors of patients' satisfaction so that appropriate remedies will be undertaken by health managers and service providers.

Conclusion

This study revealed that satisfaction of patients with outpatient health services is related to financial and interpersonal factors. The interpersonal factors might have happened due to the fact that there are student medical practitioners providing services in the hospital who do not have previous skill and experience of handling patients. Thus, the interpretation of the findings of this study may not apply to other non-teaching hospitals. Health managers and service providers should devise innovative ways to reduce waiting time, ensure existence of good dialogue with patients, and maintain privacy of patients in order to improve the level of satisfaction of patients.

ACKNOWLEDGMENTS

The authors would like to acknowledge Hawassa University for funding this study. Next, our heartfelt thanks goes to all study participants, and data collectors who participated in the study.

REFERENCES

Abdosh B (2006). The quality of hospital services in Eastern Ethiopia: patient's perspective. Ethiop J. Health Dev. 20(3):199-200.

Aldebasi YH, Ahmed MI (2011). Patients' Satisfaction with Medical Services in the Qassim Area. J. Clin. Diagn. Res. 5(4):813-817.

Bekele A, Taye G, Mekonnen Y, Girma W, Degefu A, Mekonnen A, Dejene A (2008). Levels of outpatient satisfaction at selected health facilities in six regions of Ethiopia. Ethiop J. Health Dev. 22(1):42-48.

Birhanu Z, Assefa T, Woldie M, Morankar S (2010). Determinants of satisfaction with health care provider interactions at health centres in central Ethiopia: a cross sectional study. BMC Health Serv. Res.10:78.

Bjertnaes OA, Sjetne IS, Iversen HH (2011). Overall patient satisfaction with hospitals: effects of patient-reported experiences and fulfilment of expectations. BMJ Qual. Saf. 21: 39-46.

Campbell SM, Rolland MO, Buetow SA (2000). Defining quality of care. Soc. Sci. Med. 51:1611-1625.

Crow R, Gage H, Hampson S, Hart J, Kimber A, Storey L, Thomas H (2002). The measurement of satisfaction with healthcare: implications for practice from a systematic review of the literature. Health Technol Assess. 6(32):1-244.

Ethiopia (2010). *Health Sector Development Program IV.* Addis Ababa: Ministry of Health.

Masood AB, Samaa A, Abdulla MU (2008). Healthcare quality and moderators of patient satisfaction: testing for causality. Int. J. Health Care Qual. Assur. 22:382–410.

McKinley RK, Stevenson K, Adams S, Manku-Scott TK (2002). Meeting

patient expectations of care: the major determinant of satisfaction with out-of-hours primary medical care? Fam. Pract. 19(4):333-338.
Mirzaei M, Aspin C, Essue B, Jeon YH, Dugdale P, Usherwood T, Leeder S (2013). A patient-centred approach to health service delivery: improving health outcomes for people with chronic illness. BMC Health Serv Res. 13:251.
Muhondwa EPY, Leshabari MT, Mwangu M, Mbembati N, Ezekiel MJ (2008). Patient satisfaction at the Muhimbili national hospital in Dar Es Salaam, Tanzania. East Afr. J. Public Health 5:67–72.
Net N, Sermsri S, Chompikul J (2007). Patient Satisfaction with Health Services at the Out-Patient Department Clinic of Wangmamyen Community Hospital, Sakeao Province. Thailand J. Pub. Health Dev. 5 (2):33-43.
Oljira L, Gebreselassie S (2010). Satisfaction with outpatient health services at Jimma Hospital, Southwest Ethiopia. Ethiop J. Health Dev. 15(3):179-184.
Rosenheck R, Wilson NJ, Meterko M (1997). Influence of patient and hospital factors on consumer satisfaction with inpatient mental health treatment. Psychiatr Serv 1997, 48:1553-1561.
Rozenblum R, Lisby M, Hockey PM, Levtizion KO, Salzberg CA, Lipsitz S, Bates DW (2011). Uncovering the blind spot of patient satisfaction: an international survey. BMJ Qual. Saf. 20:959-965.
Ruiz RM, Torres LA, Jaramillo MI (2007). The effect of patients' met expectations on consultation outcomes. A study with family medicine residents. J. Gen. Intern. 22(1):86-91.
Tateke T, Woldie M, Ololo S (2012). Determinants of patient satisfaction with outpatient health services at public and private hospitals in Addis Ababa, Ethiopia. Afr. J. Prm. Health Care Fam. Med. 4(1):1-11.
Thi PL, Briancon S, Empereur F, Guillemin F (2002). Factors determining inpatient satisfaction with care. Soc. Sci. Med. 54:493-504.
Udonwa NE, Ogbonna UK (2012). Patient-Related Factors Influencing Satisfaction in the Patient-Doctor Encounters at the General Outpatient Clinic of the University of Calabar Teaching Hospital, Calabar, Nigeria. Int. J. Fam. Med. 2012:517027.
United States of America. Institute of Medicine (2001). *Crossing the Quality Chasm*: *A New Health System for the 21st Century*. Washington, DC: National Academy Press.
Williams S, Weinman J, Dale J, Newman S (1995). Patient expectations: what do primary care patients want from the GP and how far does meeting expectations affect patient satisfaction? Fam. Pract. 12(2):193-201.
Yildlz Z, Erdogmus S (2004). Measuring patient satisfaction of the quality of health care: a study of hospitals in Turkey. J. Med. Syst. 28(6):581-589.

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

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
academicJournals