Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: C

Microbiology and Pathology

Circulating HBV Genotypes

Susceptibility of Enterobacteriaceae

Highlights

Squamous Odontogenic Tumour

Chemical Quality of Swimming Water

Discovering Thoughts, Inventing Future

VOLUME 16 ISSUE 2 VERSION 1.0

Global Journal of Medical Research: C
Microbiology and Pathology

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Volume 16 Issue 2 (Ver. 1.0)

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Contents of the Issue

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 2 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Squamous Odontogenic Tumour of Anterior Mandible – A Rare Case in Unusual Location

By Dr. Jain Himanshu, Dr. Singhsardar Gunadhar, Dr. Roychowdhury Anadi & Dr. Bandyopadhyay Goutam

R. G. Kar Medical College and Hospital

Abstract- *Background:* Squamous odontogenic tumour (SOT) is a rare benign locally infiltrative epithelial neoplasm of periodontium. The tumour originate from rests of Malassez, gingival surface epithelium or from remnants of the dental lamina. Tumour may present as painless swelling or toothache and tooth mobility.

Case Report: We report a case of 35year male presented with swelling in anterior mandible and recurrent gum bleeding an unusual site and unusual presentation.

Conclusion: Being a rare tumour SOT should be differentiated from other similar looking tumour i.e. acanthomatous ameloblastoma, SOT like islands arises from cystic wall and many others for definite therapy

Keywords: *squmous odontogenic tumour, benign infiltrating epithelial neoplasm, acanthomatos ameloblastoma.*

GJMR-C Classification : *NLMC Code: QW 4*

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Squamous Odontogenic Tumour of Anterior Mandible – A Rare Case in Unusual Location

Dr. Jain Himanshu [α], Dr. Singhsardar Gunadhar [σ], Dr. Roychowdhury Anadi [ρ] & Dr. Bandyopadhyay Goutam [ω]

Abstract- Background: Squamous odontogenic tumour (SOT) is a rare benign locally infiltrative epithelial neoplasm of periodontium. The tumour originate from rests of Malassez, gingival surface epithelium or from remnants of the dental lamina. Tumour may present as painless swelling or toothache and tooth mobility.

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I. Introduction

Squamous odontogenic tumour (SOT) is rare intraosseous benign epithelial neoplasm with locally invasive nature. SOT was first described by Pullon et al. (1975). In ensuing 40 year less than 50 cases reported till now. SOT is defined as locally infiltrative neoplasm consisting of islands of well differentiated squamous epithelium in a fibrous stroma.[1] The age range is between 8-74 years with mean of 38.7 years[2] with slight male predominance (M:F 1.4:1).[1]

SOT occurs intraosseously and develops in the periodontal ligament between the roots of vital erupted permanent teeth. The tumour location is slightly more common in posterior mandible than anterior mandible[1]. Usually patients present with asymptomatic gingival swelling or local pain, mobility of teeth, osseous expansion and mild gingival erythema.[1]

Radiographically tumour present as well defined triangular radiolucency adjacent to roots of teeth.[3] Occasional calcification and cystic degenerationcan occur.

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Histologically, the tumour composed of multiple islands of squamous epithelium surrounded by mature connective stroma without peripheral palisading. Occasionally calcification and central cystic degeneration also observed.

Conservative surgical treatment is sufficient and recurrences are rare.

II. Case Report

A 35 year male presented with recurrent gum bleeding since last 10 days. He had history of gradually increasing gingival swelling, pain and mobility of lower incisors over anterior mandible from last one year.

Non contrast MDCT-Denta scan imaging study revealed a well-defined mixed radio opaque radiolucent space occupying lesion involving lower mandible measuring 27.8 x 25.3 x 19.8 mm. Periphery of the lesion was partly sclerotic and partly surrounded by radiolucent halo. The lesion was composed of granular septation with concomitant sign of multifocal calcification. Extensive expansion, distortion and destruction of the buccolingual cortex of bone and knife edge type of root resorption is also observed. [Figure1]

Previous incisional biopsy from the tumour tissue reported asacanthomatous ameloblastoma.

Anterior segmental mandibulectomy done. On gross examination a greyish white irregular solid-cystic structure measuring 5 x 3 x 2cm. Outer surface of which was smooth and covered with gingival mucosa. Inner surface was friable. Histopathological examination revealed presence of islands of squamous cells lined at its periphery by flattened cells without categorical pleomorphism or atypical mitotic figure surrounded by fibrous stroma. Occasional cells show clear cytoplasm. Palisading of cells and ameloblastic stroma is not noted anywhere with serial sections. Focal calcification and central cystic changes also observed. [Figure 2, Figure 3, Figure 4]

III. Discussion

In 1975 Pullon et al. first described 6 cases of previously unnamed oral lesions as squamous odontogenic tumour.[4]

Bansal et al descried a table of 44 cases showed that tumour location is slightly more common in mandible than maxilla. Posterior mandible is more prone

than anterior for the lesion and anterior maxilla is far more common location than the posterior maxilla. Only few cases were multicentric and only one case was bilateral in posterior maxilla.[5] Though SOT in maxilla is more aggressive than mandible and required more radical surgery.[6,7] Only one case is reported till now tumour localised between roots of central incisor of mandible[5]. In our case tumour was localised below all the incisors of mandible.

Clinically tumour presents as painless gradually increasing swelling of mandible or maxillary bone, mobility of teeth, pain and erythema of the lesion. Though SOT may be asymptomatic and detected in routine intraoral radiograph. Our case is unique it also presents with recurrent gum bleeding.

Radiographically tumour present as well defined triangular radiolucency adjacent to roots of teeth.[3] Occasional calcification and cystic denegation can occur. The lesion is usually central but sometimes it may be peripheral[3] which may produce some saucerization of the underlying bone- a result of pressure from tumour expansion rather than neoplastic infiltration.[1]

Due to calcified material SOTs may be misdiagnosed as acathomatous ameloblastoma, desmoplastic ameloblastom, well differentiated squamous cell carcinoma or pseudoepitheliomatous hyperplasia. Other possible differential diagnosis may be "squamous odontogenic tumour like islands arising in the walls of odontogenic cyst".[1] SOT can differentiate from ameloblastomaby observing absence of peripheral palisading and cytoplasmic vacuolation. In addition, stellate reticulum like cells and ameloblastic stroma, which are always present in ameloblastoma, are never seen in SOT.[8]

CH Siar et al showed positive reactivity of varying intensity in the neoplastic epithelial for Notch1, Notch3, Notch4 and their ligands Jagged1 and Delta1. No immunoreactivity was detected for Notch2 and Jagged2.[9]

IV. Conclusion

It is a rare tumour mimicking other more common odontogenic tumour, intracystic squamous cell carcinoma and some benign proliferative lesions. It should be bear in mind that SOT is a locally aggressive tumour which is curative with careful surgery and should be differentiated from the other mentioned tumour and tumour like lesions for specific therapy.

Abbreviations

SOT – Squamous odontogenic tumour

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Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 2 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

The S-Gene Mutations in the Circulating HBV Genotypes/Sub-Genotypes Associated with Hepatitis B Infection in Uganda and their Effects on Cytokines Expression in Liver Disease Progression

By Hussein Mukasa Kafeero, Abubaker Kawooya, Mariam Namusoke, Saad Atiku & Joseph Mugambwa

Makerere University

Abstract- The causal agent for hepatitis B is called hepatitis B virus (HBV). It is a partially double stranded circular DNA virus of the family Hepadnaviridae. It has been implicated as the leading cause of hepatocellular carcinoma and only second to tobacco among the global human carcinogens. Liver damage as a result of HBV infection is due to host immune response and is modulate by cytokines. The HBV is classified into 10 genotype denoted as A, B, C, D, E, F, G, H, I and J together with several sub-genotypes which have diverse geographical distribution. These genotypes influence liver disease progression and severity as well as response to antiviral therapies. Mutations in the S-gene have been implicated in the paradoxical coexistence of HBsAg and the anti-HBs antibodies which is associated with advanced liver diseases including hepatocellular carcinoma and liver cirrhosis.

Keywords: *hepatitis B virus, genotypes, cytokines, mutations.*

GJMR-C Classification : *NLMC Code: QW 170*

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Hussein Mukasa Kafeero [α], Abubaker Kawooya [σ], Mariam Namusoke [ρ], Saad Atiku [ω] & Joseph Mugambwa [¥]

Abstract- The causal agent for hepatitis B is called hepatitis B virus (HBV). It is a partially double stranded circular DNA virus of the family Hepadnaviridae. It has been implicated as the leading cause of hepatocellular carcinoma and only second to tobacco among the global human carcinogens. Liver damage as a result of HBV infection is due to host immune response and is modulate by cytokines. The HBV is classified into 10 genotype denoted as A, B, C, D, E, F, G, H, I and J together with several sub-genotypes which have diverse geographical distribution. These genotypes influence liver disease progression and severity as well as response to antiviral therapies. Mutations in the S-gene have been implicated in the paradoxical coexistence of HBsAg and the anti-HBs antibodies which is associated with advanced liver diseases including hepatocellular carcinoma and liver cirrhosis. Management of HBV is by using antiviral therapy but there is no treatment that can cure HBV. Therefore the practical alternative is vaccination but this is genotype specific. It therefore absolutely necessary to match vaccine strains with field strains. Success on this subject is contingent upon accurate diagnosis and routine genotyping. The concept paper also explicates the need for more elucidation of cytokine profiles in HBV virus infection since liver disease progression is cytokine modulated especially in the scenario where mutations are common yet they influence cytokine profiles.

Keywords: hepatitis B virus, genotypes, cytokines, mutations.

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I. Section One: Back Ground

Hepatitis B virus (HBV), a member of the *Hepadnaviridae*, is a circular, partially double-stranded DNA virus and is one of the major causes of chronic liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Matsuura et al., 2009, Khamduang et al., 2013). The HBV genomic structure has been exclusively reviewed by Coleman (2006), Suppiah, Mohd Zain, Bahari, Haji Nawi, & Saat (2014), Ding, Miao, Li, Dai, & Yu (2015) as a partially double stranded DNA of genomic size of approximately 3.2kb with four open-reading frames (ORF). The ORFs encode four genes including the polymerase gene designated as P gene, core (C) gene, large, medium and small surface antigen proteins (S gene) and the X protein (Coleman, 2006, Kahila Bar-Gal et al., 2012). The HBV genome S gene is paramount importance in the molecular genetics of the virus since it is concerned with the expression of the surface antigens and classification of the viral strains (Suppiah et al., 2014) as well as the antigenic variation of the virus. The coexistence of the HBsAg and anti-HBs Antibodies is implicated on the mutation in the S-gene encoding for the surface antigen as a result of HBV immune escape election mutations (Ding et al., 2015). This in turn associated with more advanced liver diseases including hepatocellular carcinoma and liver cirrhosis or chronic hepatitis B infection (Seo, Choi, & Choi, 2014). This is consistent with the earlier study by Svicher, Cento, & Salpin, (2011) who found out that mutations in the S-gene affect pathogenicity and oncogenic potential which in turn affects cytokine profile in HBV infection. Cytokine are critical molecules in progression of the liver disease as reported earlier by Akpolat, Yahsi, Godekmerdan, Demirbag, & Yalniz (2005) and

Frodsham et al (2006). Damage to the liver as a result of HBV infection is due to immune response as reported in earlier studies by Racanelli & Rehermann (2006) and in recent studies by Wang & Zhang (2009) and is cytokine modulated but cytokine profiles in HBV virus infection need more elucidation especially in the scenario where mutations are common. The virus interferes with the functioning of liver cells (hepatocytes) causing the innate immunity to release immune mediators particularly chemokines and cytokines to combat the infectious agent (Keating et al., 2014) culminating into a pathological damage to the liver which becomes inflamed. In the studies by Nora Alma Fierro et al.,(2011), Keating et al., (2014) and Nora A Fierro et al., (2015). a panel of cytokines including IFN-γ, IL-5, IL-6, IL-13 and TNF-α were up regulated after the acute phase of infection.

Arauz-Ruiz, Norder, Robertson, & Magnius, (2002) previously classified HBV into 8 genotype identified as A-H based on an intergroup divergence of 8% or more in complete nucleotide sequence whose geographical distributions was previously extensively studied by Sanchez-Tapias, Costa, Mas, Bruguera, & Rodes, (2002) who documented that genotype A is pandemic, B and C are predominant in Asia, D in southern Europe, E in Africa, F in United States of America, G in France while H in Central America. However recent studies by McMahon (2009), Cao (2009), Kurbanov, Tanaka, & Mizokami, (2010) have introduced two new genotypes designated as I and J giving a total of 10 genotypes together with several sub-genotypes.

In a recent study by Dunford et.al., (2012), genotype variations in HBV together with specific viral mutations have been implicated in influencing the clinical outcome of HBV infection. A similar study by Singhatiraj, Suri, & Goulston, (2012) has documented that genotype A is the most common in co-infection, genotypes B and C are associated with higher viral loads when compared to A and D. Selection such as vaccination, antiviral therapy especially in HIV co-infection, as well as host immune responses have resulted into emergency of viral variants associated with severity of the liver diseases (Dunford et al., 2012). Multiple studies by Thio, (2009) and Matthews et al., (2013) have shown that the use of highly active anti-retroviral therapy (HAART) have resulted into hepatotoxicity and has been implicated as the major cause of mortality in HBV-HIV co-infection.

According to the world health organization (WHO), countries of Africa, Asia and South America have carrier rates as high as greater than 8% (Franco et al., 2012) with Sub-Saharan Africa accounting for 20% of global burden (Khamduang et al., 2013). In Uganda the burden of the disease varies from region to region with Nothern Uganda having the highest prevalence of 17.6% as reported in the study by (Ochola et al., 2013). However the press release from the ministry of health revealed that 10% (more than 3.5 million Ugandans) are living with chronic Hepatitis B infection and the prevalence is region specific with North East 21.7%, North Central 19.4%, West Nile 18.7%, Western 10%, Kampala 5.8%, Central 5.8%, while South West with 2.9%. (MOH), 2015). This challenge is precipitated by lack of advanced clinical laboratory for routine and accurate patient testing (Franco et al., 2012) as well as the limited knowledge about the circulating genotypes and sub genotypes in the developing world (Singhatiraj et al., 2012). Previously, a novel field deployable, rapid, simpler, single temperature, nucleic acid amplification method, termed loop-mediated isothermal amplification (LAMP), has been developed for laboratory diagnosis of many infections (Notomi *et al.,* 2000). However no study has been reported to evaluate the use of LAMP in the diagnosis of HBV in Uganda. It has been used for the timely diagnosis of hepatitis C virus (Nyan et al., 2014), malaria (Hopkins et al., 2013), African Swine fever Virus (Atuhaire et al., 2014), foot-and-mouth disease virus (Kafeero et al., 2016) and Human African Trypanasomiasis (Matovu, Enock,Kuepfer, Boobo, Kibona, & Burri, 2010). This concept paper underpins the urgent need to use rapid diagnostic assays such as LAMP and comparing its diagnostic sensitivity and specificity with the commonly used assays of ELISA and polymerase chain reaction in order to come up with recommendations to the policy makers and the Ministry of Health about the potential benefits of the assay that has received a lot of attention in the recent times.

This concept paper is divided into four sections. After introducing the key conceptual issues in section one, there is section two which provides the conceptual objectives and hypothesis that underpin the development of the whole concept. Section three explicates the conceptual problem which this paper is trying to address. Section four summarizes the literature that informed the design of the concept. The objective of this paper is to elicit Ugandan scientists, physicians and policy makers to appreciate the magnitude of the current and future effect of HBV in our country and think outside the box, using evidence based practice to manage HBV. This is in line with the recently ushered in sustainable development goals as stipulated in goal 3 target 3.3 which is aimed at eliminating hepatitis B by 2030.

II. Section Two: Conceptual Objectives and Hypothesis

We hypothesize that mutations have occurred not only in the S-gene implicated in the antigenicity of the virus but also in the entire genome of the hepatitis B virus resulting into evolution and emergency of several HBV genotypes which influence cytokine profiles. Mutations in the S-gene are of paramount significance

because they have been implicated in the paradoxical coexistence of the HBsAg and the anti-HBs antibodies. This in turn affects the severity of the liver diseases. Immigrants into our country from all other parts of Africa in particular and World over in general have profound effect on the HBV circulating genotypes yet some are issued with immigration documents without valid HBV vaccination documents. This re-affirms our hypothesis of existence of novel genotypes in our region. Liver disease progression, vaccination and antiviral therapy are genotype specific. Unfortunately there is no clear documentation on the circulating genotypes in Uganda underscoring the burden of the disease and escalating the devastating effects of the epidemic.

a) General objective

The main aim of the conceptual paper is to establish the S-gene mutations in the circulating HBV genotypes/ sub-genotypes associated with hepatitis B infection and the role of cytokines in liver disease progression.

b) Specific objectives

1. To determine the prevalence of HBV DNA and antibodies in blood/serum of the study subjects.
2. To establish the circulating genotypes and sub-genotypes of HBV in Uganda.
3. To determine the mutations in S gene of the HBV genome.
4. To profile the panel of cytokines in patients infected with HBV.

III. Section Three: Conceptual Problem

Management of HBV requires the knowledge of mutations particularly the S-gene mutations as well the genotypes and the sub-genotypes because they affect antiviral therapy (Kurbanov, F., Y. Tanaka, K. Fujiwara, F. Sugauchi, D. Mbanya, N. Ndembi, C. Ngansop, L. Kaptue, T. Miura, E. Ido, M. Hayami, & Ichimura, 2005). According to CY Wai, Chu, Hussain, & Lok, (2002) Kobayashi, Arase, & Ikeda, (2002) Kao, Chen, Lai, & Chen, (2002), Yuen et al., (2003), Tanaka et al (2004), Kurbanov, F. et al (2005), Cooksley, (2010), Ferreira & Tenore, (2010), Lin & Kao, (2011), Singhatiraj et al., (2012), Dunford et al., (2012), Matthews et al., (2013). There have been increasing lines of evidence to indicate influences of HBV sub-genotypes on the outcome of liver disease, HBeAg seroconversion, severity of the disease and the response to antiviral therapies as previously reviewed by Singhatiraj et al., (2012) and Dunford et al., (2012).

To date there is no curative treatment to eradicate the HBV but the commonly used therapies such as interferon, pegylated interferon as well as nucleoside / nucleotide analogs prolong the suppression of viral replication and establish host immune control over the virus (Alazawi & Foster, 2008). The success of these therapies is contingent upon the specific viral genotype infecting the host (Kao et al., 2002, Janssen, van Zonneveld, & Senturk, 2005) as well as the mutations in the genome.

There is paucity of information about HBV genotypes, S-gene mutations, cytokine profiles in HBV infections elsewhere as reported elsewhere in a series of studies by Arauz-Ruiz et al., (2002), Sanchez-Tapias et al., (2002), McMahon, (2009), Coleman, (2006), Kahila Bar-Gal et al., (2012), and Zhang et al., (2016). However no study in Uganda to date on the molecular genomics of the circulating genotypes/ subtypes, mutations and their effects on disease progression in terms of cytokine profiles in pathological conditions. Since migrations and variations in selection pressures affect the circulating genotypes and the subsequent mutations (Kahila Bar-Gal et al., 2012), these affect the efficacy of the antiviral drugs. These challenges underscore the burden of the disease which is a public health concern. The recently ushered in sustainable development goals (SDGs) have emphasized the need to eliminate hepatitis B virus infection by 2030. Therefore this concept paper retaliates for an urgent need for studies on molecular biology of the HBV in order to provide physicians and other health workers with evidence based information particularly in areas of molecular genetics of the virus required in the management of HBV in Uganda.

IV. Section Four; Literature Review

a) Hepatitis B genotypes and subtypes and their effect on liver disease progression

The HBV genome is composed of approximately 3,200 nucleotides (Matsuura et al., 2009). Arauz-Ruiz, Norder, Robertson, & Magnius, (2002) previously classified HBV into 8 genotype identified as A-H based on an intergroup divergence of 8% or more in complete nucleotide sequence whose geographical distributions was previously extensively studied by Sanchez-Tapias, Costa, Mas, Bruguera, & Rodes, (2002) who documented that genotype A is pandemic, B and C are predominant in Asia, D in southern Europe, E in Africa, F in United States of America, G in France while H in Central America. However recent studies by McMahon (2009), Cao (2009), Kurbanov, Tanaka, & Mizokami, (2010) have introduced two new genotypes designated as I and J giving a total of 10 genotypes together with several sub-genotypes. With the exception of the newly identified genotypes, the other genotypes and sub-genotypes have well characterized ethnic and geographical distribution (Lin & Kao, 2011). According to the study by Lin & Kao, (2011) genotype A has three sub-genotypes (A1-3) with A1 having prevalence in Sub-Saharan Africa whereas A3 located in West Africa (Table 1). Genotype B has six sub-genotypes B1-6 with none of them isolated in Africa. Genotype, C with five sub-genotypes C1-5 and none of them in Africa. Genotype

D, with sub-genotypes D1-5, and none of them in Africa. Genotype E has no reported sub-genotype up to date and it is restricted in West Africa. Genotype F has four sub-genotypes and none of them is distributed in Africa.

Table 1 : Distribution of genotypes and sub-genotypes among some African countries

Country	Genotypes	Sub-genotypes	Reference
Tunisia	D, F	-	(Ayari et al., 2012)
Gambia, Nigeria, Congo, Rwanda, Cameroon	A	A4, A5, A6, A7	(Shi, 2012)
Egypt	D	D1	(Ragheb et al., 2012)
Central African	A, D, E	A1,D4	(Komas et al., 2013)
South Africa	D	D3	(Yousif & Kramvis, 2013)
Morocco	D,A	D1, D7, A2	(Baha et al., 2012)

The other genotypes G- J have no sub-genotypes and none of them has been reported in Africa. Countries in Africa where genotyping is routine and thence information about the circulating genotypes and sub-genotypes is available include Tunisia, Gambia, Nigeria, Congo, Rwanda, Cameroon, Egypt, Central Africa, South Africa and Morocco (Table 1). Uganda and many other countries are missing on this list. The current epidemiology has been extensively studied by Ott, Stevens, Groegar, & Wiersma, (2012) as shown in fig 1.

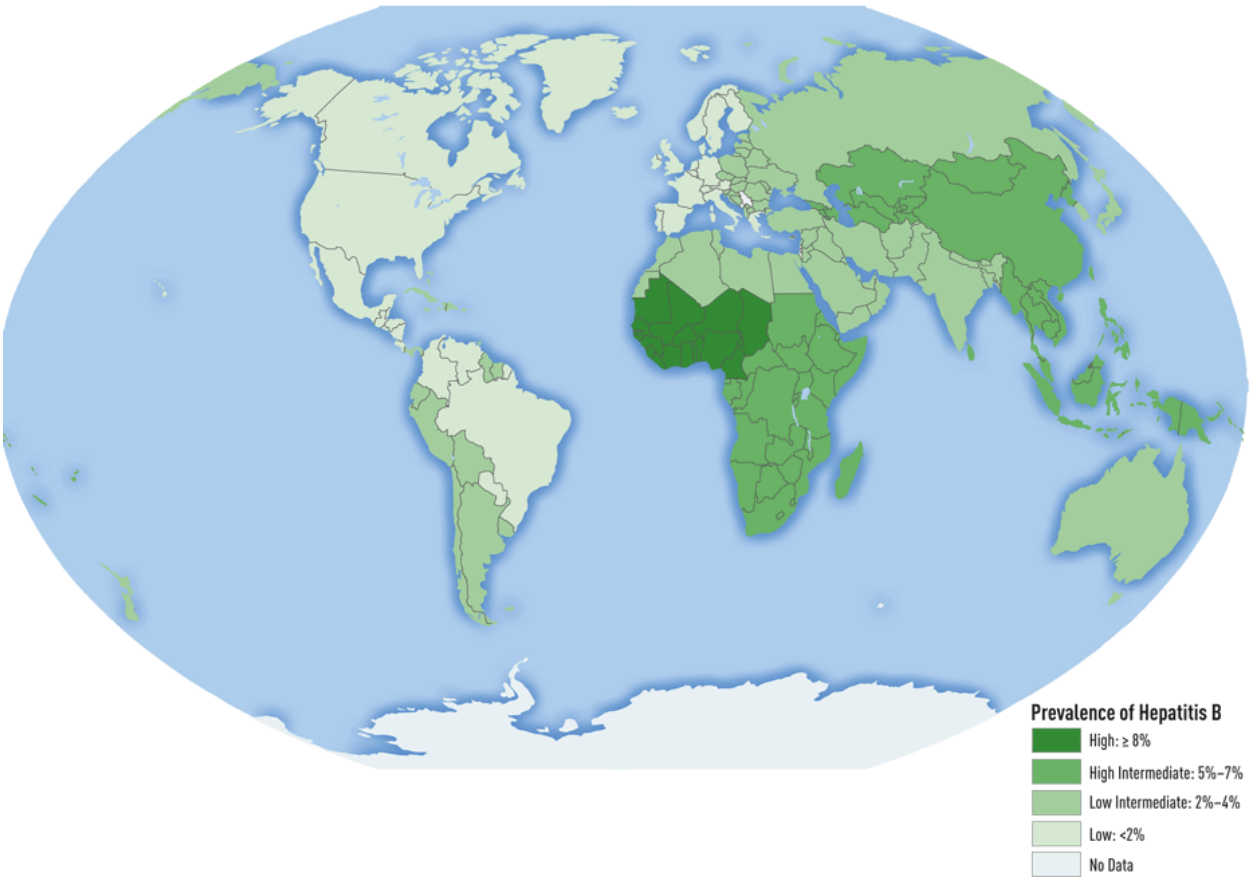

Fig. 1 : Global prevalence of chronic hepatitis B virus infection among adults

According to Tanaka et al (2004), Kurbanov, F. et al (2005), there have been increasing lines of evidence to indicate influences of HBV sub-genotypes on the outcome of liver disease and the response to antiviral therapies. From previous studies by Jia-horng Kao, Chen, Lai, & Chen (2002), hepatitis B virus genotypes have been linked to staging of the disease progression. According to their study, young patients with hepatocellular carcinoma are likely to be infected with HBV genotype B than genotype C whereas older

patients with more advanced liver disease are more likely to be infected with genotype C than genotype B. In an earlier study by Yuen et al., (2003), it was established that patients with HBV genotype B had more severe presentation of the disease and at a more risk of hepatic decompensation as compared to HBV/C infection. In a related study by Kobayashi et al., (2002) it has shown that patients with HBV/B have more serious liver disease than patients with HBV/C. The HBV genotypes have also been implicated in variations in seroconversion to hepatitis B e antigen (HBeAg) antigen. Studies by Chu, Hussain, & Lok, (2002) have shown that patients with genotype B achieve HBeAg seroconversion 10 years ealier than patients with genotype C. Variations in response to treatment are also affected by genotypes. Evidence from studies by Wai, Chu, Hussain, & Lok, (2002) have shown that patients with genotype B respond better to IFN-γ as compared to patients with genotype C.

b) The HBV S gene mutations and the paradoxical coexistence of HBsAg and anti-HBs in chronic infection with HBV

The HBV genomic structure has been exclusively reviewed by Coleman (2006), Suppiah, Mohd Zain, Bahari, Haji Nawi, & Saat (2014), Ding, Miao, Li, Dai, & Yu (2015) as a partially double stranded DNA of genomic size of approximately 3.2kb with four open-reading frames (ORF). The ORFs encode four genes including the polymerase gene designated as P gene, core (C) gene, large, medium and small surface antigen proteins (S gene) and the X protein (Coleman, 2006, Kahila Bar-Gal et al., 2012). Studies on the full genome analysis of hepatitis B genome have given a paucity of information including identification of mutations reported world over in all the four ORFs.(Quer et al., 2008).

The HBV genome S gene is paramount importance in the molecular genetics of the virus since it is concerned with the expression of the surface antigens and classification of the viral strains (Suppiah et al., 2014) as well as the antigenic variation of the virus. These genetic mutations in the S-gene enable the virus to escape the host's immune system as well other selection pressures such as antiviral drugs and vaccines. The immune escape S-gene mutations against the imposed selection pressures have been implicated in the coexistence of the HBsAg and anti-HBs antibodies (Ding et al., 2015) especially in advanced liver damage such in cases of liver carcinoma, fibrosis ,cirrhosis or chronic liver (Seo et al., 2014). Therefore mutations in the S-gene are considered as the culprits in pathogenicity and oncogenicity of viral hepatitis B, an argument consistent with the earlier findings by Svicher, Cento, & Salpin, (2011). The challenge in giving health care services to chronic HBV infections as a result of the antigenic variations of the virus is reduced sensitivity and specificity of the assays used in the diagnosis of the virus giving false negatives, failure of medication, and vaccination if the mutations are not timely identified.

c) Cytokine profile in HBV infection

Cytokine are critical molecules in progression of the liver disease as reported earlier by Akpolat, Yahsi, Godekmerdan, Demirbag, & Yalniz (2005), Frodsham et al (2006) and it common knowledge that damage to the liver as a result of HBV infection is due to immune response as reported in earlier studies by Racanelli & Rehermann (2006) and in recent studies by Wang & Zhang (2009) and is cytokine modulated but cytokine profiles in HBV virus infection need more elucidation especially in the scenario where mutations are common.

d) Rapid detection of HBV

The hepatitis B infection is a global public health concern. This is aggravated in countries where health care facilities are poor due to the shrinking resource allocation to the health care services in the national budgets (Nyan et al., 2014). This problem is worsened by the natural coincidence of the disease being endemic in these poor countries (Nyan et al., 2014). According to the world health organization (WHO), countries of Africa, Asia and South America have carrier rates as high as greater than 8% (Franco et al., 2012). This challenge is precipitated by lack of advanced clinical laboratory for routine and accurate patient testing (Franco et al., 2012) as well as the limited knowledge about the circulating genotypes and sub genotypes in the developing world (Singhatiraj et al., 2012). In many countries, HBV diagnosis is based on screening for HBV surface antigen, antibodies to the core HBV, and HBV DNA (Nyan et al., 2014). These tests are performed with enzyme-linked immunosorbent assay (ELISA) and real time polymerase chain reaction (rRT-PCR). These tests are slow and require expensive laboratory equipment such as the ELISA reader, real time PCR machine in addition to specially trained laboratory staff (Caliendo, Valsamakis, & Bremer, 2011, Kao,JH 2008). A recent study in Uganda by Mullis et al., (2013) revealed a high frequency of false-positive hepatitis C virus in Rakai. In their study, the high prevalence of false positive was due to clearance of HCV RNA but not the antibody. However this explanation is invalid since in their study, there was no single sample that was positive by both the HCV RNA Abbot real time HCV assay and ELISA assay suggesting that the positives by ELISA are most likely to be false positive. These studies provide a basis for adopting the use of alternative assays in the detection of HBV with rapidity, high sensitivity, specificity and at lower cost without the need of sophisticated laboratory equipment and trained staff in Uganda.

V. Discussions and Conclusions

Uganda is a country of high intermediate HBV prevalence (Fig.1) with a prevalence of 5-7% suggesting that the disease burden is high and needs attention. However little research has been conducted to provide information required to evidence based management of the epidemic. This prevalence may even underestimate the current prevalence in the country because it is not from a local study and was done close to four years ago (Ott et al., 2012). The previous HBV epidemiological survey in Uganda by Pido & Kagimu, (2005) among health workers put the prevalence at 8-11%. This study was conducted 10 years ago and the prevalence must be certainly different though almost consistent with a preceding report from the world health organization (WHO) which reported a prevalence of 8% (WHO, 2004), being the highest in highly endemic countries of sub-Saharan Africa. In the previous study by Watson-Williams & Kataaha, (1990), the prevalence of hepatitis B virus surface antigen in the Ugandan population was then between 6 and 15% among blood donors when screening was introduced. Fortunately, the WHO-recommended strategy for HBV control vaccine was introduced in Uganda in 2002 as part of the expanded Program on Immunization (EPI) and is given at 6, 10 and 14 weeks of age (WHO 2004, Pido & Kagimu 2005). The high prevalence then could have been due to inadequate access to the vaccine, limited awareness of the disease, and the value of vaccination against HBV. For the past 20 years massive campaign to vaccinate pregnant mothers and the new borne has been on in our country to prevent parenteral transmission to the new borne. However success of vaccination is largely dependent on matching the vaccine strains with the field strains. This is anchored on the knowledge of the circulating genotypes and sub-genotypes as well as the mutations in the S-gene which influence the HBsAg. This information is not available in Uganda. In case of the sero-positive cases, management is by use of antiviral drugs. However these are also genotype specific. The developing world is challenged with inadequate clinical research in the rapid and accurate diagnosis of HBV, a key feature in the management of the epidemic.

The concept paper has underlined the need to investigate the S-gene mutations in the circulating hepatitis B viral strains in Uganda. The S-gene encodes for the surface protein coat with has been implicated in antigenicity of the virus which in turn influences the effectiveness of antiviral therapy. It has also highlighted the need to for routine HBV genotyping in order to match vaccine strains with field strains for effective immunization programs in our country. The concept paper has underpinned the need to screen immigrants using HBV genotype specific assays in order to inform the physicians so as to adopt evidence based HBV management.

The paper has outlined the need for rapid and accurate detection of HBV which is paramount in management of the disease. The paper has quoted studies which left several questions unanswered hence leaving knowledge gaps.

Competing interests

We declare that we have no any competing interests.

Authors' contributions

Hussein Mukasa Kafeero, Kawooya Abubakar, Namusoke Mariam, Atiku Saad and Mugambwa Joseph contributed to the conception of the idea, drafting and writing of the manuscript and manuscript preparation.

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Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 2 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Prevalence and Susceptibility of *Enterobacteriaceae* Isolated from the Saliva of Students from the Northeast of Brazil

By Maria Gerusa Brito Aragão, Francisco Isaac Fernandes Gomes, Francisco Ruliglesio Rocha, Vicente Teixeira Pinto & Francisco Cesar Barroso Barbosa

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Abstract- The aim of this study was to investigate the prevalence and antimicrobial susceptibility of *Enterobacteriaceae* isolated from the saliva of high-school students from Sobral-CE, Brazil Public schools in Sobral-CE were randomly selected to participate of the investigation. Saliva samples were collected from 30 volunteers aging 15 to 19 years. The samples were inoculated into MacConkey agar, and then the microorganisms isolated were submitted to identification and antimicrobial susceptibility tests. It was found a prevalence of 23.3% of Enterobacteriaceae isolated from the saliva samples. The most common isolated microorganism was Serratia liquefaciens (31.8%), followed by *Enterobacter Cloaceae* (18.1%). Out of 55% of the samples showed resistance to amoxicillin with clavulanic acid. However, all the samples were sentitive to imipenem. The prevalence of *Enterobacteriaceae* isolated from the saliva samples was elevated, which is a concern because of the multidrug resistance character that these microorganisms presented.

Keywords: *enterobacteriaceae, oral cavity, saliva, adolescents.*

GJMR-C Classification : *NLMC Code: QW 190*

Strictly as per the compliance and regulations of:

Prevalence and Susceptibility of *Enterobacteriaceae* Isolated from the Saliva of Students from the Northeast of Brazil

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I. Introduction

The microflora of the mouth is different from that present in other sites of the body because it owns exclusive biologic and physical properties(Marsh & Percival 2006) Despite the diversity that the oral flora has, the majority of the microorganisms from elsewhere in the body are not able to colonise the oral cavity (Poeta et al. 2009).The homeostasis of the mouth depends on the harmonic relationship existent among the extensive range of microorganisms present in the oral environment. This balanced interaction can be affected by several factors such as alimentation habits, systemic diseases, debilitated conditions, immunological suppression, or the prolonged use of drugs (Reddy et al. 2013).

When an imbalance occurs in the oral environment, some opportunistic microorganisms can colonise the oral cavity. *Enterobacteriaceae* is an example of a family that includes some pathogenic bacteria that may colonise the oral cavity under uncommon physiological conditions. Escherichia, Klebisiela and Serratia are some of the representative microorganisms of this group, and they can cause diseases such as meningitis, dysentery, and food poisoning (Hejazi & Falkiner 1997),(Paju et al. 2003), (Bremer et al. 2005)

These pathogens are able to invade either the oral cavity of those patients systemically compromised and of those undergoing cytotoxic drugs or a broad-spectrum antibiotic therapy (Hejazi & Falkiner 1997). Nonetheless, investigations have been demonstrating that species of *Enterobacteriaceae* can be isolated from the oral cavity of subjects systemically healthy(Slots et al. 1990), (Ali et al. 1994), (Barbosa et al. 2001). Whenentericbacilli are isolated from the oral cavityorfluids, thisoccurrence may also be correlated to deficient sanitary conditions, to the consumption of contaminated water or food, or to an inadequate personal hygiene (Ali et al. 1996)

Since the mentioned microorganisms are involved in severe infections, it is relevant to highlight their presence in the oral cavity as concern when considering the potential that these bacteria have to cause disease (Hejazi & Falkiner 1997), (Paju et al. 2003), (Bremer et al. 2005). Moreover, infections caused by these microorganisms can be difficult to treat due to the resistance that they show to a variety of antibiotics, including β-lactams, amoxicillin with clavulanic acid, cephalosporin, aminoglycosides, carbapenems, chloramphenicol, aztreonam, trimethoprim/ sulfamethoxazole and tetracycline(Karlowsky et al. 2002), (Lee et al. 2005), (Okimoto et al. 2005), (Park et al. 2005).

The characterization of the prevalence of Enterobacteriaceae in the oral cavity is extremely important, since the mouth can serve as a reservoir for opportunistic pathogens that may cause severe systemic infections. Therefore, the aim of this study is to investigate the prevalence and antimicrobial

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susceptibility of Enterobacteriaceae isolated from saliva of high-school students from Sobral-CE, Brazil.

II. Materials and Method

This study was a cross-sectional microbiology investigation, conducted on saliva samples of 30 adolescents aged 15 to 19 years old. Public schools from Sobral, a city in the Northeast of Brazil, were randomly selected to participate of the research. Those subjects presenting any medical illness, undergoing antibiotic therapy, or who were pregnant were excluded from the investigation. In regards of the ethical aspects, the Ethics Committee of the Universidade Estadual Vale do Acaraú approved this investigation under the protocol number FR-186159. Additionally, the volunteers were informed about the aims of the research, and signed a consentient term for the collection of the material to be analysed.

To collect the saliva, it was given to each volunteer a sterile bottle containing 10 ml of a phosphate-buffered sterile saline (PBS; 0.1 M, pH 7.2). The subjects were instructed to perform a mouth rinsing for 60 seconds with the saline solution, and then they were asked to expectorate the rinsing into a labelled sterile bottle. The samples were transported to the laboratory within no more than 24 hours. To isolate the microorganism, an aliquot of 0.1 ml of the sample was inoculated on MacConkey agar medium culture using a sterile Drigalski handle. Then, the plates were incubated at 37 °C for 24 hours.

After 24 hours, the samples were removed from the incubator, and the bacterial growth was examined. The lactose fermenting colonies were identified as those that appeared pink in colour, and the non-lactose fermenting as those in a pale red colour. The colonies were submitted to the Gram's stain, examined under microscope for gram-negative bacilli, and tested to the activity of the cytochrome oxidase (Newprov, Pinhais, Brazil). The samples that were oxidase negative and gran-negative were classified as Enterobacateriaceae. The final sample identification of the bacteria was performed using the NEWPROV (Brazil) kit for Enterobacteriaceae, as well as with the BBL CRYSTAL ENTERIC/NONFERMENTER system (Becton Dickinson Micronbiology Sistems, Cockeysville MD, USA).

The susceptibility to antimicrobial agents was tested through the disk diffusion method(Bauer et al. 1966)and according to the recommendations of the Clinical and Laboratorial Standards Institute (CLSI 2015). The concentrations of bacterial cultures grown overnight in BHI broth were adjusted to a standard density of 0.5 McFarland (10^8CFU/mL) and seeded with a swab on petri plates containing Mueller-Hinton agar (Acumedia Manufacturers, Inc., Baltimore, MD, USA). Antibiotic discs (Sensifar-Cefar, Sao Paulo, Brazil) were distributed over the surface of the inoculated agar, and the diameters of the inhibition zones were measured after 18 hours of aerobic incubation at 37 ° C. The antimicrobials used for the susceptibility test included: amoxicillin (10mg), amoxicillin/ clavulanic acid (20 / 10mg), doxycycline (30µg), tetracycline (30µg), tobramycin (10mg), imipenem (10mg), cefotaxime (30µg) and ciprofloxacin (5µg). The isolated samples were classified as sensitive, intermediate or resistant according to the rules of CLSI. *Serratia marcescens* CDC 4112 was standardized as control.

III. Results

In this research, out of 33.3% of the subjects were men, while women accounted for 66.6% and were more numerous than men in all age groups, except among the volunteers aged 18, who were all male (Table 1). Seven of the 30 samples analysed were infected (23.3%) with Enterobacteriaceae. Enteric bacilli were cultured from 3 (30%) out of 10 men, while 4 (20%) out of 20 women were infected. (Table 2).

Thus, Entereobacteriaceae were prevalent in 6 (85%) out the 7 contaminated samples. The same volunteer could be contaminated with more than one type of bacterium. Serratia liquefaciens and *Entero-bactercloacae* were the most commonly isolated microorganisms, corresponding to 31.8 and 18.1%, respectively (Table 3).

The antimicrobial susceptibility tests showed that al the isolates of Serratia liquefaciens, the most prevalent microorganism, were susceptible to tobramycin and ciprofloxacin, while 4 out of the 7 (57%) isolates of this bacterium demonstrated an intermediate susceptibility to imipenem and ciprofloxacin. *Entero-bactercloacea* exhibited resistance to cefotaxime, tetracycline, and amoxicillin associated with clavulanic acid. Overall, the most effective antibiotic against the isolated microorganisms was tobramycin, since 80% of the samples demonstrated susceptibility to this drug. Moreover, 90% of the *Enterobacteriaceae* isolated in this investigation demonstrated an intermediary level of resistance to ciprofloxacin, and 65% to imipenem. More than half of the microorganisms evaluated were resistant to amoxicillin associated with clavulanic acid. The least effective antibiotic was ciprofloxacin, since only 5% of the samples demonstrated susceptibility to this drug. (Chart 01).

IV. Discussion

Although studies of the prevalence of Enterobacteriaceae in the oral cavity are not usual, investigations of this type are of extreme importance because of the multi-resistant character that these bacteria show to a wide range of antibiotics. Furthermore, the mouth can be a reservoir for these pathogens, and as soon as the organism of the carrier faces an imbalance, these bacteria can act as

opportunists and intensify existent illness. Hosting enterobacteria in the oral cavity is a predisposing and aggravating factor for many oral and systemic diseases, as well as it can be a way from which these pathogens can be spread to the environment.

In this study, the prevalence of isolation of Enterobacteriaceae from saliva of healthy subjects was 23.3%, which was higher than the 18.7% found in similar a study with workers of a hospital in Sao Paulo-Brazil (Leão-Vasconcelos et al. 2015).The prevalence obtained in this study was three times higher than that found by Barbosa et al. (2001) in the biofilm of individuals with periodontitis and more than twice of that found by Sedgley et al. (1996), who analysed samples of mouth rinses of monks in Hong Kong. Our findings were almost two times higher than the results presented by Sedgley et al. (1997), who analysed the prevalence of enteric bacilli in the oral cavity of Chinese children. As long as *Enterobacteriaceae* is not an indigenous microorganism of the oral flora, its isolation from saliva can be related to aging or poor hygiene habits. Moreover, most of the studies present in the current literature associate the occurrence and prevalence of this type of microorganism with immunocompromised patients, and our investigations highlights the fact that these pathogenic and multi-resistant bacteria might be present in the saliva even before the diagnose of some serious illness, which is a risk factor for complications during the treatment.

Among the isolated bacteria, Serratia liquefaciens represented 31,8% of the microorganisms isolated, while *Enterobater cloacae* was 18,1%. Some studies have been relating the oral carriage of these tow microorganisms to systemic diseases. Back-Brito *et al.* (2011) observed a significantly higher number such pathogens in the oral cavities of HIV positive patients compared to HIV negative patients(Back-Brito et al. 2011).

In regards of the antimicrobial susceptibility, among the microorganisms studied 90% of them were susceptible to tobramycin, which was different from the results obtained by Barbosa et al. (2001) who observed that ciprofloxacin, the antibiotic with the least effectiveness in this investigation, was the most effective in inhibiting the growth of such organisms In our study. This study and other published studies showed that the initial choice of treatment of antibiotic resistant Gram-negative infections is carbapenems (English & Gaur 2010). However, there has been the emergence of resistance to carbapenems (Gupta et al. 2006), which was also observed in the present study.

V. Conclusion

The occurrence of *Enterobacteriaceae* in the saliva of healthy individuals must be seen as a sign of alert, since the mouth can serve as reservoir, and those who carry these pathogenic and multidrug resistant microorganisms in the mouth might spread them in the community.

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Tables

Table 1 : Subject Distribution per Age and Sex

Age (years)	Men N*	%	Women N	%	Total N	%
5	3	10	7	23,3	10	33,3
16	2	6,6	3	10	5	16,6
17	3	10	9	30	12	40
18	2	0	0	0	2	6,6
19	0	0	1	3,3	1	3,3
Total	**10**	**33.3**	**20**	**66.6**	**30**	**100**

*N = Number of subjects % = Percentage of the total of subjects

Table 2 : Distribution of Bacterium Species Isolated from 10 Men and 20 Women

Microorganism	Men N *	%	Women N	%
Enterobacteriaceae	3	30	3	15%
No microorganism	7	70	16	80
Other Gram-negative microorganism	0	0	1	5
Total of subjects of both genders	**10**	**100%**	**20**	**100%**

*N = Number of subjects % = Percentage of subjects infected according to the sex

Table 3 : Microorganism isolated from saliva of 30 adolescents

Specie	Number of isolated
Serratia liquefaciens	7 (35%)
Enterobacter cloacae	4 (20%)
E Scherichia coli	3 (15%)
Enterobacter aerogenes	2 (10%)
EEnterobacter gergoviae	1 (5%)
Serratia marcescens	1 (5%)
C Citrobacter freundii	1 (5%)
Proteus mirabilis	1 (5%)
Total	20 (100%)

Charts

Chart 1 : Antimicrobial Susceptibility of *Enterobacteriaceae* Isolated from the Saliva of High School Students

( ) = Number of strains isolated S = Sensitive; I = Intermediate sensitivity; R = Resistant

The values of sensitivity (μg/ml) were interpreted according to the CLSI (2015) standards.

AMC(Amoxicillin + Clavulanic Acid): S = ≥18; I = 14-17; R = ≤13; IPM (Imipenem): S = ≥23; I = 20-22; R = ≤19 CTX

Microorganism	AMC	IPM	CTX	TOB	DOX	TET	CIP
Serratia liquefaciens (7)	S(4) I(1) R(2)	S(1) I(4) R(2)	S(1) I(4) R(2)	S(7)	S(5) I(1) R(1)	S(4) I(2) R(1)	I(7)
Enterobacter cloacae (4)	S(1) R(3)	I(4)	R(4)	S(3) R(1)	S(1) I(1) R(2)	I(1) R(3)	I (3) R(1)
Escherichia coli (3)	S(2) R(1)	S(1) I(1) R(1)	S(2) I(1)	I(2) R(1)	S(1) R(2)	S(1) I(1) R(1)	I(3)
Enterobacter gergoviae (2)	S(1) R(1)	I(2)	I(1) RI(1)	S(2)	I(1) R(1)	S(1) I(1)	I(2)
Enterobacter aerogenes (1)	R(1)	I(1)	I(1)	S(1)	S(1)	S(1)	I(1)
Serratia marcescens (1)	R(1)	S(1)	S(1)	S(1)	I(1)	I(1)	I(1)
Citrobacter freundii (1)	R(1)	S(1)	S(1)	S(1)	R(1)	R(1)	S(1)
Proteus mirabilis (1)	R(1)	I(1)	I(1)	S(1)	I(1)	S(1)	I(1)
Total (20)	S(8)=40% I(1)= 5% R(11)= 55%	S(4)=20 % I I (13)=65% R(3)= 15%	S(5)=25 % I(8)=40% R(7)= 35%	S(16)=80 % I(2)=10% R(2)= 10%	S(8)=40 % I(5)=25% R(7)= 35%	S(7)=35 % I(6)=30% R(7)= 35%	S(1)=5 % I(18)=90% R(0)= 0%

(Cefotaxime): S = ≥26; I = 23-25; R = ≤22; TOB (Tobramycin): S= ≥15; I = 13-14; R = ≤12; DOX (Doxycycline): S = ≥14; I = 11-13; R = ≤10; TET (Tetracycline): S = ≥15; I = 12-14; R = ≤11 (Ciprofloxacin): S = ≥31; I = 21-30; R = ≤20.

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Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 2 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Microbiological, Physical and Chemical Quality of Swimming Water with Emphasize Bacteriological Quality

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Abstract- Recreational use of inland and marine waters is increasing in many countries. It is estimated that foreign and local tourists together spend many days annually at coastal recreational resorts. Swimming is considered to be a healthy leisure activity for both the young and old. Swimming is even advised as the most appropriate sport for asthmatic children, mainly on the grounds that inhaling moist air is less conducive to triggering exercise-induced asthma. Swimming pools may be supplied with fresh, marine or thermal water. Swimming pools may be located indoors, outdoors or both; they may be heated or unheated. Swimming pools can be categorized as public, semi-public, and residential pools.

A quality of swimming water takes into account physical, chemical and microbiological quality information and shall be maintained these water quality standards at all times. Pool water clarity must be maintained in a clean, clear condition so that a 150 mm diameter matt contrasts with the color of the bottom of the swimming pool, is clearly visible when viewed through the pool water at the deepest part of the swimming pool. There must have a minimum chemical criterion by which a swimming pool should be operated to minimize health risks to bathers to acceptable levels. The microbiological quality of water must not present risk to the health of bathers.

GJMR-C Classification : NLMC Code: QY 100

MICROBIOLOGICALPHYSICALANDCHEMICALQUALITYOFSWIMMINGWATERWITHEMPHASIZEBACTERIOLOGICALQUALITY

Strictly as per the compliance and regulations of:

Microbiological, Physical and Chemical Quality of Swimming Water with Emphasize Bacteriological Quality

Tebelay Dilnessa [α] & Gebreselassie Demeke [σ]

Abstract- Recreational use of inland and marine waters is increasing in many countries. It is estimated that foreign and local tourists together spend many days annually at coastal recreational resorts. Swimming is considered to be a healthy leisure activity for both the young and old. Swimming is even advised as the most appropriate sport for asthmatic children, mainly on the grounds that inhaling moist air is less conducive to triggering exercise-induced asthma. Swimming pools may be supplied with fresh, marine or thermal water. Swimming pools may be located indoors, outdoors or both; they may be heated or unheated. Swimming pools can be categorized as public, semi-public, and residential pools.

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A variety of microorganisms can be found in swimming pools, which may be introduced in the pool water in a number of ways. In many cases, the risk of illness or infection has been linked to fecal contamination of the water, due to feces released by bathers or contaminated source water or, in outdoor pools, may be the result of direct animal contamination. If swimming pool is not managed properly, bathers acquire bacterial, viral, parasitological and fungal infection. To minimize/avoid these risks of infection a variety of disinfection techniques are used. Disinfection methods include filtration to remove pollutants, disinfection to kill infectious microorganisms, swimmer hygiene to minimize the introduction of contaminants into pool water, and regular testing of pool water, including chlorine and pH levels. Re-circulating pool water is disinfected during the treatment process, and the entire water body is disinfected by application of a disinfectant residual, which inactivates agents added to the pool by bathers.

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I. Introduction

Recreational use of inland and marine water is increasing in many countries. It is estimated that foreign and local tourists together spend around two billion days annually at coastal recreational resorts. The World Tourism Organization predicts that by 2026, 346 million tourists will visit Mediterranean destinations annually, representing about 22% of all arrivals worldwide. It has been estimated that 129 million people visited the beach or waterside in the United States of America between 2000 and 2001, an increase of 6% from 1995. In United Kingdom it is estimated that over 20 million people use British coast each year, in addition to inland waters and their surrounding areas, for a variety of reasons [1].

Swimming is generally considered to be a healthy leisure activity for both the young and old. Swimming is even often advised as the most appropriate sport for asthmatic children, mainly on the grounds that inhaling moist air is less conducive to trigger exercise-induced asthma [2]. The growing popularity of swimming and other "in-the-water" activities for sport, fitness, therapy or just enjoyable relaxation has led to the increased use of swimming pools and the establishment of a variety of specific-use pools such as spa pools, waterslides, and more recently, hydrotherapy and wave pools. These pools are used by a variety of people of various ages, health status and standards of hygiene [3]. Swimming pools may be supplied with fresh, marine or thermal water. Swimming pools may be located indoors, outdoors or both; they may be heated or unheated [4].

A variety of microorganisms can be found in swimming pools, which may be introduced in the pool water in a number of ways. Some of the various sources of bacteria and microbes in pool include people swimming in the pool, animals, dead wildlife and debris from around the property, such as leaves, grass and dust. In many cases, the risk of illness or infection has been linked to fecal contamination of the water, due to feces released by bathers or contaminated source water or, in outdoor pools, may be the result of direct animal contamination. Non-fecal human shedding (e.g. from vomit, mucus, saliva or skin) in swimming pool is also a potential source of pathogenic organisms [5].

Swimming pool sanitation refers to methods for ensuring healthy conditions in swimming pools, hot tubs, plunge pools and similar recreational water venues. Proper sanitation is needed to maintain the visual clarity of water and to prevent the transmission of infectious diseases. Sanitation methods include filtration to remove pollutants, disinfection to kill infectious microorganisms, swimmer hygiene to minimize the introduction of contaminants into pool water, and regular testing of pool water, including chlorine and pH levels [5]. Re-circulating pool water is disinfected during the treatment process, and the entire water body is disinfected by application of a disinfectant residual, which inactivates agents added to the pool by bathers. Effective swimming pool disinfection will take account of the need to inactivate any potential pathogens with the need to provide a pleasant swimming experience without adverse long term health effects [6, 7].

II. Type and Quality Standards for Swimming Water

The growing popularity of swimming and other "in-the-water" activities for sport, fitness, therapy or just enjoyable relaxation has led to the increased use of swimming pools and the establishment of a variety of specific-use pools such as spa pools, waterslides, and more recently, hydrotherapy and wave pools. These pools are used by a variety of people of various ages, health status and standards of hygiene. Swimming pools are a common feature in most middle to upper income bracket homes, with a strong design focus on outdoor living areas [3].

Swimming pools may be supplied with fresh, marine or thermal water. Swimming pools may be located indoors, outdoors or both; they may be heated or unheated. Swimming pools can be categorized as public, semi-public, and residential pools. A public pool is usually a larger pool (more than 1800 square feet) that is owned or operated by some legal entity and is made available to anyone who pays a small entry fee. Semi-public pool is similar to the public pool but has specific entry restrictions like that of a fitness center, country club, or hotel/motel pool. Residential pools are private pools that are not regulated by the health department or some other regulatory agency. Residential pools are not intended for commercial use and are not owned by more than three families. Public pools are usually inspected by a health official, whereas residential pools are not. More specifically, the basic components of a swimming pool and its circulation system include swimming pool basin, balancing tank, pump, hair and lint strainer, filter, heater and chemical disinfectant systems [4].

Essentially a swimming or spa pool that has balanced water meets the chemical criteria and should be relatively free from pathogenic organisms. Therefore, the potential risk of disease transmission for the pool is negligible [8]. The quality of swimming water takes into account epidemiology, microbiology and water quality information. The water in swimming pools shall be maintained at the following water quality standards at all times.

a) Physical Quality

Pool water clarity must be maintained in a clean, clear condition so that a 150 mm diameter matt black disc, or a 150 mm diameter disc that contrasts with the color of the bottom of the swimming pool, is clearly visible when viewed through the pool water at the deepest part of the swimming pool. The pool water shall be clear and clean. No scum or floating impurities shall be allowed to accumulate. The color of the water shall not exceed 5 Hazen units and the turbidity shall not exceed 5 NTU (nephelometric turbidity unit) [9].

b) Bacteriological Quality

The microbiological quality of water must not present risk to the health of bathers. When water samples are taken directly by pool operators, a copy of all results should be forwarded directly to the local health authority. Poor microbiological testing results will often mean immediate corrective action is needed as required by the health authority. The local health authority will assist in deciding on a routine sampling schedule sampling regime, and may require this as part of the conditions on an operating permit. Routine testing of water for bacteriological quality can provide evidence of the effectiveness of disinfection systems and sanitation schedules [9].

a. *E. coli* shall not be present in any 100 ml sample of water taken from the pool.
b. Not more than 10 coliform organisms shall be present in any 100 ml of water taken.
c. Not more than one out of five consecutive samples of the water, taken monthly, shall contain any coliform organisms in 100 ml of the water sample.
d. No sample shall contain more than 200 bacteria per ml as determined by the 24-hour plate count at 37 ° C or by the membrane filter method.

c) Chemical Quality

These specify the minimum chemical criteria by which a swimming pool should be operated to minimize the public health risks to bathers to acceptable levels. It is important for people responsible for pool operation to maintain their pools at a standard equal to or greater than these guidelines at all times the pool is open to the public. The level of one chemical parameter can adversely affect another that is if the pH is too high or too low the disinfectant properties of chlorine are decreased [8].

- A free chlorine residual of not less than 1.0 mg per liter and not more than 3.0 mg per liter shall be maintained in the pool.

- If copper sulfate is used as an algicidal agent, copper sulfate concentration of the water determined as copper shall not exceed 0.2 mg per liter.
- The pool water shall have a pH value of between 7.2 and 7.8.
- If cyanuric acid is used as a stabilizer for chlorine, its maximum concentration shall not exceed 100 mg/liter in the swimming pool water [10].

The first reviews of the incidence of disease associated with the use of recreational waters were undertaken by the American Public Health Association in the early 1920s. They investigated the link between bathing and illness. The findings concluded that there was an appreciably higher overall illness incidence rate in people who swam in Lake Michigan, the United States, in 1948 and on Ohio River at Dayton, United States, in 1949 compared with non-swimmers, regardless of the levels of coliform bacteria found in the water quality tests. It was concluded that, based upon the results of this study, the stricter bacterial quality requirements could be relaxed without a detrimental effect on the health of bathers. A study based on five years of investigation of 43 beaches in the United Kingdom and concluded that there was only a 'negligible risk to health' of bathing in sewage polluted sea water even when beaches were 'aesthetically very unsatisfactory' and that a serious risk would only exist if the water was so fouled as to be revolting to the senses. They insisted that pathogenic bacteria which were isolated from sewage contaminated sea water were more important as indicators of the disease in the population than as evidence of a health risk in the waters [1].

A study in Iran showed that the mean of the physicochemical parameters, except in temperature, was standard in more than 60 % of the pools. Average temperature was higher than standard. The highest chlorine level was recorded in summer. Coliform bacteria were found to be positive in 3 % of the samples. Prevalence of saprophytic and opportunistic fungi was 27 %. Twelve species of fungi were isolated; the most common were *Aspergillus, Penicillium sp*, *Rhizopus sp*, and *Fusarium sp*, and the highest fungi pollution was observed in the summer. Prevalence of bacterial contamination was 9 %; bacteria isolate included *Staphylococcus epidermidis*, *Bacillus subtilis* and *Escherichia coli*. There was a significant association found between fungal and bacterial contamination with residual chlorine [11].

Quantitative microbial risk assessment (QMRA) is used to estimate the probability of becoming infected by a specific pathogen after an exposure. QMRA uses densities of particular pathogens, assumed rates of ingestion, and appropriate dose-response models for the exposed population to estimate the level of risk. QMRA can be useful in determining the risk of infection from the use of recreational water. QMRA and epidemiological studies provide complimentary information and should be used together to provide better overall estimates of risk. The process of QMRA produces a statistical estimate of adverse effects associated with exposure to particular hazards. The process consists of hazard identification, exposure assessment, dose-response assessment and risk characterization. One of the main problems with risk assessment is that a number of assumptions need to be made with respect to exposures. Assumptions need to be validated through research under similar conditions to those being modeled. Slight changes in for example, pathogen concentration or die-off may lead to widely varying results [1].

Water contact time is a prime factor influencing the amount of exposure to pathogens in water. The longer a person is in the water the more they can be exposed to pathogens in the water through ingestion, inhalation or penetration of the skin. Some activities are likely to pose greater risk of water ingestion than others. In most cases, monitoring for potential microbial hazards is done using indicator microorganisms which are easy to enumerate and would be expected to be present in greater numbers than pathogens. The traditional role of indicator parameters was to show the presence or absence of fecal pollution in water supplies. The indicator should be absent in unpolluted environments and present when the source of pathogenic microorganisms of concern is present [1].

- The indicator should not multiply in the environment
- The indicator should be present in greater numbers than the pathogenic microorganisms.
- The indicator should respond to natural environmental conditions and water treatment processes
- The indicator should be easy to isolate, identify and enumerate
- Indicator tests should be inexpensive, thereby permitting numerous samples to be taken

d) Microorganisms in swimming water

Microorganisms that are used to assess the microbial quality of swimming pool and similar environments include heterotrophic plate count (HPC) (a general measure of non-specific microbial levels), faecal indicators (such as thermotolerant coliforms, *E. coli*), *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Legionella* species. HPC, thermotolerant coliforms and *E. coli* are indicators in the strict sense of definition. As health risks in pools and similar environments may be faecal or non-faecal in origin, both faecal indicators and non-faecally-derived microorganisms (e.g. *P. aeruginosa*, *S. aureus* and *Legionella* spps) should be examined. Faecal indicators are used to monitor for the possible presence of fecal contamination; HPC,

Pseudomonas aeruginosa and *Legionella* species can be used to examine growth, and *Staphylococcus aureus* can be used to determine non-fecal shedding. The absence of these organisms, however, does not guarantee safety, as some pathogens are more resistant to treatment than the indicators, and there is no perfect indicator organism [12].

Campylobacter jejuni is more likely to be found in recreational waters contaminated by animal and human waste. *Escherichia coli* O157 associated with food, a number of outbreaks have been reported from recreational use of waters, particularly in pools that are not adequately chlorinated. *Helicobacter pylori* have been implicated as one mode of transmission through swimming pools. *Legionella* species are a number of reports of Legionnaires' disease associated with the use of, and proximity to, hot tubs in particular. *Mycobacterium avium* complex is clear evidence for the association of *Mycobacterium avium* complex with recreational waters [1].

Shigella species exist with the association of recreational use of water and self-limiting infection with *Shigella* bacteria. The species responsible for the more severe illness, *Shigella dysenteriae,* is more common in tropical regions but no cases associated with recreational waters were found in the literature. The infective dose for *Shigella* species is usually between 10 and 100 organisms. *Vibrio vulnificus* bacterium commonly occurs in marine and estuarine environments. Evidence exists for the association of recreational use of water and infection with *V. vulnificus* where the user has a pre-existing open wound [12]. *Pseudomonas aeruginosa* is an organism that usually causes disease in immunecompromised individuals. These bacteria can live in many places and are common inhabitants of soil, water, plants, and animals in addition to swimming pools, whirlpools, and hot tubs [13, 14].

Cryptosporidium parvum fecal accidents are implicated in most of the cases as the cause of the outbreaks of cryptosporidiosis, which have primarily occurred in swimming pools. The risk of death and probability of developing long-term sequelae from this infection can be prolonged and moderately severe especially in immunocompromised persons. *Giardia lambilia* is a proven risk factor for infection in immunocompromised patients. *Naegeria fowleri* has been shown to colonize warm freshwater habitats, such as swimming pools and natural hot springs and there is a high risk of death in infected persons. *Schistosoma* species cause serious pathology associated with infection by *Schistosoma mansoni* occurs and can lead to long-term health issues. Schistosoma is only a potential hazard in certain geographic areas. Evidence shows that exposure to schistosomes is difficult to avoid but it has been shown that towel-drying after exposure to infested water can markedly reduce the risk of infection [12, 14].

Viral diseases transmission in recreational waters, primarily inadequately chlorinated swimming pools, has been documented via faecally-contaminated water and through droplets. Adenovirus and Hepatitis A virus has been isolated from surface waters which may be used for recreational purposes and a number of cases of HAV have been documented associated with recreational water users. Hepatitis E virus has been isolated from surface waters which may be used for recreational purposes [1].

e) Disinfection of swimming water

The importance of available chlorine and redox potential as indicators of the kill of *Escherichia coli* has previously been studied in laboratory experiments. Different chlorine compounds are tested in chlorine demand free water. The reduction of bacteria within 3 minutes is relatively well correlated with both available chlorine and redox potential for each pure chlorine compound, but the available chlorine needed for total kill is about 10 times higher for inorganic chloramines than for free available chlorine. The five keys to maintaining water quality in swimming pool include filtration, chlorination, pH level, total alkalinity and calcium hardness [15].

f) Filtration

The water in pool is pumped through a filter to remove debris and particles. How long you need to run the filter depends on the size of the swimming pool and the horsepower of the pool pump. Remember that even when you are filtering your pool according to specifications, about 35 percent of the water still won't be filtered. An electrically operated water pump is the prime motivator in re-circulating the water from the pool. Water is forced through a filter and then returned to the pool. Using the filtration method requires a constant electrical supply, with the typical pool pump using 500 watts to 2,000 watts. Coated mesh filters and sand filters are two basic types [16].

Coated mesh filters can be broken into two types - diatomaceous earth and cartridge filters. Diatomaceous earth is obtained from mining the skeletons of diatoms, minute creatures that lived millions of years ago. Diatomaceous Earth filters consist of a set of pads or filter elements that are coated with diatomaceous earth before use. After the filter becomes dirty the precoat, with the sediment, is backwashed to the drain. Cartridge filters come in various sizes to suit particular volumes of water. A cartridge filter usually consists of a container, which should include an automatic pressure bypass valve, and a manual release valve, in which a replaceable cartridge of porous material such as polyester or paper is fitted and sealed. Sand filters design is to secure maximum reduction in suspended and colloidal matter, long runs between backwashes, effective cleaning during the backwash cycle itself and a long life of the filter medium itself. This

is achieved through careful selection of the sand, design of the washing equipment and under drainage system. Sand filters are generally available in three separate types the open gravity filter, pressure filter and hi-rate sand filter. Water is diffused, softening the water flow, in the filter over the top of the sand bed and through into the under drain in the bottom of the filter tank [17].

g) Chlorination

Chlorine is a chemical that disinfects the water and helps to remove debris. You should use a chlorine stabilizer to extend the chlorine's half-life. Generally, the longer the filtration cycle, the less chlorine you will need. Similarly, the more chlorine you use, the shorter the required filtration cycle. Chlorine requirements will be affected by a range of factors, including the pump and filter system, water temperature, water level, amount of debris and the number of swimmers in the pool [6]. Chloramination employs a mixture of ammonia and chlorine, the reaction products of both compounds are still active for killing microorganisms, but they are much less available for formation of disinfection by-products [16].

h) Free chlorine

A pool operated at a low free chlorine level is going to be a much healthier, non smelly pool for chloramine production is also minimized, and so too is the amount of chemicals used. Bathers want good clean water to swim in with no risk of cross-infection. The free chlorine residual should be at the lowest concentration that gives satisfactory microbiological quality; this should be possible at less than 1mg/l. The absolute minimum free chlorine level given that there needs to be a residual of active disinfectant to prevent the risk of cross infection is 0.5 mg/l. When operating at low free chlorine levels the pH value of the water must be around 7.2 to 7.4 to obtain the greatest disinfection effect [16].

- Ideally free chlorine should be no more than 1.5 – 2.0mg/l
- Free chlorine residuals above 2mg/l should not be used
- Free chlorine residuals above 3mg/l are unlikely to be necessary
- Above 5mg/l free chlorine, chlorination should be stopped immediately
- Above 10mg/l bathing should cease

i) Combined chlorine

The reactions between hypochlorite and nitrogenous matter or ammonia in the pool are complex and it is dangerous to over-simplify the meaning of test results. The combined chlorine residual should be as low as possible. It should always be at least half the free chlorine residual and never more than 1mg/l, where the pool water treatment is operating well.

j) PH level

For hypochlorite disinfectants to work properly, the pH value of the pool is critical. The pH level indicates how acidic or alkaline the water is at any given time. The pH value should be maintained between the acceptable range of 7.2 and 7.8; the bottom of the range should be the target, as disinfection will be more effective as may be coagulation. This is particularly important when operating at low levels of free chlorine. If, however, there is a "chlorine" smell or irritation, the pH value may have to be raised towards the upper part of the range and any bathing overload corrected. If the water pH is higher than 8, anyone who swims in the pool is at risk of skin rashes, while a pH of lower than 7 can sting the swimmers' eyes. Some of the many factors that can affect your pool's pH level include heavy rain, lots of swimmers and pool chemicals [16].

k) Total alkalinity (TA)

Total alkalinity means the sum of all alkaline chemicals in water. Total alkalinity is the measurement of the ability of pool water to resist changes in pH. Total alkalinity is the governor of pH. If TA is too low, the pH balance can become unstable. Concrete and painted pool surfaces will also deteriorate over time. TA and pH are interconnected. For example, raising the TA could also raise the pH. Make sure you don't disrupt your pool's pH when adjusting the TA and vice versa. The recommended range in public pools is 80 -120 ppm.

l) Calcium hardness

Calcium hardness refers to the amount of the mineral calcium dissolved in pool water. Low calcium levels will deteriorate pool surfaces, while high calcium levels will leave a 'scum' or scale on surfaces and equipment. The recommended range in public pools: 200 -300 ppm.

m) Bromine

Bromine has always been considered a disinfectant with similar properties to chlorine but in the context of swimming pool water treatment it is superior. In chlorine treated water, by-products are often formed which cause eye irritation and sometimes offensive odors. In bromine treated pools, although combined bromines are formed, the bromamines, eye irritation is virtually totally absent as these, unlike the chloramines, are good disinfectants with their activity almost being equal to free chlorine or free bromine. The use of elemental bromine however is very uncommon as it is a heavy red liquid which is very corrosive and gives off pungent fumes [17].

n) Ozone

Ozone is the most rapid disinfectant and most powerful oxidizing agent available for water treatment. It is a highly active gas which reacts immediately on contact with bacteria or other contaminants and impurities found in swimming pool water. Ozone is not a

stable gas and it reverts quickly back to oxygen. This is why it has to be generated on-site and immediately introduced into the circulating pool water. The most efficient commercial production method is to pass dried air through a corona discharge ionizing field. Ozone's powerful oxidizing properties prevent the buildup of undesirable and odor producing by-products of the chlorination of human-based organic pollution [17].

o) UV Disinfection

UV is now a well-established method of swimming pool water treatment, from hydrotherapy spas to full-sized competition pools. This growth in popularity has been largely due to UV's reliability and ease of use. Another major factor is the reduced reliance on traditional chemical treatments it affords, particularly chlorine. UV is also highly effective at destroying chlorine-resistant microorganisms like *Cryptosporidium parvum* and *Giardia lambilia*. UV disinfection has many advantages over alternative methods. Unlike chemical treatment, UV does not introduce toxins or residues into process water and does not alter the chemical composition, taste, odor or pH of the fluid being disinfected. Another major benefit of UV is that it significantly reduces the need for backwashing and dilution, saving hundreds of pounds a month for pool operators [18].

p) Swimming water in Ethiopian

In Ethiopia, swimming water is rarely used, but there are some swimming pools in the country including both indoor and outdoor pools. Among the well known swimming pools Sheraton Addis, Ghion Hotel, Hilton Hotel, Intercontinental Addis Hotel, Elelly Hotel and Emibilta Hotel can be mentioned. Technical workers use different chemicals (HCl, Cl, $CuSO_4$, H_2SO_4, etc) to remove/inhibit microorganisms and as the same time the pools are supported by continuous pumping and filtration system [19]. Some of the managers supervising these pools do not have enough knowledge on about the physical, chemical and microbiological quality of their pools. For example, in Emibilta Hotel the technical worker disinfects the pool by simply adding un-weighted amount of HCl, H_2SO_4 i.e. without following WHO guidelines for swimming pools. From the interview I understand that they know nothing the proportion of disinfectant and volume of water in the well as well as the impact of disinfection by-products (DBP).

q) Laboratory assessment of swimming water

Poor microbiological testing results will often mean immediate corrective action is needed as required by the health authority. Routine testing of water for bacteriological quality can provide evidence of the effectiveness of disinfection systems and sanitation schedules. Pool water that is found to have poor microbiological quality could indicate there is a problem with the disinfection and recirculation system, or it could also indicate a health risk to patrons [9].

a. *E. coli* shall not be present in any 100 ml sample of water taken from the pool.
b. Not more than 10 coliform organisms shall be present in any 100 ml of water taken.
c. Not more than one out of five consecutive samples of the water, taken monthly, shall contain any coliform organisms in 100 ml of the water sample.
d. No sample shall contain more than 200 bacteria per ml as determined by the 24-hour plate count at 37 ° C or by the membrane filter method.

For microbiological procedures, water samples are collected, when no bathers are in the pools. All samples are collected into sterile dark glass bottles with capacities 250, 500, 1000 and 2000 ml. Sufficient (100 mg/l) sodium thiosulfate pentahydrate is added to each bottle for de-chlorinization. The samples are collected from a depth of 30 cm, at a point about 40 cm away from the pool edge and they were transferred to the laboratory at 4°C within 1–2 h from collection, using appropriate insulated coolers and they are processed immediately after arrival at the laboratory. Bacteriological samples are analyzed by the membrane filter technique, using 0.47mm diameter, 0.45 mm pore size filters as specified in standard methods to determine the following parameters: Total heterotrophic plate counts (THC) per 1ml at 20 °C and 36°C [5].

r) Sample collection

- Sample collection for rapid methods is the same as for traditional methods
- Environmental/aquatic samples are complex; methods may have to be optimized for specific water types
- At low indicator bacteria numbers, detection limit for many methods becomes an issue
- Collection can include filtration, size fractionation, ultra-concentration, and enrichment in order to further, select/concentrate organisms of interest
- Filtration captures unwanted compounds that can inhibit detection of target organisms

Most detection technologies are based on measuring sample volumes less than 1 ml. The recommended marine bathing water standard is 35 *Enterococci* per 100 ml, which equates to less than one cell per ml. Thus, detectors measuring only a 1 ml volume, even if they are capable of detection of one cell per ml, will necessarily produce unacceptable sensitivity and poor precision at concentrations near the standard. There are two possible approaches to overcoming inadequate sensitivity. The first is to improve detector technology to allow measurement of larger volume samples. The preferred option at the present time is pre-concentration, which can enhance sensitivity several fold by increasing the number of target organisms per unit volume at a relatively modest cost [20].

s) Analytical techniques for indicator bacteria

The analytical techniques for indicator bacteria include membrane filtration, multiple tube fermentation and detection using chromogenic substances.

t) Membrane filtration

Indicator bacteria can be cultured on media which are specifically formulated to allow the growth of the species of interest and inhibit growth of other organisms. Typically, environmental water samples are filtered through membranes with small pore sizes and then the membrane is placed onto a selective agar. It is often necessary to vary the volume of water sample filtered in order to prevent too few or too many colonies from forming on a plate. Bacterial colonies can be counted after 24–48 hours depending on the type of bacteria. Counts are reported as colony forming units per 100 mL (cfu/100 mL) [20].

u) Multiple tube fermentation

Multiple tube fermentation technique is used to determine the presence of a member of the coliform group in ground water and surface water. The coliform group, as analyzed for in this procedure, is defined as all aerobic and facultative anaerobic, gram-negative, non-spore-forming, rod shaped bacteria that ferment lactose with gas formation within 48 hr at 35°C.

Presumptive Stage: A series of lauryl tryptose broth primary fermentation tubes are inoculated with graduated quantities of the sample to be tested. The inoculated tubes are incubated at 35 $\pm$ 0.5°C for 24 $\pm$ 2 hrs, at which time the tubes are examined for gas formation. For the tubes in which no gas is formed, continue incubation and examine for gas formation at the end of 48 $\pm$ 3 hrs. Formation of gas in any amount within 48 $\pm$ 3 hrs is a positive presumptive test.

Confirmed Stage: The confirmed stage is used on all primary fermentation tubes showing gas formation during 24 hrs and 48 hrs. Fermentation tubes containing brilliant green lactose bile broth are inoculated with medium from the tubes showing a positive result in the presumptive test. Inoculation should be performed as soon as possible after gas formation occurs. The inoculated tubes are incubated for 48 $\pm$ 3 hr at 35 $\pm$ 0.5°C. Formation of gas at any time in the tube indicates a positive confirmed test.

Completed Test: The completed test is performed on all samples showing a positive result in the confirmed test. It can also be used as a quality control measure on 20% of all samples analyzed. One or more plates of eosin methylene blue are streaked with sample to be analyzed. The streaked plates are incubated for 24 $\pm$ 2 hr at 35 $\pm$ 0.5°C. After incubation, transfer one or more typical colonies (nucleated, with or without metallic sheen) to a lauryl tryptose broth fermentation tube and a nutrient agar slant. The fermentation tubes and agar slants are incubated at 35 $\pm$ 0.5°C for 24 $\pm$ 2 hrs or for 48 $\pm$ 3 hrs if gas is not produced. From the agar slants corresponding to the fermentation tubes in which gas formation occurs, gram-stained samples are examined microscopically. The formation of gas in the fermentation tube and the presence of gram-negative, non-spore-forming, rod-shaped bacteria in the agar culture may be considered a satisfactorily completed test, demonstrating the positive presence of coliform bacteria in the analyzed sample.

v) Detections using chromogenic substances

One technique for detecting indicator organisms is the use of chromogenic compounds, which are added to conventional or newly devised media used for isolation of the indicator bacteria. These chromogenic compounds are modified to change color or fluorescence by the addition of either enzymes or specific bacterial metabolites. This enables for easy detection and avoids the need for isolation of pure cultures and confirmatory tests [20, 21].

w) Intervention for swimming water

Intervention is a deliberate entry into a situation or dispute in order to influence events or prevent undesirable consequences to improve a situation that are intended to relieve illness or injury. The most important intervention strategies include [22]:

- Increase public awareness about water safety
- Increase awareness and enforcement of pool and spa safety act requirements
- Address the role of swimming lessons as a layer of protection
- Familiarize technical workers/ the community how to disinfect swimming pools
- Increase public awareness that standard cardiovascular pulmonary recitation (CPR)
- Emphasize the importance of pool barriers as a layer of protection
- Expand statistical reporting throughout state

III. Conclusion

The growing popularity of swimming water activities for sport, fitness, therapy or just enjoyable relaxation has led the increased use of swimming pools and the establishment of a variety of specific-use pools such as spa pools, waterslides, and hydrotherapy. The difficulties associated with attributing an infection to recreational water use are numerous and the majority of research in this field has focused on infections. It is also increasingly apparent that a number of micro-organisms or their products are directly or indirectly associated with secondary health outcomes or sequelae and a number of these sequelae may result from waterborne infections.

Proper sanitation is needed to maintain the visual clarity of water and to prevent the transmission of infectious diseases. Sanitation methods include filtration

to remove pollutants, disinfection to kill infectious microorganisms, swimmer hygiene to minimize the introduction of contaminants into pool water and regular testing of pool water, including chlorine and pH levels. The five keys to maintaining water quality in swimming pool include filtration, chlorination, pH level, total alkalinity and calcium hardness. The water in pool is pumped through a filter to remove debris and particles. Chlorine is a chemical that disinfects the water and helps to remove debris.

Microorganisms that are used to assess the microbial quality of swimming pool include heterotrophic plate count, faecal indicators, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Legionella* species. The development of rapid microbial indicator methods is moving quickly and they will likely become available for use allowing managers to take action toward protecting swimmers from exposure to waterborne pathogens. Different intervention strategies are used to prevent undesirable consequences, increase public awareness about water safety, increase awareness and enforcement of pool activities.

IV. Recommendations

From the article review on microbiological and quality of swimming water, thereby would like to recommend some points to laboratory professionals, the concerned bodies and other stakeholders to investigate swimming pool in our country and apply the knowledge to improve the quality of swimming pool interms of physical, chemical and microbiological perspectives. It requires attention to minimize the risk of infection acquired through pools and the Government Issue policies, manuals and guidelines for appropriate/safe use of swimming pools at all levels from personal to public level at large. The population also should aware the merit/demerits of swimming pool and follow guidelines for safe swimming as well as strictly apply precautions. Since the impact of microorganisms and harmful residues of chemicals (disinfectant by products) is so devastating for health of the community, technical workers of swimming waters should be well trained about pool disinfection mechanisms.

List of abbreviations

AFR: Accidental fecal release;
CFU: Colony-forming unit;
CPR: Cardiovascular pulmonary recitation;
DBAA: Dibromoacetic acid;
DBP: Disinfection by-products;
DCAA: Dichloroacetic acid;
DMH: Dimethylhydantoin;
HPC: Heterotrophic plate count;
HUS: Haemolytic uraemic syndrome;
ISO: International Organization for Standardization;
MCAA: Monochloroacetic acid;
NOEL: No-observed-effect level;
NTU: Nephelometric turbidity unit;
ORP: Oxidation–reduction potential;
PFU: Plaque-forming unit;
Ppm: Parts per million;
QMRA: Quantitative microbiological risk assessment;
TCAA: Trichloroacetic acid;
TCAN: Trichloroacetonitrile;
TDS: Total dissolved solids;
UV: Ultraviolet

Conflict of interest

The authors declare that they have no competing interests.

V. Acknowledgments

The authors would like to acknowledge Debre Markos University for internet access.

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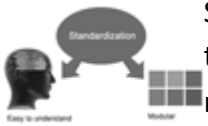

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"

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3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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ISSN 9755896