ISSN 1595-689X

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY

MAY 2009 **VOLUME 10** **NUMBER 2**

Official Publication of the African Society for Clinical Microbiology

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY (ISSN 1595-689X)

GENERAL INFORMATION

Aims and scope

African Journal of Clinical and Experimental Microbiology is the official Journal of the African Society for Clinical Microbiology. It publishes original research, review papers, case reports/series, short communications and letters to the editors, in all aspects of Medical Microbiology including Bacteriology, Virology, Rickettsiology and Chlamydiology, Mycology, Mycobacteriology and Actinomycetes, Parasitology, Clinical Microbiology, and Clinical Veterinary Microbiology

Subscription information

African Journal of Clinical and Experimental Microbiology is an OPEN ACCESS JOURNAL CC BY VERSION 4.0 INTERNATIONAL, and publishes two or three times a year. Free downloads can be made from the website of the world"s largest online library of peer reviewed, Africa published scholarly journals, African Journals OnLine (AJOL): https://www.ajol.info/index.php/ajcem. Subscription is however still open to individuals, libraries, University Departments, Research Institutes and other Multi-reader institutions who may want to have hard copies of the Journal. For each volume (4 issues), subscription rate is £400 (United Kingdom), US $800 (USA/Canada), US $600 (African Countries), US $800 (Other Countries), N28,000 (Nigeria). Additional charges will be made for postage and packaging. A copyright for these is with African Journal of Clinical and Experimental Microbiology.

Subscription enquiries and all other matters relating to the Journal including manuscripts, adverts booking and sponsorship should be addressed to:

Prof Boaz Adegboro (MD)
Editor, African Journal of Clinical and Experimental Microbiology,
Department of Medical Microbiology, Faculty of Health Sciences,
University of Ilorin, Nigeria.
Phone: 031 – 222076-9
Email: ajcem2002@yahoo.com

It is a condition of publication that manuscripts submitted to this Journal have not been published and will not be simultaneously submitted to be published elsewhere except as conference abstracts, for which authors must disclose at the point of manuscript submission. Authors should be aware that electronic journals issues/articles can be accessed free (Open Access) online at the AJOL website: https://www.ajol.info/index.php/ajcem

Responsibility for accuracy of manuscripts lies entirely with the authors. All submissions must conform to the International Committee of Medical Journal Editors (ICMJE) uniform recommendations for manuscripts submitted to biomedical journals (http://www.icmje.org/recommendations/) and follow the guidelines of Committee on Publication Ethics https://publicationethics.org/guidance/Guidelines

Manuscripts should be typewritten with double line spacing and wide margins, following the conventional form: Title, Author's name and full correspondence address, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgment(s), References, Tables, Figures and Legends to Figures. Short Communications and Letters to The Editor are also entertained, and need not follow the above format.

If the research involves the use of human subjects, including collection of human blood or other human specimens, an institutional ethical clearance document should be submitted with the manuscripts. Alternatively, a statement should be made in the "Materials and Methods" section that informed consent of the experimental subjects and the approval of the appropriate ethical committee had been obtained.

All necessary illustrations should accompany the manuscripts, but should not be in the text. The illustrations should be numbered consecutively in the order in which they are referred to in the text. The top of illustration should also be indicated if this is not clear. All x-ray films must be clear and should be in photographic prints. Legends to figures should give sufficient information to make the illustration comprehensive without reference to the text.

References should be listed in their order of appearance in the text; and be indicated in the text by Arabic numbers in brackets e.g. (1), (2, 3, 4), etc (Modified Vancouver style). Accuracy of the references is the responsibility of the authors. The authors‟ names and initials should be followed by the title of the paper, abbreviated name of the journal, which should conform to those used in Index Medicus, year of publication, volume, and the first and last page numbers. Note the following examples.

For Journals:

1. Nsanze, H. Recommendation for management of gonorrhoea and genital ulcers in Africa. Afr J Sex Transm Dis. 1984; 1:5-7

2. Odugbemi, T. O., and Arko, R. J. Differentiation of *Kingella denitrificans* and *Neisseria gonorrhoeae* by growth on a semi solid medium and sensitivity to amylase J Clin Microbiol. 1983; 17: 389-391

For books:

3. Arya, O. P., Osoba, A. O., and Bennett, P. Tropical Venereology, Churchill Livingstone, Edinburgh, 1980 OR when referring to a chapter in a book and where the names of authors are also given, the reference should be as follows:

4. Easmon, C. S. F. Host-Parasite relationship in experimental staphylococcal infections. In: Macdonald, A., and Smith, G. (eds). The Staphylococci. University Press, Aberdeen 1981: 63-72

General:

a. To ensure rapid and accurate publication, it is essential that manuscripts conform to all instructions. Manuscripts, which are not in accordance with these specifications, may be returned.

b. An electronic copy of manuscript typed in Microsoft Word should be sent via email to ajcem2002@yahoo.com

c. An estimation of page charges will be mailed to the author(s) after the paper has been accepted for publication.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2009 ISBN 1595-689X VOL 10(2)
AJCEM/2008112/20909 -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 10(2): 64 - 79

OPTIMIZATION OF RNA EXTRACTION IN *MYCOBACTERIUM TUBERCULOSIS* FOR STUDYING INTRACELLULAR GENE EXPRESSION.

Alli [1,2] , O.A.Terry, Mangan[3,] J.A., Butcher[3], P.D. andSpreadbury[1], C.L.

[1]Department of Biomedical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, [2]Department of Infection, University of Birmingham, Birmingham, UK B15 2TT, [3]Department of Medical Microbiology, St George's Hospital Medical School, Tooting, London, UK SW17 0RE.

Correspondence: O.A. Terry Alli, [1]Department of Biomedical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo.
E mail: alliot@hotmail.com

Abstract

***Mycobacterium tuberculosis* is the leading cause of death due to infectious disease after Human immunodeficiency virus. There has been an upsurge in the incidence of tuberculosis since 1980s. In order to reverse this trend, there is need to understand the biology of the organism. This can be brought about by studying gene expression at transcriptional level. The success of this hinges on RNA of good quality. In this paper, five methods (hot phenol, sonication with guanidinium thiocyanate (GTC) solution, beadbeating method with Trizol, FastPrep machine with Divolab as detergent and GTC solution, and FastPrep machine with Trizol) of extracting RNA from bacteria were compared to find which of the method would be suitable for mycobacteria. The study found that physical method of lysing bacteria was necessary for extraction of RNA from mycobacteria. FastPrep machine gave the highest yield and also provided the speed necessary for optimum RNA extraction. FastPrep and Trizol as reagent for extraction of RNA was applied to macrophage infected with *M. tuberculosis* (H37Rv) after removing the macrophage RNA. We were able to demonstrate the expression of *dnaK* gene in both intracellular and broth grown bacilli. The expression of *dnaK* gene was found to be downregulated in macrophage compared to broth**.

Introduction

Mycobacterium tuberculosis is ranked among the leading cause of mortality and morbidity due to infectious disease. In 2006, 9.2 million people was estimated to be new cases of tuberculosis (TB) worldwide – an increase of 0.6% from previous year with 1.7 million people died from the disease [1]. Even despite the availability of anti-tuberculosis drugs, this bacterium still continue to claim more lives than any other infectious agents put together with the exception of Human Immunodeficiency virus (HIV). The resurgence in the incidence of tuberculosis since 1980s is partly due to HIV infection and the emergence of multi-drug resistance *M. tuberculosis* complex. To make matter worse, there is no effective vaccine that can curb the menace. The present Bacille Calmette Guerin (BCG) vaccine has been found to have variable vaccine efficacy [2]. For example, the BCG vaccine efficacy has been found to be 0% in India and close to 90% in United Kingdom[2-4]. This variability in vaccine efficacy has been attributed to environmental mycobacteria.

Some countries are not predisposed to the use of the vaccine for some reasons like the inability to monitor the immune status of the individuals exposed to mycobacteria.

In view of these, there is need for more anti-tuberculosis drug and new effective vaccine. But in order to do this, there is need for novel way of identifying drug target(s) and vaccine candidate. Identifying possible drug targets and vaccine candidates have been made easy with completion of *M. tuberculosis*'s genome [5] and other species of mycobacteria [6, 7]. The genetic system for studying basic biology of mycobacteria is not well developed and where it is developed, it is not easy to apply. This has been the cog in the wheel of progress. Studying gene expression of mycobacteria could provide an alternative in the sense that there is no need for well developed genetic system. Gene expression can be studied either at transcriptional level or translational level. RNA is a basic ingredient for studying gene expression at transcriptional level. RNA extraction with good quality and yields is the cornerstone of transcriptional study. There are many RNA extraction methods available but majority of them are not applicable to mycobacteria because of the inherent toughness of mycobacteria to lysis solutions that are used for lysing cells. Some of the methods do not take into consideration of the short half life of bacterial RNA. For example, the half life of *Escherichia coli*'s mRNA has been found to be less than 1 minute. Mangan et al. (1997) developed RNA extraction for *M. tuberculosis* using a special detergent and guanidium thiocyanate [8], which might not be applicable to some of the transcription-based studies like microarray. The aim of this study was therefore to optimise RNA extraction methods for studying gene expression in *M. tuberculosis* that can be found applicable for functional genomics like microarray and individual gene expression studies.

Materials and Methods

Maintenance and culture of mycobacteria

Mycobacterial species used in this study were *M. bovis* BCG (Statens Seruminstitut vaccine strain ST1077, obtained from Evans Medical Ltd., Leatherhead, UK) and *M. tuberculosis* H37Rv (NCTC 7416/ATCC 9360) obtained from the National Culture Type Collection, Colindale, UK. Mycobacteria were grown to mid log or late log phase in Middlebrook 7H9 broth (Difco Laboratories Ltd., West Molesey, UK) supplemented with 10% albumin-dextrose-catalase enrichment (ADC; Difco) and 0.02% Tween 80 (Sigma, Poole, UK) at 37^{o}C (in the presence of 5% CO_2 for *M. tuberculosis)* before harvesting at 0.5-1.0 OD_{600}. *M. tuberculosis* H37Rv cultures were also grown for 3 weeks at 37^{o}C on thick 7H10 agar plates supplemented with 10% oleic acid-

albumin-dextrose-catalase enrichment (OADC; Difco) and 0.2% glycerol and then stored at 4°C. For long term storage, *M. bovis* BCG broth cultures were stored at -80°C. All work involving *M. tuberculosis* was performed in a Class I biohazard safety cabinet under Category 3 containment conditions.

Hot phenol method

Several RNA extraction methods were used to determine the optimal method for extracting RNA from mycobacteria. All equipment and solutions used in these procedures were treated with 0.1% diethyl pyrocarbonate DEPC (Sigma) as described before [9].

The hot phenol method of RNA extraction was based on the method of Von Gabain *et al.* [10]. Five millilitres of *M. bovis* BCG bacilli representing 1 × 10^8 colony forming units (cfu) per ml were pelleted by centrifugation at 5,000 ×g for 1 min and the supernatant was discarded immediately. The cell pellet was resuspended in 400 µl RNA lysis solution (0.15 M sucrose, 10 mM sodium acetate [pH 5.2], 1% (w/v) SDS) and quickly transferred to an Oakridge tube containing 4 ml of hot (65°C) phenol. The bacterial suspension was incubated for 10 min at 65°C with inversion of the tube twice during the incubation period. The sample was aliquoted into 1.5 ml Eppendorf tubes and centrifuged at 10,000×g for 5 min at 4°C. The supernatants were transferred to fresh tubes containing 500 µl of phenol. The tubes were inverted several times and incubated at 65°C and centrifuged as before. The supernatant was transferred to a fresh tube containing 500 µl of phenol: chloroform: isoamyl alcohol (25:24:1), vortexed briefly and centrifuged. The resultant supernatant was transferred to fresh tubes containing 500 µl chloroform: isoamyl alcohol (24:1), vortexed briefly and centrifuged at 10,000×g for 5 min. The supernatants were pooled and 100 µl of 3 M sodium acetate (pH 5.2; final concentration 0.3 M) was added prior to RNA precipitation. The RNA was precipitated with 2.0-2.5 volumes of absolute ethanol and placed at –20°C for at least 30 min before centrifugation at 10,000×g for 10 min at 4°C. The ethanol was discarded and the RNA pellet was suspended in 75% ethanol, centrifuged briefly, and discarded. The RNA was air dried for 15 min before being dissolved in 10 µl of DEPC treated water.

Sonication method

This method was based on the methods of Patel *et al*. [11] and Chomcynski and Sacchi [12]. One and half millilitres of *M. bovis* BCG broth culture representing 5 × 10^8 cfu/ml were pelleted by centrifugation at 10,000 ×g for 30 s. The bacterial pellet was immediately resuspended in 1 ml of 4 M guanidinium thiocyanate (GTC) lysis solution (containing 0.5% sodium N-

laurylsarcosine, 25 mM sodium citrate [pH 7], and 0.1 M 2-mercaptoethanol) by vigorous vortexing followed by immediate sonication using a sonicator (Heat Systems Ultrasonics) with an ultra-thin probe (4mm diameter) for 45 s with the control set at position 2. Following sonication, each tube was immediately subjected to continuous vortex mixing for 10-15 min. The lysate was left at room temperature for 3-4 hr with intermittent vortex mixing every 30 min for 3 min. Separation of RNA after bacterial lysis was performed essentially as described before [12]. The lysate was split between two 1.5 ml Eppendorf tubes and 50 µl of 2 M sodium acetate (pH 4), 0.5 ml of water saturated phenol (Invitrogen, UK) and 0.1 ml of chloroform-isoamyl alcohol (49:1) were added to the homogenate with thorough mixing by inversion after the addition of each reagent. The final suspension was shaken vigorously for 10 s and cooled on ice for 15 min. Samples were centrifuged at 10,000 ×g for 20 min at 4°C. After centrifugation, RNA present in the aqueous phase (DNA and proteins are present in the interphase and phenol phase) was transferred to a fresh tube and mixed with 0.5 ml of isopropanol to precipitate the RNA. The RNA was pelleted by centrifugation at 10,000 ×g for 20 min and dissolved in 150 µl of GTC solution and precipitated with 1 volume of isopropanol at –20°C for 1 hr. After centrifugation in a microcentrifuge for 10 min at 4°C, the RNA pellet was resuspended in 75% ethanol, sedimented, air-dried, and dissolved in 20 µl DEPC treated water at 65°C for 10 min.

Beadbeating method

The sonication method was unsuitable for RNA extraction from *M. tuberculosis* for safety reasons due to the generation of aerosols. The use of a beadbeater circumvented this problem, as it is a closed system and could be placed in a Class I safety cabinet during its operation. One millilitre of *M. bovis* BCG culture was centrifuged at 12,000 ×g for 1 min. The cell pellet was resuspended in 1 ml of Trizol™ (Life Technologies). The suspension was transferred to a 2 ml skirted tube containing approximately 0.5 ml of 0.1 mm-size zirconia/silica (Biospec, Luton, UK) to aid the disruption of the mycobacterial cells. This suspension was beadbeater at 5,000 r.p.m for 40 s. Immediately the tube was placed on ice for 5 min before adding 0.25 ml of chloroform and mixed by vortexing for several seconds. The lysate was allowed to stand for 2 min before centrifugation at 12,000 ×g for 15 min. The upper aqueous phase was transferred to a fresh 1.5 ml Eppendorf tube and the RNA was precipitated with 0.5 ml of isopropanol. The solution was left at room temperature for 10 min before centrifuging at 12,000 ×g for 10 min. The supernatant was discarded and the RNA pellet was resuspended in 75% ethanol and centrifuged for 5 min at 8,000 ×g. The

supernatant was removed and the RNA pellet was air dried before dissolving in 20 µl of DEPC treated water.

FastPrep™ method

The FastPrep™ machine FP120 (BIO 101 Savant, obtained from Anachem Ltd., Luton, UK) was used as a physical means of disrupting the mycobacterial cells. The advantage of this machine over the bead beater is that multiple samples can be processed at any one time. RNA was extracted using a modification of Cheung *et al.'s* method [13]. *M. tuberculosis* H37Rv bacilli in mid-log phase were centrifuged at 10,000× g for 1 min and the pellets were resuspended in Trizol™ reagent. The cell suspensions were transferred to a 2 ml microcentrifuge tube to which 0.5 ml of zirconia/silica beads (0.1 mm-size) had previously been added. The tubes were shaken in a FastPrep™ machine at 6000 rpm for 20 s. Two hundred microlitres of chloroform were added to the lysate, shaken for 15 s and left at room temperature for 3 min. The mixture was then centrifuged at 12,000× g for 15 min at 4°C. The RNA in the aqueous phase (top layer) was precipitated with 0.5 ml of isopropanol and pelleted (12,000× g for 10 min). The pellet was washed with 75% ethanol, air dried for 10 min and resuspended in 10 µl of DEPC treated water.

FastPrep™/Divolab™ method

This method was developed by Mangan *et al*. [8] and it also makes use of the FastPrep™ machine but a different detergent (Divolab™) is used. This method enabled efficient lysis of the mycobacteria and recovery of RNA from the lysate resulting in a higher yield of RNA and lower DNA contamination compared to the previous methods. A culture of *M. tuberculosis* bacilli was harvested by centrifugation at 12,000 ×g for 20 s in a microcentrifuge, washed in 0.5% Tween 80 solution, repelleted and resuspended in 200 µl DEPC treated water. To a FastRNA.Kit-Blue tube [a 2 ml skirted screw-capped microcentrifuge tube with 'O' ring seal, containing acid washed 0.1 mm silica/ceramic beads] (BIO 101, obtained from Anachem Ltd., Luton, UK) were added: 500 µl detergent solution (9.6 ml Divolab™ No.1 [Diversey Hygiene, Northampton, UK], 24 ml 500 mM sodium acetate pH 4.0, 66.4 ml DEPC treated water), 500 µl acid phenol (water saturated phenol equilibrated with 50 mM sodium acetate pH 4.0), 100 µl chloroform/isoamyl alcohol (24:1) and 200 µl resuspended bacterial pellet. The tube was immediately processed in the FastPrep™ machine for 45 s at a 6.5 speed setting and left on ice for 10 min. The cell debris was removed by centrifugation at full speed in a microcentrifuge for 10 min. The aqueous phase was removed and the RNA precipitated by the addition of 500 µl

solution (0.3 ml 3 M sodium acetate pH 4.0, 49.7 ml isopropanol) at -80°C for 30 min. The sample was pelleted by centrifugation for 15 min, air dried and the RNA was dissolved in 20 µl of DEPC treated water.

DNase I treatment and testing for DNA contamination

All RNA samples were DNase I treated to destroy any contaminating DNA by incubating the RNA samples in the presence of DNase I (Pharmacia Biotech, St Albans, UK), 50 µg/ml bovine serum albumin (BSA), 60mM Tris-HCl [pH 7.5], and 10 mM $MgCl_2$ at 37°C for 30 min. The samples were transferred to a fresh Eppendorf tubes and the DNase I was inactivated by incubating the samples at 65°C for 15 min. All RNA samples were then stored at -80°C. To confirm that there was no DNA contamination, a PCR (40 µl reaction volume) was performed (see section 2.5) with the RNA samples using primers $P_{71KDTB1}$ (5'-ATTGTGCACGTCACCGCCAA-3') and $P_{71KDTB2}$ (5'-ACCGCGGCATCAACCTTGTT-3') to amplify a 275 bp fragment of the *M. tuberculosis dnaK* as previously described [11]. PCR was conducted for 30 cycles of denaturation (94°C, 1 min), annealing (55°C, 2 min) and extension (72°C, 3 min) followed by 1 prolonged cycle of extension (72°C, 7 min) using a Hybaid thermal cycler. The PCR products were fractionated through a 1% agarose gel.

Determination of the yield and integrity of RNA

RNA was quantified spectrophotometrically as previously described [14]. Five microlitres of RNA was diluted 1:200 in 1 ml of distilled water. The OD was read at 260 nm and 280 nm with water as the reference. The concentration of RNA was calculated based on an OD_{260} reading of 1.0 corresponds to approximately 40 µg /ml. Thus, the concentration of RNA per ml = OD_{260} reading × dilution factor × 40 µg/ml. The purity of the RNA was determined by the OD_{260}/OD_{280} ratio where a ratio of 2.0 indicates pure RNA. The integrity of the RNA was determined by agarose gel electrophoresis against a RNA marker (BDH) comprising the eukaryotic 28S, 18S, and prokaryotic 23S, 16S, and 5S rRNA species.

RNA/DNA samples were fractionated through 1-2% agarose gels. The samples were loaded onto agarose gel with the aid of 10% of 10 × loading buffer (0.05% bromophenol blue, 0.05% xylene cyanol, 5% sucrose, and 50% glycerol) with appropriate DNA/RNA size markers. Electrophoresis was performed with 0.5 × TBE buffer typically for 1 hour at 75 v in a horizontal gel electrophoresis apparatus (Invitrogen, UK) before visualisation/photography of the RNA/DNA samples with UV transillumination at 302 nm. Where appropriate, formaldehyde agarose gel

electrophoresis was performed to analyse some of the RNA samples.

Macrophage infection conditions and preparation of RNA

J774.2 macrophages infected overnight with 10:1 non-opsonised *M. tuberculosis* bacilli were used (typically, 5 x 75cm^2 tissue culture flasks). A control comprised *M. tuberculosis* bacilli growing in Middlebrook 7H9/ADC/Tween 80 broth. RNA was prepared from macrophages infected with *M. tuberculosis* using a method based on a differential lysis method kindly provided by Prof. P. D. Butcher (St. George's Hospital Medical School, London). The culture medium was discarded and the infected macrophage monolayer was resuspended in 25 ml guanidine thiocyanate solution per flask to lyse the macrophages. In order to reduce the viscosity of the solution, a long thin nosed plastic Pasteur pipette was used to squirt the lysate solution in and out with force to shear the macrophage nucleic acids. The lysates were transferred to 30 ml sterile universal tubes and centrifuged at 2,500×g for 20 min to concentrate the intracellular bacilli. The pellets of intracellular bacilli were combined using 1 ml of wash solution (1 ml 0.5 % Tween 80) and transferred to a 1.5 ml Eppendorf tube and centrifuged in a microcentrifuge at 12,000×g for 30 s. The supernatant (wash solution) was saved and placed in a fresh Eppendorf tube and stored at -80^{o}C. The pellet of bacteria was resuspended in 200 µl of sterile DEPC treated water. The RNA was extracted from the harvested intracellular bacilli and the extracellular bacilli control using Trizol (Invitrogen, UK) following manufacturer's instructions.

Reverse transcription polymerase chain reaction (RT-PCR)

Reverse transcription PCR was used to determine the presence of RNA transcripts in samples of interest. Total RNA (typically 250 ng) was reverse transcribed in a 20 µl reaction volume containing 0.5 mM each of dATP, dGTP, dCTP and dTTP with 1 µM of gene specific downstream primer and 100 U Superscript™ RNase H$^-$ reverse transcriptase (Invitrogen, UK) in a reaction buffer of 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$ and 10 mM dithiothreitol following the manufacturer's instructions. Briefly, the RNA sample, and the primer were incubated together for 15 min at 70^{o}C and chilled on ice before adding the dNTPs (total concentration in the reaction was 0.5 mM), reaction buffer. This was incubated at 42^{o}C for 2 min before the addition of the reverse transcriptase and cDNA synthesis was then performed at 42^{o}C for 50 min. The reaction was stopped by incubating the samples at 70^{o}C for 15 min. One tenth of the cDNA was then used in the PCR. The cDNA was added to the PCR reagents containing 1 µM of each gene specific primer (upstream

and downstream primers). PCR was performed as before.

Results

Several RNA extraction methods (a 'standard' method and some specifically designed to extract RNA from mycobacteria) were evaluated to determine which gave the best yield of RNA from mycobacteria. The main objective was to obtain a high yield, non-degraded RNA that would provide good transcripts for cDNA synthesis for RT-PCR analyses. The fact that prokaryotic mRNA has a very short half life of 1-3 minutes [15] was an important factor in terms of harvesting the bacilli as rapidly as possible to stabilise the mRNA and prevent as much as possible changes in gene expression. The quality of the RNA obtained using the different extraction methods are shown in Figures 1a-d and the yields are summarised in Table 1. Spectrophotometric analysis to determine purity and yield was used but where this was not possible (where small volumes were obtained, for example), agarose gel electrophoresis of the sample along side a known amount of RNA was used to determine the concentration of RNA. Difficulty in assessing the RNA yield by spectrophotometry was particularly problematic when using FastPrep™/Divolab™ method of extraction. Unreliable readings were generated and this may have been due to the detergent or the lysis buffer used to lyse the bacilli.

Table 1. Differences in the yields of RNA using different RNA extraction methods

Method	RNA yield (μg)	Number of mycobacteria used cfu/ml
Hot phenol	Nil	5×10^8
Sonication	0.50 -1.00	5×10^8
Beadbeater	0.75 - 1.50	5×10^8
Fastprep□□/Trizol□□	1.75 - 2.00	5×10^8
Fastprep□□ /Divolab□□	4.50 - 8.00	4×10^8

The RNA yields obtained with the various methods ranged from 1 μg per 5×10^8 cfu/ml of mycobacteria using the sonication method to approximately 6 μg using the FastPrep™/Divolab™ method (see Table 1). No RNA was detected using the standard method of extracting RNA from *E. coli* (the hot phenol method) reflecting the differences in the nature of the cell wall components of mycobacteria. It was clear that for efficient extraction, rapid lysis of the mycobacteria and inactivation of the

ribonucleases was essential and this could only be achieved by using the mechanical means of disrupting the bacilli in a solution containing ribonuclease inhibitors such as phenol or guanidium thiocyanate. No RNA was obtained from the methods that did not use a mechanical means of lysing the bacteria. The mark of a successful RNA extraction method is that the 16S rRNA and 23S rRNA species should be visible following formaldehyde or standard agarose gel electrophoresis. Even the RNA extraction method that produced the lowest yield of RNA (see Table 1) revealed the 16S rRNA and 23S rRNA as determined by formaldehyde gel electrophoresis (see Figure 1a). None of the methods used gave RNA free of indigenous DNA contamination (as determined by PCR of the RNA for the constitutively expressed *dnaK* gene), but contaminating DNA could be readily removed with DNase I. This step was very important in order to rule out false positive results that might be generated in the subsequent gene expression studies. To determine the integrity of the RNA extracted, RT-PCR was performed for *dnaK* and its expression was demonstrated in all samples from all the RNA extraction methods (Figure 2). RT-PCR was not performed with RNA obtained using methods that failed to reveal the presence of 16S rRNA and 23S rRNA bands.

In order to determine the main factor that influenced the efficient recovery of RNA from mycobacteria, a comparative assessment was carried out on all the RNA extraction methods using Trizol™ as the sole reagent to lyse *M. tuberculosis* and the samples were subjected to the different mechanical means of disrupting the cells (i.e. sonication, beadbeater and the Fastprep™ machine). RNA was then extracted using the procedure outlined in the beadbeating method. The FastPrep™ machine gave the highest recovery of RNA from mycobacteria while sonication method gave the least amount of RNA (Figure 3) and this efficiency could be increased further with the use of the Divolab detergent as described in Mangan *et al.'s* method [8]. Furthermore with this method, the RT-PCR for *dnaK* showed there were more transcripts from the same volume of RNA input used in the cDNA synthesis and subsequent PCR step (Figure 3). The disadvantage of this method was the fact that only a few of the samples could be used to generate cDNA because the reverse transcriptase appeared to be inhibited by the Divolab™ detergent (data not shown). Any effort to remove the detergent resulted in the loss of RNA, which suggests that a chemical complex formation between the RNA and the detergent. Furthermore, spectrophotometric analysis to determine the RNA concentration was not possible because of this inherent problem. However, the FastPrep™/Divolab™ method was

chosen over the other methods because of the safety aspect of the operation, the higher recovery of RNA, and the consistency of the method in obtaining RNA from mycobacteria. The FastPrep™/Trizol could be used instead of FastPrep™/Divolab™ since this method did not give much inhibition of the reverse transcription process. Based on this fact, the FastPrep™/Trizol was used in extracting RNA from infected macrophages. The result (Figure 4) showed that the presence of 16S and 23S was visible on ethidium bromide stained agarose gel from RNA extracted from intracellular bacilli, but not as visible as broth grown bacilli. To ascertain the validity of using RNA from infected macrophages, competitive RT-PCR was performed to compare the expression of *dnaK* gene during intracellular infection of macrophages with broth grown *M. tuberculosis* at transcriptional level, which had previously been shown to be upregulated during intracellular infection at the protein (translational) level (Lee and Horwitz, 1995) was studied. The *dnaK* expression was investigated by RT-PCR to confirm that this gene was expressed intracellularly prior to proceeding with a competitive RT-PCR. The result showed that there was expression of *dnaK* gene by the intracellular and extracellular broth-grown bacilli (Figure 5a). Further experiment was carried out to compare the expression of *dnaK* after equalising the RNA by competitive PCR for 16S gene, results showed that there was no much difference in the level of *dnaK* expression between the intracellular and broth-grown bacilli (Figure 5b) as the native *dnaK* amplicon shared equal intensity with the second dilution of the competitive *dnaK* amplicon in both the broth-grown and intracellular bacilli, respectively. .Quantitatively, the level of expression was found to be 1.6×10^{-4} pg in broth grown bacilli while that of intracellular macrophage was slightly less than 1.6×10^{-4} pg but more than 7.8×10^{-5} pg. In fact, if anything the gene appeared to be downregulated in intracellular bacilli after the overnight (15 hr) infection period compared to broth grown bacilli.

Discussion

RNA extraction is the cornerstone of RNA based gene expression analysis. Working with RNA can be very problematic because of the ubiquitous nature of the ribonucleases that destroy the RNA. Over the past decades this major problem has been solved with the introduction of ribonuclease inactivating agents such as DEPC, RNasin, vanadyl-ribonucleoside complexes, guanidium hydrochloride, and guanidium thiocyanate [9].

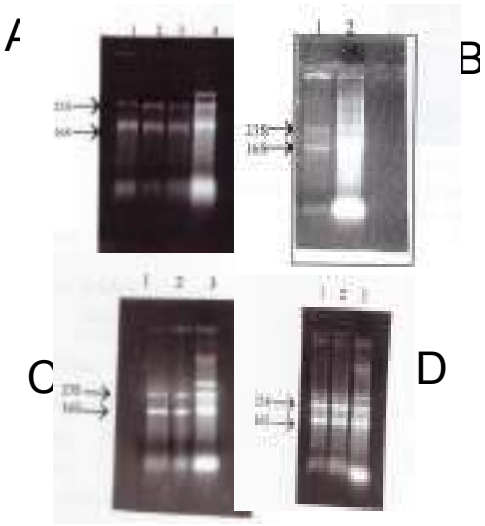

Figure 1a. RNA extracted using sonication method. Formaldehyde agarose gel electrophoresis of RNA. Lanes 1-3: RNA extracted from 9.6 × 10^9 *M. bovis* BCG cfu/ml (~ 2 µg RNA); lane 4: RNA size marker (5 µg).

Figure 1b. RNA extracted by the beadbeater/Trizol™ method. Formaldehyde agarose gel electrophoresis of *M. bovis* BCG RNA. Lane 1: RNA extracted from 1.0 × 10^8 BCG cfu/ml (~0.7 µg); lane 2: RNA size marker (2 µg).

Figure 1c. RNA extracted by the FastPrep™/Trizol™ method. Electrophoretic analysis of RNA extracted by the FastPrep™/Trizol™ method. Lanes 1 and 2: RNA extracted from 1.2 × 10^{10} *M. bovis* BCG cfu/ml (~1.5 µg); lane 3: RNA size marker (2 µg).

Figure 1d. RNA extracted by the FastPrep™/Divolab™ method. Electrophoretic analysis of RNA extracted by FastPrep™/Divolab™ method. Lanes 1 and 2: RNA extracted from 1.6 × 10^8 *M. bovis* BCG cfu/ml (~3 µg); lane 3: RNA size marker (2 µg).

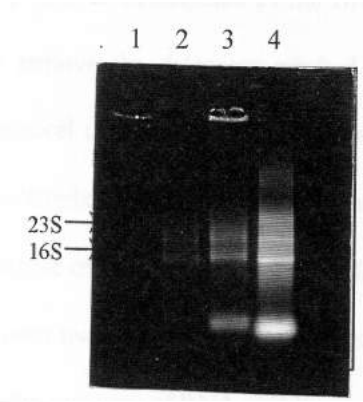

Figure 2. RNA extracted from *M. bovis* BCG using different physical methods of cell disruption. Electrophoretic analysis of RNA extracted from 1.5 × 10^8 cfu/ml of *M. bovis* BCG showing the yields of RNA obtained using the sonication method (lane 1: traces), beadbeating (lane 2: 0.5 µg) and FastPrep™ method (lane 3: 1.2 µg) Lane 4 : RNA size marker (2 µg).

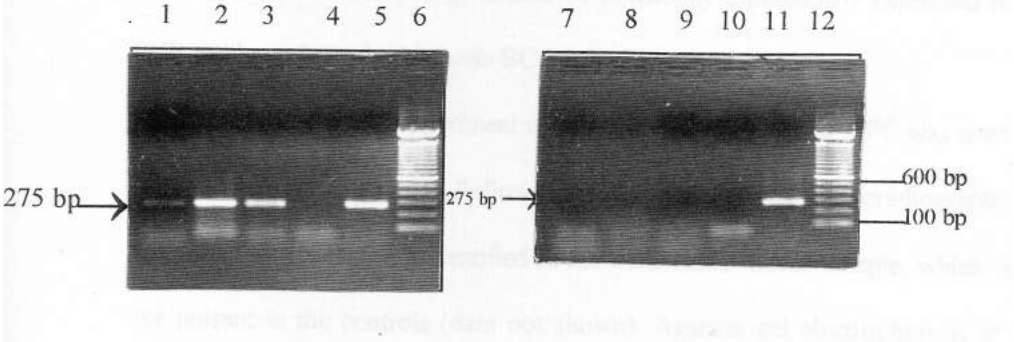

Figure 3. RT-PCR for demonstration of *dnaK* expression using RNA extracted by the different physical methods of cell disruption. Electrophoretic analysis of the RT-PCR products generated with RNA prepared from the sonication, FastPrep™, and beadbeating methods, respectively. Lanes 7, 8, and 9: DNA contamination controls for the sonication, FastPrep™, and beadbeating methods, respectively. Lanes 4 and 10: negative PCR controls; lanes 5 and 11: positive controls; and lanes 6 and 12: 100 bp DNA ladders. The arrows indicate *dnaK* 275 bp product.

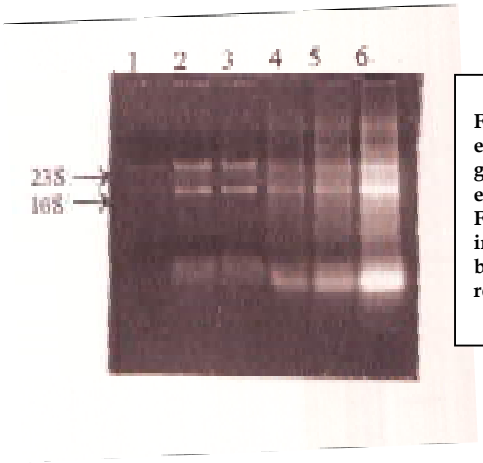

Figure 4. Typical yield of RNA from intracellular versus extracellular broth-grown M. tuberculosis (H37Rv). Agarose gel electrophoresis of RNA extracted from intracellular and extracellular broth grown *M. tuberculosis* by the FastPrep™/Trizol™ method. Lane 1: RNA from intracellular bacilli; lanes 2 and 3: RNA from broth grown bacilli; lanes 4-6: RNA size markers (1 µg, 2 µg, and 5 µg, respectively).

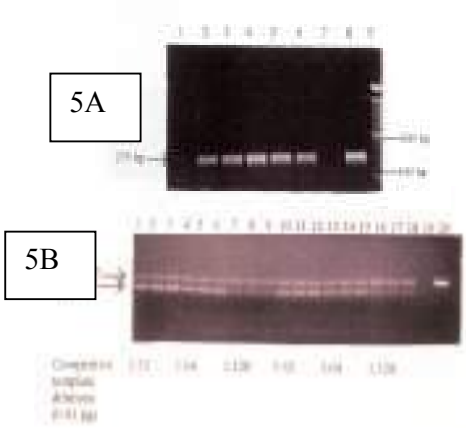

Figure 5a. Expression of *M. tuberculosis dnaK* gene during intracellular infection of macrophages. Electrophoretic analysis of RT-PCR products for *dnaK*. Lanes 1, 2, and 3: PCR products from ½, ¼, and ⅛ dilutions of the cDNA from extracellular broth grown bacilli, respectively. Lane 7: negative PCR control; lane 8: positive PCR control and lane 9: 100 bp DNA ladder. The arrow indicates the 275 bp *dnaK* product.

Figure 5b. Quantification of *dnaK* gene expression in intracellular and broth grown *M. tuberculosis* by competitive RT-PCR. Electrophoretic analysis of competitive RT-PCR products for *dnaK* gene. Various amounts of *dnaK* competitive DNA competed against a fixed concentration of native *dnaK* cDNA. Lanes 1-9 intracellular bacilli (in triplicate dilutions of the competitive template); lane 10-18: extracellular broth-grown bacilli (in triplicate dilutions of the competitive template); lanes 19 and 20: PCR negative and positive controls, respectively. The 275 and 195 bp products are the native and competitive amplicons, respectively.

Bacterial mRNA has a very short half life of approximately 1-3 min [15] which means speed is essential for the successful recovery of intact mRNA and the protection of RNA from ribonuclease (RNAse) is very important. Gene expression is accompanied by the transcription of a segment of DNA into mRNA, which later undergoes translation to protein. The way bacteria respond to their new environment is always accompanied by the expression of a new set of genes [16]. Therefore, the ability to isolate the necessary mRNA representing the genes expressed is very important.

A good RNA extraction method should be able to extract mRNA representing the genes being expressed at a particular point in time. However, not all the extraction methods can achieve this because of differences between microorganisms. The time taken to lyse mycobacteria by standard enzymatic methods (such as the use of lysozyme and detergent) will take longer than spontaneous lysis afforded by any of the machines used in this study. For example using lysozyme to lyse *E. coli*

with the hope of recovering RNA and the time involved will result in changes in gene expression and longer exposure times to RNAses and thus will not reflect true gene expression at a given time point. It is therefore important to use a method, which could lyse the mycobacteria rapidly. The first method used in this study was the hot phenol method devised for organisms such as *E. coli* and is based on the use of detergent to lyse the organism and phenol to protect the RNA from degradation. However, the fact that the RNA obtained was degraded and of a poor yield indicated that this method was inappropriate for extracting RNA from mycobacteria. The main reason for this probably lies in the differences in the mycobacterial cell wall architecture, which make mycobacterial cells more resistant to passive lysis. RNA extracted using mechanical means of lysing the bacilli was far more successful. The sonication method [11] gave good yields but failed to address the safety aspect of sonicating Category 3 pathogens and this was problematic when it comes to using *M. tuberculosis.* Moreover, the technique was time consuming. This problem was overcome by the use of the beadbeater (which also gave a better yield than the sonicator) as its compact size means it can be operated within the biohazard safety cabinet. The major drawback is that only one sample can be processed at a time. The introduction of the Fastprep™ reciprocal shaker several years ago by Savant Instruments offered a means to process many samples at a time. However, the use of this equipment still requires the use of an appropriate lysis solution to facilitate the maximum recovery of RNA from mycobacteria. The use of the lysis solution developed by Mangan *et al.*, [8] which comprised the detergent Divolab™ was found to improve the RNA yield considerably. The major disadvantage of this method was that spectrophotometric estimation of RNA concentration was not possible, which meant that other methods of estimating the RNA yield had to be used. The *M. tuberculosis dnaK* gene encoding the HSP71 protein was chosen to evaluate the use of RNA obtained by FastPrep/Trizol method for studying gene expression. The fact that there was no increase in the level of *dnaK* expression suggests that *dnaK* is not upregulated after 15 hr of infection. This is contrary to a report where it was found to be one of the most abundantly expressed genes during intracellular infection of macrophages [17]. The discrepancy between these results probably reflects the fact that these authors studied the differential expression of *dnaK* at the translational level whereas this study determined its expression at the transcriptional level. This suggests that the control of

expression of *dnaK* is most likely to be regulated at the translational level. The HSP71 protein is a stress protein and has shown to be upregulated in response to increase in temperature *in vitro* [11]. If *dnaK* was upregulated during intracellular infection it would be expected to have a specific function in relation to the intracellular environment of macrophages. It has been shown for a wide variety of bacterial pathogens that virulence and other factors, which play a role in host-parasite interactions, are co-ordinately regulated with heat shock proteins. These stress proteins are expressed as overlapping subsets of proteins in response to environmental stimuli likely to be encountered within the infected host [16, 18].

The improvements in extracting RNA from *M. tuberculosis* made throughout the study made it possible to achieve the objective of studying the gene expression of *M. tuberculosis* during intracellular infection of macrophages as demonstrate by the ability to determine the expression level of *dnaK* gene. With this RNA extraction method (FastPrep/Trizol), it is now possible to study whole genome expression by microarrays or individual gene expression, which could provide more information in annotating some of the genes of unknown function in completed genome sequence of *M. tuberculosis* [5] and other species from the same genus. Recent study from the authors' laboratory has shown it is possible to use the RNA extracted with FastPrep/Trizol to study individual gene expression [19].

Acknowledgements

This work is funded by Glaxo-Wellcome as part of action TB initiative.

References

1. WHO: **Global Tuberculosis Control 2008: Surveillance, planning, Financing**. In. Geneva: World Health Organization; 2008.
2. Fine PEM: **Bacille Calmette Guerin Vaccines: A rough guide.** *Clinical Infectious Diseases* 1995, **20**:11-14.
3. MRC: **BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life.** *Bulletin of the World Health Organization* 1972, **46**:371-385.
4. Paramasivan CN, Govindan D, Prabhakar R, Somasundaram PR, Subbammal S, Tripathy SP: **Species level idenification of non-tuberculous mycobacteria from South Indian BCG trial area during 1981.** *Tubercle* 1985, **66**:9-15.
5. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE, 3rd *et al*: **Deciphering the biology of**

Mycobacterium tuberculosis from the complete genome sequence. *Nature* 1998, **393**(6685):537-544.

6. Garnier T, Eiglmeier K, Camus JC, Medina N, Mansoor H, Pryor M, Duthoy S, Grondin S, Lacroix C, Monsempe C *et al*: **The complete genome sequence of Mycobacterium bovis**. *Proc Natl Acad Sci U S A* 2003, **100**(13):7877-7882.
7. Stinear TP, Seemann T, Harrison PF, Jenkin GA, Davies JK, Johnson PD, Abdellah Z, Arrowsmith C, Chillingworth T, Churcher C *et al*: **Insights from the complete genome sequence of Mycobacterium marinum on the evolution of Mycobacterium tuberculosis**. *Genome Res* 2008, **18**(5):729-741.
8. Mangan JA, Sole KM, Mitchison DA, Butcher PD: **An effective method of RNA extraction from bacteria refractory to disruption, including mycobacteria.** *Nucleic Acids Research* 1997, **25**:675-676.
9. Wilkinson M: **Purification of RNA**. In: *Essential Molecular Biology - A Practical Approach.* Edited by Brown TA, vol. I. Oxford: Oxford University Press; 1991: 69-87.
10. Von Gabain A, Belasco JG, Schottel JL, Chang ACY, Cohen SN: **Decay of messenger RNA in Escherichia coli - investigation of the fate of specific segments of transcripts.** *Proceedings of The National Academy of Sciences of the United States of America* 1983, **80**:653-665.
11. Patel BKR, Banerjee DK, Butcher PD: **Extraction and characterization of mRNA from mycobacteria: implication for virulence gene identification**. *Journal of Microbiological Methods* 1991, **13**:99-111.
12. Chomczynski P, Sacchi N: **Single-step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction.** *Analytical Biochemistry* 1987, **162**:156-159.
13. Cheung AL, Eberhardt KJ, Fischetti VA: **A method to isolate RNA from Gram-positive bacteria and mycobacteria.** *Analytical Biochemistry* 1994, **222**:511-514.
14. Sambrook J, Fritsch EF, Maniatis T: **Molecular Cloning: A laboratory manual.**: Cold Spring Harbor Laboratory Press; 1989.
15. Lewin B: **The messenger RNA template.** In: *Genes IV*. Oxford: Oxford University Press; 1990: 171-184.
16. Guiney DG: **Regulation of bacterial virulence gene**

expression by the host environment. *Journal of Clinical Investigation* 1997, **99**:565-569.

17. Lee BY, Horwitz MA: **Identification of macrophage and stress-induced proteins of Mycobacterium tuberculosis**. *J Clin Invest* 1995, **96**(1):245-249.

18. Buchmeier NA, Heffron F: **Induction of Salmonella stress proteins upon infection of macrophages.** *Science* 1990, **248**:730-732.

19. Alli OAT, Hingley-Wilson S, Ogbolu DO, Spreadbury CL: **The Mycobacterium tuberculosis homologue of the Mycobacterium avium mig gene is not specifically expressed in the macrophage.** *African Journal of Biomedical Research* 2008, **11**:173-181.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2009 ISBN 1595-689X VOL 10(2)
AJCEM/200903/20910 -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 10(2): 80- 87

STUDIES ON THE DISTRIBUTION OF CLINICALLY DIAGNOSED PULMONARY TUBERCULOSIS IN EBONYI STATE, NIGERIA

***Obi, R.K[1], Amadi, A.N.[2], Idika I.M [3], Nwanebu, F.C[1] Departments of Microbiology[1], and Public Health Technology[2], Federal University of Technology, Owerri, Imo State and Presbyterian Joint Hospital[3], Uburu, Ebonyi State, Nigeria.**
**Author for correspondence: robertobi_2003@yahoo.ca*

Abstract
A study was conducted between January, 2005 to December, 2006 to ascertain the prevalence of pulmonary tuberculosis among patients who attended chest clinics in some randomly selected hospitals, Clinics and Health Centers in Ebonyi State, Nigeria. Investigations were carried out using the two popular diagnostic criteria for pulmonary tuberculosis namely the specific Ziehl Neelsen (ZN) methods and chest X-ray. A total of 962 patients with clinical signs and symptoms of tuberculosis were studied. Out of this figure, 559 (58.1%) had pulmonary tuberculosis with 1:1 male/female ratio. There was no significant difference between ZN sputum smear positive and chest x-ray in the diagnosis of pulmonary tuberculosis in the studied population at 95% confidence level. The possible reasons for the high prevalence of pulmonary tuberculosis may be attributed to increase in the incidence of HIV/AIDS, high rate of poverty, emergence of drug-resistant strains of *Mycobacteria* and to a lesser extent, smoking and diabetes.
Key words: Pulmonary tuberculosis, sputum, chest X-ray, Ziehl Neelsen.

INTRODUCTION

Pulmonary tuberculosis (TB) is a common, deadly infectious disease caused by *Mycobacteria*, mainly *Mycobacterium tuberculosis.* Tuberculosis most commonly attacks the lungs but can also affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, bones, joints and even the skin.

Other *Mycobacteria* such as *Mycobacterium bovis, Mycobacterium africanum, Mycobacterium canetti* and *Mycobacterium microti* can also cause tuberculosis especially in individuals with defective immune systems [1, 2]

Tuberculosis can develop after inhaling droplets sprayed into the air from a cough or sneeze by someone infected with *M. tuberculosis.* The risk of contracting TB however increases with frequency of contact with people who have the disease, with crowded or unsanitary living conditions and with poor nutrition[2]. Others at risk of contracting the disease include intravenous drug abusers, medically under-served and low-income populations, and patients immunocompromised by conditions such as HIV/AIDS, and health care workers serving these high risk patients[3].

Initial infection of TB (known as primary pulmonary tuberculosis) occurs when *Mycobacteria* reach the pulmonary alveoli and invade and

replicate within alveolar macrophages [4]. This primary stage of the infection is usually asymptomatic and in most cases patients recover from the infection without further evidence of the disease [5]. However in a few number of cases, especially when the immune system does not successfully contain the primary infection, the latent or asymptomatic TB always progresses to the active case. Symptoms of active cases of TB include chest pain, coughing up blood (hemoptysis) and a productive, prolonged cough for more than three weeks. The symptoms could also be systemic and may include fever, chills, night sweats, appetite loss, weight loss, pallor and often, a tendency to fatigue easily [6]. If untreated, active TB disease kills more than half of its victims [7].

Over one third of the world's population are carriers of the bacterium, with new infections occurring at a rate of one per second[6]. This increase in TB resurgence may be attributable to increase in HIV infection and neglect of TB control programs, poor environment, poor nutrition and the emergence of drug resistant strains of the *Mycobacterium*[8;9]. As a result of the above factors, which also have worsened treatment efforts, TB has now been declared a global health emergency by the World Health Organization [10].

Materials and methods

Sampling

A total of 962 patients with suspected cases of pulmonary tuberculosis who presented to different chest clinics in some randomly selected hospitals and clinics in Ebonyi State were used for this two-year study which spanned from January 2005 to December 2006. This included 648 males and 314 females. The purpose of the study was fully explained to them by the medical officers and their informed consents obtained prior to the study as recommended by the World Health Organization [11].

Diagnosis

A combination of chest x-ray and ZN techniques was used to screen the patients after physical examinations were made to assess their general health status. Sputum samples were obtained from patients showing signs of prolonged coughing to investigate for the presence of *Mycobacterium tuberculosis* using the Zieehl–Neelson's acid fast staining method as applied by[12]. In the process each sputum specimen was swirled in its neat screw – capped container and with the help of a wire loop, smeared at the middle of a clean glass slide. The smear was air-dried, heat-fixed over the bunsen burner flame and flooded with strong carbol fuchsin solution with the application of gentle heat for five minutes, on a staining rack. The smear

was next washed with water and decolourized by covering with 3% acid – alcohol for 10 minutes. It was washed again with water and counter stained using Loeffler's methylene blue solution for 30 seconds. The stained smear was allowed to air-dry and examined microscopically using oil immersion (x1000) objective. The acid – fast bacteria appeared as bright red coloured rods that stood out clearly against the blue background of methylene blue, indicating positive cases. A parallel chest x–ray test was run on the same patients. The chest x-ray which involved the standard posterior – anterior view was used as an extra confirmatory test for tuberculosis as recommended by[13]. In the test, cavitary lesions in the upper lobe of the lungs or mediastinal lymphadenopathy accompanied by patchy irregular consolidations indicated active pulmonary TB cases. Inactive cases were observed as fibrocalcific scar within the lungs.

Results

The results (Table 1) showed that a total number of 559 (58%) out of the 962 were diagnosed as PTB in the study. This is the sum of the occurrence in 2005 with 260 cases (27%) and 2006 with 299 cases (31%). There was no significant difference ($p > 0.05$) between the rate of PTB occurrence in years 2005 and 2006. The results as seen in Table 2 also showed that 324 (34%) males and 235 (24%) females tested positive for PTB.

TABLE 1: DISTRIBUTION OF PULMONARY TUBERCULOSIS AMONG PATIENTS IN UBURU, EBONYI STATE.

YEAR	TOTAL SCREENED	ACTIVE PTB	INACTIVE PTB	PERCENTAGE ACTIVE PTB
2005	461	260	201	27
2006	501	299	202	31
TOTAL	962	559	403	58

TABLE 2: DISTRIBUTION OF PTB AMONGST PATIENTS ACCORDING TO AND SEX IN UBURU, EBONYI STATE.

YEAR	Males	Female
2005	152	108
2006	172	127
TOTAL	324 (34%)	235 (24%)

Discussion

In 2005, 260 (27%) active TB cases were diagnosed in the community under survey. The figure rose to 299 (31%) in 2006. This represents an increase of 39 (4%) showing that more people may have been exposed to the pre-disposing factors that lead to the observed increase in the incidence of the disease in a space of only one year (Table 1).

Similarly, 152 (27.2%) men were diagnosed as active TB cases in 2005 while the number of women was 108 (19.3%) showing a difference of 44 (7.9%) more men than women diagnosed of the infection in the year. In 2006, the number of men diagnosed rose significantly to 172 (30.8%) representing an increase of 6.2 in the diagnosis of men made in 2005 and 2006, while that of women also increase to 127 (22.7%) similarly representing an increase of 8.1% in the diagnosis of women in 2005 and 2006. The difference between diagnosis made of women and men was observed to be 45 (8.1%) in the year (Table 2).

Incidence of TB was indeed on a steady increase in Uburu with more men suffering from the ailment than women probably because they were known to indulge more in the pre-disposing factors to the infection than the latter. This observation has been corroborated by that made by the World Health Organization, who reported that the incidence of TB was on the increase worldwide with nearly 2 billion people, representing one-third of the World's population suffering from the disease 14 . In addition, the Centre for Disease Control and Prevention 15 reported that about 8 million people are added to this figure with about 2 million deaths resulting ailment annually.

Some factors which have been implicated in the re-emergence and subsequent epidemics of TB cases all over the world were also observed to be responsible for the increase in the incidence of the disease in Uburu. These include increase in the incidence of HIV/AIDS; individuals with immune systems damaged by HIV/AIDS have a higher risk of developing active TB – either from new exposure to the disease or a reactivation of dormant *Mycobacteria*. With the destruction of the immune system, treatment will become more difficult with the result that the disease will become more resistant to therapy[10]. Furthermore, the rate of emergence of drug – resistant strains of *Mycobacteria*, was enhanced by

inadequate treatments through not taking the prescribed regimen appropriately, or using low quality medications [16;2]. Drug resistant *Mycobacteria* caused an increase of about 20% in TB resistance to standard treatments and 2% resistant to second-line drugs recorded between 2000 and 2004[5]. In addition there was a high level of poverty observed among the people in Uburu which must have contributed to the high prevalence of TB in the area. Poverty has been reported to be a very important factor in disease progression and prevalence in developing countries especially Sub-Saharan Africa where majority of the twenty countries of the world with the highest rates of TB cases are said to be located[10]. Finally[17] reported that in addition to other factors, smoking more than 20 sticks of cigarette every day could increase the risk of succumbing to TB infections, while[18] identified diabetes mellitus as the greatest predisposing risk factor to this disease that is growing in importance in developing countries. Similar factors may also have been responsible for the rising cases of TB noticed in Uburu.

With the incidence of HIV/AIDS pandemic which was reported[19] to be highly prevalent in Nigeria still wreaking havoc on the health status of many individuals, the re-emergence of TB epidemics in addition may initiate calls for a declaration of a state of emergency in the health sector. Fortunately, the WHO has already declared the disease a global health emergency and has instituted several measures to assist developing countries to control the infection and spread of *Mycobacteria*. The Nigerian government on its part is complementing this effort through its adequate provision of preventive BCG vaccines for infant vaccination in various hospitals and health centers; and numerous radio, newspaper, and television advertisements, urging anyone with suspected signs and symptoms of the disease or who is positive for the HIV virus to report for immediate diagnosis for TB. Positive individuals would commence early treatments with antituberculous drugs distributed freely at government-owned hospitals, health centers and clinics.

However, the persistence and upsurge in TB cases today despite this generosity from government, calls for stringent managerial policies to be instituted to handle cases and contain the epidemic before the greater population in this country falls victim to the infection. To this effect, efforts should be made to improve the standard of living of every

Nigerian through adequate provision of basic and essential amenities such as potable water, good food and affordable housing. Also in any situation where a patient has been diagnosed as a TB case, adequate control measures should be instituted to break the chain of possible transmission of infection to others by isolating such a patient compulsorily in a health center or clinic where intensive care could be given. Antituberculous drugs administered to such a patient should be closely monitored to ensure strict compliance to prescriptions and duration of treatments and thus prevent any form of drug resistance developing as a result of treatment failures. Furthermore, since inactive TB cases could progress to active forms later in life, especially after a reactivating infection of HIV, it will only become reasonable to subject all inactive cases to appropriate treatment with antituberculous drugs. In addition, campaigns on the treatment, prevention and control of HIV/AIDS should be intensified. Government must create adequate awareness on the aetiology and epidemiology of TB infection, stressing the need for healthy living involving good feeding, clean environment and adequate housing. However, government should provide relevant health institutions with diagnostic equipment and materials to detect *Mycobacteria*. Trained environmental personnel should be posted to the Local Government Areas to monitor public compliance to laid-down policies on control and early detection of any outbreaks of the disease. Finally adequate protection should be given to all medical personnel and healthcare providers who handle TB patients, through immunization, payment of adequate hazard allowances and training since their constant contact with these high risk patients is a danger, not only to themselves but to the larger part of the society.

REFERENCES

1. Raviglione, M.C and O'Brien, R.J (2004). Tuberculosis in Kasper, D.L, Braunwald, E., Fauci, A.S., Hauser, S.L., Longo, D.L., Jameson, J.L., Isselbacher, K.J. eds: Harrisons Principles of Internal Medicine, 16th ed., McGraw-Hill Professional, 953-66.
2. Ferrara, G., Loci M, Meacci, M (2005). Routine Hospital use of a New Commercial Whole Blood Interferon – (gamma) Assay for the Diagnosis of Tuberculosis Infection. *Am J. Respir Crit Care Med.* **172(5):** 631-5

3. Griffith, D., and Kerr, C 91996). "tuberculosis: disease of the past, disease of the present." *J. Perianesth Nurs* **11(4):** 204-5.

4. Onyebujoh, P., and Rook G.A.W (2004). World Health Organization Disease Watch: Focus: Tuberculosis. http://en:wikipedia.org/wiki/ tuberculosis.

5. Madison, B. (2006). Emergence of Mycobacterium tuberculosis with extensive resistance to second line drugs worldwide, 2000-2004. *MMWR Morb Mortal Wkly Rep* **55(11):** 301-5.

6. World Health Organization (2006). Tuberculosis fact sheet No:14 Global and regional incidence. http://www.who./mediacenter/factsheets/fs14/en/.

7. Sobero, R., Peabody, J. (2006). Tuberculosis control in Bolivia, Chile, Colombia, and Peru: why does the incidence vary so much between neighbours? *Int. J. Tuberc. Lung Dis* **10 (11)**:1292-5.

8. Iademarco, M.F., and Castro, K.G. (2003). Epidemiology of tuberculosis. *Seminars in respiratory infections* **18(4):** 225-40.

9. CDC (2003) Division of Tuberculosis elimination. Core Curriculum on tuberculosis: What Clinicians should know. 4th edition. http:// www. Cdc/gov/ncidod/dbmd/diseaseinfo/tuberculosi_g.htm.

10. World Health Organization (2006) Global tuberculosis control – surveillance, planning, financing. http://en.wikipedia.org/wiki/ surveillance_planning_financing

11. TDR (2002) work look for investigators UNDP / World Bank / WHO Special Programme for Research and Training on Tropical Diseases (TDR / PRD / GCP / 02). Pp30

12. Madison, B (2001). Application of stains in clinical microbiology *Biotech Histochem* **76(3):** 119-25.

13. Nahid, P., Pai, M., Hopewell, P. (2006). Advances in diagnosis and treatment of tuberculosis. *Trends Microbiol* **6(3):**107-12.

14. National Institute of Allergy and Infectious Diseases (NIAID) (2005). Microbes in sickness and in health. http://www.niaid.nih.gov/factsheets/microb_sickness_health.htm.

15. CDC (2006). 2005 Surveillance Slide

Set.http://www.Cdc/gov/ncidod/dbmd/diseaseinfo_surveillance/tuberculosi_g.htm.

16. O' Brien, R. (1994). Drug – resistant tuberculosis: etiology, management and prevention. *Semin Respire Infect.* **9(2):** 104-12.
17. Davies, P.D.O, Yew W.W., Ganguly, D. (2006). "Smoking and tuberculosis: the epidemiological association and pathogenesis." *Trans R Soc Trop Med Hyg* **100**:291-8.
18. Restrepo, b.I. (2007). Convergence of the tuberculosis and diabetes epidemics: renewal of old acquaintances. *Clin Infect Dis* **45:**436-8.
19. Obi, R.K, Iroagba, I.I, Ojiako, O.A, (2007). Prevalence of human immunodeficiency virus (HIV) infection among pregnant women in an antenatal Clinic in Port Harcourt, Nigeria. *Afr. J. Biotechnol* **6(3):** 263-266.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2009 ISBN 1595-689X VOL 10(2)
AJCEM/200908/20911 -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 10(2): 88-91

SENSITIVITIES OF *CITROBACTER*, *PROTEUS* AND *PROVIDENCIA* ISOLATES TO SULBACTAM-AMPICILLIN,TRIMETHOPRIM-SULFAMETHOXAZOL AND TICARCILLIN-CLAVULANIC ACID ANTIBIOTICS

GUVEN URAZ[1], TURHAN KOK[1] , HANDE D. BINNET[1]
Gazi University, Faculty of Science and Arts, Department of Biology , Teknikokullar ANKARA/TURKEY

Corresponce: Hande D. Bannet, Gazi University, Faculty of Science and Arts, Department of Biology, Teknikokullar ANKARA-TURKEY. E mail: handebinnet@yahoo.com

ABSTRACT

Gram negative bacterias which belong to Enterobacteriaceae family which is critically important as a matter of human health, are comperatively prevalent in nature and foods. Infections formed by bacterias resistant to antibiotics significantly cause mortality and economical losses. Sensitivities of gram-negative bacterias isolated from miscellaneous samples to sulbactam-ampicillin, trimethoprim-sulfamethoxazol and ticarcillin-clavulanic acid were analysed. In this study, *Proteus* is primary isolated microorganism with % 52,08. It was determined that *Proteus* types are proportionally 86,66% sensitive to Trimethoprim-Sulfamethoxazol and 76% resistant to Sulbactam-Ampicillin . *Citrobacter* is the secondary isolated microorganism (31,25%). It was determined that *Citrobacter* types were 86,66% sensitive to Trimethoprim-Sulfamethoxazol and % 73,3 resistant to Sulbactam-Ampicillin. *Providencia* types which are thirdly isolated microorganism (16%), For *Providencia* types, Trimethoprim-Sulfamethoxazol were determined as the most effective antibiotic again with 86,66% sensitivity. Resistance to Sulbactam-Ampicillin was however, found to be 76%. In conclusion, it is very important to conduct sensitivity tests in choosing antibiotics for chemotherapy of infections. Trimethoprim-Sulfamethoxazol is recommended in the empiric treatment of urinary tract infections in our environment.

Key Words: Enterobacter, sensitive, SXT, TİM, SAM

INTRODUCTION

Gram negative bacteria belonging to *Enterobacteriaceae* family, which is critically important as a matter of human health, are prevalent in nature and foods. They especially exist in water and materials contaminated by excrement (1). Consequently, they are important sources of infection for humans when they consume contaminated foods. Infections caused by bacteria resistant to antibiotics significantly cause mortality and economical losses. Trimethoprim-sulfamethoxazol antibiotics and beta lactam inhibitors are frequently used in the treatment of Citrobacter, Proteus and Providencia, which belong to the family *Enterobacteriaceae*. For this reason, sensitivities of gram-negative bacteria isolated from miscellaneous samples to sulbactam-ampicillin, trimethoprim- sulfamethoxazol and ticarcillin-clavulanic acid were analyzed.

MATERIALS AND METHODS

In research, microorganisms isolated from 200 raw milk samples were identified as API 20 E kit (bioMeriux).In study,antibiotic sensitivities of isolated microorganisms,in accordance with NCCLS criterias, had been tested by the method of Kirby-Bauer disc diffusion in culture of Müeller-Hinton Agar (1).

In study, as a result of terming done with API 20 E kit from 200 samples, overall 48 (% 24) gram-negative isolate were obtained. It was determined that 15 (% 31,25) of these 48 isolates is *Citrobacter*, 25 (%52,08) of 48 is *Proteus* and 8 (%16,67) of 48 is *Providencia* type (Table 1).

Table 1: Distribution of isolated gram-bacteria

Type	Family	number of isolate
Citrobacter	*Citrobacter amaloniticus*	**2**
	Citrobacter braaki	**1**
	Citrobacter diversus	**1**
	Citrobacter freundi	**11**
Protes	*Proteus mirabilis*	**13**
	Proteus penneri	**6**
	Proteus vulgaris	**6**
Providencia	*Providencia rettgeri*	**6**
	Providencia stuarti	**1**
	Providencia alcalifaciens	**1**

RESULTS

In study, 2 (4,17%) *C.amaloniticus*, 1 (2,08%) *C.diversus*, 1 (2,08%) *C.braaki*, 11 (22,92%) *C. freundii* isolates belonged to *Citrobacter* type.Thirteen (%27,08) *P. mirabilis*, 6 (%12,5%) *P.penneri* 6 (%12,5) *P.vulgaris* isolates belonged to *Proteus* type. Six (12,5%) *P. rettgeri*, 1 (2,08%) *P. stuarti*, 1 (2,08%) *P.alcalifaciens* isolates belonged to *Providencia* type. Antibiotic sensitivities of isolated bacterias are shown in table 2, table 3 and table 4. Sulbactam-Ampicillin was found to be more effective than Trimethoprim-Sulfamethoxazol and Ticarcillin-Clavulanic acid in the treatment of urinary tract infections.

Table 2: Sensitivity proportions of isolated *Citrobacter* bacterias to tested antibiotics

Antibiotic	Sensitive (number of isolate)	medial sensitive (number of isolate)	resistant (number of isolate)
SAM	**2 (%13,3)**	**2 (%13,3)**	**11 (%73,3)**
SXT	**13 (%86,66)**	**0 (%0,0)**	**2 (%13,33)**
TİM	**3 (%20)**	**2 (%3,66)**	**10 (%66,6)**

SAM:Sulbactam-Ampicillin;SXT:Trimethoprim-Sulfamethoxazol;TİM:Ticarcillin-Clavulanic acid

Table 3: Sensitivity proportions of isolated *Proteus* bacterias to tested antibiotics

Antibiotic	Sensitive (number of isolate)	medial sensitive (number of isolate)	Resistant (number of isolate)
SAM	2 (%8)	4 (%16)	19 (%76)
SXT	24 (%96)	0 (%0,0)	1 (%4)
TİM	6 (%24)	6 (%24)	13 (%52)

SAM:Sulbactam-Ampicillin;SXT:Trimethoprim-Sulfamethoxazol;TİM:Ticarcillin-Clavulanic acid

Table 4: Sensitivity proportions of isolated *Providencia* to tested antibiotics

Antibiotic	Sensitive (number of isolate)	medial sensitive (number of isolate)	Resistant (number of isolate)
SAM	2 (%25)	1 (%12,5)	5 (%62,5)
SXT	2 (%25)	2 (%25)	4 (%50)
TİM	1 (%12,5)	3 (%37,5)	4 (%50)

SAM:Sulbactam-Ampicillin;SXT:Trimethoprim-Sulfamethoxazol;TİM:Ticarcillin-Clavulanic acid

DISCUSSION

In treating bacterial infections, determining antimicrobial sensitivity is very important for success. Urinary tract infections caused by *Proteus* bacterias were declared as chronic

in nature and difficult to treat (2). In this study, *Proteus* was the most frequently isolated microorganism (52,08% of isolates). It was determined that *Proteus* types were highly sensitive to Trimethoprim-Sulfamethoxazol -SXT

(86,66 %) and resistant to Sulbactam/Ampicillin –SAM (76%). *Citrobacter* types mostly couse urinary tract infections (3). In study, *Citrobacter* was the second most frequently isolated microorganism (31,25% of isolates). It was determined that *Citrobacter* types were 86,66% sensitive to Trimethoprim-Sulfamethoxazol SXT and % 73,3 resistant to Salbactam SAM antibiotic. On the other hand, *Providencia* types which were the thirdly isolated microorganism (16,66% frequency) are known to cause lower and upper urinary tract infections and kidney stone formation. Within the *Providencia* types, SXT were determined as the most effective antibiotic again with 86,66% sensitivity. Resistant of Sulbactam antibiotic however,were determined as 76%. In different studies done in our country, it was determined that Sulbactam resistance amongst gram-negative bacterias is between 80 and 100% and Trimethoprim-Sulfamethoxazol SXT resistance is

between 38 and 100 (4,5). As per the results of our research, sensitivity of acterias to Trimethoprim-Sulfamethoxazol SXT antibiotic were found to be significanly higher than those of Sulbactam SAM and Ticarcillin-Clavulanic acid. In conclusion, it is very important to conduct sensitivity tests in choosing antibiotics for chemotherapy of infections. Trimethoprim-Sulfamethoxazol is recommended in the empiric treatment of urinary tractm infections in our environment.

REFERENCES

1. National Committee for Clinical Laboratory Standarts.Performance Standarts for anti- microbial suscebility testing.Ninth Informational Supplement.1999, M100-S9 19;1.

2. McLean, R.J.C Nickel JC, Cheng K.J, Costerton J.W. (1988) The ecology and pathogenicity of urease producing bacteria. in the urinary tract.CRC Crit Rev Microbiol. 16:37-39

3. Abbot SL:Klebsiella, Enterobacter, Citrobacter, Serratia and other enterobacteriaceae,"Murray PR, Baron EJ, Pfaller Ma, Jargensen Yh, Yolken RH (2003) (eds). Manual of Clinical Microbiology 8.edit. ASM pres,Washington,DC 684-695

4. Urbarlı A, Arı A, Erdenizmenli M, Fidan N, Özgenç O (2001) Abstracted gram negative bacterias and their resistant proportions in urine samples Infecs. Magazine 15:249

5. Türkmen L (2002) Sensitivity to different antibiotics of gram negative bacterias isolated from urine samples. İnönü University Medical Facul. Magazine 9-185

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY2009 ISBN 1595-689X VOL 10(2)
AJCEM/200915/20912 -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 10(2): 92-98

CANDIDA SPECIES AMONGST PREGNANT WOMEN IN BENIN CITY, NIGERIA: EFFECT OF PREDISPOSING FACTORS

[1]Oviasogie F.E, and [2*]Okungbowa F.I. Departments of [1]Microbiology and [2]Botany, University of Benin, P.M.B, 1154, Benin City, Nigeria.

*Author for correspondence: Department of Botany, University of Benin, P.M.B, 1154, Benin City, Nigeria. Email: fiokun2002@yahoo.com Tel: **08055376204.**

Abstract

Genital samples from pregnant women were examined to determine the *Candida* species present and how some predisposing factors would affect the frequency of isolation of species. A total of 147 women (87 volunteer asymptomatic pregnant women and 60 asymptomatic non-pregnant women) were examined. High Vaginal Swabs (HVS) were collected in pairs while demographic data (age of individual, age of pregnancy, occupation and use of contraceptive pill) on each subject were obtained alongside. Samples were cultured on Sabouraud Dextrose Agar plates and incubated for 72 hours at 37°C. Results revealed five *Candida* species (*C.albicans, C.glabrata, C.tropicalis, C.stellatoidea, C.parapsilosis*) with 61.5, 17.9, 7.7, 5.1, 2.6 % frequency of isolation respectively, as against 73.7, 7.7, 1.4, 11.3, and 0.0% for control. Women in the second trimester of pregnancy had the highest occurrence (68.8%) while the age groups 24-30 and 31-37 years had the highest occurrence in the control (46.2%) and in the pregnant ones (51.7%) respectively. Traders had 68.8%, and full-time housewives, 26.7% occurrence. More women who used contraceptives had *Candida* (58.3%) as against non contraceptive users (35.3%). The results support literature reports that there are high rates of colonization of the vagina of pregnant women by *Candida*. They also give credence to earlier reports that the factors determined here affect the frequency of isolation of *Candida* species from the vagina.
Keywords: *Candida*, pregnancy, women, genital specimens, Benin City.

Introduction

Though members of the normal flora of the skin and mucosal surfaces of humans, several species of *Candida* are capable of causing candidiasis, as infection caused by *Candida* is called (1). Where they occur as normal flora, *Candida* species may gain dominance when there is a disruption of the normal flora balance of the body (as in the immunosuppressed, for example) producing progressive systemic disease (1). Some *Candida* species are also found in the soil and food (2).

The fungus *Candida* lives in small numbers in a healthy vagina, rectum and mouth (3, 4). About 75% of women generally harbour this fungus without it causing harm to them (5,6). Some of the factors which predispose women to vaginal candidiasis are change in pH, use of oral contraceptives, tight clothing, and personal hygiene (4,7). However, it is reported that there is increase in occurrence of vaginal candidiasis during pregnancy due to increased levels of hormones such as

oestrogen and steroid hormones (8). *Candida* infection in pregnancy does not usually harm the unborn child but causes great discomfort to the mother, which includes increased discharge, redness, itching, and burning sensation in the vulva area (9). If the disease is not treated the baby can get infected (oral thrush) at birth which can be a very serious health problem in premature babies. Also, untreated vaginal infections can lead to pelvic inflammatory disease, a condition which can scar the fallopian tube and cause infertility (10). The aim of this study was to determine the *Candida* species present in the vaginas of pregnant women attending two private hospitals in Benin City, and examine the role of age of subject, occupation, age of pregnancy and use of oral contraceptives on the occurrence of *Candida*.

Materials and Methods

***Specimens*:** A total of 87 volunteer pregnant women attending antenatal clinic and 60 non-pregnant women at Suyi Hospital and Lahor Clinic both in Benin City, were screened. High Vaginal Swabs (HVS) were collected in pairs from individuals while demographic information such as age of individual, age of pregnancy, occupation, use of contraceptive pill, were also collected from the women alongside the specimens. For the controls (non pregnant women) absence of pregnancy was confirmed with the HCG Pregnancy Kit (Quimica Clinica Aplicada,S.A. Amposta,, Spain).

Microscopic examination of specimens

One swab from each pair was used for wet preparation direct mount under the x40 power of an optical microscope to detect the presence of blastospores and pseudohyphae, while the other swab was used to streak Sabouraud Dextrose Agar SDA (Oxoid, England) plates containing 0.5ml Chloramphenicol (500mg,Pfizer) and incubated at 37^0C in triplicates. Uninoculated plain agar plates served as control for the inoculated ones. Discrete yeast colonies were subcultured on fresh SDA slants in McCarthney bottles for identification and storage.

***Identification of Candida species*:** Specimens were identified using CHROMagar *Candida* (France) and API 20C System (Analytab Products, USA) according to earlier procedures (11, 12).

Statistical analysis: Chi-Square test was used to analyze the results.

Results

Five *Candida* species were isolated with varying distributions, namely, *C .albicans* (61.5, 73.7), *C. glabrata* (17.9, 7.7), *C. tropicalis* (7.7, 1.4), *C. stellatoidea* (5.1, 11.3), *C. parapsilosis* (2.6, 0.0 %) for pregnant and non-pregnant women (control), respectively (Fig. 1). The mean frequencies of isolation of *Candida* from women in the first, second and third

trimesters were 33, 68 and 30% respectively (Tab. I).

FIGURE 1: DISTRIBUTION FREQUENCY OF *CANDIDA* SPECIES IN SAMPLED POPULATION

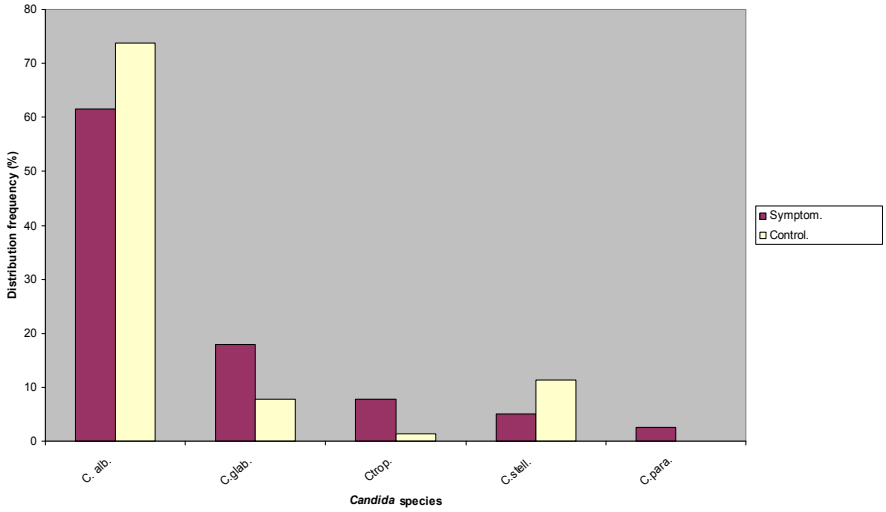

TABLE I: OCCURRENCE OF *CANDIDA* SPECIES ACCORDING TO AGE OF PREGNANCY.

Trimester (weeks)	Sample size	positive cultures		Positive cultures
		No.	%	% in total (87) samples
First (0 -12)	6	2	33.3	2.3
Second (13 -26)	32	22	68.8	25.3
Third (27 -40)	49	15	30.6	17.2

TABLE II: AGE DISTRIBUTION OF PATIENTS WITH *CANDIDA* SPECIES

Age (years)	Sample size		No. of positive cultures		% of positive cultures	
	Exper..	Control	Exper.	Control	Exper.	Control
17-23	29	20	15	7	51.7	35
24-30	46	26	20	12	43.5	46.2
31-37	11	13	4	6	36.4	46.2
38-44	1	1	-	-	-	-

TABLE III: OCCURRENCE OF *CANDIDA* SPECIES IN VARIOUS OCCUPATIONAL GROUPS

Occupation	Sample size		Positive cultures (No. and %)	
	Exper.	Control	Exper.	Control
Traders	16	33	11 (68.8)	14 (42.4)
Unskilled workers	13	9	8 (61.5)	6 (66.7)
Professionals	43	12	18 (41.9)	4 (33.3)
Full –time housewives	15	6	4 (26.7)	1 (16.7)

Table IV: Occurrence of *Candida* species in women who used contraceptive and those who did not.

Index	Sample size	No. of positive cultures	% of pos. cultures
Used contra.	36	21	58.3
Never used contra.	51	18	35.3

Age distribution of patients with *Candida* showed isolation rates of 51.7,43.5, and 36.4% among age 17-23, 24-30 and 31-37 years, respectively (Tab. II). Among occupational groups, traders had the highest occurrence of *Candida* species (68.8%) while full-time housewives had the lowest (26.7%) as shown in Tab. III. Women who used contraceptive pill had higher occurrence (58.3%) than those who never used contraceptive (35.3%, Tab. IV).

Discussion

Out of the 87 samples 44.8% were positive while for controls 46.7% were *Candida*-positive. These rates are high and compare well with earlier reports (13, 14). *Candida albicans* was most frequently isolated; this distribution is close to the result reported before (15, 16). Although *C. albicans* is widely reported in literature to be the most frequently isolated *Candida* species (4, 17) other *Candida* species are now being isolated with high rates (12, 14). *Candida albicans* produces protease, phosphatase and carbohydrates which enhance its attachment to human epithelium (18,19). Although such factors are also being studied in other *Candida* species, information on non-*albicans Candida* species is still scanty (19, 20).

Women in the second trimester had the highest occurrence of *Candida* infection (68.8%). It is likely that the conditions, such as pH and temperature, which encourage colonisation by *Candida,* are enhanced as age of pregnancy increases.

The distributions of infection among all age groups except 38 - 44 years were similar. These three age groups with high occurrence of infection were also the ones reported earlier to be the most affected; 20-25 years (21) and 21-30 years (12). Although, these authors' reports were based on non-pregnant women, the same factors namely, high sexual activity and child-bearing are attributable. This is corroborated by our results showing no significant difference (p=0.05) between age distribution of occurrence among pregnant women and non-pregnant women (control).

Traders had the highest occurrence; this agrees with earlier report (22). Traders usually have cash and due to their busy work, they might easily yield to self-medication and drug misuse which is a factor that increases the rate of colonisation by *Candida.* The difference in results for women who used contraceptives and those who never used was significant (p = 0.05). Use of oral contraceptive pill has been reported to increase the occurrence of *Candida* infection of the vagina (4, 5, 7).

There are indications that the factors studied here affect the rate of isolation of *Candida* species from pregnant women.

Acknowledgement

Authors are grateful to the staff of Suyi Hospital and Lahor Clinic for their assistance during the collection of specimens.

References

1. Ako-Nai A.K., Kassim O.O., Adeniran M.O. and Taiwo O.. A study of urinary tract infections at Ile-Ife, Nigeria: East Afr. Med. J. 1993:70 (1): 10-14.

2. Brooks GF Butel JS Morse SA. Medical Microbiology, Third Edition. Appleton and Lange, Connecticut. 2004.

3. Buckley C.H. Fox H. Pathology for Gynaecologists, Second Edition. Edward Arnold, London.1991.

4. Carlsen G. The *Candida* Yeast Answer. *Candida* Wellness Center, Provo. 2001.

5. Dam M. Poch F. Levin D. High rate of vaginal infections caused by non *Candida albicans Candida* species among asymptomatic women: Med. Mycol. 2002, 40 (4): 383-386.

6.Ellepola A.N.B. Samaranayake L.P. The effect of limited exposure to antimycotic on the relative cell-surface hydrophobicity and the adhesion of oral *Candida albicans* to buccal epithelial cells: Arch. Oral. Biol. 1998, 43**:** 879 – 887.

7. Enweani I.B. Gugnani H.C. Okobia R.

Ojo S.B. Effect of contraceptives on the prevalence of vaginal colonization with *Candida* species in Edo State, Nigeria: Rev. Iberoam. Micol. 2000, 18: 171 – 173.

8. Enweani I.B. Ogbonna C.I. Kozak W. The incidence of candidiasis amongst the asymptomatic female students of the University of Jos, Nigeria: *Mycopathologia* 1987, 99: 135 – 141.

9. Falagas M.E. Betsi G.I. Athaiasiou S. Probiotics for prevention of recurrent vulvovaginal candidiasis: a review: J. Antimicrob. Chemother. 2006, 58 (2): 266-272.

10. Garcia H.M. Garcia S.D. Copolillo E.F. Cora E.M. Banata A.D. Vay C.A. Torres R.A. Tiraboschi N. Famiglietti A.M. Prevalence of vaginal candidiasis in pregnant women, identification of yeast and susceptibility to antifungal

agent: Rev. Appl. Microbiol. 2006, 38 (1): 9 -12.

11. Grigoriu D., Delacretaz J. and Borelli D. Medical Mycology, Hans Huber, Toronto.1987.

12. Gugnani H.C. Nzelibe F.K. Gini P.C. Chukwudebelu W.O. Njoku-Obi A.N. Incidence of yeasts in pregnant and non-pregnant women in Nigeria: *Mycoses*, 1989, 32 (3): 131-135.

13. Houang E.T. Chu K.C. Koehler A. Chen A.T.F. Use of CHROMagar – Candida for genital specimens in the diagnostic laboratory. *J Clin Pathol* 1997,50 (7): 563-565.

14. Kuhn D.M. Mukherjee P.K. Clark T.A., Pujol C., Chandra J., Hayjjeh R.A., Warnock D.W., Soll D.R. Ghannoum M.A *Candida parapsilopsis* characterization in an outbreak setting. Emerg. Infect. Dis. 2004, 10(6): 1074 – 1081.

15. Okonofua F.E. Ako-Nai K.A. Dighitoghi M.D. Lower genital tract infections in infertile Nigerian women compared with controls. Genit. Med.1995, 71(3): 163-168.

16. Okungbowa F.I. Biology and diversity of *Candida* species isolated from the human genitourinary tract in some Nigerian cities. PhD Thesis, University of Benin,Nigeria. 2002.

17. Okungbowa F.I. Isikhuemhen O.S. Dede A.P.O. The distribution frequency of *Candida* species in the genitourinary tract among symptomatic individuals in Nigerian cities: Rev. Iberoam. Micol. 2003, 20: 60 – 63.

18. Pichova I. Pavikova L. Dostal J. Dolej E. Hrukova-Heidingsfeldova O. Weber

J. Rumi T. Souek M. Secreted aspartic proteases of *Candida albicans,C. tropicalis, C. parapsilosis and C. lusitaniae*; inhibition with peptidomimetic inhibitor. Europ J Biochem 2001, 268: 2669-2677.

19. Shwebke K. Hitlier S.L. Oxford textbook of medicine. Fourth Edition. Oxford Publishers, London. 2003.

20. Slope J.D.Hellman D.B. Ladensen P.W. Petty G.B. Traut A.T. The principles and practice of medicine. Third Edition. Appleton and Lange, Connecticut. 1996.

21. Vidotto V. Mantoan B. Pugliese A. Ponton J. Quindos G. Aoki S. Ito-Kuwa S. Adherence of *Candida albicans* and *C. dublinienses* to buccal and vaginal cells: Rev. Iberoam. Micol. 2003, 20: 52-54.

22. Wenjin Q. Yifu S. Epidemiological study on vaginal *Candida glabrata* isolated from pregnant women: Scand. J. Infect. Dis. 2006, 38 (1): 49-54.

REVIEW ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2009 ISBN 1595-689X VOL 10(2)
AJCEM/200922/20913 -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 10(2): 99-116

RISK FACTORS, THREATS AND PREVENTION OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) IN AFRICAN COUNTRIES

[1]Musa*, O. I., [1]Salaudeen A. G., [2]Akanbi II, A. A. and [1]Bolarinwa, O. A.
Departents of [1]Epidemiology & Community Health,and Medical Microbiology, College of Medicine, University of Ilorin, Ilorin, Nigeria
***Correspondence:** E-mail: mtosh2002@yahoo.com**

Abstract

Highly pathogenic avian influenza (HPAI) is a viral disease that affects the digestive, nervous and respiratory systems of all domestic and wild birds with high morbidity and mortality. It is highly contagious disease which can be fatal in humans. The avian influenza viruses (AIVs) are classified as types A, B and C with 15 subtypes of the type A. To date, all disease causing HPAI Viruses belong to H5 or H7 subtypes; and affect pigs and humans with the pigs serving as a mixing vehicle for re-assortment of the virus. The domestic ducks get infected without showing clinical signs and serve as a source of infection for domestic poultry.

Outbreaks of HPAl in Europe, Asia and Turkey are reported to be associated, with the presence of wet lands and lakes where migratory birds rest. In some African countries like Nigeria, such wet lands exist with free flying wild birds and domestic ducks visiting and resting. The possible source of introduction into a country could be through importation or smuggling of infected poultry products across the borders and through migratory birds that fly through identified pathways. The status of HPAl in many African countries including Nigeria is still under investigation so that appropriate strategies / measures to prevent introduction of the disease into the country can be implemented and / or strengthened through restriction of importation of poultry and poultry products from high risk countries, effective disease surveillance, functional National Veterinary services, quarantine and community based participatory epidemiological system for HPAI surveillance and control.

This article reviewed the global epidemiology and risk factors of HPAI infection in Nigeria and other African countries with emphasis on specific preventive measures that can reduce introduction of the virus into the country and the epidemiological surveillance for case detection / identification, screening and management. This review provides useful information and updates for health workers in tropical countries on the trends of AIVs and HPAI, diagnostic criteria using case definitions for both community and health facility levels and management protocols for confirmed cases as recommended by the World health Organization.

Introduction

Avian influenza, or "bird flu", is a contagious disease of animals caused by viruses that normally infect only birds and, less commonly, pigs. Avian influenza viruses (AIVs) are highly species-specific, but have, on rare occasions, crossed the species barrier to infect humans. In domestic poultry, infection with avian influenza viruses causes two main forms of disease, distinguished by low and high extremes of virulence. The so-

called "low pathogenic" form commonly causes only mild symptoms (ruffled feathers, a drop in egg production) and may easily go undetected. The highly pathogenic form is far more dramatic. It spreads very rapidly through poultry flocks, causes disease affecting multiple internal organs, and has a mortality that can approach 100%, often within 48

hours (1,2). The role of migratory birds in the spread of highly pathogenic avian influenza is not fully understood. Wild waterfowl are considered the natural reservoir of all influenza A viruses. They have probably carried influenza viruses, with no apparent harm, for centuries. They are known to carry viruses of the H5 and H7 subtypes, but usually in the low pathogenic form. Considerable circumstantial evidence suggests that migratory birds can introduce low pathogenic H5 and H7 viruses to poultry flocks, which then mutate to the highly pathogenic form. In the past, highly pathogenic viruses have been isolated from migratory birds on very rare occasions involving a few birds, usually found dead within the flight range of a poultry outbreak. This finding long suggested that wild waterfowl are not agents for the onward transmission of these viruses (2,3).

Direct or indirect contact between domestic flocks and wild migratory waterfowl has been implicated as a frequent cause of epidemics in poultry populations. It is generally accepted that migratory waterfowl, most notably wild ducks, are the natural reservoir of AI viruses, which can be transmitted to domestic bird populations and to commercial poultry (2,4).

Avian Influenza Viruses

Avian influenza virus (AIV) is a member of the family *Orthomyxoviridae* and genus influenza A. The influenza viruses that constitute this family are classified into type A, B, or C based on the differences between their nucleoprotein and matrix protein Antigens. AIVs belong to type A, which are further categorized into subtypes according to the Antigens of the haemagglutinin (H) and neuraminidase (N) projections on their surfaces. There are 16 haemagglutinin subtypes and 9 Neuraminidase subtypes. Only viruses of the H5 and H7 subtypes are known to cause the highly pathogenic form of the disease. However, not all viruses of the H5 and H7 subtypes are highly pathogenic and not all will cause severe disease in poultry. On present understanding, H5 and H7 viruses are introduced to poultry flocks in their low pathogenic form. When allowed to circulate in poultry populations, the viruses can mutate, usually within a

few months, into the highly pathogenic form. This is why the presence of an H5 or H7 virus in poultry is always cause for concern, even when the initial signs of infection are mild (2,3, 5,6).

AIVs are susceptible to warm environmental condition, short term exposure to ultraviolet radiation and desiccation. Common detergents and disinfectants such as calcium & sodium hypochloride and Virlcox can readily inactivate it. However the virus can survives for 35-44 days in faeces at 4^{o}C but for 4days at 25^{o}C. Within poultry farms the virus can survive for up to 35 days. AIVs can be isolated from Lake Water where they can persist and retain infectivity for more than 30 days at 0 ^{o}C and for only 4days at 22 ^{o}C (7,8). AIVs are shed in faeces and respiratory tract of infected birds for at least 14 days and can be recovered from refrigerated carcasses for 23 days. Birds processed during the viraemic stage can contaminate other carcasses with blood and faeces containing the virus. Eggs laid in the early stage of AI infection could contain the virus on the egg yolk, albumen and the eggshell. The virus can also penetrate cracked or even intact eggshells (1,2,8).

HPAI Virus is present in all secretions and excretions of infected birds. Transmission may be direct inhalation of contaminated aerosol, dust as droplets and droplets nuclei or indirectly via primarily attributed to movement of ingestion of contaminated water, feeds or infected carcasses. Transmission of HPAI between flocks is infected birds and action of humans through movement of feeds/personal equipment and vehicles into or out of contaminated premises. Spread of the disease into counties could be through importation or smuggling of infected poultry and poultry products across the border and through migratory birds (9). The virus is infective for almost all commercial, domestic and wild avian species. Chickens and turkeys are highly susceptible. Ducks and geese though susceptible to infection with all AIV strains but suffer clinical disease from only virulent strain. Other birds are susceptible at varying degree, while Pigs and humans are equally susceptible to infection by the HPAI virus (1,2,10).

Prevalence and distribution of AIV

AI is an infectious disease of birds caused by type A strains of the influenza virus. The disease, which was first identified in Italy more than 100 years ago, occurs worldwide. In the history of man, it is responsible for the largest pandemics of the last century especially “Spanish flu” pandemic of 1918-1919 which claimed over 40 million lives (1,10,11). From 1998 to

2002 avian flu pandemic with onset in China has spread round the world resulting in avoidable human death and over 20million chicken culled world-wide (1,10,11). The current outbreak of highly pathogenic AI A(H5N1), which began in Southeast Asia in mid-2003, is the largest and most severe affecting many countries simultaneously. Despite the death and destruction of over 150 million birds, the virus is now considered endemic in many parts of Indonesia and Viet Nam and in some parts of Cambodia, China and Thailand (10–12) . The risk of a pandemic will persist until the disease is controlled in birds, which may take several years.

The first human cases of AI A (H5N1) associated with the current outbreak in birds were confirmed in January 2004 in two children and one adult admitted into hospital in Hanoi with severe respiratory illness who tested positive for this strain. Since then, additional human cases have occurred in several countries, and the clinical spectrum of AI A (H5N1) infection in humans ranges from asymptomatic infection to severe disseminated disease (2,3,12). In the African Region, little is known about the burden of avian influenza because few countries have laboratory capacity for virus isolation. No documented H5N1 infection has been reported in the region. However, an outbreak of H5N2 influenza was reported among ostriches in South Africa in 2004 and in December 2005 in Zimbabwe (13). In order to address the threat of a pandemic of influenza, in African region, WHO have tried to improve and strengthen laboratory capacities of African counties for influenza and promoting the framework of Integrated Disease Surveillance and Response (IDSR) strategy (13).

There is increasing concern that Africa is at great risk of pandemic influenza. Firstly, as winter sets in Northern hemisphere, migratory birds can bring the deadly virus to Africa. Secondly, with increasing global travel there is a high risk of dissemination of the H5N1 virus. Thirdly, the poor health infrastructure in Africa and the non-availability of vaccines or drugs means that millions of people would be at high risk of pandemic influenza (1– 4).

In January 2006 AI infection was reported in Sambawa poultry Farm in Kaduna state of Nigeria and this was confirmed on 7th February 2006 by international office of Epizootic (IOE) reference Lab in Padova, Italy (9). Since then several focal outbreaks in poultry farms have been recorded in 13 Northern states of Nigeria including Abuja, the Federal Capital Territory (FCT). More than 2 million chickens were culled, poultry farms closed down

and huge some of money paid as compensation to poultry farmers (9). Although, only Four (4) human morbidity cases and one death were recorded in the country (9). This figure probably represents a tip of ice berg because of the prevailing poor reporting and incomplete data that characterized the disease surveillance and notification system in Nigeria and indeed other developing African countries.

Risk factors for AI infection

Since 1878 when HPAI was first reported in Italy, the infection had spread across all international boundaries due to increase international travels, migratory birds, poultry and products exports, hence assuming a rapid pandemic trend in the last decade with pockets of epidemics world over. AIVs are probably ubiquitous in wild water birds. Pathogenic strains could emerge and cause disease in domestic poultry in any country at any time without warning. The occurrence of human cases creates enormous challenges to the health systems. Laboratory confirmation and management of human H5N1 viral infections is technically challenging, expensive, and demanding on human and financial resources. The major risk factors for AI infection and outbreak of HPAI in developing countries can be grouped into 2:

i. Risk of introduction of AIVs through migratory birds: Nigeria lies in the East Africa/West Asian and the North Atlantic flyways of the migrating birds, and hence is at risk of the introduction of the virus. Also, increasing trade and human traffic between Nigeria and other countries in South East Africa and South Africa is another major threat to having the infection (9). Expansion of infection zone of Avian influenza due to globalization and relative ease of movement and transportation through porous borders that encourage informal livestock movement, Smuggling / illegal movement of poultry and products from infected countries coupled with inadequate veterinary quarantine facilities and manpower are the major risk factors for introduction of AIV into other countries especially those within the flyways of these migratory birds.

ii. Risk of sustenance and maintenance of HPAI in African countries: Risk factors under this category are associated with poverty, ignorance and socio-cultural believes and practices relating to keeping of pets and domestic animals for economic

and spiritual purposes. Also, lack or poor implementation of veterinary policies and inefficient public veterinary services are other issues that may encourage sustenance of the virus. Some of the specific factors that arise from above issues are (1, 9):

- Backyard poultry, peri-urban and urban commercial poultry production with little or no bio-security and constant introduction of new birds from relatively unknown and unverified sources.

- Uncontrolled livestock and poultry movement within and outside the country due to lack of enforcement of animal disease control laws and regulation, including registration and Licensing of poultry farms & hatcheries

- Increased close contact between poultry and human, and lack of organized poultry marketing which encourage open live poultry markets characterized by interspecies mixing and poor sanitary condition.

- Inadequate experience of most vet and health workers in the recognition & diagnosing of HPAI.

- Inadequate / lack of funding for veterinary services, compensation of Livestock/ flocks owners in the events of mass culling of birds and poor disposal facilities for culled or dead birds thus encouraging sale and consumption of sick and dead birds

Threats and Consequences of HPAI

AIVs do not infect humans because of host barriers to infection, such as cell receptor specificities. However, they can occasionally cross the species barrier and directly infect humans, including highly pathogenic strains that have caused fatal disease in humans.(1,2) In 1997, AI A(H5N1) caused an outbreak in domestic poultry in Hong Kong and also infected 18 people and causing 6 deaths (11,14). Since then, other AI outbreaks have resulted in human infections. The outbreaks of highly pathogenic H5N1 avian influenza in poultry which began in Asia in mid-2003 have led to over 120 confirmed human cases of which more than half have died (1,2). The majority

of cases occurred in previously healthy children and young adults. Historically, the number of deaths during a pandemic has varied greatly. Death rates are largely determined by four factors: the number of people who become infected, the virulence of the virus, the underlying characteristics and vulnerability of affected populations, and the effectiveness of preventive measures.

Presently, avian influenza A (H5N1) is endemic in birds in many parts of the world. The widespread persistence of H5N1 in bird populations poses two main risks to human health. The first is the risk of infection when the virus spreads directly from birds to humans. The second risk, which is of even greater concern, is that there will be increased possibilities for the widely circulating virus to infect humans and possibly re-assort into a strain that is both highly infectious for humans and spreads easily from human-to-human. Such a change could mark the start of a pandemic (15).

One important feature of AIV is the tendency to acquire new properties through adaptation, mutation and recombination that may lead to emergence of a highly virulent virus with many serotypes. The fear is that in nearest future a more highly virulent virus with high possibility of man to man transmission could be produced if the spread of HPAI is not curtailed. The implication of the pandemic nature is such that stringent international and National regulations on Quarantine and veterinary Laws have to be put in place which may affect human movements, poultry and diary products exports. This will invariably affects tourism, international trade and the world economy. For example, over 2million chicken were culled and about 160,000 US dollars paid as compensation to poultry farmers following January / February 2006 outbreaks of AI in Nigeria. These outbreaks also caused loss of jobs due to closure of poultry farms (9). At the global level, it is estimated that 20million chicken were culled causing the world over 2Billion USD loss (1,2). This huge amount of resources could have been used for other health conditions.

Nigeria has an estimated poultry productions of around 140million birds largely concentrated in the South-western part of the country and 60% of these poultry production takes place in small backyard flocks, making families, associates and visitors at risk (9). Large scale commercial farming of poultry occurs mainly in the Northern States where outbreak had been confirmed. Rapid spread of the virus within Nigeria has raised concern over possible spread to neighboring countries in the

sub-region because of porous borders and restriction of movement of people and poultry are difficult to enforce, thus putting the neighboring countries at risk.

Another implication of HPAI outbreaks and pandemic is that Medical supplies will be inadequate. Vaccines and antiviral drugs (the two most important medical interventions for reducing illness and deaths during a pandemic) will be inadequate in all countries at the start of a pandemic and for many months thereafter. Inadequate supplies of vaccines are of particular concern, as vaccines are considered the first line of defense for protecting populations. On present trends, many developing countries will have no access to vaccines throughout the duration of a pandemic; and because of this, large numbers of deaths will occur (1, 2). Economic and social activities will be disrupted due to High rates of illness and worker absenteeism and schools may be shot down. Social and economic disruptions would be worse if workers absenteeism involve people in essential services, such as power, transportation, health and communications.

Prevention and Control

AIV usually spreads when live birds carrying infection are bought and sold, and by contact of birds with bird droppings on dirty equipment, cages, feed, vehicles or shoes/clothing (9,16). Good hygienic practices (bio-security) is therefore an extremely important safety measure to prevent infection entering domesticated poultry; and all persons keeping or working with poultry should play their role in this. Therefore, promoting and encouraging good health habit would go along way in curtailing the spread of the virus in human population. Health education and awareness campaign to the public, poultry workers, diary products handlers, families with backyard poultry will go a long way to prevent spread of the virus. Specific preventive measures are:

a. Prevention of introduction of HPAI into the country

This can be achieved by adopting WHO recommended standard measures and strategies which include (6,16,17):

- Ban on importation of poultry and poultry products from countries where the disease is known to exist especially from the Asian continent
- Adopting effective animal disease surveillance involving

- high-risk areas such as poultry markets, wet lands and poultry located along known migratory birds routes, poultry abattoirs, borders and targeted farms should be carried out.

- Creation or revitalization of functional National veterinary Quarantine services (NVQS) including immediate rehabilitation and revitalization of existing veterinary quarantine infrastructure, control post and enhancing manpower capabilities.

- Targeted community based training of rural backyard poultry farmers in various aspects of HPAI recognition and control including bio-security procedures applicable to rural small-scale poultry enterprises and the role of animal disease vigilante in the control of HPAI.

- Introduction and enforcement of regulatory policies and requirements for import permits for poultry and poultry products by the Federal authorities or agency concern with live stock services.

- Development of a traceable mechanism for animal and strict monitoring of movement of poultry and poultry products through registration and Licensing of poultry farms, hatcheries and other poultry enterprises.

- Development of a community based, participatory rural livestock and poultry disease surveillance system and integrating it into the existing epidemiological surveillance network.

- Training of veterinarian, auxiliaries, others

- ategories of poultry farmers on HPAI prevention and control strategies including aspect of bio-security and early recognition / detection of the disease.

- Effective public enlightenment and awareness programmes on HPAI policy and preventive services available to the public

b. Specific control measures in Birds and man

Preventive strategies and activities under this category are (2, 3, 9, 10, 16, 17):

- Importation restriction/ regulation of birds

- Proper care of poultry farm that include nutrition, vaccination, isolation of infected birds, mass destruction of infected birds and disinfection poultry farms. Control of the disease in birds by early diagnosis & treatment

- Proper regulation and inspection of poultry farms and products by the regulatory authorities. Food Hygiene involving proper meat and chicken inspection, farm inspection, thorough cooking of meat and chicken. Avian flu does not survive high temperature.

- Prompt response to disease outbreak in birds' population e.g. Proper notification, isolation and veterinary surveillance.

- Observing universal precautions and passionate personal hygiene among poultry workers before and after handling poultry birds and poultry products. Provision and use of personal protective measures (like apron, coats, gloves, boots, goggles and masks etc) for high risk groups like poultry workers, vet officers.

- Immunization against HPAI among people at high risk, e.g. veterinary officers, poultry farmers & workers, Diary handlers etc.

- Avoidance of unnecessary contact and proximity to poultry area especially among backyard poultry, free ranging flock and birds aspects.

c. Early Diagnosis & Screening

Diagnosis involves clinical signs and symptoms with confirmatory Laboratory tests which include: virus isolation and gene sequence detection at the National veterinary laboratory (where available) and further characterization of isolates like heamoglutinin and neuraminidase typing could be done at national Lab and other regional and world reference laboratories. The WHO recommended standard procedures for the screening and diagnosis of suspected human influenza A H5NI cases are: (9, 16, 18-20)

1. Surveillance case definition for community and Health workers

Community base alert definition consists of fever, cough and history of contact with sick /dead birds or history of contact with another person with similar complaints. Members of the community are expected to report and/ or send a. Rapid antigen detection using any of the following tests: Immuno-fluorescence assay which is a widely used, sensitive method for diagnosis of influenza A and B virus infections and five other clinically important respiratory viruses; and Enzyme immunoassay for influenza A nucleoprotein (NP). The results of these tests can be obtained within 30 minutes.

b. Virus culture provides results in 2–10 days. Both shell-vial and standard cell-culture methods may be used to detect clinically important respiratory viruses. Positive influenza cultures may or may not exhibit cytopathic effects but virus identification by immuno-fluorescence of cell cultures or haemaglutination-inhibition (HI) assay of cell culture medium (supernatant) is required.

c. Polymerase chain reaction

(PCR) and Real-time assays. Primer sets specific for the haemaglutinin (HA) gene of currently circulating influenza A/H1, A/H3 and B viruses are becoming more widely used. Results can be available within a few hours from either clinical swabs or infected cell cultures.

Samples required for birds are tracheal ad cloacae swabs, faecal samples, and paired sera from live birds. Intestinal contents, samples of trachea, lungs, air sacs, spleen, kidneys, brain, liver, pancreas and heart can also be used. Laboratory diagnosis for birds includes antigen detection through Immuno-flourescence and Direct antigen Flu A tests (Directigen TM fluA test). This is followed by Virus Isolation and identification using Reverse transcriptase polymerase chain reaction (PT – PCR), Virus characterization through Haemaglutinin typing, neuraminidase typing, gene sequence detection and Serological diagnosis using Haemaglutination Inhibition test (HIT), Agar Gel Immuno-diffusion test (AGID), Competitive Enzyme linked Immunosorbent Assay (C-ELISA). Infected animals could be differentiated from vaccinated by serological test called Differentiating Infected from Vaccinated Animal System (DIVA).cases meeting this definition to nearest health facility. Health care workers must be at alert to suspect cases of avian flu by using health facility based case definition which is (9):

- Fever > 38^{o}c with History of exposure in last 7 days before onset of illness and one or more of the following symptoms / signs: Cough, sore throat, Breathing difficulties.

- Any person requiring hospitalization for an influenza-like illness and living in, or coming from an area with avian influenza outbreak..

- Any person having died of an unexplained respiratory illness.

2. Notification of suspected cases

Any case that meets the suspected case definition should be immediately notified to the Disease Surveillance and Notification Officer (DSNO) at the district, regional or national health office using duly completed Immediate case based reporting form (IDSR 001) and must be sent by the fastest means (e.g. fax, email, radio, telephone etc). Following this reporting, a Rapid Response team would be dispatched to conduct detailed investigation and re-assessment of the suspected cases and fresh specimen collected. The team also arrange for the transfer of patient to

avian influenza treatment centre (if

vailable) using dedicated vehicle; and help the local / district health unit / facility or department in producing plan to identify and follow up close contacts.

3. Laboratory diagnosis of AI and HPAI infection in birds and man

Laboratory identification of human influenza A virus infections is commonly carried out by direct antigen detection, isolation in cell culture, or detection of influenza-specific RNA by reverse transcriptase–polymerase chain reaction. The optimal specimen for influenza A virus detection is a nasopharyngeal aspirate obtained within 3 days of the onset of symptoms. Nasopharyngeal swabs and other specimens including serum can also be used. Assays available for the diagnosis of influenza A virus infections include (18–22):

d. Case management

Case management at the hospitals (or avian influenza treatment centre) includes patient isolation, barrier nursing and maintenance of precautionary measures. The patients and family must be educated on the risk of transmission and the need to notify health Authorities of other suspected cases.

Standard precautions should be used for all patients receiving care in hospitals, regardless of their diagnosis or presumed infection status. Some of the precautionary measures include (19, 23–25):

i) Hand washing with either a plain or antimicrobial soap and water to prevent possible self-inoculation of the nose, mouth, and conjunctivae and the transfer of micro-organisms to the environment or other patients by contaminated hands. The use of an alcohol-based hand rub for routine hand antisepsis is recommended. Many studies have demonstrated that influenza, an enveloped virus, is susceptible to alcohols when tested in vitro and in vivo testing with a 95% ethyl alcohol.

ii) Use of gloves when touching mucous membranes and non-intact skin, blood, body fluids, secretions, excretions, and contaminated items. Change gloves between tasks and procedures on the same patient after contact with material that may contain a high

concentration of micro-organisms. Remove gloves promptly after use and wash hands immediately to avoid transfer of micro-organisms to other patients or environments.

iii) Wear mask and eye protection or a face shield to protect mucous membranes of the eyes, nose, and mouth during procedures and patient-care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, and excretions.

iv) Wearing of gown: a clean, non-sterile gown is required to protect skin and prevent soiling of clothing during procedures, patients' care and other activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions. Soiled gown should be removed as promptly as possible and wash hands to avoid transfer of microorganisms to other patients or environments.

e. Drug treatment and use of vaccine

Two drugs (in the neuraminidase inhibitors class), oseltamivir (commercially known as Tamiflu) and zanamivir (commercially known as Relenza) are available; and both can reduce the severity and duration of illness caused by seasonal influenza. The choice of drug may depend on its availability and cost. The efficacy of the neuraminidase inhibitors depends on their administration within 48 hours of onset of symptom (26). For cases of human infection with H5N1, the drugs may improve prospects of survival, if administered early, but clinical data are limited. The dosage treatment with a neuraminidase inhibitor such as oseltamivir is 75 mg orally, twice daily for 5 days. Reference must be made to manufacturer's instruction for children dosage (9,16). Supportive cares with oygen, antibiotics and antipyretics may be required. Administration of salicylates (such as aspirin) in children less than 18 years of age must be avoided because of the risk of Reye syndrome, rather paracetamol or ibuprofen can be used (9,16, 26).

Vaccines effective against a pandemic virus are not yet

available. Vaccines are produced each year for seasonal influenza but will not protect against pandemic influenza. Although, vaccine against the H5N1 virus is under development in several developed countries, none is ready for commercial production. Some clinical trials are now under way to test whether experimental vaccines will be fully protective and to determine whether different formulations can economize on the amount of antigen required, thus boosting production capacity (16,27).

f. Discharge policy

Studies are ongoing for better understanding of viral excretion patterns in humans infected with the influenza A(H5N1) viruses. However, WHO recommends that adult patients remain in hospital (if admitted) or confined to a location (un-hospitalised) for 7 days after resolution of fever. Previous human influenza studies have indicated that children younger than 12 years can shed virus for 21 days after onset of illness (5,27). Therefore, infection control measures for children should ideally remain in place for this period. Where this is not feasible the family should be educated on personal hygiene and infection control measures; and the affected child should not attend school during this period.

ACKNOWLEDGEMENTS

We thank, Prof. D. B. Parakoyi, of the Dept of Epid & Community Health, University of Ilorin Teaching Hospital, Nigeria for going through the manuscript and providing useful comments. We also acknowledge the support of the World Health Organization, Kwara State office in Nigeria for providing relevant literatures / documents.

Reference:

1. Zeitlin G, Maslow M. Avian Influenza. Curr Infect Dis Rep 2005; 7:193-199

2. WHO; Avian influenza A (H5N1) infection in humans. N Engl J Med 2005; 353: 1374-85

3. Hayden F, Croisier A. Transmission of Avian Influenza Viruses to and between Humans. J Infect Dis 2005; 192: 1311-4

4. Nicholson KG, Wood JM, Zambon M (2003). Influenza. Lancet, 362:1733–1745

5. World Health Organization (WHO); Selected guidelines on influenza H5N1. http://www.who.int/csr/disease/avian_influenza/guidelines/en/

6. International Food Safety Authorities Network (INFOSAN); Highly pathogenic H5N1 avian influenza outbreaks in poultry and in humans: Food safety implications; INFOSAN Information Note No. 7/2005 (Rev 1. 5 Dec) Decemmber 2005; pg 1 – 4

7. Lu H, Castro AE, Pennick K, et al. Survival of avian influenza virus H7N2 in SPF chickens and their environments. Avian Dis 2003; 47: 1015-21

8. Swayne D., Beck J. (2004); Heat inactivation of avian influenza and Newcastle disease viruses in egg products. Avian Pathology 33(5), 512-518

9. Federal Ministry of Agric (Dept of Live stocks & pest control services); Report of technical committee on the prevention & control of HPAI in Nigeria; December 2005, p 5-18.

10. Horimoto T, Kawaoka Y. Influenza: lessons from past pandemics, warnings from current incidents. Nat Rev Microbiol 2005; 3:591-600

11. Chan PK. Outbreak of avian influenza A (H5N1) virus infection in Hong Kong in 1997. Clinical Infectious Diseases, 2002, 34:S58–S64

12. WHO. Inter-country-consultation: influenza A/H5N1 in humans in Asia: Manila, Philippines, 6-7 May. 2005

13. WHO Regional Director for Africa, Opening remarks at the regional meeting of experts on pandemic of avian influenza. January 2006; Brazzaville DRC

14. Olsen S.J., et al. (2005) Poultry-handling practices during avian influenza outbreak, Thailand. Emerging Infectious Diseases, Vol. 11, No. 10

15. Ungchusak K, Auewarkul P, Dowell SF et al; Probable person to person transmission of avian influenza A (H5N1). N. Engl J. Med 2005; 352: 33 – 40.

16. WHO (Western Pacific regional office): Avian Influenza,

17. including Influenza A (H5N1), in Humans: WHO Interim Infection Control Guidelines for Health Care Facilities. Published on 9th February, 2006; by WHO WPRO, Manila Philippine. Pg 6-43

18. European Commission; Introducing Community measures for the control of avian influenza. Council Directive 92/40/EEC of 19 May 1992 Official Journal of the European Union, L 167, 22.6.1992, p.1

19. WHO, Recommended laboratory tests to identify avian influenza A virus in specimens from humans WHO Geneva, June 2005. http://www.who.int/csr/disease/avian_influenza/guidelines/referencelabs/en/index.html

20. Yuen KY, Chan PK, Peiris M, et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet 1998; 351:467-71

21. Fouchier RA et al. (2000); Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. Journal of Clinical Microbiology, 38:4096–4101.

22. Lee CW, Suarez DL (2004). Application of real-time RT-PCR for the quantitation and competitive replication study of H5 and H7 subtype avian influenza virus. Journal of Virological Methods, 119: 151–158..

23. Wright KE et al. (1995). Typing and sub-typing of influenza viruses in clinical samples by PCR. Journal of Clinical Microbiology, 33:1180–1184.

24. Garner J. Guideline for Isolation Precautions in Hospitals. Infect Control Hosp Epidemiol; 1996; 17: 53-80

25. Suarez D, Spackman E, Senne D, Bulaga L, Welsch A and Froberg K. The effect of various disinfectants on detection of avian influenza virus by real time RT-PCR. Avian Dis 2003;47:1091-1095

26. Swayne D., Beck J. (2005) Experimental study to determine if low-pathogenicity and high-pathogenicity avian

27. influenza viruses can be present in chicken breast and thigh meat following intranasal virus inoculation. Avian Diseases 49:81-85

28. Hayden FG, Atmar RL, Schilling M, et al. Use of the selective oral neuraminidase inhibitor oseltamivir to prevent influenza. N Engl J Med 1999; 341:1336-43.

29. World Health Organization (WHO); Guidelines on the use of vaccines and antivirals during influenza pandemics. Geneva, 2004. (Document WHO/CDS/CSR/RMD/2004.8 .)

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2009 ISBN 1595-689X VOL 10(2)
AJCEM/200925/20914 -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 10(2): 117-127

PHYSICO-CHEMICAL QUALITY OF DRINKING WATER AT MUSHAIT, ASEER, SOUTH-WESTERN SAUDI ARABIA.

***[1]Al-Otaibi,Eed Lafi and **Zaki, Mahmoud Samy Ahmed. Remote Sensing and Geographical Information Systems Section*; and Epidemiology and Environmental Health Section**, Department of Applied Medical Sciences, Khamis Mushait University, Saudi Arabia.**

***Correspondence: E mail elalotaibi@nu.edu.sa Tel/Fax: 009665441861, Mobile: 009503592622/ 00966556588595**

Abstract

The physico-chemical quality study of different drinking water sources used in Khamis Mushait, southwestern, Saudi Arabia (SA) has been studied to evaluate their suitability for potable purposes. A total of 62 drinking water samples were collected randomly from bottled, desalinated and groundwater located around the study area. The parameters determined in this study were Turbidity; Conductivity; Total solids; pH; Chlorides; Hardness; Sulphate; Ammonia; Nitrite; Nitrate; Iron; Manganese; Copper and Zinc. Most examined parameters were higher in groundwater, followed by desalinated water except for Manganese and Zinc levels. Manganese level was highest in desalinated, followed by bottled water, while Zinc was higher in groundwater than bottled and desalinated water. Most examined groundwater samples had the highest physico-chemical levels as compared with guideline of international and Saudi standards, followed by desalinated and lastly bottled water. Desalinated water showed the higher Manganese value more than the recommended level followed by bottled water, while all the examined water samples had Zinc content lower than the guideline values of international and Saudi standards. Strict hygienic measures should be applied to improve quality of drinking water and to avoid deleterious effect on human health. This could be achieved by working towards a proper monitoring program of drinking water supply and sources.
Key Words: physico-chemical quality, Drinking Water Quality, Khamis Mushait, Saudi Arabia.

Introduction

Safe and good quality of a drinking water source is the basis for good human health. Water provides essential elements, but when polluted it may become a source of undesirable substances dangerous to human health.

Quality of drinking water is strongly influenced by the quality of the corresponding natural water, from which drinking water derives [1]. There are still water quality problems related mainly to the effective water treatment and defects in the distribution networks of drinking water or during transportation and distribution stages in general, which may expose the consumers to the risk of adverse health effect [2].

Bottled water consumption is widely and rapidly increasing because its accessibility, availability, tastes better, contains fewer impurities and confers a higher social status on consumer than tap water. Poor quality control during production and\or distribution can contaminate this widely consumed water resource [3].

Groundwater is an important resource for human water supply and in Asia alone some one billion people are directly dependent on it. In Saudi Arabia, groundwater is the main source for safe and reliable drinking water consumption particularly in rural areas. Water taken from groundwater (wells) is often of better quality than surface water. This is true only if the soil or rock is fine-grained and does not have cracks, crevices and bedding plants, which permit free and faster passage of pollution such as of polluted surface water [4]. The inorganic chemical quality of these waters is however, rarely adequately tested before the wells are put into production. Due to variation in the regional geology and water/ rock interactions, high concentrations of many chemical

elements can occur in such water source. During the last 10 years several studies have shown that wells in areas with particular geological features yield water that does not meet established drinking water standards without any influence from anthropogenic contamination [5,6].

For the last four decades, many countries in the arid regions had been depending on desalination of seawater to meet their growing domestic needs. The Kingdom of Saudi Arabia is the biggest producer of freshwater by desalination with an installed capacity of more than 1000 million USGPD accounting for 24.4% of the world desalinated water production [7,8]. Contamination of desalinated water with various chemical parameters may occur and end up in drinking water sources threatening human lives and surrounding environment.

The main objective of this study was to assess physico-chemical parameters of drinking water samples from different sources (bottled water, desalinated water and groundwater) from Khamis Mushait area and to compare these results with the drinking water standards set by Saudi Arabia Standards Organization (SASO) and other international regulatory standards.

Materials and Methods

A total of 62 drinking water samples were collected randomly from bottled water from local stores, desalinated water and groundwater (wells) from Khamis Mushait, area, south western SA. Sixteen brands of bottled water consisting of spring and purified bottled water types were purchased from different supermarket stores at Khamis Mushait governorate. Concerning desalinated water, 31 water samples were obtained from public & regional distributing points known as *Ashiab*, water trailers; houses; water networks; fish markets and slaughterhouse. From groundwater, 15 water samples were taken from different types of wells located at and around Khamis Mushait governorate using point network technique utilizing digital satellite maps of Googlearth engine search to map and sample groundwater network of study area in order to determine and locate sampling points in the field of both houses and wells [9].

All the brands of bottled water samples were in plastic container with capacity of 1.5liter and plastic screw caps except purified water samples, which collected in 1.5liter colorless glass container with metal screw caps. Water samples from desalinated and groundwater were collected in colorless labeled glass bottles provided with glass stopper with capacity of 1.5liter. For physico-chemical examination of desalinated and groundwater, samples were taken after flushing water for at least 5 minutes [10]. All water samples were dispatched to laboratories of Medical Lab. Technology Department, Khamis Mushait Community College, King Khalid University to perform physico-chemical examination for the collected drinking water samples. All the collected drinking water samples were purchased and analyzed between March and June, 2007. All water samples were transported to the laboratory, where most water quality constituents were determined within 2-6 hours after collection. Furthermore, all collected water samples did not contain particulates. Thus, water samples were not filtered prior to analysis for various parameters.

The physico-chemical parameters analyzed in this study were Turbidity (FAU); Conductivity (µS/ cm); Total solids (mg/l); pH; Chlorides (mg/l); Hardness as Ca Co_3 (mg/l); Sulphate (mg/l); Ammonia (mg/l); Nitrite (mg/l); Nitrate (mg/l); Iron (mg/l); Manganese (mg/l); Copper (mg/l) and Zinc (mg/l).

Conductivity (µS/cm) was measured by the Dist

Family II meter (Hana Instrument, An ISO9001 Certified Co., Portugal), after calibration with conductivity standard solutions. pH was measured by using pH meter (HI8014, Hana Instrument, Romania), after calibration with standard pH buffers. Total solids and Chloride contents were determined according to the methods previously described [10].

Turbidity; Hardness as $CaCo_3$ (mg/l); Ammonia (mg/l); Nitrite (mg/l); Nitrate (mg/l); Sulphate (mg/l); Copper (mg/l); Manganese (mg/l); Zinc (mg/l) and Iron (mg/l) were determined by using DR/ 890 Colorimeter (HACH Company, 1997-2004, USA). The procedures used for measuring these parameters were fully described in the procedure manual of DR/ 890 Colorimeter (Table 1).

Statistical analysis of the obtained results was done using a one-way analysis of variance (ANOVA). The one way parametric ANOVA procedure (at 5% level of significance) was adopted since it has been adjudged to be preferred method compared to non-parametric procedures, especially when the percentage of non-detects is less than 15% [11].

Results

Generally, average turbidities (FAU) of all examined water sources were 2.85±0.4 (Table 2). However, the variation of turbidity within each source was large. The highest mean being recorded in groundwater, which had an average of 5.47±0.98, followed by desalinated water (2.23±0.46). The lowest mean turbidity value (0.56±0.18 FAU) was reported in samples taken from bottled water source. The higher turbidity levels recorded in groundwater samples were significantly correlated with bottled and desalinated water samples ($p \leq 0.05$). There was also a significant correlation at $p \leq 0.05$ in the turbidity level between desalinated and bottled water (Table 2). Generally, 12 water samples did not meet the international and Saudi standards for turbidity in drinking water, which set as 5 NTU. Three (9.68%) and 9 (60%) from desalinated and groundwater samples were higher than the maximum limit for turbidity (Table 3). Higher values were previously recorded [12], while a lower turbidity level was also reported [13].

It is evident from Table (2) that the overall range of conductivity (µs/cm) in all collected water samples were 120.00-2460.00 with an average of 296.49±63.20. The highest conductivity value (µs/cm) was detected in groundwater samples (937.67±199.13), followed by those determined in desalinated water (152.90±3.05) and lastly bottled water samples (136.25±12.84). From the result obtained in the present study, conductivity had large variation within the different water sources. The higher turbidity levels recorded in groundwater samples were significantly correlated with bottled and desalinated water samples ($p \leq 0.05$). Table (3) declared that 10 out of 15 (66.67%) examined groundwater samples had conductivity values exceeding the permissible limit set by international and Saudi standards (400 µs/ cm). On the other hand, all water samples collected from bottled and desalinated water sources were lower than the permissible limit.

Generally total solids (mg/l) in all collected water samples considering range values were found to be 68.00-3640.00 with a mean value of 481+ 98.56 (Table 2). In terms of the mean value obtained in all water samples, the minimum value was 130.31±13.79 mg/l for bottled water, followed by desalinated water 203.48±8.97 mg/l. However, the highest total solids (mg/l) was recorded in samples taken from groundwater with an average of 1373.00±314.51. The higher total solid recorded in groundwater samples were significantly correlated with bottled and desalinated water samples ($p \leq 0.05$). As presented in Table (3), 66.67% (9 out of 15) groundwater samples exceeded the permissible

levels (500 mg/ l) set by the international and Saudi standards for total solids. On the other hand, there no water samples collected from bottled and desalinated water exceeding the permissible limit of total solids.

Overall mean pH value was 7.64±0.04 in all investigated water samples (Table 2). The variation of pH values within the examined water sources was small, where the range was between 6.67 and 8.40. The lowest mean pH value (7.28±0.09) was recorded in groundwater, while the highest pH value (7.78±0.09) was found in bottled water followed by desalinated water with a mean value of 7.75±0.04. On the other hand statistical analysis presented in Table (2) revealed a significant correlation between the lowest pH value of underground water and pH value of bottled and desalinated water samples ($p \leq 0.05$). The results of pH value recorded in this study fall within the range of international and Saudi standards for drinking water, where the range lies between 6.5 and 8.5 (Table 3).

The overall mean value of chloride content (mg/l) in all drinking water samples was 64.50+18.07 (Table 2). The chloride ranges (mg/l) were 14.50-40.50; 15.20-34.00 and 11.50-914.80 in the examined bottled, desalinated and groundwater respectively. For comparison, groundwater samples showed the highest chloride value, where mean value was 206.01±63.15 mg/l, followed by bottled water (21.78±1.82 mg/l). On the other hand, desalinated water had the lowest chloride content with a mean of 18.08±0.66. There was a significant variation at $p \leq 0.05$ in the amount of chloride between groundwater and bottled as well as desalinated water (Table 2). Only 40% (6 out of 15) having chlorides exceeding the guideline value (250 mg/l) as recommended by international and Saudi Standards (Table 3).

Total hardness is expression of total Ca and Mg content of water expressed in equivalent of $CaCo_3$. Table (2) shows that overall mean of total hardness as $CaCo_3$ (mg/l) in all investigated drinking water samples was 36.68±7.59. The mean values of total hardness were 36.46±6.19; 4.82±1.58 and 102.75±22.81 in samples taken from bottled, desalinated and groundwater, respectively. It is indicated from Table (2) that total hardness showed a significant higher values at $p \leq 0.05$ in water samples taken from bottled water more than recorded in desalinated water. However, A significant difference was also present in the total hardness level between groundwater and desalinated or bottled water ($p \leq 0.05$). It is clear from Table (3) that out of 15 examined groundwater samples, 7 (46.67%) had higher hardness levels more than the guideline level of hardness (100 mg/l as $CaCo_3$) as recommended by international and Saudi standards.

It is presented from Table (2) that the overall mean value of sulphate (mg/l) in all examined drinking water samples was 158.84±39.76. The highest sulphate concentration was recorded in samples collected from groundwater (524.20±125.59), followed by desalinated water with an average of 55.13±2.84 and bottled water which had a mean value of 21.3±4.73. A statistically significant difference at $p \leq 0.05$ was very clear between the higher sulphate level in groundwater and bottled as well as desalinated water. Of 15 groundwater samples, 9 (60.00% were above permissible limit of sulphate (200 mg/ l) as reported by international and Saudi standards. On the other hand, there was no water samples had sulphate content above the permissible limit (Table 3).

It is shown from Table (2) that the overall mean value of ammonia (mg/l) in all examined water samples was 0.03+0.01. The mean values of

ammonia (mg/l) in the examined bottled;, desalinated and groundwater samples were 0.002±0.001, 0.02±0.005 and 0.09±0.03, respectively. Ammonia levels were varied significantly between groundwater and bottled or desalinated water ($p \leq 0.05$), where groundwater had more ammonia levels than recorded in desalinated and bottled water. All examined water samples had ammonia levels below international and Saudi standards of ammonia in drinking water, which should not exceed a level of 0.5 mg/ l (Table 3).

It is obvious from Table (2) that nitrite was non-detectable in all investigated drinking water samples in this study. Regarding nitrate, Table (2) revealed that the average of nitrate (mg/l) in all examined water samples was 16.74±1.78. The highest nitrate value was recorded in samples taken from groundwater with an average of 26.94±5.85 mg/l, followed by desalinated and bottled water, where the respective values were 13.98±1.35 and 12.54±2.11 mg/l. Moreover, nitrate showed significant high levels in groundwater than in desalinated or bottled water samples ($p \leq 0.05$). Table (3) declared that in comparison the results of nitrate concentrations with the international and Saudi standards, it was found that only one out of 31 (3.23% and 4 out of 15 (27.67%) water samples collected from desalinated and groundwater exceeded the maximum permissible limit for nitrate (45 mg/ l).

The overall mean of iron (mg/l) for all investigated drinking water samples was 0.17±0.05 (Table 2). The iron ranges (mg/l) in the examined bottled, desalinated and groundwater samples were 0.00-0.28; 0.00-0.39 and 0.00-2.20, respectively. On the other hand, the highest mean value of iron (Fe) was recorded in samples taken from groundwater (0.41±0.18 mg/l), followed by desalinated water (0.11±0.02 mg/ l) and bottled water with a mean of 0.07±0.02 mg/l. A statistically significant difference at $p \leq 0.05$ was very clear between the higher iron levels in groundwater and desalinated as well as bottled water. It was found in Table (3) that 9.69% (3 out of 31) and 26.67% (4 out of 15) of desalinated and groundwater samples present high iron concentrations exceeding the maximum permissible limit set by the international and Saudi standards for iron (0.3 mg/ l).

It is evident from Table (2) that manganese (Mn) value (mg/l) recorded in all examined water samples was 0.29±0.03. The mean Mn values recorded in bottled, desalinated and groundwater samples were 0.16±0.04; 0.39±0.05 and 0.23±0.07, respectively. The recorded Mn level in desalinated water was significantly higher than bottled and groundwater samples ($p<0.05$). However, there was no significance variation of Mn values between bottled and groundwater samples. As indicated from Table (3) that most examined water samples had higher Mn levels more than the guideline value (0.05 mg/l) as recommended by international and Saudi standards. Water samples exceeding the maximum permissible limit of Mn were obtained from bottled, desalinated and groundwater with frequencies of 68.75% (11 out of 16); 87.10% (27 out of 31) and 53.33% (8 out of 15), respectively.

The overall mean of copper (Cu) concentration (mg/l) in all examined water samples was 0.78±0.22 (Table 2). Higher copper value (mg/l) was recorded in samples taken from groundwater with an average of 2.95±0.68, followed by desalinated water (0.09±0.03) and bottled water, which showed copper levels ranged from 0.00 to 0.7 with an average of 0.08±0.04. It is indicated also from Table (2) that copper levels showed significant higher levels in groundwater than in desalinated or bottled water ($p<0.05$). However, there was no significant variation in copper levels between desalinated and bottled water. Copper levels

\hould not exceed 1.0 mg/l in drinking water supplies, as recommended by the international and Saudi standards. As presented in Table (3), water samples exceeding maximum permissible limit were obtained from groundwater with a frequency of 60.00% (9 out of 15).

Zinc (Zn) levels in the examined water from bottled, desalinated and groundwater are presented in Table (2), where ranges (mg/l) were 0.20-0.90; 0.00-0.26 and 0.0-2.42 with mean values of 0.22±0.05; 0.10±0.01 and 0.83±0.16, respectively. Moreover, the overall mean Zn value (mg/l) was 0.31±0.06 for all examined water samples. Zinc values showed significant differences between higher Zn levels in groundwater and Zn levels in bottled and desalinated water. ($p<0.05$). Table (3) showed that all the examined bottled, desalinated and groundwater samples had Zn content lower than the guideline values as recommended by international and Saudi standards (5.0 mg/ l).

Table 1: Physico-chemical constituent results of drinking water samples

Water source/ Physicochemical parameter	Bottled water (n=16)		Desalinated water (n=31)		Groundwater (n=15)		TOTAL (n=62)	
	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE
Turbidity (FAU)	0.00-2.00	0.56± 0.18	0.00-13.00	2.23[a]± 0.46	0.00-10.00	5.47[a b]± 0.98	0.00-13.00	2.85 ± 0.40
Conductivity (µS/cm)	90.00-290.00	136.25± 12.84	110.00-200.00	152.90± 3.05	120.00-2460.0	937.67 [a b]± 199.13	120.00-2460.0	296.49± 63.20
Total solids (mg/l)	68.00-270.00	130.31± 13.79	110.00-340.00	230.48± 8.97	150.00-3640.00	1373.0[a b]± 314.51	68.00-3640.0	481.05± 98.56
PH	7.03-8.29	7.78± 0.09	7.40-8.40	7.75± 0.04	6.67-7.90	7.28[a b]± 0.09	6.67-8.40	7.64± 0.04
Chloride (mg/l)	14.50-40.50	21.78± 1.82	15.20-34.00	18.08± 0.66	11.50-914.80	206.01[a b]± 63.15	11.50-914.80	64.50± 18.07
Total hardness as Ca co_3 (mg/l)	3.07-85.40	36.46± 6.19	2.46-52.28	4.82 [a] ± 1.58	11.52-299.93	102.75[a b]± 22.81	2.46-299.93	36.68± 7.59
Ammonia (mg/l)	0.00-0.02	0.002± 0.001	0.00-0.10	0.02± 0.005	0.00-0.40	0.09 [a b]± 0.03	0.00-0.40	0.03± 0.01
Sulphate (mg/l)	3.00-72.00	21.13± 4.73	17.00-82.00	53.13± 2.84	7.00-1400.0	524.20 [a b]± 125.59	3.00-1400.0	158.84± 39.76
Nitrite (mg/l)	ND	-	ND	-	ND	-	ND	-
Nitrate (mg/l)	2.66-41.17	12.54± 2.11	3.98-49.13	13.98± 1.35	0.00-64.63	26.94 [a b]± 5.83	0.00-64.63	16.74± 1.78
Iron (mg/l)	0.00-0.28	0.07± 0.02	0.00-0.39	0.11± 0.02	0.00-2.20	0.41 [a b]± 0.18	0.00-2.20	0.17± 0.05
Manganese (mg/l)	0.00-0.60	0.16± 0.04	0.00-1.00	0.39 [a] ± 0.05	0.00-0.70	0.23± 0.07	0.00-1.0	0.29± 0.03
Copper (mg/l)	0.00-0.68	0.08± 0.04	0.00-0.70	0.09± 0.03	0.01-6.54	2.95 [a b]± 0.68	0.00-6.54	0.78± 0.22
Zinc (mg/l)	0.20 0.90	0.22± 0.05	0.00-0.26	0.10± 0.01	0.00-2.42	0.83 [a b]± 0.16	0.00-2.42	0.31± 0.06

(Fieldwork and sample analysis have extended from February-July 2007)

ND= Not Detected.

[a]= Variation against bottled water samples (the mean difference is significant at 0.05 level).

[b]= Variation against desalinated water samples(the mean difference is significant at 0.05 level).

Table 2: Percentage of water samples exceeding the permissible limits of physical, and chemical parameters of the examined drinking water sources

Parameter	Permissible limit	Bottled water (n=16)		Desalinated water (n=31)		Groundwater (wells) (n=15)	
		Over Permissible Limit		Over Permissible Limit		Over Permissible Limit	
		No.	%	No.	%	No.	%
Turbidity (NTU)	5**	0	0.0	3	9.68	9	60.00
Conductivity µS/ cm	400***	0	0.0	0	0.00	10	66.67
Total solids (mg/l)	500*	0	0.00	0	0.0	9	60.00
Ph	6.5-8.5*	0	0.0	0	0.0	0	0.0
Chloride (mg/l)	250*	0	0.0	0	0.0	6	40.0
Total hardness as Ca co_3 (mg/l)	100**	0	0.0	0	0.0	7	46.67
Sulphate (mg/l)	200*	0	0.0	0	0	9	60.0
Ammonia (mg/l)	0.5*	0	0.0	0	0.0	0	0.0
Nitrite (mg/l)	0.2**	0	0.0	0	0.0	0	0.0
Nitrate (mg/l)	45*	0	0.0	1	3.23	4	26.67
Iron (mg/l)	0.3*	0	0.0	3	9.68	4	26.67
Manganese (mg/l)	0.05**	11	68.75	27	87.10	8	53.33
Copper (mg/l)	1*	0	0.0	0	0.0	9	60.0
Zinc (mg/l)	5.0*	0	0.00	0	0.00	0.0	0.00

(fieldwork and sample analysis have extended from February-July 2007)
ND=Not Detected. *=Saudi standards for drinking water.
**=Highest desirable limit for drinking water [33].
***= European Union [35]

Discussion

The main problems related to drinking water quality are associated with the conditions of the water supply networks of the study area. The hygienic condition of the three major water resources (bottled, desalinated and groundwater) used in this study area was assessed.

Turbidity of water less than 5 NTU (FAU) is usually accepted for consumers, although this may vary with local circumstances. From the hygienic point of view, the consumption of high turbid water may constitute a health risk as excessive turbidity can protect pathogenic microorganisms from the effects of disinfectants, and also stimulate the growth of bacteria during storage [20].

The results of conductivity recorded in this study were nearly similar with those of previously reported [2, 7]. lower conductivity values were previously reported [21], while higher levels were also recorded [22]. It was concluded that taste was objectionable and soap consumption deemed high for the water with highest conductivity, while for water with lowest conductivity, taste was satisfactory and soap consumption deemed normal [18].

Regarding total solids, lower figures were previously recorded [23]. From the hygienic aspects, high levels of total solids may affect taste, hardness and corrosion properties of water. On the other hand, water with extremely low levels of total solids may also be unacceptable of its flat and insipid taste [20].

In case of pH, nearly similar results were obtained [18, 19, 22, 23]. Lower pH values were also recorded [7, 21]. It was suggested that low pH results in corrosion and high pH would result taste

complaints [20].

The chloride levels recorded in our study are supported with those previously recorded [2, 22, 23]. Lower chloride levels were also reported [24]. In previous study carried out in Saudi Arabia on water samples taken from Zamzam well water and Zamzam pipe water, the mean chloride values were 163.3 and 159.7 mg/l, respectively [25]. Bottled and desalinated water samples had chloride levels lower than the maximum permissible limit of chloride. The highest chloride content is related to evaporation process [7], However, enriched water with salt may occur either naturally or due to over pumping, resulting in intrusion of marine waters in groundwater [2]. It should be underlined that the parametric value established especially regarding chloride, are not health related, but set in order to avoid unpleasant taste and corrosion effect in pipes. The high salt content in water supplies is also not suitable for drinking. It may reduce their palatability and can cause distress in livestock [26]. A higher hardness level was previously recorded [23].

Calcium and magnesium are known to occur naturally in water due to its passage through mineral deposits and rock strata and contribute to its total hardness [2]. It has been suspected that there is a causal link between water hardness and cardiovascular disease and mortality [27].

The obtained sulphate results are supported with those previously recorded [2, 22]. Lower sulphate figures were also reported [7, 21, 24, 25]. Sulphate occurs naturally in many water sources coming in contact with particular rock strata and mineral deposits [28]. Moreover, excess in sulphate indicates many causes of pollution either surface or ground water. Considering groundwater in particular, such substance may associated with health risk. Diarrhea may be associated with the consumption of water polluted with sulphate [2].

In comparison the results of ammonia obtained in this study with other previous results, it was found that higher levels were previously recorded [2, 23, 25, 29]. The presence of ammonia at higher levels is an important indicator of recent faecal pollution from sewage, imposing a serious threat against public health. This may be attributed to the fact that ammonia may result also from fertilizers, although in this case it is relatively easily oxidized in soil to nitrite and finally to nitrate [2].

Higher nitrate levels more than the obtained results of this study were previously recorded [2, 21, 22, 23, 24, 29]. However, lower nitrate figure was also reported [7]. Nitrates originate from fertilizers of potassium and/or ammonium nitrate. Since these salts are very soluble and do not bind to soils, nitrates migrate easily to groundwater [30]. The presence of nitrates indicates older events of pollution and excessive nitrate levels in drinking water have caused illness and sometimes death. Nitrate and nitrite at higher levels have an potential to cause the diuresis, increased starchy deposits and haemorrhage of spleen as well as causing infant methaemoglobinaemia like nitrates, a disease characterized by bluish coloration of skin [26].

In previous studies, lower iron figures were reported [7, 19, 22, 24]. Iron is mostly a naturally derived metallic pollutant, which owes its origin in waters mainly to the sources derived from soil and rocks [26]. Iron is essential in low concentration. Moreover, iron poisoning is very clear in children less than 5 years of age. Gastrointestinal tract and liver are the main target iron toxicity. Iron produces coagulative necrosis, bleeding and death [32].

Higher Mn value was previously reported [19], while lower Mn figures were also recorded [7, 21, 22, 24]. Excessive Mn concentration may exist in groundwater from soil and rocks as well as decaying organic matter. Manganese does not

appear to have any toxicological significance in drinking water at the quantities generally occur in raw waters. In some cases, chronic poisoning by Mn can result by extended exposure to very high levels in drinking water, manifested by progressive deterioration of central nervous system, lethargy and symptoms stimulating Parkinson›s syndrome [26, 33]. Nearly similar results were previously reported [22]. Lower copper figures were also recorded [2, 7, 21, 24]. The recorded copper levels in desalinated and bottled water were lower than the maximum permissible levels for copper.

The results of copper levels showed significant higher levels in groundwater than in desalinated or bottled water. Copper is essential at low concentration, but it is toxic at high levels in drinking water. Ingestion of water with high copper concentrations may lead to gastrointestinal distress, jaundice and Wilson›s disease which characterized by destruction of new cells, liver cirrhosis, ascitis, oedema and hemolytic anaemia, cardiovascular collapse and hepatic failure [34].

From the obtained zinc results, the highest value was recorded in samples taken from groundwater, followed by bottled and lastly desalinated water. Zinc is an essential component for at least 8 enzyme systems. Moreover, when Zn ingested in water with high amounts may lead to gastrointestinal irritation with nausea, vomiting and watery diarrhea. Also, it is associated with central nervous system depression and tremors [33, 34].

Conclusion

In conclusion, the physico-chemical analysis of water sources used in Khamis Mushait governorate has allowed determining and characterizing their quality and pointing out its suitability for human consumptions with regards of national and international standards. Water derived from groundwater (wells) showed increases in most of the investigated physico-chemical parameters as compared to bottled and desalinated water. However, this may be attributed to the fact that groundwater is at risk contamination as indicated by the higher levels of these parameters and has to be considered for more of investigations. Moreover, groundwater is exposed to point pollution sources as septic wells, domestic and farming effluents as well as soil of high humus content. The lower physico-chemical characteristics in bottled water samples indicates their satisfactory for human drinking water purposes. Desalinated water is considered also satisfactory at its regional distributing point. However, contamination of desalinated water may occur during transportation or storage in house reservoir. Improving and expanding existing water treatment as well as sanitation system is more likely to provide safe and sustainable sources of water over the long term. However, private drinking water systems are not regulated by local health and environmental agency. So, strict hygienic measures by local authorities of the study area should be applied to improve quality of drinking water and to avoid deleterious effect on human health. This could be achieved by working towards a proper monitoring program of drinking water supply and sources for Aseer province in general. Sanitary criteria and regulations should be also established\to provide necessary protection of drinking water consumption.

REFERENCES

1. Nash, H. and McCall, G.J.H. Groundwater quality. 17th Special Report. Chapmann and Hall, London United, 1995.

2. Karavoltsos, S.; Sakellar, A.; Mihopoulos, N.; Dassenakis, M. and Scoullos, M. J. Evaluation of the quality of drinking water in regions of Greece. Desalination 2008; 224: 317-329.

3. Mancini, G.; Roccaro, P. and Vagliasindi, F.G.A.

Water intended for human consumption. Part II: Treatment alternatives, monitoring issues and resulting costs. Desalination. 2005; 176: 143-153.

4. Misund, A.; Frengstad, B.; Siewers, U. and Reimann, C. Variation of 66 elements in European bottled mineral waters. The Science of the Total Environment. 1999; 243/ 244: 21-41.

5. Frengstad, B.; Skrede, A.K.M.; Banks, D.; Krog, J.R. and Siewere, U. The chemistry of Norwegian groundwaters: III. The distribution of trace elements in 476 crystalline bedrock groundwaters, as analysed by ICP-MS techniques. The Science of the Total Environment. 2000; 246: 21-40.

6. Ozturk, N. and Yilmaz, Y.Z. Trace elements and radioactivity levels in drinking water near Tuncbilek coal-fired power plant in Kutahya, Turkey. Water Research. 2000; 34: 704-708.

7. Galindo, G.; Sainato, C.; Dapena, C.; Fernández-Turiel, J.L.; Gimeno, D.; Pomposiello, M.C and Panarello, H.O. Surface and groundwater quality in the northeastern region of Buenos Aries Province, Argentina. Journal of South American Earth Sciences. 2007; 23: 136-145.

8. Pip, E. Survey of bottled drinking water available in Manitoba, Canada. Environmental Health Perspectives. 2000; 108: 863-866.

9. Robles, E.; Ramirez, P.; Gonzalez, E.; Sainz, G.; Martinez, B.; Duran, A. and Marinez, E. Bottled water quality in metropolitan Mexico City. Water Air Soil Pollution. 1999; 113: 217-226.

10. Al-Saleh, I. and Al-Doush, I. Survey of trace elements in household and bottled drinking water samples collected in Riyadh, Saudi Arabia. The Science of the Total Environment. 1998; 216: 181-192.

11. Allen, H.E.; Halley-Henderson, M.A. and Hass, C.N. Chemical composition of bottled mineral water. Archive of Environmental Health. 1989; 44: 102-116.

12. Meijerink, A.M. Remote Sensing Applications to Hydrology: Groundwater, 1997.

13. Wangnick, K. IDA worldwide Desalting Plant Inventory. Report No. 12, IDA, Topsfield, USA, 1992.

14. Kutty, P.C.M.; Nomani, A.A.; Al-Sulami, S. and Al-Rabeh, A. Monitoring of trace metals in desalinated drinking water and their permissible levels. IDA conference, Abu Dhabi (18-24 November). 1995; 1180-1190.

15. Rango, A. Application of remote sensing methods to hydrology and water resources. Hydrological Science Journal. 1994; 39: 309-319.

16. American Public Health Association (APHA). Standards Methods for Examination of Water and Wastewater. 20th Ed. Washington, D.C., USA, 1998.

17. McBean, E.D. and Rovers, F.A. Statistical procedures for analysis of environmental monitoring data and risk assessment. New Jersy 07458: Prentice Hall PTR, 1998.

18. Hoko, Z. An assessment of water quality of drinking water in rural districts in Zimbabwe. The case of Gokwe South, Nkayi, Lupane, and Mwenezi districts. Physics and Chemistry of the Earth. 2005; 30: 859-866.

19. Ortiz, R.M. Assessment of microbial and chemical water quality of individual and small system groundwater supplies in Arizona. Department of Soil and Environmental Science. ph D. Thesis, University of Arizona, 2007.

20. WHO. World Health Organization. Guidelines for Drinking Water Quality. 2nd ed. Health Criteria and Other Supporting Information. Vol. 2 WHO, Geneva, Switzerland, 1996.

21. Flaten, T.P. A nation-wide survey of the chemical composition of drinking water in Norway. The Science of the total Environment. 1991; 102: 35-73.

22. Ikem, A.; Odueyungbo, S.; Egiebor, N.O. and Nyavor, K. Chemical quality of bottled waters from three cities in eastern Alabama. The Science of the Total Environment. 2002; 285: 165-175.

23. Zaki, M.S.A.; Byomi, A.M. and Hussein, M.M. Hygienic evaluation of water used in some broiler farms around Sadat governorate in Menoufia governorate. 6th Vet. Med. Zagazig Conference (5-7 September). 2002; 209-224.

24. Reimann, C.; Bjell, K.; Frengstad, B.; Melaku, Z.; Tarele-Haimanot, R. and Siewers, U. Drinking water quality in Ethiopian section of the East African Rift Valley I-data and health aspects. The Science of the Total Environment. 2003; 311: 65-80.

25. Al-Zuhair, N. and Khounganian, R.A comparative study between the chemical composition of potable water and Zamzam water in Saudi Arabia. Saudi Dental Journal. 2006; 18 (Si). Abst. 071.

26. Goel, P.K. Water Pollution; Causes, Effects and Control. Published by Poplai, H.S. for New Age International (P) Limited, New Delhi-110 002, India, 1997.

27. COMA. Nutritional aspects of cardiovascular disease No. 46. London: Committee on Medical Aspects of Food Policy, HMSO, 1994.

28. Beamonte, E.; Bermuder, J.D.; Casino, A. and Veres, E. A statistical study of the quality of surface water intended for human consumption near Valencia (Spain). Journal of Environmental Management. 2007; 83: 307-314.

29. Alaa-El-Din, M.N.; Madany, I.M.; Al-Tayaran, A.; Al-Jubair, A.H. and Gomaa, A. Quality of Water from Wells in S.A. Water, Air, and Soil Pollution. 1993; 66(1-2): 135-143.

30. Papa, G. Hygienic importance of chemical parameters in drinking water. Hellenic Ministry of Health and Welfare, National School of Public Health (in Greek), 2001.

31. US EPA. Consumer fact sheet on: Nitrates/Nitrites. United States Environmental Protection Agency, 2003.

32. Bryson, P.D. Comparative Review in Toxicology. 2nd Ed. Aspan Publishers Inc. Rockville, Maryland. Royal Cambridge Wells. Chap. 39. pp. 456-484, 1989.

33. WHO. World Health Organization. Guidelines for Drinking Water Quality. Geneva, Switzerland, 2004.

34. Gossel, T.A. and Bricker, J.D. Principles of Clinical Toxicology. 2nd Ed. Raven Press Ltd New York, USA, 1990.

35. Kiely, G. Environmental engineering. Maidenhead, England; McGraw-Hill Publishing Company, 1997.

Acknowledgement

The authors would like to express their great thanks to admin. colleagues; staff of Dept. of Applied Medical Sciences, Khamis Mushait Community College, King Khalid University, KSA, for their help and handy support and advises during the course of this study.

ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY MAY 2009 ISBN 1595-689X VOL 10(2)
AJCEM/200032/20915 -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 10(2): 128-135

SEXUAL BEHAVIOUR AND CONDOM USE AMONG NIGERIAN SOLDIERS IN ILORIN, KWARA STATE, NIGERIA.

* Hussain, N.A.A. and ** Akande, T.M. *

Department of Preventive Health, 68 Nigerian Army Reference Hospital, Yaba- Lagos, Nigeria and ** Department of Epidemiology and Community Health, University Teaching Hospital, Ilorin, Nigeria.

* Correspondence: Dr N.A.A. Hussain. Mobile Tel: 08055606166. E-mail: naahussain@yahoo.co.uk

ABSTRACT

Background/Objective: Studies have shown that military personnel are aware and knowledgeable about HIV/AIDS and its impact on combat preparedness and efficiency. However, this knowledge did not appear to have translated to reduced risky sexual behaviours. This study, therefore aimed at determining the sexual behaviour and condom use among Nigerian Army personnel in Ilorin, Nigeria.
Materials and methods: A descriptive cross-sectional study using a multistage sampling technique to select 400 participants. A pre-tested semi-structured questionnaire was administered.
Results: Out of 386 respondents (response rate 96.5%) males constituted 91.2%. Two hundred and seventy two (70.5 %) were married while 42 (15.4%) of the married did not live with their spouses. A significant proportion of the respondents (45.5%), especially those within the age of 18 to 34 years (p=0.000), those who had participated in foreign military operations (p=0.030) and those who did not live with their spouses (p=0.000) engaged in pre/extramarital sexual activities. Regular condom use among the respondents was low.
Conclusion/Recommendations: There was a high risky sexual behaviour with irregular condom use among the study population. A continuous information, education and communication on HIV/AIDS to effect safe sex behaviours and regular condom use among this population are recommended.

Key Words: Sexual, Behaviour, Condom, Nigerian, Soldiers, Ilorin

INTRODUCTION

By the dictates of their profession, military personnel are at higher risk of contracting sexually transmitted infections (including HIV/AIDS) than the general population[1]. Their frequent mobility, involvement in international peacekeeping operations, intravenous drug use, patronage of commercial sex workers and proneness to injuries which may necessitate urgent blood transfusion make them more at risk.[2,3,4] The higher vulnerability to HIV/AIDS could probably also be attributed to the fact that, globally, the military culture, at least until recently, had traditionally fostered the habit of heavy smoking, high alcohol consumption and increased risk-taking[5].

The Nigerian Army (comprising about 80,000 personnel) is saddled with the Constitutional roles of defending the nation from external aggression, maintaining its territorial integrity and securing its borders on land, sea and air[6]. In addition, the

personnel also participate in international peacekeeping missions in many conflict-ridden countries in Africa. Their duties demand optimum physical fitness which is could be sustained by the adoption of healthy lifestyle. Considering the adverse impact of HIV/AIDS on military preparedness and effectiveness, concerted efforts have been made by the Nigerian military authorities to arm their personnel with the needed information.

Researches have shown that soldiers are well aware and sensitized on HIV; its modes of transmission, risk factors for transmission, prevention and control measures.[7,8,9] However, there is dearth of research on Nigerian soldier's sexual behaviours and use of condoms especially in casual sex. This study therefore aimed at determining the sexual behaviours and condom use among this population.

MATERIALS AND METHODS

Study Site

This study was carried out in Sobi Barracks, in Ilorin East Local Government Area of Kwara State. The barracks is made up of 3 autonomous military units; Headquarters 22 Armoured Brigade, 222 Battalion and the Nigerian ArmSchool of Education. With a total of about 1703 soldiers, the barracks has a well staffed Medical Centre, a "Mammy market", 2 primary schools and a day secondary school. The barracks is a multiethnic one, with seemingly homogenous representation of all religious and ethnic groups in Nigeria. It has 2 messes (one each for the Commissioned Officers and Non Commissioned Officers and a soldier's Canteen) where the personnel recreate after work. The barracks has an HIV Control Committee which is headed by the Doctor in charge of the Medical Centre with other members drawn from the 3 autonomous military units.

Methodology

A minimum sample size of 384 was obtained using the appropriate formula and a sexual behavioural prevalence of 50%. To allow for a non-response of 4%, four hundred soldiers were sampled for the study. A multistage sampling strategy was used. An initial sampling proportionate to sizes of the three army units was followed by systematic sampling technique using each unit's register as a sampling frame and a calculated sampling interval. Then the respondents in each of the units were randomly selected using the ballot method.

Self-administered, semi-structured and pre-tested questionnaires were administered to the selected soldiers in order to determine their sexual behaviours, perception of condom and its use in casual sex. The administered questionnaires were collated and analyzed using EPI Info 2002 version software package. Frequency distribution and cross tabulations were done. Appropriate test statistics were used where

necessary at 95% confidence limit.

RESULTS

Out of the four hundred questionnaires administered, 386 were returned, giving a response rate of 96.5%. The age range of the respondents was 18 and 60 years with 241 (62.4%) of them within 18 and 34 years. There was a significant difference between the mean age of the male respondents and that of females (p=0.0027). Males constituted 91.2% of the respondents. Two hundred and seventy two (70.5%) were married and out of these 42 (15.4%) did not live with their spouses. Two hundred and twelve (54.9%) respondents were Christians while 174 (45.1%) were Muslims. About 92.5% had at least secondary education. (Table 1)

One hundred and seventy four (45.5%) respondents engaged in pre/extramarital sexual activities. Out of this, 129 (33.7%) were within the age of 18 and 34 years (p=0.000). There were significant relationships between respondent's participation in foreign military operations (p=0.030), marital status (0.002), not living with spouses (p=0.000) and engagement in pre/extramarital sex (Table 2). Among the 174 respondents who engaged in pre/extramarital sexual activities, 107 (61.5%) use condoms regularly. For respondents who were within 18 to 34 years of age 79 (45.4%) used condoms regularly while 32(18.4%) and 18(10.3%) either used it occasionally or did not use it at all. Among the married respondents who engaged in casual sex, only 41.4% of them used condoms regularly. There were no significant relationships between the respondent's educational qualification, rank and marital status and condom use.

TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

Characteristics	No of respondents (N =386)		Total
	Male (%)	Female (%)	
●Sex Distribution	352(91.2)	34(8.8)	386(100)
●Age distribution (years)			
18-24	51(13.2)	9(2.3)	60(15.5)
25-34	164(42.5)	17(4.4)	181(46.9)
35-44	99(25.6)	5(1.3)	104(26.9)
45-54	28 (7.3)	3(0.8)	31(8.1)
55-60	10 (2.5)	-	10(2.5)
Mean ages	33.5 ±5.4	30.5± 6.8	P value =0.003
●Religious distribution			
Christianity	189(49.0)	23(6.0)	212(55.0)
Islam	163(42.1)	11(2.8)	174(44.9)
●Educational levels			
Primary	26(6.7)	3(0.8)	29(7.5)
Secondary	255(66.1)	28(7.3)	

Post-secondary	**71(18.3)**	**3(0.8)**	**283(73.3)**
●Rank distribution			**74(19.2)**
Corporals and below	**213(55.2)**	**24(6.2)**	
Senior Non-Commissioned Officers	**126(32.6)**	**9(2.3)**	**237(61.4)**
			135(34.9)
Commissioned Officers	**13(3.4)**	**1(0.3)**	
●Marital status			**14(3.6)**
Single	**95(24.6)**	**13(3.4)**	
Married	**254(65.8)**	**18(4.7)**	**108(28.0)**
Widowed	**-**	**-**	**272(70.5)**
Divorced	**3(0.8)**	**1(0.3)**	**-**
Separated	**2(0.5)**	**-**	**4(1.1)**
●Live with spouse			
Yes	**217(77.8)**	**13(4.8)**	**2(0.5)**
No	**37(13.6)**	**5(1.8)**	
			230(82.6)
			42(15.4)

DISCUSSION

The age range of the surveyed population was 18 to 60 years and this reflects the minimum entry and retirement ages in the Nigerian Army. The preponderance of males (91.2%) among the respondents reflects the fact that the military is a male-dominant profession. Two hundred and forty one respondents (62.4%) were within the highly vulnerable age group of 18 to 34 years. This is an important target age group in the military that needs to be continuously sensitised on HIV/AIDS because of their probable increased mobility, increased risky sexual behavior and substance use.[2,4,7,8] Out of 272 (70.5%) married respondents, 42 (15.4%) did not live with their spouses. That less than one-sixth of the married respondents did not live with their spouses could be attributed to the frequent redeployment of soldiers without a prompt accommodation so that their families could join them.[1,2] This temporary family separation removes the contact with their spouses and regular sexual partners thereby encouraging sexual escapades and other harmful activities as a way of relieving boredom or easing tension.

TABLE 2: PRE/EXTRAMARITAL SEXUAL ACTIVITIES AMONG THE RESPONDENTS

Characteristics	Pre/extramarital sexual activities				
	Yes (%)	No (%)	Total	X^2	P value
●Pre/extramarital sexual activities	174(45.5)	208(54.5)	382		
●Age distribution (years)					
18-24					
25-34	46(12.0)	13 (3.4)	59		
35-44	83 (21.7)	95(24.9)	178		
45-54	31 (8.1)	73(19.1)	104	38.96	0.000
55-60	9 (2.4)	22(5.8)	31		
●Educational levels	5(1.3)	5(1.3)	10		
Primary					
Secondary	13(3.4)	15(3.9)	28		
Post-secondary	127(33.2)	152(39.8)	279		
●Distribution by Rank	34(8.9)	41(10.9)	75	0.01	0.000
Corporals and below					
Senior Non-Commissioned Officers	103(27.0)	130(34.0)	233		
Commissioned Officers	62(16.2)	73(19.1)	135	2.16	0.339
●Marital status	9(2.4)	5(1.3)	14		
Single					
Married	61(15.9)	46(12.0)	107		
Divorced	108(28.3)	161(42.5)	269	12.28	0.002
Separated	3(0.8)	1(0.3)	4		
●Live with spouse	2 (0.5)	-	2		
Yes					
No	81(21.2)	146(38.2)	227	21.96	0.000
●Involved in foreign operations	93(24.3)	62(16.2)	155		
Yes					
No					
	73(19.1)	65(17.0)	138		
	101(26.2)	143(37.4)	244	4.70	0.030

Nearly half of the surveyed respondents (45.5%), with 33.7% of them in the age group18-34 years engaged in premarital / extramarital sexual activities. Equally evident was the fact that a significant proportion of the respondents with secondary education (33.2%) and those who were involved in foreign operations (41.4%) also engaged in pre/extramarital sexual activities ($p<0.05$).

These findings corroborated surveys in other military communities, which revealed

TABLE 3: CONDOM USE AMONG RESPONDENTS WHO ENGAGED IN PRE/EXTRAMARITAL SEXUAL ACTIVITIES.

Characteristics	Regular use	Occasional use	Do not use	Total	X^2	P value
●Frequency of condom use	107(61.5)	44(25.3)	23(13.2)	174		
●Age distribution (years)						
18-24	26(14.9)	13(7.5)	7(4.0)	46		
25-34	53(30.5)	19(10.9)	11(6.3)	83	3.10	0.795
35-44	21(12.1)	8(4.6)	2(1.1)	31		
45-54	4(2.3)	4(2.3)	1(1.1)	9		
55-60	3(1.7)	-	2(1.1)	5		
●Educational levels						
Primary	6(3.4)	4(2.3)	3(1.7)	13		
Secondary	87(50.0)	23(13.2)	17(9.8)	127	6.71	0.152
Post-secondary	14(8.0)	17(9.8)	3(1.7)	34		
●Distribution by Rank						
Corporals and below	56(32.2)	29(16.7)	18(10.3)	103		
Senior Non-Commissioned Officers	45(25.9)	13(7.5)	4(2.3)	62	6.54	0.161
Commissioned Officers	6(3.4)	2(1.1)	1(0.6)	9		
●Marital status						
Single	34(19.5)	19(10.9)	8(4.6)	61		
Married	71(40.8)	24(13.8)	13(7.5)	108	1.78	0.410
Divorced	2(1.1)	-	1(0.6)	3		
Separated	-	1(0.6)	1(0.6)	2		

that young soldiers and those who were deployed for foreign operations were more likely to engage in risky sex than the older ones, or those who never participated in foreign operations.[12,13,14]

Condom promotion: its frequent and correct use in risky sex is an important intervention in HIV/AIDS prevention and control.

Among those who engaged in pre/extramarital sexual activities, the overall prevalence of regular condom use was 61.5%. Regular condom use among respondents within the age range 18-34 years was low (45.4%) while 10.3% of them did not use it. However, consistent condom use was higher among respondents with secondary education although this was not significant (p=0.301). This finding on condom use among the surveyed respondents is in agreement with a previous study among military personnel in Nigeria, which showed that about 72.7% of the respondents were aware that condom use could be protective against HIV/AIDS, but

only 55% of them reported using condom with non-regular partners[14]. Although reduction in sexual sensation, outright non-interest in condoms and its messiness have been documented for low condom use among civilian populations[15,16], further research is necessary to ascertain the reasons for its irregular or non-use among this surveyed population..

CONCLUSION

Pre/extramarital sexual activities were high and frequent condom use among these respondents was low. A continuous information, education and communication on HIV/AIDS to effect safe sex behaviours and regular condom use among this population are recommended.

References

1 Joint United Nations Programmes on HIV/AIDS. Uniformed Services, HIV/AIDS and National Security. *UNAIDS*. Feb 2002:1- 4

2. Lovgren Stefan. African Army Hastening HIV/AIDS Spread. *Jenda; A Journal of Culture and African Women Studies.* 2001; 1 (2):11-3

3. Yeager R, Hendrix C.W, Kingma S. International Military HIV/AIDS Policies and Programmes, Strengths and Limitation. *Military Medicine* Feb 2000.165 (5):87-92.

4. Okulate G T, Osibogun A, Obe J. HIV/AIDS Fear, Knowledge and Alcohol Use in a Military Population. *The Cliniscope: Journal of Military Hospital Lagos, Nigeria.* 2001; 1 (3): 14-19.

5. Chikwem J O. Impact of HIV on the Military. A Paper Presented at the National War College Abuja-Lagos. *Sponsored by the US Department of Defence and Lincoln University USA.* March 21, 2002.

6 Egbewunmi O O. AIDS Ravages the Nigerian Military. *A speech delivered during the Armed Forces Programme on AIDS Control (AFPAC) week*. Feb 2002.

7. Lamptey P R. Reducing Heterosexual Transmission of HIV in Poor Countries. *British Medical Journal.* Jan 2002; 324:207-211

8. WHO/GPA. Current and Future Dimensions of HIV Pandemic. *WHO/GPA Geneva* 1990:1-15.

9. Joint United Nations Programme on HIV/AIDS. Reports on the Global HIV/AIDS Epidemic. Geneva. *UNAIDS*:2000.

10. Newsdesk International Action. AIDS; World Health Organisation effort falters. *African Health* 1992; 14 (5):7

11. Akande TM. Willingness of Health Workers to Undergo HIV Screening. *Nigerian Quarterly Journal of Hospital Medicine*. 1999; 9 (4) :303-6

12. Ekong E. Comparison of Knowledge, Attitude and Practice on HIV/AIDS among Medical and Infantry Corp of

the Nigerian Army. *12th World AIDS Conference, Geneva- Switzerland* .June28-July 3, 1998.

13. Van der Ryst, Jombert G, Steyn F, Heunis C, La-roux J, Williams C. HIV/AIDS related Knowledge, Attitude and Practices among South African Military Recruits. *South African Medical Journal*. Jul 2001; 91 (7): 587- 91.
14. Adebajo S, Egbewunmi OO, Jerome M, Scott M, Nancy M. Knowledge, Attitude and Sexual Behaviour Among the Military Concerning HIV/AIDS/STIs. *Technical Report by the Armed Forces Programme on AIDS Control (AFPAC)*.Aug.2001.
15. Armed Forces Programme on AIDS Control (AFPAC). Armed Forces HIV/AIDS Policy Guidelines. 2004 (revised):10-12

The views expressed in this paper are the individual views of the authors and do not reflect that of the Nigerian Army or Ministry of Defence.