Editorial

We are in the midst of a constitutional crisis in Sri Lanka at present and it is very hard for me to write an Editorial for the Journal. However, as reminded by Jawaharlal Nehru

‘Most of us seldom take the trouble to think. It is a troublesome and fatiguing process and often leads to uncomfortable conclusions. But crises and deadlocks when they occur have at least this advantage, that they force us to think’.
The Unity of India: Collected Writings, 1937-1940 (1942), p. 94

This is true in all of life and is reflected in many of the contributions to this issue of SLJID. Ill health due to infectious diseases continue to cause personal as well as societal crises and we are forced to think of new and creative ways of diagnosing, treating as well as preventing them.

Several of the papers in this issue remind us of the continuing problems caused by parasitic infections in Sri Lanka. Though there has been much work on soil transmitted parasites as discussed in the invited review, these parasites continue to be a cause of significant morbidity in estate communities. The need to rethink strategies to reduce this burden is emphasized by the authors of the study on soil-transmitted helminth infections in a Sri Lankan estate community.

Antimicrobial resistance, diagnostic challenges and availability of required antimicrobials continue to be issues, not only in Sri Lanka but throughout much of the world. The Perspective on the continuing worldwide threat of antimicrobial resistance takes on a local Sri Lankan perspective in the report on invasive hospital acquired vancomycin resistant enterococci in oncology patients at the National Cancer Institute of Sri Lanka. The 3 case reports in this issue continue to remind us of diagnostic challenges due to unusual or unexpected pathogens and the need for continuous alertness of diagnostic laboratories.

We are all agreed that knowledge is essential. However, the conversion of knowledge to effective preventive strategies is challenging as seen in the submissions on HIV, HBsAg and HCV co-infections among patients with tuberculosis and the study of knowledge, attitudes and practices on dengue among hospitalized patients from Northern Sri Lanka.

We hope that you will continue to find the contents of this issue useful and thought provoking. Do let us have your feedback as well as contributions for publication in forthcoming issues. Please visit the journal’s submission and peer review website at http://www.sljol.info/. We would also invite you to register as a Reviewer, as the availability of a wide pool of subject specialists for this purpose would assist us in our task of continuously improving the quality of the Journal.

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Invited Review

The soil-transmitted helminths in Sri Lanka: a review of the recent literature

NR de Silva

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Abstract

The major soil-transmitted helminth (STH) infections caused by *Ascaris lumbricoides*, *Trichuris trichiura* and *Necator americanus* have been recognized as endemic in Sri Lanka for over a century. Although prevalence rates have declined drastically over this period because of mass deworming programmes and improved housing, these infections are still found in high risk communities with poor access to sanitation. The available scientific literature published on STH infections in Sri Lanka from around the year 2000 onwards is reviewed here in three broad areas: prevalence of STH infections and factors affecting transmission, impact of control activities on prevalence and drug resistance, and the impact of STH infections on the health of infected individuals. In conclusion, an overview of the current control strategy adopted by the Ministry of Health in Sri Lanka is presented.

Keywords: Soil-transmitted helminths, Ascariasis, Trichuriasis, Hookworm, Epidemiology, Morbidity, Control strategies

Introduction

The soil-transmitted helminths are among the most common parasites of humans. The aetiological agents include the common roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*), the hookworms (*Necator americanus* and *Ancylostoma duodenale*), and the threadworm (*Strongyloides stercoralis*), all of which are primarily parasites of humans. They are known as soil-transmitted helminths (STH) because their continued transmission is dependent on contamination of soil with the microscopic eggs or larvae of these helminths, as a result of open defaecation.

Ascariasis, trichuriasis and hookworm infections (the major STH infections) have been identified by the World Health Organization as common and important causes of morbidity among children. It is estimated that worldwide in 2010, approximately 820 million persons were infected with *A. lumbricoides*, 460 million with *T. trichiura*, and 440 million with *A. duodenale* or *N. Americanus*.2

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Infections are known to result in a range of clinical manifestations including malnutrition, intestinal and biliary obstruction, dysentery and anaemia. The highest prevalence occurs in areas where sanitation is inadequate and water supplies are unsafe. The strategy recommended by WHO to control morbidity from STH involves the periodic administration of anthelmintic medicines to populations at risk of disease, which includes pre-school age children, school age children and women of reproductive age.3

New infections of *A. lumbricoides* and *T. trichiura* are acquired by ingestion of eggs containing infective larvae, via direct ingestion of contaminated soil, or fruits and vegetables contaminated with infected soil. Infection with the hookworms occurs when the free-living larvae, which are found on the surface of contaminated soil, penetrate bare skin.

As a developing country with a warm, wet, tropical climate, Sri Lanka is also endemic for all three types of STH (excluding *A duodenale*). Hookworm infections were first identified in this country over one hundred years ago, in the late 19th century.4 The public health impact of all three STH infections was well recognized5-11 and extensive de-worming programmes were conducted over much of the 20th century. As a result of these programmes, combined with gradual improvements in housing and sanitation, as well as general health literacy, prevalence rates have drifted down steadily, albeit slowly. STH infections have, however remained endemic in Sri Lanka, and there is a significant body of work that has been published over the years, summarized by de Silva in 2005.12

This review summarizes the available scientific literature published on STH infections in Sri Lanka, from around the year 2000 onwards. The review is organized into three broad areas: prevalence of STH infections and factors affecting transmission; impact of control activities on prevalence and drug resistance; and the impact of STH infections on the health of infected individuals. In conclusion, an overview of the current control strategy adopted by the Ministry of Health in Sri Lanka is presented.

**Prevalence of STH infections and factors affecting transmission**

A nationwide survey of the health 9 – 10 year old school children, carried out in 2003, included examination for STH infections.13 A total of 2,173 children from all nine provinces were examined using a single Kato-Katz smear of faecal samples. The cumulative prevalence (percentage of children with any STH infection) was estimated to be 6.9% among those examined. Whipworm was the most prevalent infection (4.0%), followed by roundworm (2.8%) and hookworm (1.2%). Cumulative prevalence was highest in the Eastern (12.3%), Northern (11.1%) and Western provinces (10.0%).

Other studies which have examined prevalence in more restricted populations around the same time include a survey of 743 schoolchildren attending Grades 1 – 5 in four schools in the Moneragala District in 1997.14 Fresh stool samples were examined using the Kato-Katz methods. The prevalence of ascariasis, trichuriasis and hookworm infections were 2.0%, 0.7% and 5% respectively. In 2016, a survey of children attending 9 schools in the Kaduwela area which had been severely affected by floods, found that none of the 156 faecal samples examined by normal saline smears were positive for STH eggs.15

A detailed study conducted in the estate sector, which remains a high-risk community for STH infections in Sri Lanka, has examined in depth, factors that affect the prevalence of STH
infections, to facilitate greater effectiveness in control activities. Different aspects of this study have been presented in sequential papers.

The first paper examines the relationships between the prevalence and intensity of human infection with *Ascaris* and the availability of sanitary facilities, socio-economic status and personal health habits.16 During the period July – December 2000, 176 subjects, who lived on a low-country tea plantation, were investigated using Kato-Katz smears. Half (50.0%) of the subjects were found to be excreting *Ascaris* eggs. Almost all (96.6%) of the subjects lived in terraces of one-room houses built by the plantation owners, and only 30.7% had access to a latrine. Most (90.3%) obtained their drinking water from common taps, and 48.8% boiled their drinking water. The subjects who only drank water that had been boiled and those who washed their hands before meals were relatively unlikely to be infected. The authors concluded that even in congested living conditions with poor sanitary facilities, good hygiene and the boiling of all drinking water can reduce the risks of *Ascaris* infection.

The second paper examines the effect of seasonal variations in climatic conditions on transmission of *Ascaris* infections in two selected low-country plantations, during the period March 2000 – June 2001.17 Faecal samples from 477 persons aged between 2 and 74 years were tested using the Kato-Katz method and the prevalence and intensity of infection determined. Monthly follow-ups were undertaken with similar stool examinations and treatment given with a single dose of mebendazole, if found positive. Infection and re-infection rates were calculated each month. Rainfall and temperature were recorded each day. Total rainfall, number of wet-days and mean temperature was calculated for each month. The prevalence of *Ascaris* infection was 53.4% and 51.0% at Maliboda and Ayr estates respectively. Highest infection and re-infection rates at Maliboda (37.7%, 37.2%) occurred in June and at Ayr (13.3%, 25.9%) in October 2000 respectively. During the study period, the mean rainfall was 28.1 cm (range 7.4-63.9 cm) and mean temperature 27.6 degrees C (range 22.1 degrees -34.4 degrees C). Significant correlations were found between the re-infection rate and rainfall, temperature and the number of wet-days. Similar correlations were observed with the infection rate and temperature and the number of wet-days. *Ascaris* infections were found to correlate significantly only with the number of wet-days in a month. The authors concluded that the number of wet-days appeared to be a better indicator of *Ascaris* infections than total rainfall or mean temperature.

The third paper examines the climatic, socio-economic and behavioural factors influencing *N americanus* infection in the same study population.18 The baseline prevalence of hookworm infection was 28.5%, while the intensity of infection ranged from 0-4,828.5 eggs/g faeces, with a mean of 128.4 eggs/g. The monthly incidence of hookworm infection was found to show three peaks at Maliboda (September 2000 (21.3%), January 2001 (20.8%) and May 2001 (17.5%)), while two peaks were seen at Ayr (September 2000 (25.0%) and February 2001 (29.2%)). The authors found that the incidence of hookworm infection showed statistically significant correlation with mean temperature. Bathing and washing with water from rock-pools formed by waterfalls, the use of wells, and a lack of toilets were also found to increase the risk of hookworm infection significantly.

Some years later, data from these three studies were re-analysed to evaluate patterns of co-infection and changes in egg deposition.19 This analysis found positive associations between *T. trichiura* and both *N. americanus* and *A. lumbricoides*, but no association between *N. americanus* and *Ascaris*. It was shown that *N. americanus* and *Ascaris* infections had lower egg depositions when they were in single infections than when they were co-infecting. There
was no clear evidence, however, of a similar effect of co-infection in *Trichuris* egg deposition. The authors concluded that associations in prevalence and egg deposition in STH species may vary, possibly indicating that effects of co-infection are species dependent.

In a separate, cross sectional study conducted in 2013, 258 children aged 1 - 6 years, living on Uduwela tea plantation in the Kandy District were examined to determine the prevalence of ascariasis and factors associated with it. Data regarding socio-demographic and hygienic habits were collected from heads of households via an interviewer administered structured questionnaire. Wet mount preparation, formaldehyde-ether sedimentation and Kato-Katz techniques were used to examine stool samples for *Ascaris* eggs, and prevalence was found to be 37.8%. On multivariate logistic regression analysis, factors significantly associated with *Ascaris* infections included living in attached houses, shared toilet facilities, de-worming period more than three months, maternal education level and living in the “top” government administrative division in the study area. As in the other studies from the estate sector, poor sanitation facilities and poor health education were found to be important factors associated with *Ascaris* infections.

**Impact of control programmes on STH prevalence**

Mass deworming programmes were widely pursued by public health authorities in Sri Lanka in the first half of the 20th century, even though the anthelmintics were not very effective. More recently however, anthelmintics have not been used on a mass scale, except in relation to certain defined, target populations.

For example, a biannual mass deworming programme with single dose mebendazole (500mg) targeting school aged children was implemented in the plantation sector from 1994 to 2005, after it was shown that the prevalence of STH infection among these children was over 90%. In 2009, five years after the deworming programme had been abandoned due to lack of funds, faecal samples from 1,890 children attending 114 estate sector schools in five districts (Kandy, Nuwara Eliya, Badulla, Ratnapura and Kegalle) were examined using single Kato-Katz smears. Although the prevalence was nowhere near as high as in the 1990s, the overall combined prevalence was still high (29.0%), while 24.4% had ascariasis (found in all five districts), 5.9% had trichuriasis (also seen in all five districts), and 4.7% had hookworm infections (not found in Nuwara Eliya and Badulla districts). This study demonstrated that even after ten years of mass chemotherapy, prevalence can bounce back after cessation of preventive chemotherapy, if the initial force of transmission is strong and other long-term control measures are not concomitantly implemented.

The above results present a stark contrast with findings from the rest of the country. During the period 2002 – 2006, the mass drug administration (MDA) programmes conducted by the Ministry of Health to eliminate lymphatic filariasis (LF) as a public health problem from Sri Lanka, involved the annual administration of diethylcarbamazine citrate (DEC) and albendazole to all those living in the nine LF-endemic districts along the Western and Southern coastal belt. Since albendazole is very effective against STH infections too, several studies examined the effect of the LF MDA programme on STH infections.

In the first of these, children attending six selected schools in the Ragama Medical Officer of Health area were examined just before and after the MDA programme in July 2002. Of the 265 children examined in the baseline survey, only 12 had any STH infection (prevalence 4.5%). In the follow-up survey only 2/252 children (2.0%) were infected. Like overall
prevalence, the mean egg counts (as estimated by the examination of single Kato-Katz smears) also declined from baseline to follow up but the differences were not statistically significant in either event.

The second survey was conducted in 2006 to assess the impact of LF MDA in the Western Province. Faecal samples from 448 children who attended 17 schools selected for the national survey conducted in 2003 (just after the 1st round of LF MDA) were examined using single Kato-Katz smears. Roundworm prevalence was found to be marginally lower in 2006 (4.0%) than in 2003 (4.7%), as was hookworm (0.2% vs 0.4%), whereas whipworm prevalence was higher (13.8% vs 9.4%). However, these differences, as well as that between the geometric mean egg counts, were not statistically significant. Compliance with MDA in 2006, however, as reported by the schoolchildren examined, was only 59%. The findings suggested that four annual rounds of MDA with DEC and albendazole had virtually no effect on STH infections among school children in the Western Province.

A third survey, conducted in 2012, explored the practicality of integrating surveillance for STH, (assessed by Kato-Katz) with transmission assessment surveys for LF in two evaluation units (EUs) in the Gampaha district. Each transmission assessment survey tested children (N = 1,462 inland EU; 1,642 coastal EU) sampled from 30 primary schools. Low filarial antigenemia rates (0% and 0.1% for the inland and coastal EUs) suggested that LF transmission was very low in the Gampaha district. The STH rates were also low: 0.8% (inland) and 2.8% (coastal). Most STH detected were low or moderate intensity T. trichiura infections. The added cost of including STH testing was estimated at approximately $5,000 per EU, and the authors concluded that the results suggest that it is feasible to integrate school-based surveillance for STH and LF.

**Drug resistance**

Large-scale treatment with the benzimidazoles (albendazole and mebendazole) has been adopted globally as a major control strategy against STH infections. Prolonged and repeated treatment with the same anthelmintics has led to the emergence of widespread benzimidazole resistance in veterinary parasites, caused by a single nucleotide polymorphism at codon 200,167 or 198 in the β-tubulin gene. Concern that prolonged use of these anthelmintics may select for resistant parasites has promoted efforts to develop genotyping assays to screen for β-tubulin polymorphisms in *N. americanus*. Single nucleotide polymorphism assays were developed based on the Smart amplification method (SmartAmp2) to target the above codons in the β-tubulin isotype 1 gene and applied to 110 *N americanus* larval samples from the Ratnapura district in Sri Lanka. None of the larval samples showed significant levels of polymorphisms either at positions 167 or 200, but a polymorphism was identified at codon position 198A/C in some samples, the first time that this mutant type has been detected in *N americanus*. The results suggest that although full-blown benzimidazole resistance was not present in the samples tested, the mutations associated with its emergence are detectable.

**Impact of STH infections on health**

As summarized previously, the early literature from the last century bears ample testimony to the morbidity and even mortality caused by heavy STH infections: severe anaemia, reduction in physical fitness, acute intestinal obstruction, rectal prolapse, etc. As prevalence rates and the intensity of infections have declined, these manifestations have become increasingly rare and in the current context, STH infections are often asymptomatic. As a result, the studies
published over the last two decades on the association between STH infection and ill health have focussed on more subtle manifestations such as physical growth, anaemia and cognitive development in children.

During the national survey of the health status of school children conducted in 2003, children with STH infections were found to have a significantly higher prevalence of anaemia than those who were uninfected.\textsuperscript{13} There was however no significant association between STH infection and stunting of growth as assessed by height for age z-scores and body mass index.

A prospective placebo-controlled randomized study was conducted in 2009-2010 to assess the impact of de-worming and iron supplementation on the cognitive abilities and educational achievement of school-age children in the plantation sector.\textsuperscript{27} The treatment group (n = 615) received de-worming (mebendazole 500 mg single dose) and weekly iron supplementation (tablets containing 200 mg of ferrous sulphate equivalent to 60 mg of elemental iron) for six months, while the control group (n = 575) received placebo for both the anthelmintic and iron. The prevalence of soil-transmitted helminth (STH) infection was reduced in the treatment group, with significant differences between treatment and control groups in the levels of \textit{Ascaris} and \textit{Trichuris}. The results also suggested that the anthelmintic treatment was effective against roundworm and whipworm infections, but not hookworm infection. In contrast to the prevalence of roundworm which dropped from 20.8\% at baseline to 14.3\% at follow-up 6 months later, and whipworm, which also dropped from 7.5\% to 4.9\%, hookworm infection rose from 5.5\% to 8.0\% in the treatment group. This is consistent with the evidence from other efficacy trials that mebendazole is not as effective in treating hookworm as it is in treating roundworm or whipworm infections.\textsuperscript{28} Final analyses found no impact on haemoglobin levels, nor any significant impact on concentration levels or on educational test scores. The authors concluded that decline in STH prevalence alone, in the absence of improved haemoglobin status, produced no evidence of impact on concentration levels or educational test scores.

A study conducted in 2013, on 489 children aged 1-12 years, living on Uduwela tea estate in the Kandy District, examined the association between ascariasis and physical growth of the children.\textsuperscript{29} The prevalence of ascariasis was 38.4\% and most children (51\%) had light intensity infections, 30\% had infections of moderate intensity, and 19\% had heavy infections. The prevalence of undernutrition was 61.7\%; 45\% per cent were underweight, while 24.1\% and 21.5\% of children were stunted and wasted respectively. No significant association was found between \textit{Ascaris} infections status and undernutrition, but heavy intensity infections were associated with decreased values of weight-for-height z-scores (WHZ).

The commensal flora of the gut is known to play key roles in human health, including nutrient metabolism, protection against pathogens and regulation of both innate and adaptive immune responses. Given that the soil-transmitted helminths and the gut microbial flora share the same environment within the human host, it is thought possible that parasite-microbiota interactions may impact on the health of helminth-infected individuals. A study conducted on 76 subjects from 9 villages in four districts of Sri Lanka explored qualitative and quantitative differences between the microbial community profiles of those infected with one or more STH, and uninfected subjects and volunteers who had been subjected to regular prophylactic anthelmintic treatment.\textsuperscript{30} High-throughput sequencing of the bacterial 16S rRNA gene, followed by bioinformatics and biostatistical analyses of sequence data revealed no significant differences in alpha diversity (Shannon) and richness between groups. However, beta diversity was significantly increased in infected and treated subjects when individually compared to uninfected volunteers. Among others, bacteria of the families \textit{Verrucomicrobiaceae} and
Enterobacteriaceae showed a trend towards increased abundance in infected persons, whereas the Leuconostocaceae and Bacteroidaceae showed a relative increase in the uninfected and treated respectively.

Current control strategy in Sri Lanka

In 2012, the Family Health Bureau of the Ministry of Health issued a general circular letter (Number 02-172/2012) with guidelines for de-worming children and pregnant women in the community setting during the period 2013-2016. These guidelines divided the country into areas of high risk (Uva, Sabaragamuwa and Central Provinces) or moderate risk (all other provinces), based on the national survey conducted in 2003, together with the 2009 survey in the plantation sector. The guidelines recommend that all children in high risk areas should be treated twice a year with a single dose of mebendazole (500 mg), while children in moderate risk areas should be treated once a year.

The guidelines issued by the Ministry of Health address community-based de-worming of both children and pregnant women. The latter group is included because hookworm infections are known to result in iron deficiency anaemia, which has particularly adverse health consequences for pregnant women, and because the age prevalence of hookworm infections, unlike that of ascariasis and trichuriasis, continues to increase until adulthood. Hence the recommendation, first issued by the Ministry of Health in 1994, is that all pregnant women should be given a single dose of mebendazole 500mg after completion of the first trimester of pregnancy.

In 2017, a second national survey of STH infections was conducted, which showed that overall prevalence has declined in all parts of the country, including the high risk communities in the estate sector and urban low income settlements. Based on these findings, the Family Health Bureau is currently in the process of revising the de-worming guidelines issued by the Ministry of Health.

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**Perspectives**

Despite huge investment to contain AMR, recent reports of increased occurrence of superbugs are terrifying

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**Abstract**

Antimicrobial resistance (AMR) which is considered a significant global health crisis continues to evolve and spread across the globe. If no adequate strategies are put in place to halt its rapid spread, routine surgical and medical procedures would become impossible without effective antimicrobials. By the year 2050, AMR is estimated to cost 10 million lives and about US$100 trillion annually. Growing concerns due to the escalating spread of multidrug resistant organisms have forced the World Health Organisation (WHO), the Centre for Disease Control and Prevention (CDC), European Commission of the EU, the UK Department of Health and the African Academy of Sciences to put strategies against AMR in place. However, despite efforts to halt AMR, recent reports show disturbing trends of difficult-to-treat deadly infections including gonorrhoea, typhoid, pneumonia, influenza, HIV, malaria and tuberculosis. This article bolsters the need for a comprehensive, real-time and improved surveillance of multidrug resistant organisms, sustained and coordinated action against AMR, development of new antibiotics and vaccines, prevention and control of diseases, improved sanitation and hygiene, sustained research, development of rapid diagnostics as well as provision of affordable and accessible health services.

**Keywords:** Antimicrobial resistance, Multidrug resistant organisms, Antibiotic resistance, AMR, MDR

**Introduction**

Antimicrobial resistance (AMR) is a major global public health threat. At present, the global annual mortality rate due to AMR is estimated to be 700,000 and by the year 2050, if no adequate strategies are put in place to halt its rapid spread, it is estimated to cost 10 million lives and about US$100 trillion annually.¹ The World Health Organization, the Centre for Disease Control, the Institute of Medicine and the Infectious Diseases Society of America have all declared AMR as a global public health crisis.² There is no doubt that the threat posed by AMR is significant. Unfortunately, many countries of the world are yet to fully recognise how

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significant the threat posed by AMR is. This is evident by the fact that significant gaps have been identified in the area of surveillance, standard methodologies and data sharing especially in Africa and South East Asia as the regions without established AMR surveillance systems. Although the burden of AMR is more significant in the developing countries with poor sanitation facilities and indiscriminate use of antibiotics in medicine and agriculture, it is a problem that affects every country irrespective of its level of income and development since resistant pathogens are cosmopolitan.

As an evolutionary response to the deadly impact of antimicrobials, microorganisms develop resistance thereby rendering existing antimicrobials ineffective. The development of resistance has resulted in infections becoming more difficult to treat and modern medical interventions becoming more dangerous. As a result, if appropriate and timely actions are not taken, there is a serious concern that the role of antibiotics in reducing the mortality and morbidity rates of infectious diseases is threatened.

**Efforts to tackle the menace of AMR**

Growing concerns due to the escalating spread of superbugs have forced the global health community to put strategies against AMR in place. In 2011, the European strategic action plan on antibiotic resistance developed and put in place strategies to help European member countries address the complex factors that cause AMR. In order to meet the objectives of this action plan, both the European Commission of the EU and the UK Department of Health came up with their own strategies. The European Commission came up with 12 recommendations against AMR for its member States to implement in 2011. The recommendations were supplemented in 2013, and later updated in 2016. The UK developed a new and improved 5-year AMR strategy covering the period 2013–18, which went beyond that of the European Commission, incorporating aims to increase awareness, promote stewardship of current treatments, and stimulate the development of new treatments. The global community endorsed these strategies for combating AMR and some of the strategies were adopted beyond Europe. The Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) was established in 2011 to enhance Europe and the USA working together against AMR. Similarly a number of recommendations have been published on tackling the spread of AMR. Additionally, to help hospitals promptly identify drug resistant infections and halt their spread, the CDC has in 2016 set up a country-wide laboratory network. The African Academy of Sciences and the South African Medical Research Council announced a research investment of $2 million to tackle resistance in 2018.

Despite these efforts and recommendations and increasing political will to halt AMR, it continues to evolve and spread globally.

However, in comparison with developed regions such as Europe and US, African and South East Asian regions have not put adequate strategies in place to combat AMR. In these regions, the problem of AMR is not only understudied, but also aggravated by factors such as high rate of infections, suboptimal disease surveillance programs, lack of AMR stewardship, and importantly, indiscriminate use of antibiotics. Additionally, in many developing countries, healthcare facilities are lacking and where available, they are mostly in dilapidated conditions characterised by inadequate diagnostic capacity and lack of reliable supply of reagents, leaving the population with limited access to formal healthcare services. To make the situation even worse, drug retailers some of whom with no form of medical training, diagnose and prescribe all sorts of antibiotics. Patients patronise them since they are cheaper,
quick to access and offer negotiable health services. Some remote communities have these types of facilities as the only available health facilities. By implication, inadequate laboratory diagnosis means that resistant pathogens go undetected, untreated and may continue to be spread. In many cases, diseases due to resistant pathogens are detected only when treatment failure became eminent.  

**Difficult-to-treat superbugs are on the increase globally**

Resistance shown by some potentially life-threatening infections to even last resort antibiotics is alarming. In 2017, the European Centre for Disease Prevention and Control (ECDC) report on antimicrobial resistance showed increasing trend of antimicrobial resistance among various bacterial pathogens. A noteworthy observation is that the overall resistance rates among *E. coli* isolates skyrocketed from 2013 to 2016. For the second consecutive year, a high percentage of carbapenems, aminoglycosides, and fluoroquinolones resistant *Acinetobacter* species were isolated from Baltic countries as well as southern and south-eastern Europe. *Acinetobacter* spp have been linked to drug resistant pneumonia. Additionally, resistance has been reported in deadly diseases such as influenza, HIV, malaria, tuberculosis, and gonorrhea. A 2017 WHO report on AMR indicates that difficult-to-treat gonorrhea is on the increase in 77 countries. Endemic typhoid caused by an extensively drug-resistant (XDR) *Salmonella Typhi* strain heightened worries that the viability of treating such infections, particularly in developing countries will be highly limited or even impossible, leading to the possible re-emergence of pre-antibiotic era when typhoid was a death sentence.

A recent WHO report published in January, 2018 indicated that terrifying high levels of AMR was found worldwide. In Korea, increased incidence of TEM-135 β-Lactamase-producing *Neisseria gonorrhoeae* has recently been documented. In 2017, the Centre for Disease Control identified more than 220 cases of drug resistant bacteria that were not only resistant to almost all available drugs, but also capable of spreading antibiotic resistant genes. These types of superbugs are especially deadly in people with impaired immune system and up to 50% of infection with such bacteria are fatal. An estimated 2 million Americans fall sick due to these terrifying superbugs. In Africa, a high level of multidrug-drug resistant bacteria to frequently prescribed antibiotics have been documented in a recent systematic review. Sometime in March this year, the UK government agency, Public Health England, in a press release, announced an extreme case of high level resistant gonorrhoea for the first time being resistant to a combination of azithromycin and ceftriaxone. This had sparked serious fear. 

These rapid and widespread cases of difficult-to-treat infections indicate limited antimicrobial treatment options and further indicate superbugs are on the verge of taking over. The CDC principal deputy director, Anne Schuchat, once said, ‘as fast as we run to tackle antibiotic resistance, some microbes have moved fast ahead of us’. The director of WHO’s Antimicrobial Resistance Secretariat, Dr Marc Sprenger, recently said reports of worldwide high level antimicrobial resistant superbugs confirm a serious situation.

**Conclusion**

The problem of AMR may continue to linger since the issue of widespread consumer abuse of antibiotics, which is a major driver of AMR is very difficult (though not impossible) to confront, especially in remote localities of developing countries with no health facilities. To prevent superbugs from taking over, it is necessary to come up with sustainable local and international interventions. The World Health Organisation has warned that AMR is a major
global health crisis that must be urgently tackled through coordinated action. A comprehensive, real-time and improved surveillance of AMR at both international and national levels are necessary. The traditional practice of prescribing antibiotics before diagnostic outcome, must stop. Equally, the search for new antibiotics and development of vaccines must continue. The United Nations has recognised that prevention and control of diseases in both humans and animals, improved sanitation and hygiene, sustained research and development, development of rapid diagnostics, affordable and accessible health services, enhanced capacity-building and technology transfer, as keys to tackling AMR.

References

Research article

A preliminary study on Naegleria species in water bodies of Kurunegala district, Sri Lanka

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Abstract

Introduction and Objective: Species belonging to the genus Naegleria are free-living ubiquitous protozoa. They have been isolated from most regions of the world. N. fowleri causes an acute, fulminant and rapidly fatal infection involving the central nervous system (CNS) in humans. It is known as primary amoebic meningoencephalitis (PAM). Infection is generally acquired while swimming, diving and total submersion for bathing in freshwater-lakes and ponds. Many inland fresh water bodies are present in Sri Lanka. These water bodies are frequently used by people for their daily needs. However, studies have not yet been conducted to determine the prevalence of Naegleria species occurring in local water bodies. The present study was therefore, carried out to isolate Naegleria species from selected water bodies located in four Divisional Secretariat (DS) divisions in the Kurunegala district, Sri Lanka.

Methods: Two different sites (clear and turbid water) of each tank were selected for sampling. Two water samples (surface water and deep water) were collected from each site (4 samples from one tank). Altogether, eighty water samples were collected from 20 tanks. Culture, enflagellation test and staining were done to detect Naegleria species. ArcGIS 10.3 and MINITAB (14) software were used for the data analysis.

Results: Flagella transformation was observed in 19 (47.5%) surface water samples and 11 (27.5%) deep water samples. Of 20 tanks, 10 were positive for Naegleria species.

Conclusions: Findings of the present study suggest that more specific genotyping studies are needed to confirm the presence of pathogenic N. fowleri in the study area.

Keywords: Primary amoebic meningoencephalitis, Naegleria fowleri, Tanks, Sri Lanka

Introduction
Naegleria species are free-living amoebae which inhabit warm fresh water bodies (rivers, lakes and hot springs) and soil. There are several species in the Genus Naegleria. However, Naegleria fowleri is the only species known to cause infection in humans. It causes fulminant and rapidly fatal primary amoebic meningoencephalitis (PAM). While PAM is a rare disease, it could be acquired while swimming, diving and total submersion for bathing in freshwater-lakes and ponds. Other Naegleria species such as N. australiensis and N. italica are known to cause PAM in experimentally infected animals. In addition to PAM, humidifier fever can be caused by Naegleria spp. It is a nonlethal hypersensitivity reaction caused due to antigenic material of Naegleria spp. present in humidifier systems.

Three distinct stages (amoeba, flagellate and cyst) of this organism can be identified in the life cycle. Trophozoites (10μm-25 μm) multiply by binary fission and encyst in response to unfavourable conditions. The cyst is round in shape with a single wall and size is varied (8μm-12 μm).

Studies have shown 92.9%, 35.3% and 15.0% prevalence of Naegleria species in environmental water samples, natural hot springs and recreational water in China, Thailand and Iran respectively.

There are many fresh water bodies (locally known as “tanks”) in the dry zone of Sri Lanka. These tanks are primarily built for agricultural purposes. However, these water bodies are frequently used by people for their daily needs (mainly for washing and bathing). Conditions of these water bodies are ideal for the growth of Naegleria species. Except for one report, the occurrence of Naegleria spp. in local water bodies has not yet been investigated.

This preliminary study was therefore carried out to isolate Naegleria species from 20 water bodies of four Divisional Secretariat divisions in the Kurunegala district, Sri Lanka using culture techniques and the enflagellation test.

Methods

Study area

The Kurunegala district is located in the North-Western province of Sri Lanka. The total population of the study area was approximately 142,078 in a land area of 719 square kilometres with a mean elevation of approximately 76 m from sea level. The annual rainfall is around 2,316.1 mm with the highest rainfall occurring in October and November during the North-East monsoon. The mean annual temperature is approximately 27.4 °C and the annual relative humidity varies from 71-87%.

The Kurunegala District consists of 30 Divisional Secretariat (DS) divisions from which the Maho, Nikaweratiya, Kotawehera and Abanpolu DS divisions were selected for the study, based on the density of water-bodies. There are approximately 654 water bodies in the study area. A standard random number table was used to select the tanks. Of the 654 water bodies, 50 were randomly selected from the four DS Divisions.
As some of the randomly selected tanks were located in rural localities with no proper road access, 20 of the 50 tanks were selected for the study based on the convenience of collection and transportation of samples.

**Collection of water sample**

There were several entryways to a tank in the study area. Two different entryways were selected for sampling. The bathing area frequently used by people was selected as the first sampling site. A turbid area (frequented by cattle and buffaloes) was selected as the second sampling site. Two water samples were collected from each site (4 samples from one tank). One sample was collected from the surface water approximately 0.5 m away from the edge of the dam. The second water sample was collected by opening the lid of the container at a depth of 1 m. The purpose of sampling from surface and deep water was to determine the presence of *Naegleria* species in either deep or surface water in the study area.

Samples were collected during day time (12.00 noon to 14.00 pm) in sterile universal glass containers (30 ml). Sample collection was carried out during a warm and dry month (September 2016) of the year with a minimum rainfall. Containers were capped and labelled immediately after collection. The samples were transported at room temperature to the Department of Parasitology, Faculty of Medicine, University of Peradeniya for further investigations.

**Global positioning system (GPS) coordinates**

Geographical coordinates (Latitude and Longitudes) of sampling sites were recorded using an android GPS receiver.

**Culture**

The water samples were mixed well, and 15 ml of water transferred into new conical tubes. The tubes were centrifuged at 2000 rpm for 2 minutes (International centrifuge; GEC A4378x1). The supernatant was discarded, and the sediment was used for inoculation. Cultures were carried out on non-nutrient agar plates with *Escherichia coli* (NCTC10418) as described by Ash and Orihel (1987). Plates were sealed with parafilm to prevent contamination. Culture plates were incubated at 37°C overnight. The plates were observed for five consecutive days using an inverted microscope (Leitz Diavert). Positive growth was identified by increased localised amoebic count in the culture plate. Localised areas were marked on the plate.

**Examination for flagellates (enflagellation test)**

Scrapings from culture plates with growth were inoculated into 1 ml of distilled water and incubated at 37 °C for 30 minutes. Wet smears were prepared and observed for transformation of trophozoites into pear shaped bi-flagellates or multi-flagellates. Preservation of flagellates was done using polyvinyl alcohol (PVA) fixative for trichrome staining. Trichrome stained trophozoites and flagellate forms were examined with a light microscope separately. Dimensions of trophozoites and flagellate forms were measured using a calibrated micrometer at (x100) magnification.

**GIS analysis**

GIS analysis was done using ArcGIS 10.3 software which works with maps to compile geographic information.
**Statistical analysis**
The results were analysed using MINITAB (14) statistical software. Two proportions and correlation tests were performed. Positivity in surface water vs deep water and positivity in first site vs second site were considered as variables.

**Results**

Eighty water samples were collected from 20 tanks. From each tank, sampling was done from two sites (clear and turbid water). From each site, two samples were collected (surface and deep). Flagella transformation was observed in 19 surface water samples (47.5%) and 11 (27.5%) deep water samples. Of 20 tanks, 10 (50%) were positive for *Naegleria* spp. (Table 1, 2 and Figure 1).

**Table 1**: Results of enflagellation test

<table>
<thead>
<tr>
<th>Name of the Tank</th>
<th>GPS N</th>
<th>GPS E</th>
<th>1st site positivity</th>
<th>2nd site positivity</th>
<th>Overall remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hulugallewewa</td>
<td>7.785488</td>
<td>80.14305</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Magollewewa</td>
<td>7.740626</td>
<td>80.12363</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diwullawewa</td>
<td>7.765771</td>
<td>80.13234</td>
<td>S,D</td>
<td>S</td>
<td>S,D</td>
</tr>
<tr>
<td>Mahagirilllawewa</td>
<td>7.829951</td>
<td>80.11597</td>
<td>S</td>
<td>S</td>
<td>S,D</td>
</tr>
<tr>
<td>Udagirilllawewa</td>
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<td>80.13112</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thabarambuwamahawewa</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mahakirindewewa</td>
<td>7.814126</td>
<td>80.11163</td>
<td>S,D</td>
<td>S,D</td>
<td>S,D</td>
</tr>
<tr>
<td>Olupeliyawawewa</td>
<td>7.805042</td>
<td>80.11689</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tubullawewa</td>
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<td>80.09382</td>
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<td>0</td>
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<tr>
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<td>80.11006</td>
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<td>S,D</td>
<td>S,D</td>
</tr>
<tr>
<td>Ipalogamamahawewa</td>
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<td>80.209</td>
<td>S</td>
<td>S,D</td>
<td>S,D</td>
</tr>
<tr>
<td>Thammitagamawewa</td>
<td>7.825202</td>
<td>80.22933</td>
<td>S,D</td>
<td>S,D</td>
<td>S,D</td>
</tr>
<tr>
<td>Uduweriyawewa</td>
<td>7.849772</td>
<td>80.24091</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kaburupitiyawewa</td>
<td>7.794035</td>
<td>80.20798</td>
<td>S</td>
<td>S,D</td>
<td>S,D</td>
</tr>
<tr>
<td>Ithewwewa</td>
<td>7.805821</td>
<td>80.18694</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PahalaManingamuwawewa</td>
<td>7.832664</td>
<td>80.21649</td>
<td>0</td>
<td>S,D</td>
<td>S,D</td>
</tr>
<tr>
<td>Ehetuwewa</td>
<td>7.78442</td>
<td>80.15906</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hithkadawalawewa</td>
<td>7.856582</td>
<td>80.26146</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Dalupotawewa</td>
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<td>80.21903</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mahowewa</td>
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<td>80.2798</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

S: Enflagellation test positive-surface water  D: Enflagellation test positive-deep water  0: Enflagellation test negative
Table 2: Sample positivity in DS Divisions

<table>
<thead>
<tr>
<th>DS Divisions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample type</td>
<td>Mahawa</td>
</tr>
<tr>
<td>Surface water</td>
<td>9</td>
</tr>
<tr>
<td>Deep water</td>
<td>5</td>
</tr>
<tr>
<td>Total tanks (surface or deep)</td>
<td>5</td>
</tr>
<tr>
<td>1st site (clear water)</td>
<td>5</td>
</tr>
<tr>
<td>2nd site (turbid water)</td>
<td>9</td>
</tr>
</tbody>
</table>

There was no significant positive correlation (p>0.05) between clear and turbid water. However, we found a significant positive correlation between surface water and deep water (p<0.05).

The shape and size of the flagellate, trophozoite, and cyst were evaluated on trichrome stained slides for confirmation.

The trophozoites were active and constantly changing their size and shape. The length varied from 18-22 μm. The cytoplasm was finely granular and contained a conspicuous clear nuclear halo and a dense central nucleolus. Few vacuoles were visible in the cytoplasm.

The trophozoites were motile and moved by extending blunt rounded lobopodia (Figure 2). Bi-flagellates were pear-shaped (Figure 3). They were 10-12 μm in length and 5-7 μm in width. Flagella was fixed into the conical edge of the organism and showed sluggish circular movements. Multi-flagellates were not observed in cultured samples. Cysts were rounded and spherical in shape with smooth outer coverings. The length of the cyst varied between 6-9 μm. In most cases, the cytoplasm was granular (Figure 3).
Discussion

Epidemiologically, *Naegleria* has a worldwide distribution. Primary amoebic meningoencephalitis was first described by Malcolm Fowler in Australia. Thereafter, cases of PAM were identified in many parts of the world. Over 440 cases of PAM were reported worldwide. Among them, nearly half of the cases were reported from the United States.18 In Asia, the highest number of cases has been reported in India followed by Pakistan. A single case of PAM has been reported due to freshwater pearl diving in Vietnam.

The main method of contracting PAM is by swimming in *N. fowleri* contaminated fresh water collections. However, recent reports suggest that the use of “neti pots” for nasal irrigation can also predispose to PAM. In addition; *N. fowleri* has been isolated from the nares of apparently healthy children.

Neither PAM nor *N. fowleri* has been reported in Sri Lanka. However, the climatic conditions prevailing in Sri Lanka is favourable for growth of *Naegleria* species in local water bodies. Thus, isolation and identification of *N. fowleri* in local water bodies is a necessity. However, identification of *N. fowleri* based on morphological characteristics is not easy due to the existence of several genera of amoebae with similar morphological features in the same ecological habitat. Furthermore, pathogenic *N. fowleri* and non-pathogenic *Naegleria lovaniensis* are antigenically related species.

Enflagellation test (Amoeba-to-Flagellate Transformation) was performed to detect *Naegleria* species in the present study. However, the enflagellation test is not considered the gold standard test for detecting *Naegleria* species, because few non-flagellating *Naegleria* strains have been isolated from France. In addition, trophozoites isolated in one location of Australia have failed consistently to transform into flagellates. However, non-flagellating *Naegleria* species have not been reported so far in Asia or the Indian subcontinent.

Current methods for detection and enumeration of *Naegleria* species are based on culture techniques followed by various other methods (species-specific monoclonal antibodies, PCR, enzyme electrophoresis, isoenzyme electrophoretic profiles and DNA restriction fragment length polymorphisms (RFLPs) to identify the isolate up to the species level.
Randomly amplified polymorphic DNA typing has been used to differentiate *Naegleria* spp. and could be used to detect minor variations in *N. fowleri* strains. In addition, *N. fowleri* positive cultures could be tested for pathogenicity by mice inoculation.

Records on the isolation of *Naegleria* from water bodies in tropical countries are very limited. Prevalence of 29% and 10% were reported in aquatic habitats in a human environment and in stagnant water in an industrial area in Thailand. A recent study carried out in natural hot springs in 13 provinces of Thailand showed 35% positivity for *Naegleria* indicating a high health risk to those exposed to such waters. Findings of our study also suggest a high prevalence (50%) of these free-living amoebae in aquatic habitats in the study area. These water-bodies form a network of reservoirs where water cascades and spills over to the tank situated below and finally drains into a common canal or stream. Hence, *Naegleria* can easily migrate from this network to other tanks. This could be one reason for the high prevalence of the organism in these lakes.

Usually in the dry season water is clear as no muddy rain water is drained to the tank. The most plausible reason for the turbid water in these tanks was the wallowing of agricultural animals (such as buffalos) for long hours in the water. These animals enter the tanks through specific entry points. These entry points are also used by the local people for bathing and various other purposes. The role of animals in *Naegleria* ecology and epidemiology is yet unclear. A study in Tennessee, USA, showed that 13 wild mammalian species had serum antibodies against *Naegleria* indicating past exposure. This was the reason we compared sites in the tanks having both clear and turbid water. However, we could not find a significant difference (p>0.05) between the two sites, clear and turbid water. A study done in India showed 34.5% prevalence of *Naegleria* spp in surface water. It was 7.6% in deep well water in Arizona.

The significant finding of this study was that the organism thrives more in surface water than in deep water (p<0.05). A similar result has been reported in some DS divisions in the same district previously.

Limitations: Small sample size and not performing specific tests (such as FAT or PCR) to detect *Naegleria* species were the limiting factors of this study. However, the findings of the present study would encourage more epidemiological investigations on *Naegleria* species occurring in local water bodies.

Conclusions

Our results show a high prevalence of *Naegleria* species in the water bodies of the study area. Consequently, there is a possibility of the existence of pathogenic *Naegleria fowleri* in these water bodies. More specific and accurate genotyping is required to confirm the presence of pathogenic *Naegleria* in these water bodies, as it may pose a health risk to people who use such water bodies for domestic and recreational activities. The findings of this study will be used in designing a long-term study for genotyping of isolates from different sites.

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20.19.18.16.14.13.12.11.10.9.8.7.6.5.4.3.2.1

Fowleri


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Research article

Prevalence rates of HIV, HBsAg and HCV co-infections among tuberculosis patients in Sokoto Metropolis, Northwest Nigeria

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Abstract

Introduction and Objectives: Tuberculosis (TB) and viral hepatitis are global health problems which are common in developing and undeveloped countries. The current study was aimed at determining the prevalence and co-infections of HIV, HBsAg and HCV among patients with tuberculosis in Sokoto, Northwest Nigeria.

Methods: A cross-sectional study was conducted on a total of 276 patients with tuberculosis, aged 19-60 years at Usmanu Danfodiyo University Teaching Hospital, Sokoto and Specialist Hospital, Sokoto between May and November 2015. Blood samples collected were screened for HIV, HBsAg and HCV using immunochromatographic techniques.

Results: The prevalence rates of HIV, HBsAg and HCV among TB patients were 16.3%, 14.9% and 5.4%, respectively while the prevalence of co-infections HIV-HBsAg, HIV-HCV, HBsAg-HCV and HIV-HBsAg-HCV in TB patients were 50.7%, 1.81%, 2.9% and 1.45%, respectively. Prevalence of HIV and HCV showed no relationship with age (P=0.240 and 0.987, respectively) but prevalence of HBsAg was associated with age (P=0.0304). There were no relationships of frequencies of HIV, HBsAg and HCV with gender.

Conclusion and Recommendations: The study showed high prevalence rates of HIV, HBsAg and HIV-HBsAg co-infections among patients with tuberculosis in Sokoto, while age and gender showed little or no association. HIV and HBsAg tests are recommended for every patient diagnosed with tuberculosis to guide therapeutic decisions.

Keywords: Prevalence, Viral infections, Tuberculosis patients, Sokoto metropolis

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Introduction

Tuberculosis (TB) remains a leading health problem in both developing and developed countries and it is caused by Mycobacterium tuberculosis complex. The World Health Organization (WHO) estimated 8.6 million new cases of TB in 2012 and 1.3 million deaths while two billion people have been reported to be affected worldwide with a global incidence of 219/100,000 and death rate of 25/100,000. Nigeria has been ranked 10th among the 22 high TB burden countries of the world and the fourth highest in Africa (after South-Africa, Ethiopia and DR Congo).

The World Health Organization (WHO) estimated approximately 240 million people worldwide as being chronically infected with hepatitis B virus (HBV) while areas of high prevalence are similar to the global TB epidemiological ‘hotspots’ and these include Sub-Saharan Africa and South Asia, where the prevalence is estimated to be between 8 and 20%.

Human immuno-deficiency virus (HIV) infection increases the risk of TB 20 fold compared with HIV seronegative individuals in high HIV-prevalence countries. The HIV epidemic increased the number of TB cases in countries with a high prevalence of HIV infection starting in the late 1980s, with a 3-fold increase in the number of TB case notifications over the decade, particularly in Sub-Saharan Africa. Worldwide, 14.8% of TB patients have HIV co-infection, and as many as 50-80% have HIV co-infection in parts of Sub-Saharan Africa.

Tuberculosis and hepatitis C virus (HCV) co-infection contributes to major disease mortality and morbidity. However, the causal link between HCV infection and TB risk remains unclear but a study has suggested that HCV infection is associated with a higher risk of developing active TB disease.

Injection drug use is a key factor in the transmission of blood borne pathogens. Behavioral epidemiological studies have shown that both injection-related risk factors of injecting drugs, type of drug injected, direct and indirect sharing of injection paraphernalia and sex-related risk factors (lack of condom use, multiple sexual partners, survival sex) have been associated with the spread of HIV, HBV and HCV.

HIV and TB have a synergistic interaction and each of these accentuates progression of the other. In 2010, TB was flourishing unhindered, reaching a proportion of 82% TB cases co-infected with HIV in Sub-Saharan African region. The HIV/AIDS pandemic has been linked to the resurgence of TB, leading to increased morbidity and mortality worldwide.

Viral hepatitis is life threatening liver disease caused by hepatitis B and C viruses and is a major public health problem, particularly in developing countries. Chronic liver disease increases the risk of hepatotoxicity during anti-tuberculosis treatment, which could be up to three to five times more than TB patients without viral infection. A fourteen fold increase in the risk of anti-TB hepatotoxicity has been reported in HIV and HCV co-infected patients.

The prevalence of HIV among TB patients varies in different parts of Nigeria. HIV prevalence of 44.2% in Nassarawa State, 53.3% in Benue State and Federal Capital Territory (FCT), 12.3%
in South-western Nigeria\textsuperscript{22} and 32.8\% in Edo State\textsuperscript{23} in TB patients has been reported in Nigeria while prevalence rates of hepatitis B surface antigen (HBsAg) of 8.7\% and HCV of 14.8\% among TB patients were reported in Kano, Northern Nigeria by Taura et al.\textsuperscript{24} HBsAg prevalence of 2.2-15.5\% and HCV of 0.7-7.0\% were reported in Southern Nigeria.\textsuperscript{25,26} HIV prevalence rates of 11.4\% in Ethiopia and 27.6\% in Brazil among patients with tuberculosis have been documented by other authors.\textsuperscript{27,28} The prevalence of HBsAg of 5.5\% in Pakistan, 9.5\% in Sudan and 25.6\% in Brazil have been reported amongst tuberculosis patients.\textsuperscript{27,29,30} while the prevalence rates of HCV in tuberculosis patients of 3.5-10.0\% have been documented by earlier studies.\textsuperscript{29,30}

In Northern Nigeria, the information on the prevalence of the co-existence of TB, HIV and viral hepatitis is scanty. This study on prevalence and co-infections of HIV, HBsAg and HCV among patients with tuberculosis in Sokoto metropolis is important as the findings can be utilized in making plans for the management of TB patients in the metropolis and possibly Sokoto State as a whole.

**Materials and methods**

A cross-sectional study aimed at determining the prevalence rates of HIV, HBsAg, HCV and co-infections among TB patients was done. Patients with confirmed tuberculosis attending the TB clinics of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto and Specialist Hospital, Sokoto between May and November 2015 were recruited for the study. Patients with productive cough for three or more weeks, with at least two positive sputum smears or one positive smear and consistent X-ray findings that were suggestive of tuberculosis were included in the study while non-consenting TB patients were excluded from the study.

Three ml of whole blood was collected from each patient. The sample was allowed to clot and centrifuged at 3000 revolutions per minute for 5 minutes to separate the serum for analysis.

Hepatitis B surface antigen (onsite HBsAg rapid test kit-CTK Biotech Inc., USA. Sensitivity/Specificity 96\%/99.6\%), HIV antibody (Alere Determine HIV 1/ 2 kit-Alere Medical Co. Ltd. Sensitivity/Specificity 100\%/98.9\%) and anti-HCV antibody (onsite HCV antibody rapid test kit: CTK Biotech, Inc., USA. Sensitivity/Specificity 100\%/99.6\%) tests were done on every sample.

Data on age and gender were collected from all the participants using a designed and structured questionnaire.

Data were analyzed using statistical package for social sciences (SPSS version 20) to determine the prevalence of HIV, HBsAg and HCV and co-infections among TB patients. Age and gender in relation to prevalence of viral infections among TB patients were determined using chi-square and a p-value of <0.05 was considered significant.
Results

276 confirmed TB patients aged 19-60 years were included in the study. The prevalence rates of HIV, HBsAg and HCV among TB patients are shown in Table 1. The prevalence rates of HIV, HBsAg and HCV among TB patient were 16.3%, 14.9% and 5.4%, respectively. The prevalence rates of HIV, HBsAg and HCV showed significant relationship with one another (P<0.001).

Table 1. Prevalence rates of HIV, HBsAg, HCV among TB patients

<table>
<thead>
<tr>
<th>Status</th>
<th>HIV</th>
<th>HBsAg</th>
<th>HCV</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Number</td>
<td>45</td>
<td>41</td>
<td>15</td>
<td>0.000</td>
</tr>
<tr>
<td>positive (%)</td>
<td>(16.3)</td>
<td>(14.9)</td>
<td>(5.4)</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>11.9-20.7</td>
<td>10.7-19.1</td>
<td>2.6-8.2</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>231</td>
<td>235</td>
<td>261</td>
<td>123</td>
</tr>
<tr>
<td>negative (%)</td>
<td>(83.7)</td>
<td>(85.1)</td>
<td>(94.6)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 demonstrates the prevalence rates of co-infections of HIV, HBsAg, HCV in TB patients.

Table 2. Prevalence rates of co-infections of HIV, HBsAg and HCV among TB patients.

<table>
<thead>
<tr>
<th>Status</th>
<th>HIV-HBsAg</th>
<th>HIV-HCV</th>
<th>HBsAg-HCV</th>
<th>HIV-HBsAg-HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>262</td>
<td>271</td>
<td>268</td>
<td>272</td>
</tr>
<tr>
<td>% Positivity</td>
<td>5.07</td>
<td>1.81</td>
<td>2.90</td>
<td>1.45</td>
</tr>
<tr>
<td>95% CI</td>
<td>2.47-7.67%</td>
<td>0.21-3.41%</td>
<td>0.9-4.9%</td>
<td>0.01-2.89%</td>
</tr>
</tbody>
</table>

The prevalence of co-infections of HIV-HBsAg, HIV-HCV, HBsAg-HCV and HIV-HBsAg-HCV in TB patients were 5.07%, 1.81%, 2.9 and 1.45%, % respectively.

The age distribution of HIV, HBsAg and HCV infections among the TB patients is shown in Table 3. Age group of 19-28 years had the highest prevalence of HIV (23.17%), followed by age group 49-58 years (17.64%), > 59 years (12.5%), 39-48 years (11.66%) and 29-38 (10.0%) but the prevalence of HIV showed no significant relationship with age (P=0.240).

Table 3. Age distribution of HIV, HBsAg and HCV infections among TB patients

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No of patients (n)</th>
<th>HIV positive</th>
<th>HBsAg positive</th>
<th>HCV Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>19-28</td>
<td>82</td>
<td>19</td>
<td>23.17</td>
<td>20</td>
</tr>
<tr>
<td>29-38</td>
<td>50</td>
<td>5</td>
<td>10.0</td>
<td>4</td>
</tr>
<tr>
<td>39-48</td>
<td>60</td>
<td>7</td>
<td>11.66</td>
<td>13</td>
</tr>
<tr>
<td>49-58</td>
<td>68</td>
<td>12</td>
<td>17.64</td>
<td>6</td>
</tr>
<tr>
<td>&gt; 59</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
<td>2</td>
</tr>
<tr>
<td>P-value</td>
<td>0.240</td>
<td>0.0304</td>
<td>0.987</td>
<td></td>
</tr>
</tbody>
</table>
prevalence of HBsAg among TB patients (24.39%), followed by age group 39-48 years (21.66%), > 59 years (12.5%), 49-58 years (8.82%) and 29-38 years (8.0%). The prevalence of HBsAg in TB patients showed significant association with age (P=0.0304).

The highest prevalence of HCV among TB patients was observed amongst those > 59 years (6.25%), followed by 19-28 years (6.09%), 49-58 years (5.88%), 39-48 years (5.0%) and 29-38 years (4.0%). The prevalence of HCV showed no significant relationship with age (P=0.987).

Table 4 displays the distribution of HIV, HBsAg and HCV infections by gender among TB patients. Prevalence of HIV, HBsAg and HCV amongst males with tuberculosis were 14.01%, 14.01% and 3.82%, respectively while that of females with tuberculosis were 19.32% 15.96% and 7.56%, respectively. The prevalence rates of HIV, HBsAg and HCV among TB patients showed no relationship with gender at P- values of 0.237, 0.651 and 0.175, respectively.

Table 4. Distribution of HIV, HBsAg and HCV infections by gender among TB patients

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of patients (n)</th>
<th>HIV positive</th>
<th>HBsAg positive</th>
<th>HCV positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td>157</td>
<td>56.88</td>
<td>22</td>
<td>14.01</td>
</tr>
<tr>
<td>Female</td>
<td>119</td>
<td>43.12</td>
<td>23</td>
<td>19.32</td>
</tr>
<tr>
<td>P-value</td>
<td>0.237</td>
<td>0.651</td>
<td>0.175</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Tuberculosis and viral hepatitis are global health problems which are commoner in developing and underdeveloped countries than developed countries.1,2,31 Prevalence rates of HIV, HBsAg and HCV among TB patients vary from region to region in Nigeria and internationally. This study has shown the prevalence of HIV among TB patients to be 16.3% which is at variance with that of 44.2-53.3% reported in Nassarawa and Benue States as well as FCT which are parts of North Central region of Nigeria.20,21 This striking difference in the prevalence rates may be associated with a high prevalence rate of HIV in North Central Nigeria in contrast to prevalence rates of HIV among TB patients in Southwest Nigeria and Edo State (12.3% and 32.8%, respectively).22,23 The different frequencies of HIV among TB patients are similar to the reported HIV/AIDS prevalence rates by States in Nigeria.32 In the Republic of Georgia, Kuniholm et al.33 reported HIV prevalence rate of 0.7% among TB patients while Mengesha et al.34 reported a prevalence of 15% for HIV. However, the different prevalence rates of HIV among TB patients from some parts or regions of Nigeria and among other countries20,23,33,34 may be associated with the peculiarities of the environments and the associated prevalence rates of HIV, techniques employed, and sensitivity and specificity of test methods used among other factors.

The study further showed the prevalence of HBsAg among TB patients to be 14.9% which is in contrast to the earlier report of 8.7% in Kano.24 Akhtar et al.28 and Nail et al.29 reported prevalence
rates of HBsAg among TB patients to be 5.5% and 9.5%, respectively while Kuniholm et al.\textsuperscript{33} and other researchers in Thailand reported prevalence rates of 4.3% and 9.0%, respectively.\textsuperscript{35} The varying prevalence rates of HBV may be influenced by the populations of regions and their associated economic and hygienic factors,\textsuperscript{36} and different methods of screening.\textsuperscript{37} The current study showed a prevalence of 5.4 % for HCV among TB patients. This is in disagreement with the earlier report of 14.8% in Kano.\textsuperscript{24} However, Akhtar et al.\textsuperscript{28} and Nail et al.\textsuperscript{29} reported prevalence rates of 10% and 3.5%, respectively for HCV while Kuniholm et al.\textsuperscript{33} and Sirinak et al.\textsuperscript{35} reported prevalence rates for HCV among TB patents to be 12% and 31%, respectively. The different prevalence rates of HCV have been linked to the geographical factors of various regions.\textsuperscript{38,39}\

The prevalence rates of HIV, HBsAg and HCV have shown significant relationship in this study and this may be associated with shared modes of transmission.\textsuperscript{13} In our study, the prevalence of co-infection of HIV-HBsAg-HCV in TB patients was 1.45% compared to overall prevalence TB-HIV-Hepatitis triple infection (TB-HIV-HBV-HCV) of 2.4% in Ethiopia.\textsuperscript{34} The causes of HIV-HBV-HCV co-infection has been associated with parenteral, sexual and vertical transmission, and sharing of injections by injection drug users.\textsuperscript{13} The co-existence of TB, HIV and viral hepatitis in the same patient poses a challenge to the patient and clinicians since very little information is available on impact of the triple viral infection on TB treatment outcome.\textsuperscript{34}\

There is scanty information on the prevalence of co-infection of HBsAg-HCV among TB patients. This current study has shown a prevalence of 2.9%. Co-infection with hepatitis B or C viruses among tuberculosis patients has been associated with potentiating the risk of anti-tuberculosis therapy induced hepatotoxicity.\textsuperscript{17-19}\

Prevalence rates of co-infections of HIV-HBsAg and HIV-HCV in TB patients have been scarcely examined. The current study demonstrates a prevalence of 5.07% and 1.81% for HIV-HBsAg and HIV-HCV co-infections respectively while in Ethiopia, Mengesha et al.\textsuperscript{34} reported HIV-HBsAg-TB and HIV-HCV-TB co-infections to be 8.9% and 7.1% respectively. The difference in the prevalence of co-infections may be associated with different sample numbers and peculiarities of each environment. HIV or viral hepatitis or both in patients with tuberculosis have been linked to the risk of hepatotoxicity during anti-tuberculosis treatment.\textsuperscript{17,18}\

Our study has further shown that prevalence rates of HIV and HCV in TB patients are not associated with age while the prevalence of HBsAg is related to age. The age group 19-28 years showed the highest prevalence for HIV (23.17%) and HBsAg (24.39%) while TB patients of > 59 years had the highest prevalence of HCV (6.25%). The high prevalence rates of HIV and HBsAg
in TB patients among the younger age group in this study agrees with previous findings, which may be related to these patients being in the sexually active age group.

This study has further shown that gender is not associated with the prevalence rates of HIV, HBsAg and HCV infections among TB patients which is in agreement with the earlier study in Ethiopia. Although there was a higher prevalence of HIV, HBsAg and HCV infections in female TB patients in the current study, this difference was not statistically significant and may be a reflection of the difference in sample size in each gender group.

**Conclusion and recommendations**

This study has shown lower prevalence of HIV among TB patients in the Sokoto metropolis, Northwest Nigeria compared to North Central and Southern regions of Nigeria. Age and gender showed little or no association with the prevalence rates of HIV, HBsAg and HCV infections in TB patients but the prevalence rates of the three viral infections showed significant relationship. The 19-28 year age group had the highest prevalence rates of HIV and HBsAg, probably due to their increased sexual activity.

It is therefore recommended that HIV, HBsAg and HCV screening tests be carried out in diagnosed TB patients in Sokoto metropolis to guide therapeutic decisions. This would enable improved public health education on the transmission of these viral infections and assist in reducing the complications and mortality rate among TB patients.

**References**


Research article

Bacterial pathogens causing urinary tract infections in children and their antimicrobial susceptibility patterns in a tertiary care hospital in Sri Lanka

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Abstract

Introduction and Objectives: Urinary tract infection (UTI) is one of the most common infections in childhood. The objective of our study was to ascertain the commonest organisms causing UTI in children and their ABST patterns. The study was carried out in a Teaching Hospital in Sri Lanka.

Methods: Data was collected from children who were confirmed to have UTI by positive urine culture (>105 bacteria/ml urine) between July-December 2015. The clinical presentation, presence of predisposing factors and reports of urine culture and the antibiotic susceptibility patterns were analysed.

Results: Coliforms were found to be the commonest organism followed by Enterococcus spp. Nitrofurantoin showed the highest sensitivity of the tested antibiotics against both coliforms and the enterococcus groups. However the sensitivity to most of the antibiotics showed a reduction from previously recorded values in studies done in Sri Lanka.

Conclusions: The authors emphasize the need for regular revision of the list of organisms causing UTI and the antibiotic sensitivity to improve the treatment of childhood UTI with the ideal antibiotic.

Keywords: Urinary tract infection, UTI, Children, Pathogens

Introduction

UTI is one of the most common infections in the paediatric population.1 It is a heterogeneous disease which can be categorized into lower and upper UTI, or complicated and uncomplicated UTI, depending on the clinical features and laboratory findings.2 It causes significant morbidity and mortality, especially if complicated with structural or congenital defects of the urinary tract.
tract. In children presenting with UTI, 30-40% have underlying vesico-ureteric reflux (VUR), while other congenital anomalies like posterior urethral valves, pelvi-ureteric junction obstruction, ureteroceles and duplex systems are encountered less frequently.³

Fever, increased frequency, straining on micturition, diarrhoea and offensive urine are the common presentations of childhood UTI.³,⁴ Features are often non-specific and it is therefore recommended to perform a semiquantitative urine culture to confirm the diagnosis in children with suspected UTI.⁵ Management of UTI is with either oral or parenteral antibiotics depending on the clinical need.⁶ The initial choice of antibiotics is decided on available clinical guidelines, with availability, cost and personal preferences playing a secondary role.⁷ Clinical guidelines are available in both Sri Lankan and international contexts and provide guidance for initial antibiotic choice.⁸,⁹,¹⁰ As the treatment of the acute episode is of utmost importance, the correct choice of initial antibiotic should be based on local and timely information of the responsible organisms and their ABST pattern. Renal scarring may result from improper treatment, especially in infants and very young children.¹¹ Non-response to initial treatment requires a change of antibiotic guided by the ABS results and/or further investigation.

The ABS pattern of a particular organism is known to change with time and vary from place to place, resulting in very specific ABS patterns for causative bacteria in different locations.³,¹¹ Probable reasons for varying antibiotic susceptibility include continued exposure of causative bacteria to antibiotics in both clinical and non-clinical settings, including inappropriate and improper use (wrong dose, wrong duration). A positive correlation between antibiotic usage and development of resistance has been observed in many studies.¹²

Information on local ABS pattern is therefore important to treat UTI effectively, while avoiding unnecessary use of a wide range of antibiotics which can contribute to antibiotic resistance. In many countries, updated local information is made available to clinicians for this purpose. However, although the availability of such data in Sri Lanka is increasing, information on causative bacteria and their ABS patterns in children is still limited.¹³,¹⁴

Island wide availability of systematic updated information on causative agents/ABS of UTI in the paediatric population is a national need. This study aims to contribute to this need by studying the causative bacteria and their ABS in a paediatric population with confirmed UTI in the North Colombo Teaching Hospital, Sri Lanka.

Methods

A prospective study was done in the paediatric wards of North Colombo Teaching Hospital during a five-month period from 1st July to 1st December 2015.

Collection of urine for culture was done by mid-stream clean catch sampling. The correct procedure was routinely taught to mothers by one of the medical officers in each ward before collection of urine. Positive urine culture is defined as the presence of significant bacteriuria, (> 10⁵ CFU of a single organism/ml of urine). All the children who were confirmed to have UTI by means of positive urine culture and whose parents consented were included in the study.

An interviewer administered questionnaire was used to obtain the clinical history from the patient’s mother or guardian. Thorough clinical examination was performed on the patient by
the investigators to elicit the signs. Relevant data on the organism and the ABS was extracted from the urine culture reports. The data were entered to a beta version of SPSS statistical software. The frequencies and the cross relationships were analysed where relevant.

Results

A total of 97 subjects were recruited, 47 (48.5%) males and 50 (51.5%) females. The ages varied from two months to twelve years. The mean age was 3.69 years (SD 3.3) (Table 1).

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>25</td>
<td>25.8</td>
</tr>
<tr>
<td>1 - 5 years</td>
<td>44</td>
<td>45.4</td>
</tr>
<tr>
<td>5 - 10 years</td>
<td>18</td>
<td>18.6</td>
</tr>
<tr>
<td>&gt; 10 years</td>
<td>10</td>
<td>10.3</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47</td>
<td>48.5</td>
</tr>
<tr>
<td>Female</td>
<td>50</td>
<td>51.5</td>
</tr>
</tbody>
</table>

Fever is the commonest presenting feature of UTI in children of all age groups. In 81.4% of the children with UTI, fever was the main complaint at presentation. Straining, increased frequency of micturition, diarrhoea and offensive urine were the other common complaints (Table 2).

<table>
<thead>
<tr>
<th>Category</th>
<th>&lt;1 yr</th>
<th>1 - 5 yrs</th>
<th>5 - 10 yrs</th>
<th>&gt; 10 yrs</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>84%</td>
<td>88.6%</td>
<td>77%</td>
<td>50%</td>
<td>0.04</td>
</tr>
<tr>
<td>Straining</td>
<td>56%</td>
<td>45.4%</td>
<td>11%</td>
<td>10%</td>
<td>0.004</td>
</tr>
<tr>
<td>Frequency</td>
<td>28%</td>
<td>61.3%</td>
<td>55%</td>
<td>60%</td>
<td>0.054</td>
</tr>
<tr>
<td>Dysuria</td>
<td>44%</td>
<td>59.0%</td>
<td>88%</td>
<td>100%</td>
<td>0.001</td>
</tr>
<tr>
<td>Offensive urine</td>
<td>25%</td>
<td>15.9%</td>
<td>5.5%</td>
<td>20%</td>
<td>0.308</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>20%</td>
<td>9.1%</td>
<td>5.5%</td>
<td>10%</td>
<td>0.44</td>
</tr>
<tr>
<td>Irritability</td>
<td>32%</td>
<td>13.6%</td>
<td>0%</td>
<td>0%</td>
<td>0.12</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>4%</td>
<td>11.4%</td>
<td>66%</td>
<td>90%</td>
<td>0.000</td>
</tr>
</tbody>
</table>

The known predisposing factors for UTI were present in 15 of 97 (15.5%) patients of which constipation and previously diagnosed abnormality of the urinary tract were the most common.

The commonest organisms isolated from urine cultures in children of all age groups were coliforms, accounting for 60.8% (59/97) of which Klebsiella spp. was found in 9 (9.3%) patients (Figure 1). In 23 patients (23.7%), enterococci and Staphylococcus spp in 2 (2.1%) patients respectively. There was no gender based difference in the causative organisms (Table 3).
The organisms causing UTI in different age groups of children is shown in Figure 1. In all age groups coliforms (including *Klebsiella spp.*) was the commonest isolate. Antibiotics had been started empirically prior to the availability of ABST report in all patients. Co-amoxiclav had been prescribed in 66 of the 97 patients (68%) followed by cefotaxime in 15 patients (15.5%).

Sensitivity to nitrofurantoin was seen in 40 of the 59 (67.8%) coliforms (excluding *Klebsiella spp.*) isolated in the study. Sensitivity to nalidixic acid was seen in 34 coliform isolates (57.6%). Co amoxiclav sensitivity was lower at 52.5% (31/59 isolates). None of the isolated organisms were found to be ESBL producers.

<table>
<thead>
<tr>
<th></th>
<th>Coliforms</th>
<th>Enterococcus spp.</th>
<th>Klebsiella</th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td>53.3%</td>
<td>25.6%</td>
<td>10.7%</td>
<td>4.3%</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>68%</td>
<td>22%</td>
<td>8%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Figure 1: Bacterial pathogens causing UTI in children of different age groups
The percentage sensitivity of antibiotics against enterococci were 87% (20/23), 43.5% (10/23) and 34.8% (5/23) for nitrofurantoin, nalidixic acid and co-amoxiclav respectively. Ampicillin and ciprofloxacin were the least sensitive of tested antibiotics which denotes 14.4% and 18.6% respectively for any type of organisms. Figure 2 shows the cumulative percentages of sensitivities of each tested antibiotic against any type of organism.

**Discussion**

UTI is a common childhood bacterial infection and accounts for a considerable number of admissions to paediatric wards. The symptoms of UTI vary with age. As previously described by Gunasekara 2010, fever was the most commonly encountered symptom and found to be more evident in younger children (p = 0.04) in this study. Dysuria and abdominal pain were seen in older children (p = 0.001). These findings could be used in developing clinical criteria for the diagnosis of UTI in children of different age groups.

Coliforms were the most commonly isolated organisms in the current study. However, the isolation rate of 60.8% in the current study is less than the previously described. Enterococcus spp. was the second most common organism in contrast to previous studies where Klebsiella spp. were identified as the second common infecting organism. It must be noted that although Klebsiella spp. are also ‘coliforms’, they are often reported independently and are therefore considered in a separate category in the current study.

The enterococci are considered intrinsically resistant to many commonly used antimicrobial agents. The enterococci have emerged as an important health care associated infectious agent in recent times. Although the children in the study sample did not have a history of prolonged hospital stay or other risk factors for colonization with enterococci, 23.7% of UTIs in the present study were due to enterococci. The authors believe this may be a negative outcome of the over usage of antibiotics in the community, producing more enterococci with intrinsic and acquired antibiotic resistance. This may need further evaluation in order to prevent more complicated infections with resistant organisms in the future.
Although co-amoxiclav is the most commonly prescribed antibiotic for empirical treatment of UTI in children, only 52.5% of coliforms and 34.8% of enterococci in the study sample were sensitive to this drug. Sensitivity to co-amoxiclav had shown a dramatic reduction from 86.6% reported by Abeygunawardane et al. to 52.5% in the current study. The two studies were done in different locations, Peradeniya and Ragama respectively. No similar studies done in the same study site on a paediatric population was available for comparison. Studies done in adult populations with UTI has detected similar reduction of the susceptibility to antibiotics in Sri Lanka.

A reduction of antibiotic sensitivity is observed all over the world. Overuse, misuse of antibiotics and growing genetic diversity of the organisms are the possible causes for this. Misuse of antibiotics has been identified as a global crisis since it has given rise to various issues such as increase in antibiotic resistance and spread of *Clostridium difficile* infections. Discovery of new antibiotics that can combat emerging resistance has been very slow and ineffective.

Collection of the data on antibiotic sensitivity at national and international levels is important to make stakeholders aware of the extent of the problem of antibiotic resistance. Strengthening research on newer drugs is suggested to overcome the crisis of antibiotic resistance. Some countries have reported a reduction in the misuse of antibiotics following interventions including establishment of guidelines for antibiotic use.

**Conclusions**

The results of this study emphasize the need for regular surveillance of organisms causing UTI and their antibiotic sensitivity. This information, both local and national, is essential to provide appropriate guidelines for empirical treatment of childhood UTI.

**Conflict of interest:** The authors declare that there are no competing interests.

**Ethics statement:** Approval for this study was obtained from the Ethics Review Committee of the College of Paediatricians, Sri Lanka.

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Research article

Soil-transmitted helminth infections, associated factors and nutritional status in an estate community in Sri Lanka

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Abstract

Objectives: To determine the prevalence, intensity, and risk factors associated with soil transmitted helminth (STH) infections and to explore the association between STH infections and nutritional status of an estate community.

Methods: A cross-sectional study was conducted in Hanthana Tea Estate (HTE) in Kandy, Sri Lanka, from September 2013 to November 2014. Demographic data were obtained using an interviewer-administrated structured questionnaire. Haemoglobin and serum albumin concentrations were measured in children. Faecal samples were analyzed by direct smears in saline and iodine and Kato-Katz technique using single-stool samples. Anthropometric measurements were obtained to calculate weight-for-age (WAZ), height-for-age (HAZ), and body-mass-index-for-age (BAZ) to evaluate underweight, stunting and wasting, respectively.

Results: A total of 233 children (50% female, aged between 1 and 12 years, mean age 6.2±3.4) and 98 parents (93% female, aged between 20 and 52 years, mean age 33±6.2 years) participated in this study. The prevalence of STH infections in children and adults were 27.4% and 14.3% respectively. Ascaris lumbricoides was found in children and adult populations predominantly (26.6% and 14.3% respectively) followed by Trichuris trichiura (0.8% and 1%).

Of the infected group, 57.8% of children and 92.8% of adults had a light infection. Moderate infection was found in 40.6% of the children, and 7.2% of the adults. Only one child had a heavy egg count (≥ 50000). The prevalence of STH infections was significantly higher among children than adults (p = 0.014). Not hand washing before a meal (p = 0.002) and after defecation (p < 0.001), greater de-worming period (p < 0.001), use of shared latrine facilities (p = 0.023) and lower levels of mother’s education (p= 0.035) were significantly associated with STH infections. Children with and without STH infection had comparable levels of nutritional indicators. However, 17.6% (n=41) of stunted, 19.3% (n=45) of wasted and 39.5% (n=92) of underweight

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children were identified in the present study. In addition, 20.2% (n=47) of the children had a low serum albumin level and 15.9% (n=27) had anaemia.

**Conclusions:** Prevalence of STH infections was notably high in both children (27.4%) and their parents (14.3%), indicating a high level of morbidity among the study population. Therefore, a coordinated control and prevention programme considering the diverse socio-demographic characteristics of estate communities is needed to eliminate STH infections in the study population.

**Keywords:** Soil-transmitted helminth infections, Ascaris lumbricoides, Trichuris trichiura, Intensity, Prevalence, Anthropometric measurements

**Introduction**

Soil-transmitted helminthiasis (STH) is one of the most common neglected tropical diseases (NTDs) in the world and the poorest communities are most affected.\(^1,2\) Approximately one third of the world's population is infected with STH.\(^3,4,5\) The current level of infection for ascariasis, trichuriasis and hookworm in South Asia is 29%, 24% and 23%, respectively.\(^2\) STH infections still remain endemic in many parts of the world including Sri Lanka, despite several comprehensive efforts made by respective governments and non-governmental organizations, to control these neglected tropical diseases.\(^2\) Unfortunately, STH infections have become a normal and inevitable part of one’s life in low-income communities of countries where it is endemic.\(^6,7\) However, Salam et al. (2014)\(^8\) suggested that “community based interventions are effective in reducing the prevalence and intensity of STH”.\(^8\)

The most common STH are *A. lumbricoides*, *T. trichiura*, *Necator americanus* and *Ancylostoma duodenale*.\(^3,4\) *A. lumbricoides* and *T. trichiura* are mainly transmitted by the ingestion of viable eggs in contaminated food and/or water.\(^9,10,11\) The infective larval stages of hookworms are found freely in the soil and enter the human body by skin penetration.\(^12\) Limited access to clean water, poverty, a lack of education, poor sanitary facilities and personal hygiene are major risk factors associated with the STH infections.\(^13\) Previous studies have shown that hookworm infections are common among agricultural workers in China, Vietnam and tea estate workers in Sri Lanka, Bangladesh and India.\(^14,15,16,17\) STH infections may contribute to social marginalization, malnutrition and economic instability in societies, particularly in developing nations, and a large number of children are more susceptible to STH infections than adults.\(^18,19\) The most common clinical features of STH infections in children are anaemia, stunted growth, fatigue, protein-calorie malnutrition (PCM), and poor cognitive development.\(^6,7\)

The plantation sector in Sri Lanka, with a population of 939,000, represents significantly low-income families in the country, with a high prevalence of child malnutrition and mortality in mothers.\(^20\) According to a survey conducted in 1992, 90% of children living in these areas were at risk of acquiring STH infections.\(^21\) Poor household hygiene and sanitation and poor level of maternal education were identified as risk factors associated with STH infections of the plantation sector in Sri Lanka.\(^22,23\) A mass deworming campaign commenced in 1994, which included children aged 3-18 years. The campaign continued for 11 years up to 2005. In 2011, a survey was conducted in the plantation sector communities to identify the prevalence of STH
infection. It showed 29% prevalence despite the mass de-worming campaign conducted up to 2005. The need for well planned control strategies to eradicate STH infection was emphasized.

A national survey conducted in school children in 2003, representing regions other than the plantation sector, identified only 6.9% of the population as having STH infections. However, in our opinion, this does not reflect the actual situation in the country at large, since several other studies have shown that STH infections are still endemic, in poor, urban and plantation sector communities in Sri Lanka. The need for well planned control strategies to eradicate STH infection was emphasized.

The main focus of the present study was to determine the prevalence and intensity of STH infections in the Hanthana Tea Estate (HTE) in Kandy, Sri Lanka. In addition, the relationship between STH infections and the nutritional status of children in this estate was investigated. The findings of the present study will be helpful in implementing new strategies to control STH infections in the plantation sector in Sri Lanka.

**Materials and methods**

**Study population**

The present study was conducted from September 2013 to November 2014 in the Hanthana Tea Estate (latitude and longitude coordinates: 7° 20’-7° 29’N and 80° 61’- 80° 64’E) in Kandy, Sri Lanka. This mountainous region (1130.38 hectares) is located 600m-1100 m above sea level with an estimated population of 5511 (National Census 2012: http://www.statistics.gov.lk/). In general, the socioeconomic status of people living in this estate is very low, compared to urban communities. These families live in heavily crowded houses with limited space, resources and poor sanitary facilities. The majority of residents, including children, often go to nearby jungles for defecation due to lack of latrine facilities for the study population. Major sources of drinking water were streams and unprotected wells. Most of the residents were employed in tea estates, factories and shops as unskilled laborers.

HTE consists of 7 divisions, namely, Factory division, West division, Top division, Middle division, Urawala division, Uduwela division and Kithulmulla division. Factory and West divisions were selected using the cluster sampling method considering divisions as clusters and each division was given a number from 1 to 7. Two numbers were selected randomly using a random number generator. The sample size was determined using a formula of \( n = \frac{Z^2 \cdot P \cdot (1-P)}{d^2} \). 5% margin of error (d) and 95% confidence interval was used for the calculation. Prevalence (P) of STH infections among children in the plantation sector in Kandy was considered as 23%. The calculated sample size was 272. All children aged between 1 to 12 years (335) and their parents (140) from the selected two divisions were included in the study. Children <1 year and ≥ 13 years were excluded from the study.

**Data collection**

All parents/guardians of the West and Factory divisions were summoned to a common place prior to collection of samples and methods and implications of the research were explained to them with the help of estate administrators and medical authorities. The demographic and socioeconomic data (gender, age, family members, mother’s education attainment, occupation, and household income), sanitary facilities, living conditions and behavioral characteristics (Floor
type, latrine facilities, water source, hand washing, drinking unboiled water, sucking fingers and wearing shoes) and the history of deworming were obtained from children using an interviewer administrated and structured questionnaire. These variables/data relating to parents were not assessed. The questionnaire was prepared in their native language to enable correct understanding of the purpose of the study.

**Evaluation of nutritional status**

The weight and height of the children were measured using a calibrated digital electronic balance and a height pole, respectively. All measurements were recorded on a pre-prepared data sheet. Adults were not enrolled to take the anthropometric measurements. Weight and height were measured twice to minimize errors. Average values were used for the final calculation. These measurements (age, weight and height) were used to calculate the following parameters, Height-for-age Z-score (HAZ), Weight-for-age Z-score (WAZ) and Body-mass-index-for-age Z-score (BAZ) to assess stunted growth, underweight and wasting, respectively. AnthroPlus version 1.0.4 (WHO, Geneva, Switzerland) and the Epi Info software 3.5.1 (CDC, USA) were used to calculate each indicator with the international reference values given by the WHO (World Health Organization: [http://www.who.int/](http://www.who.int/)).

**Collection of stool and identification of parasites**

Each participant was given a clean, wide mouthed labelled plastic container with a lid and a spoon. A single stool sample was collected from each participant. Stool samples were then transported in a cool box to the Department of Parasitology, Faculty of Medicine, University of Peradeniya, for laboratory investigation. First, direct smears were observed in saline and iodine. Then, the Kato-Katz technique was performed as recommended by WHO.28,29 After that, the slides were observed under a light microscope, and the number of eggs were counted for each parasite. Finally, the number of eggs per gram of faeces (epg) was determined. The intensities of infection were grouped as follows into light, moderate, or heavy infections, according to WHO guidelines as follows, for “A. lumbricoides, 1–4,999 epg, 5,000 – 49,999 epg and ≥50,000 epg; for T. trichiura, 1–999 epg, 1,000–9,999 epg and ≥10,000 epg; and for hookworms, 1–1,999 epg, 2,000–3,999 epg and ≥4,000 epg”, respectively.30

**Collection of blood and measurement of albumin and haemoglobin levels**

Two to three milliliters of blood was collected into labeled EDTA tubes using disposable syringes. The blood samples were stored in a cool box until transported to the laboratory for further investigation. Haemoglobin concentrations were measured in a private medical laboratory in Kandy using an Auto Hematology Analyzer. WHO recommended haemoglobin levels in different age categories were used to identify anaemia in children.31 Serum albumin level was measured using a commercial kit (Liquicolor, Human, Germany) according to the manufacturer’s instructions. The reference value of the serum albumin level was provided by the manufacturer (3.8-5.1 g/dl or 38-51 g/l).

**Statistical analyses**

SPSS (Statistical Package for the Social Sciences) version 17 (SPSS, Chicago, IL, USA) was used for the statistical analysis. Descriptive data were used to explain the distinctiveness of the study population.
Firstly, all variables were analyzed using the univariate model to determine the relationship between the dependent variable (prevalence of STH), and the independent variables (socio-demographic, health hygiene behavior, deworming treatment, living and sanitary condition characteristics).

Secondly, significantly associated variables identified by the univariate model were included in a multivariate logistic regression analysis using forward step-wise elimination model to determine the risk factors of STH infections.

In addition, multiple regression analysis was done to assess the predictive effect of the factors associated with the intensity of infection. For nutritional status, differences in proportions for categorical variables (e.g., stunting, thinness, underweight, anaemia and hypoalbunaemia) were calculated using Chi square test of independence. Differences in mean values of continuous variables (e.g., HAZ, WAZ, BAZ, Hb and albumin) for infection status (negative and infected) were assessed using the student t-test. One-way ANOVA was used to analyze differences in anthropometric mean Z-scores of the study population for the intensity of infections (negative, light, moderate and heavy). The \( p \)-value \( \leq 0.05 \) was considered to be statistically significant. Odds ratio (OR) and 95% confidence interval (CI) were calculated for logistic regression analysis.

**Results**

*Characteristic of the study population and sample collection (Figure 1)*

Of the 7 divisions, 2 divisions (Factory division, and West division) were selected for the study. 335 children and 140 parents were included in the study (180 children and 77 parents from the Factory division and 153 children and 63 parents from the West division). Written consent was obtained only from mothers and/or guardians. 102 children and 42 parents refused to provide faecal samples due to unknown reasons. The mean age of children was 6.2 years (SD=3.4) and 117 (50%) were females. Parent ages ranged from 20 to 52 years (mean age was 33 years (SD=6.2)), of whom 91 (93%) were females. 233 (69.55%) and 98 (70%) faecal samples were collected from children and from their parents and/or guardians, respectively. In addition, 233 blood samples (2-3 ml) were collected from children to measure the haemoglobin and serum albumin levels.
Prevalence of STH infections

Of the 233 children, 64 (27.47%) children were infected with STH. Of the STH infected children, the majority (26.6%) were infected with *A. lumbricoides* followed by *T. trichiura* (0.86%). None of the children had mixed infections. The prevalence in adults was 14.6% for *A. lumbricoides*. Mixed infection (*A. lumbricoides* and *T. trichiura*) was detected in one adult only. Among children, the highest prevalence (39.5%) was reported in the 4 to 6 year age group. In adults, 18 to 26 year age group had the highest (16.7%) prevalence. A significantly higher prevalence of STH infections was reported among children than in adults (*p*< 0.05) (Table 1).

Table 1 Prevalence of STH infections in children and parents

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Children (n = 233)</th>
<th>Parents (n = 98)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em> (AL)</td>
<td>62 (26.6%)</td>
<td>14 (14.3%)</td>
<td>-</td>
</tr>
<tr>
<td><em>Trichuris trichiura</em> (TT)</td>
<td>2 (0.86%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mixed infection (AL+TT)</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>64 (27.46%)</td>
<td>14 (14.3%)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Intensity of STH infections

Of the infected group, 57.8% (37/64) of children and 92.8% (13/14) of adults had a light infection (epg<5000). Moderate infection (epg =5000-49999) was observed in 40.6% of children (26/64) and in 7.2% (1/14) of adults. Only one child had a heavy epg count (≥ 50000) (Table 2).

<table>
<thead>
<tr>
<th>Age (years) and sex</th>
<th>Number of eggs per gram (epg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light (epg &lt; 5000)</td>
</tr>
<tr>
<td>Children</td>
<td></td>
</tr>
<tr>
<td>1 - 3 (n = 65)</td>
<td>11</td>
</tr>
<tr>
<td>4 - 6 (n = 63)</td>
<td>14</td>
</tr>
<tr>
<td>7 - 9 (n = 64)</td>
<td>6</td>
</tr>
<tr>
<td>10 - 12 (n = 41)</td>
<td>6</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>18-26 (n = 24)</td>
<td>4</td>
</tr>
<tr>
<td>27-35 (n = 44)</td>
<td>7</td>
</tr>
<tr>
<td>36-44 (n = 23)</td>
<td>2</td>
</tr>
<tr>
<td>45-52 (n = 7)</td>
<td>-</td>
</tr>
<tr>
<td>Sex (children)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
</tr>
<tr>
<td>Sex (adults)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>-</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
</tr>
</tbody>
</table>

STH infections and associated factors in children

Table 3 shows the socio demographic, personal hygiene and other factors, which may potentially be associated with STH infections. Females had a slightly higher rate of infection than their male counterparts. Children aged between 1-6 years showed a higher prevalence (29.7%) than older children (24.7%), though not statistically significant.

Univariate analysis was used to analyze the risk factors associated with STH infections in this community (Table 3). There were five risk factors identified which included those who use shared latrine facilities (OR =2.53; 95% CI =1.36-4.70; p = 0.003), getting deworming treatment before 6 months (OR =4.39; 95% CI = 2.36-8.16; p<0.001), mothers’ education level less than grade 8 (OR =3.23; 95% CI = 1.68-3.20; p<0.001), rarely washing hands with soap before a meal (OR =2.94; 95% CI = 1.31–6.62; p = 0.009) and after defecation (OR= 3.43; 95% CI = 1.86–6.33; p<0.001), although, being female (29.1%; p = 0.585), aged between 1 to 6 years (29.7%; p = 0.402), having more than 6 family members (32.4%; p = 0.266), low household income
(31.3%; \( p = 0.058 \)), having earthen floors in households (35.3%; \( p = 0.271 \)), using unprotected spring water (27.7%; \( p = 0.796 \)), drinking boiled water (36.1%; \( p = 0.209 \)), eating unwashed fruits (28.9%; \( p = 0.700 \)), sucking fingers (35.5%; \( p = 0.057 \)) and walking barefooted (31.2%; \( p = 0.130 \)) were factors that also showed higher infection rates. However, none of these variables were statistically significant.

Table 3 Risk factors associated with STH infections in children (Univariate analysis, \( n = 233 \))

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>No. examined</th>
<th>No. of positives</th>
<th>Percentage (%)</th>
<th>OR (95% CI)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>116</td>
<td>30</td>
<td>25.9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>117</td>
<td>34</td>
<td>29.1</td>
<td>1.17 (0.66-2.90)</td>
<td>0.585</td>
</tr>
<tr>
<td>Age</td>
<td>1 – 6</td>
<td>128</td>
<td>38</td>
<td>29.7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 – 12</td>
<td>105</td>
<td>26</td>
<td>24.7</td>
<td>0.78 (0.44-1.40)</td>
<td>0.402</td>
</tr>
<tr>
<td>Number of family members</td>
<td>1 – 6</td>
<td>162</td>
<td>41</td>
<td>25.3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 6</td>
<td>71</td>
<td>23</td>
<td>32.4</td>
<td>1.41 (0.77-2.60)</td>
<td>0.266</td>
</tr>
<tr>
<td>Mothers educational level</td>
<td>&gt; Grade 8</td>
<td>99</td>
<td>16</td>
<td>16.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \leq ) Grade 8</td>
<td>134</td>
<td>48</td>
<td>35.8</td>
<td>3.23 (1.68-6.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>De-worming treatments</td>
<td>( \leq ) 6 months</td>
<td>167</td>
<td>31</td>
<td>18.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 6 months</td>
<td>66</td>
<td>33</td>
<td>50.0</td>
<td>4.39 (2.36-8.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Household income (Rs./month)</td>
<td>( \leq ) 16000</td>
<td>160</td>
<td>50</td>
<td>31.3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 16000</td>
<td>73</td>
<td>14</td>
<td>19.2</td>
<td>0.52 (0.27-1.02)</td>
<td>0.058</td>
</tr>
<tr>
<td>Household characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floor type</td>
<td>Earthen</td>
<td>34</td>
<td>12</td>
<td>35.3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cement</td>
<td>199</td>
<td>52</td>
<td>26.1</td>
<td>0.65 (0.30-1.40)</td>
<td>0.271</td>
</tr>
<tr>
<td>Toilet facility</td>
<td>Separate</td>
<td>171</td>
<td>38</td>
<td>22.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shared</td>
<td>62</td>
<td>26</td>
<td>41.9</td>
<td>2.53 (1.36-4.70)</td>
<td>0.003</td>
</tr>
<tr>
<td>Water source</td>
<td>Unprotected spring water</td>
<td>213</td>
<td>59</td>
<td>27.7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wells</td>
<td>20</td>
<td>5</td>
<td>20.0</td>
<td>0.87 (0.30-2.50)</td>
<td>0.796</td>
</tr>
<tr>
<td>Eating and sanitation habits</td>
<td>Hand washing with soap before meals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Every time</td>
<td>58</td>
<td>8</td>
<td>13.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>175</td>
<td>56</td>
<td>32.0</td>
<td>2.94 (1.31-6.62)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Hand washing with soap after defecation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Every time</td>
<td>123</td>
<td>20</td>
<td>16.3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>110</td>
<td>44</td>
<td>40.0</td>
<td>3.43 (1.86-6.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Drink unboiled water</td>
<td>Yes</td>
<td>197</td>
<td>51</td>
<td>25.9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>36</td>
<td>13</td>
<td>36.1</td>
<td>1.62 (0.76-3.43)</td>
<td>0.209</td>
</tr>
<tr>
<td>Eat unwashed fruits</td>
<td>Yes</td>
<td>90</td>
<td>26</td>
<td>28.9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>143</td>
<td>38</td>
<td>26.6</td>
<td>0.89 (0.50-1.60)</td>
<td>0.700</td>
</tr>
<tr>
<td>Sucking fingers</td>
<td>Yes</td>
<td>76</td>
<td>27</td>
<td>35.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>157</td>
<td>37</td>
<td>23.6</td>
<td>0.56 (0.31-1.02)</td>
<td>0.057</td>
</tr>
<tr>
<td>Barefoot</td>
<td>Yes</td>
<td>138</td>
<td>43</td>
<td>31.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>95</td>
<td>21</td>
<td>22.1</td>
<td>0.62 (0.34-1.15)</td>
<td>0.130</td>
</tr>
</tbody>
</table>

Multivariate analysis using forward logistic regression model revealed that shared latrine facilities were 2.5 times (95% CI = 1.13–5.32; \( p = 0.023 \)), getting deworming treatment before 6 months had 5 times (95% CI = 2.28–11.14; \( p < 0.001 \)), mothers’ education level less than grade 8 had 2.4 times (95% CI = 1.06–5.58; \( p = 0.035 \)), rarely washing hands with soap before a meal
was 5.7 times (95% CI = 2.12–15.24; \(p = 0.002\)) and after defecation was 6 times (95% CI = 2.73–13.15; \(p < 0.001\)) more prone to STH infections respectively (Table 4).

Table 4 Multivariate logistic regression analysis of risk factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>OR (95% CI)</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toilet facility</td>
<td>Separate</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shared</td>
<td>2.45 (1.13-5.32)</td>
<td>0.023</td>
</tr>
<tr>
<td>De-worming treatments</td>
<td>(\leq 6) months</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 6 months</td>
<td>5.04 (2.28-11.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hand washing with soap before meals</td>
<td>Every time</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>5.68 (2.12-15.24)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hand washing with soap after defecation</td>
<td>Every time</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>5.99 (2.73-13.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mothers educational level</td>
<td>&gt; Grade 8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\leq ) Grade 8</td>
<td>2.44 (1.06-5.58)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

OR: odd ratio; CI: confidence interval

**Nutritional status of children**

Table 5 shows the assessments of nutritional indicators (HAZ, WAZ, BAZ, Hb and serum albumin) in our study population. Most of the children’s nutritional status was within the WHO recommended level. However, 17.6% (\(n=41\)) of stunted, 19.3% (\(n=45\)) of wasted and 39.5% (\(n=92\)) of underweight children were identified in the present study. In addition, 20.2% (\(n=47\)) of children had a low serum albumin level and 15.9% (\(n=27\)) had anaemia.

**Associations between STH infections and nutritional status**

The relationship between STH infections and the nutritional status of children was also assessed using an independent sample t-test. It was shown that the mean values for WAZ, HAZ, BAZ, haemoglobin and serum albumin were not significantly associated with children who are either STH negative or positive (\(p > 0.05\)). Therefore, we suggest that there was no significant association between nutritional status and STH infections in the study population.
Discussion

Studies conducted in the 1980’s and 1990’s, showed a 100%, 95% and 89.7% prevalence of STH infections in urban slum communities in Colombo (1981), Galle (1989) and plantation sector (1994), respectively.21,32,33 The results of the present study showed a much lower prevalence of STH infections compared to that of previous studies. However, in comparison with recent studies, the prevalence of STH reported in this study was higher than in the urban and rural areas, while it was lower than that in other plantation sectors in Sri Lanka.22,23,24,34,35,36 One to 6 year children showed a slightly higher prevalence (29.7%) than 7 to 12 year old children (24.7%). This difference may be due to various reasons including a lack of awareness, increased soil related activities of younger children than adults, and the prevailing poor health and personal hygiene in the plantation sector.

A. lumbricoides is the most predominant STH found in both children and adult populations. The close environment of the inhabitants of the study area may be heavily contaminated, especially by children with infected faeces, due to defecation in open areas and close to the line houses. The
eggs of *Ascaris* are more resilient and can remain infective for years in the soil. Children are therefore more likely to be infected when playing on these contaminated grounds. Soil samples were not checked for the presence of parasitic eggs in the current study.

*Trichuris* eggs were found only in 2 children and 1 adult. Similar low prevalence was reported by Gunawardena et al. (2011) in school children in the plantation sector in Sri Lanka. A low prevalence has been shown in children living in rural communities of Pakistan as well. Eggs of *T. trichiura* are not resistant to cold, drought and direct sunlight compared to *Ascaris* eggs which may be one of the reasons for the very low prevalence of *Trichuris* infection. Further investigation using a larger sample size may be indicated.

In the current study, hookworm infection was not detected in children and adults. Similar results were reported in previous studies in the Kandy area. Hookworm is not a common backyard infection, requiring shady, sandy and moist soil for its further development. The area surrounding the study participants homes were not shady which could be a contributory factor for the low detection. Gilgen et al. (2001) have previously reported a high rate of hookworm infection among tea estate workers and among their family members. Periodic deworming, improved sanitation and hygiene since 2001 may also have contributed to a decline in hookworm infection in the plantation sector.

Table 3 shows the intensity of *A. lumbricoides* infection in children and adults. Both light and moderate infections were high in children. A heavy intensity was reported in one female child. Our results indicate that the intensity of *A. lumbricoides* infection gradually decreased with age. This might be due to an improvement in hygienic practices. Similar results were reported in adolescent school girls in Sri Lanka.

Analysis of the factors associated with STH infection in children showed that age, gender, number of family members, socioeconomic status, type of drinking water, sucking fingers, walking barefooted, source of drinking water and eating unclean fruits were not significantly associated with STH infection. Although these variables may not have a direct relationship with STH infections, these findings should be further investigated using a larger sample size to get a clearer understanding of their role in these infections. Low maternal education, infrequent hand washing using soap before meals and after defecation, shared latrine facilities and longer periods between de-worming were significantly associated with STH infection. Several studies have shown that poor maternal education is a risk factor associated with STH infection. Generally, a mother plays a significant role in the health education of their children. In this study, we found that the mother’s awareness of STH was very poor. It may be a contributing factor to the high prevalence of STH in children. A shared poor quality latrine can also lead to STH infection among children. Previous studies carried out in Sri Lanka, in Nepal, and in Pakistan have shown similar results.

Nutritional indicators (HAZ, WAZ, BAZ, and haemoglobin and serum albumin levels) of children were also analyzed in the present study. The findings were compared with the general data of the country and with the WHO reference values.
Nutrition and growth status of many children were within the normal ranges. However, there were many under-weight children in the study group (39.5%). The Sri Lankan National Nutrition and Micronutrient Survey conducted in 2012 showed that 13.1% children aged between 6 to 59 months were stunted, 19.6% wasted and 23.5% underweight. In addition, the same survey reported that 15.7%, 20.2% and 24.7% were stunted, wasted and underweight respectively in the Kandy district. A comparison between the country and district data reported that the prevalence of stunting and underweight was significantly higher in our study population.

The prevalence of anaemia in children was 15.9%. This figure was slightly higher than the country’s average (15.1%) but lower than the district average (16.9%). A reasonably high prevalence of the thalassemia traits can be a reason for the high prevalence of anaemia, but we could not find any evidences to prove it. In addition, 20.2% of children showed hypoalbuminaemia. There was no significant relationship between nutritional status and STH infections in children. This is probably due to widespread poverty in the population living in the tea plantation areas in Sri Lanka.

The Kato-Katz method was used to determine the intensity of the infection which varied from light to moderate. Limitations of the study include non-use of the concentration technique to detect STH and examining only one stool sample per participant, both of which could increase the detection of STH.

The nutritional status and socio-demographic features in this community was studied for the first time.

Conclusions and recommendations

The findings of this study contribute to the epidemiological data of STH infections which could be used to plan an effective control programme. Prevalence of STH infections was notably high in both children and their parents, indicating a high level of transmission. Providing single houses instead of line houses, health education and the improvement of sanitary facilities are essential for the effective control of STH infections in the study population. Therefore, coordinated control and prevention programmes considering the diverse socio-demographic characteristics of estate communities is needed to eliminate STH infections in the study population. Government and local authorities should pay more attention to improving not only the health, housing and sanitary facilities of these marginalized communities, but also, their household income as a priority.

Acknowledgements: The authors are very grateful to Mr. N.L.S. Wijesundara, Mrs. D.R.L.N. Bandara, and other technical staff of the Department of Parasitology, Faculty of Medicine, University of Peradeniya for providing technical assistance. We would like to thank the management of the estate medical authorities, welfare officers and other healthcare workers from the respective estates for their technical assistance given during the study. Furthermore, the authors would like to express our deepest gratitude to all the children and their parents/guardians who have voluntarily participated in this study.
Funding: Financial assistance given by the Faculty of Medicine Research Grant (RG/EF/2013/11) is acknowledged.

Competing interests: The authors declare that they have no competing interests.

Ethics approval: Ethical approval was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Peradeniya, Sri Lanka. Written informed consent was obtained from parents and/or legal guardians of children before collecting data, blood and faecal samples. In addition, written permission was obtained from the estate authorities. All data were kept strictly confidential.

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Epidemiology of invasive infections caused by vancomycin sensitive and resistant enterococcal strains among oncology patients at the National Cancer Institute of Sri Lanka from 1st of July 2012 to 31st of July 2013

LS Athukorala¹, CGUA Patabendige², PJ Arumapperuma³

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Abstract

Introduction and Objectives: Enterococci have traditionally been regarded as low grade pathogens but have emerged as an increasingly important cause of nosocomial infections in the last decade. While the Enterococcus faecalis remains the predominate species in clinical infection, Enterococcus faecium isolates are increasing in proportion. This study was carried out to describe the epidemiology of invasive enterococcal infections among oncology patients at the National Cancer Institute of Sri Lanka (NCISL).

Methods: 60 patients with invasive enterococcal infections, who were treated as inward patients at the National Cancer Institute of Sri Lanka from 1.7.2012 to 31.7.21013 whose samples were sent for microbiological investigation were included in this study. Speciation of the isolates was done by using a rapid manual analytic system (RapID STR panel-Oxoid). Vancomycin sensitivity was assessed in all enterococcal isolates by disc diffusion method, agar dilution screening method (CLSI guideline 2013)¹⁶ and detection of minimum inhibitory concentration (MIC) by using Vancomycin gradient strips. Teicoplanin MIC was assessed only in vancomycin resistant isolates using teicoplanin gradient strips. Associated factors for getting an infection with vancomycin resistant strains were assessed using a data extraction sheet.

Results: The incidence of invasive enterococcal infections among oncology patients was 1.1 per 1000 admissions. The incidence of invasive enterococcal infection caused by vancomycin resistant species was 0.16 per 1000 admissions. E. faecium was the dominant species causing invasive enterococcal infections (55%). Using the gradient strip method for determination of vancomycin MIC as the gold standard, the screening agar dilution method had 100% sensitivity and specificity and the disc diffusion test had a low specificity (77.8%). Almost all the participants (96.6%) had acquired the enterococcal infection from the hospital. Vancomycin resistant infections were more common in patients who had haematological malignancies, who were treated with 3rd generation cephalosporins and cytotoxic chemotherapy drugs.

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Conclusions: *E. faecium* is the dominant species causing invasive enterococcal infections in oncology patients (55%) and phenotypically compatible with the VanA phenotype of glycopeptide resistant enterococci.

Keywords: Enterococci, vancomycin, resistant, oncology, chemotherapy

**Introduction and Objectives**

Although about a dozen *Enterococcus* species have been identified, *E. faecalis* and *E. faecium* are responsible for most human infections. Historically, the ratio of infections due to *E. faecalis* to those due to all other *Enterococcus* species was approximately 10:1. In recent years, there has been a progressive decline in this ratio. This microbiologic shift is likely to be explained in part by the emergence of vancomycin resistant enterococci (VRE) and *E. faecium* being the dominant species identified among VRE.

The most common nosocomial infections caused by these organisms are urinary tract infections followed by intra-abdominal infections, pelvic infections, surgical wound infections, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis. A major reason why these organisms survive in the hospital environment is due to a high level of intrinsic resistance to many antibiotic groups (β lactams, low concentrations of aminoglycosides, nalidixic acid, clindamycin, fluoroquinolones and trimethoprin-sulfamethoxazole). They are also able to acquire resistance to many commonly used antibiotics (glycopeptides, tetracyclines, erythromycin, fluoroquinolones, rifampicin, chloramphenicol, fusidic acid, nitrofurantoin).

Vancomycin resistant enterococci are classified according to their phenotype using vancomycin and teicoplanin MIC and genotype using the specific ligase gene. There are 5 recognized phenotypes (VanA, VanB, VanC, VanD, VanE) and 7 genotypes (VanA, VanB, VanC, VanD, VanE, VanG, VanL). Motile enterococci, *E. gallinarum* and *E. casseliflavus* have an intrinsically low level of resistance to vancomycin and belong to the VanD genotype. These two species have been identified as a clinically significant cause of bacteraemia in immunocompromised patients. Most of the risk factors for infection with VRE, namely immunosuppression, advanced age, other co-morbidities such as renal and liver failure, invasive procedures and devices and gastrointestinal surgery are present in oncology patients. Use of third generation cephalosporins, carbapenems and glycopeptides are also regarded as risk factors for acquisition of VRE and other glycopeptide resistant enterococci. These antibiotics are frequently used as empirical treatment for febrile neutropenia in oncology patients (NCISL antibiotic policy). No study has been done in Sri Lanka to describe the epidemiology of vancomycin sensitive and resistant enterococcal infection in oncology patients or screening of patients to evaluate the prevalence of colonization with VRE. This study was therefore carried out to describe invasive enterococcal infections caused by vancomycin sensitive and resistant strains among oncology patients at the National Cancer Institute of Sri Lanka.

The objectives of the current study were to describe the following characteristics of *Enterococcus spp.* isolated from patients with invasive infections in hospitalized oncology patients at National Cancer Institute, Sri Lanka.
a. Determining vancomycin sensitivity  
b. Speciation of isolates  
c. Identifying factors associated with VRE infections  
d. Assess teicoplanin sensitivity of VRE isolates.

Methods

This study was conducted as a descriptive cross sectional study from 1.7. 2012 to 31.7.2013.

Sixty patients with invasive enterococcal infections were included in this study. A case of invasive enterococcal infection was defined by the isolation of Enterococcus spp. from a normally sterile body site. Sterile sites include blood, peritoneal aspirates, pus aspirates from abscesses and sterile body cavities, aspirate from biliary tract, pleural aspirates and cerebrospinal fluid. Urine isolates were also included if there was evidence of pyelonephritis. Nosocomial infection was defined as infection obtained >72 hr. after hospital admission.

The sample size of patients with invasive enterococcal infection necessary to obtain the required precision (0.3) and confidence (95%) level was 60.

A pilot study was done from 1.1.11 to 31.7.2011 using laboratory work sheets to determine the prevalence of invasive enterococcal infection in oncology patients at NCISL and prevalence was calculated as 0.7%. Using this prevalence, the required sample size of VRE to obtain a precision of 0.3 and confidence level of 95% was determined to be 60 which could be expected from a total sample size of 8570. During the study period 9652 samples were processed which included 4051 blood cultures, 4652 urine cultures, 855 pus aspirates, 35 pleural fluid samples, 36 peritoneal fluid samples and 23 cerebrospinal fluid samples.

Enterococcal blood stream infection was defined as the isolation of an Enterococcus spp from one peripheral blood culture sample. When blood cultures from both central catheter and peripheral site were collected at the same time, if blood culture through the catheter became positive two or more hours before peripheral blood culture, it was considered as catheter associated blood stream infection. Enterococcal bacteremia occurring >60days after a previous episode was considered to be a separate blood stream infection. In urine cultures, a pure growth of ≥10⁵ colony forming units (CFU)/ml in patients with symptoms suggestive of pyelonephritis (fever with chills and rigors with loin tenderness) or patients with ultra sound scan evidence suggestive of pyelonephritis only were included in this study.

Gram stain was performed on colonies which had growth on both blood and MacConkey agar (without salt) and which were catalase negative. In urine cultures, Gram stain was performed on lactose fermenting catalase negative colonies on CLED medium. Gram positive cocci in chains were Lancefield grouped. Lancefield group D isolates were further tested for growth on 6.5% NaCl, survival at 60 °C for 30 minutes, and hydrolyzing of L-pyrrolidonyl-b-naphthlamide (PYR) to identify enterococci. All isolates which showed growth on 6.5% NaCl, a positive result for PYR and survived 30 minutes at 60 °C were included in the study.⁹
Speciation of isolates was done using a rapid manual analytic system (Rapid STR panel-Oxoid) according to the manufacture instructions. Rap ID STR panels contain 10 reaction cavities for different biochemicals and test cavities seven through ten are bifunctional. In addition to these biochemical tests, haemolysis on blood agar plate was assessed according to the manufacturer’s instructions. The microcode obtained from the test kit was compared with the rapid STR code compendium for the identification. Evaluation of motility and pigment production was also done to identify motile and pigment producing enterococci.

Associated factors for getting an infection with VRE, namely prior administration of antibiotics and chemotherapy, invasive procedures and devices, gastrointestinal surgery, renal and liver failure, diabetes mellitus and mucositis, were assessed using a data extraction sheet. Data was obtained by reviewing medical records of each study patient.

Three methods, disk diffusion method using 30 μg vancomycin discs (Oxoid), agar dilution screening method and minimum inhibitory concentration (MIC) determination using gradient strip method (Oxoid vancomycin MIC Evaluator) were used to detect vancomycin susceptibility. The gradient strip method (Oxoid teicoplanin MIC evaluator) were used to detect MIC of teicoplanin on vancomycin resistant isolates. The disk diffusion test and the agar dilution screening methods were performed according to the recommendations of performance standards for antimicrobial susceptibility testing, clinical laboratory standard institute, M100, 2013 (CLSI, 2013). According to the CLSI guideline vancomycin zone diameter criteria were ≥17 mm - sensitive, 15-16 mm - intermediate and ≤14 mm - resistant. To detect vancomycin and teicoplanin MIC, the manufacturer’s instructions were followed and CLSI 2013 MIC interpretive criteria were used to interpret the vancomycin and teicoplanin susceptibility. Vancomycin MIC interpretive criteria were ≤4 μg/ml - sensitive, 8-16 μg/ml - intermediate and ≥32 μg/ml - resistant. Teicoplanin MIC interpretive criteria were ≤8 μg/ml - sensitive, 8-16 μg/ml - intermediate and ≥32 μg/ml - resistant. Controls used were E. faecalis ATCC 29212 and ATCC 51299.

To describe the phenotype of VRE, phenotypic classification originally described by Gold et al. was used. VanA isolates have high MICs of vancomycin (MIC range 64–1,000 μg/ml) and teicoplanin (MIC range 16–512 μg/ml), whereas VanB isolates often have lower MICs of vancomycin (MIC range 4–1,024 μg/ml but usually on the low side) but typically susceptible to teicoplanin. Van D isolates have moderate susceptibility to both glycopeptides (vancomycin MIC range 2–32; teicoplanin MIC usually <0.5 μg/ml) whereas VanC and VanE isolates display low level resistance to vancomycin (MIC range 0.5-4 μg/ml) and are susceptibility to teicoplanin.

**Results**

Of the 9652 samples, there were 60 clinically and microbiologically diagnosed patients with invasive enterococcal infections.

Mean age of the participants who were in the pediatric age group and non-pediatric age group are 4.92 years (SD=3.75) and 47.7 years (SD = 18.47) respectively (Table 1).
During the period of this study there were 12688 new registration of patients and total number of admissions was 54027. The incidence of invasive enterococcal infections among oncology patients was 1.1 per 1000 admissions. The incidence of invasive enterococcal infection caused by vancomycin resistant spp. was 0.16 per 1000 admissions.

Almost all the participants (96.6%) had acquired the enterococcal infection from the hospital (Table 2). The majority of the participants were admitted in the medical wards at the time of the positive culture. The highest proportion of vancomycin resistant infections were from pediatric ICU (23.3%) followed by medical wards (17.2%) and all were hospital acquired infections (Table 3).

### Table 1: Frequency distribution of study participants according to selected socio demographic characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number (n=60)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤12 years</td>
<td>13</td>
<td>21.7</td>
</tr>
<tr>
<td>&gt;12 years</td>
<td>47</td>
<td>78.3</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>36.7</td>
</tr>
<tr>
<td>Female</td>
<td>38</td>
<td>63.3</td>
</tr>
</tbody>
</table>

### Table 2: Association of vancomycin sensitivity of invasive enterococcal infections according to acquisition location

| Location | Vancomycin total | | | |
|----------|------------------| | | |
|          | sensitive | resistant | | |
| Hospital | 49  | 9  | 84.5 | 15.5 | 58 |
| Community | 02 | 0  | 100.0 | 0.0 | 2 |

### Table 3: Association of vancomycin sensitivity of invasive enterococcus infection with location at the time of positive culture

<table>
<thead>
<tr>
<th>Location</th>
<th>Vancomycin sensitive</th>
<th>Resistant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical ward</td>
<td>24</td>
<td>5</td>
<td>17.2</td>
</tr>
<tr>
<td>Pediatric ward</td>
<td>7</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>Surgical ward</td>
<td>10</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>Medical ICU</td>
<td>2</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Pediatric ICU</td>
<td>4</td>
<td>2</td>
<td>23.3</td>
</tr>
<tr>
<td>Surgical ICU</td>
<td>4</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Enterococci were isolated mainly from patients with UTI and with bacteraemia, with *E. faecalis* the predominant species isolated from these 2 sites as well as from pus aspirates (Table 4). 90% of infections were caused by *E. faecium* and *E. faecalis*. Almost all the infections caused by VRE were caused by *E. faecium* (88.9%). Vancomycin resistant *E. casseliflavus* was isolated from a pus aspirate of a pelvic abscess (Table 5 and 6).

### Table 4: Infections caused by vancomycin sensitive and resistant *Enterococcus* species.

<table>
<thead>
<tr>
<th>Infection</th>
<th>Vancomycin sensitive</th>
<th>Vancomycin resistant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>UTI</td>
<td>23</td>
<td>82.1</td>
<td>5</td>
</tr>
<tr>
<td>Bacteraemia</td>
<td>15</td>
<td>88.2</td>
<td>2</td>
</tr>
<tr>
<td>Pelvic abscess</td>
<td>4</td>
<td>66.7</td>
<td>2</td>
</tr>
<tr>
<td>Peritoneal infection</td>
<td>3</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>3</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Other body sites*</td>
<td>3</td>
<td>75</td>
<td>0</td>
</tr>
</tbody>
</table>

\* : Infections from other body sites include a knee joint infection, subphrenic abscess and buttock abscess

### Table 5: Frequency distribution of the *Enterococcus* species according to the vancomycin sensitivity

<table>
<thead>
<tr>
<th>Infection</th>
<th>Vancomycin sensitive</th>
<th>Vancomycin resistant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>25</td>
<td>75.8</td>
<td>8</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>21</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. durans</em></td>
<td>3</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. casseliflavus</em></td>
<td>1</td>
<td>50.0</td>
<td>1</td>
</tr>
<tr>
<td><em>E. avium</em></td>
<td>1</td>
<td>100.0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>85.0</td>
<td>9</td>
</tr>
</tbody>
</table>
Of the invasive enterococcal infections in patients with haematological malignancies, 22.7% were caused by resistant strains, compared to only 10.5% among patients with solid tumors. 16% of patients who had been given 3rd generation cephalosporins had infections with resistant strains, while resistant strains were not isolated from patients who not treated with a cephalosporin. Around 22% of patients who had been given chemotherapy had infections with resistant strains. However, these findings were not statistically significant (Table 7).

Only 2/17 of patients with positive blood cultures complied with the necessary criteria to be identified as bacteraemia associated with an intravascular catheter. Vancomycin resistant isolates were not found in these 2 isolates although prevalence of vancomycin resistance among the patients who had a CVC was 22.2%.

Seven of the 9 patients with VRE had expired at the time of collecting data from medical records.

Using the gradient strip method as the standard, the disc diffusion method did not detect vancomycin resistance in 2 isolates, *E. faecium* and *E. casseliflavus*, with an MIC of 6μg/ml (Table 8 and 9). Using the same method, Vancomycin MIC of vancomycin sensitive strains ranged from 0.5-2 μg/ml. MICs of resistant strains is shown in Table 10. The vancomycin resistant *E. faecium* isolates are compatible with VanA phenotype. Of the 2 *Enterococcus casseliflavus* isolates, one had a vancomycin MIC of 6 μg/ml and teicoplanin MIC of 0.5 μg/ml. The MIC of the second isolate was 1 μg/ml (vancomycin susceptible).

<table>
<thead>
<tr>
<th>Species</th>
<th>Vancomycin sensitive</th>
<th>Vancomycin resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td>Urine</td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td><em>E. durans</em></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>E. casseliflavus</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>E. avium</em></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 7: Association of the vancomycin sensitivity of invasive enterococci with patient characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vancomycin sensitive</th>
<th>Vancomycin resistant</th>
<th>Total</th>
<th>Significance Fisher’s exact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>%</td>
<td>No (%)</td>
<td>%</td>
</tr>
<tr>
<td>Malignancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td>34 89.5</td>
<td>4 10.5</td>
<td>38</td>
<td>P = 0.267</td>
</tr>
<tr>
<td>Haematological</td>
<td>17 77.3</td>
<td>5 22.7</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Hospital stay¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short stay</td>
<td>27 87.1</td>
<td>4 12.9</td>
<td>31</td>
<td>P = 0.727</td>
</tr>
<tr>
<td>Long stay</td>
<td>24 82.8</td>
<td>5 17.2</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>ICU admission²</td>
<td></td>
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<td>2 12.5</td>
<td>49</td>
<td>P = 1.00</td>
</tr>
<tr>
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<td>7 15.9</td>
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</tr>
<tr>
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<td>9 21.9</td>
<td>41</td>
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</tr>
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<td>0 0.0</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>9 16.7</td>
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</tr>
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<td>2 12.5</td>
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<td>≥1000</td>
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<td>4 16.7</td>
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</tr>
<tr>
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<tr>
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<td>3 75.0</td>
<td>1 25.0</td>
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<td>No</td>
<td>2 92.3</td>
<td>1 7.7</td>
<td>13</td>
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</tr>
</tbody>
</table>

¹The median days of hospital stay (18.5 days) was taken as the cutoff; ²ICU admission within one month of positive culture; ³Within 6 months of chemotherapy treatment; ⁴mucositis among who received chemotherapy; ⁵Genito-urinary and gastrointestinal surgery within 1 month; ⁶only in patients with positive blood culture; CVC-central venous catheter
A prospective study in North India identified *E. faecium* as the commonest blood culture isolate while *E. faecalis* predominated in pus and urine samples. Other species isolated in this study were *E. mundtii, E. dispar, E. durans, E. raffinosus* and *E. gallinarum*. In another study from New Delhi, *E. faecium* (66%) was the most common isolate in blood samples followed by *E. faecalis* (20%). However, *E. faecalis* (55%) followed by *E. casseiflavus* (24%) and *E. faecium* (12%) were reported from Chandigarh from urinary isolates. Perera et al. at Sri Jayewardenepura General Hospital, Sri Lanka reported that isolation of *E. faecium* was 45% while *E. faecalis* remained the predominant species. In this study *E. faecium* was the predominant species which caused invasive enterococcal infections (55%) among oncology patients. There are no local or national surveillance data on enterococcal infections in Sri Lanka for comparison.

Most risk factors for VRE infections including prolonged hospital stay and previous antibiotic therapy with multiple antibiotics are common in oncology patients. According to the antibiotic policy at NCISL since mid-2006, ceftazidime or carbapenems are used as empirical antibiotic therapy in febrile neutropenic patients, depending on the absolute neutrophil count of the patient, after initiating a septic screen. If the patient is hypotensive and in septic shock, a glycopeptide is added after consideration of renal function. Records maintained by the hospital pharmacist during the year 2012 show that NCISL used 69.6 kg of ceftazidime, 3.2 kg of cefotaxime, 4.6 kg of vancomycin, 0.5 kg of teicoplanin 13.2 kg of imipenem and 11.2 kg of meropenem (personal communication). The selective pressure of these empirical antibiotics may be a predisposing factor.
for *E. faecium* to become the predominant species and there is a need to conduct a proper case control study to identify the risk factors for VRE.

All the VRE infections were hospital acquired in this study population. However, the study was not designed to elicit an association between the duration of hospital stay and getting an infection with VRE.

The first vancomycin resistant enterococci (VRE) isolate that harboured the vanA transposon was identified in 1988 by Uttley et al. which was reported 30 years after vancomycin was clinically introduced. In 2003, 28.5% of enterococcal strains in the United States were vancomycin resistant. In Australia, in 2005, VRE was low at 0.73%. In 2007, 8.5% of enterococcal isolates in the United Kingdom were found to be vancomycin resistant. Those reports included all enterococcal isolates from all patients. In this study the incidence of vancomycin resistant enterococci causing invasive infections in oncology patients was 15%.

In a study evaluating the accuracy of eight currently available test methods (agar dilution, disk diffusion, Etest, agar screen plate, Vitek GPC-TA and GPS-101, and MicroScan overnight and rapid panels) it was shown that VanA VRE were detected by all methods but vanB VRE were often not detected by Vitek GPS-TA and MicroScan rapid (sensitivities 47% and 53% respectively). All methods except Etest and agar screen continue to show problems in the detection of VanC1 and VanC2 VRE. The sensitivity of the disc diffusion test in detecting VanA resistant was 100% but was lower in detection of VanB, VanC1 and vanC2 (93%, 52% and 63% respectively).

MIC detection by the broth dilution method is considered the gold standard to determine antibiotic sensitivity/resistance according to CLSI guidelines 2013. Due to practical difficulties in using the broth dilution method, the gradient vancomycin strip was used as the standard in this study. The detection of vancomycin resistance by disc diffusion test remained lower (77.8%) than the agar screening test (100%).

MICs of teicoplanin in vancomycin resistant isolates varied from 6-24 μg/ml making these isolates of intermediate sensitivity to teicoplanin. Of 8 vancomycin resistant *E. faecium* isolates 7 isolates were phenotypically compatible with VanA phenotype of glycopeptide resistant enterococci.

Of the 2 *E. casseliflavus* isolates, one was vancomycin susceptible according to the CLSI 2013 criteria. Since *E. casseliflavus* isolates are intrinsically resistant to vancomycin, it is not recommended to treat infections with vancomycin even though the organism shows MICs in the sensitive range. It is therefore important to speciate enterococcal species to detect such anomalies to prevent treatment failures.

**Limitations**

With this small sample size, precision of findings was low and power to detect the associations between risk factors and vancomycin sensitivity is low. A case control study is better to identify the risk factors.
Conclusions and recommendations

*E. faecium* was the dominant species (55%) causing invasive enterococcal infections in oncology patients at the National Cancer Institute of Sri Lanka in the study period (2012/13). 78% of vancomycin resistant *E. faecium* in the study were VanA phenotype of glycopeptide resistant enterococci. It is important to speciate enterococci to identify the intrinsically resistant *Enterococci spp* to prevent treatment failures by using inappropriate antibiotics.

Almost all the participants (96.6%) had acquired the enterococcal infection from the hospital. As most risk factors for VRE infections are common in oncology patients, it is recommended that surveillance of VRE is continued with inclusion of a case control study to identify risk factors.

The disc diffusion test was shown to have lower sensitivity in comparison with the agar dilution screening and E test. We therefore recommend one of the latter 2 tests in clinical laboratories investigating patients with invasive enterococcal infections.

Conflict of interest: The authors declare that there are no competing interests.

Ethics statement: Approval from this study was obtained from the Sri Lanka Medical Association ERC/12-016.

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   doi: https://doi.org/10.1086/517774
   doi: https://doi.org/10.1056/nejm199611073351907


Research article

A study on knowledge, attitude and practices regarding dengue among hospitalized patients from Northern Sri Lanka

T Kumanan¹, D Logeswaran²


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Abstract

Introduction and Objectives: Dengue fever is recognized as one of the major vector borne diseases and causes significant morbidity in tropical countries. Thus, evaluation of knowledge, attitude and practices of the population is of great importance to improve integrated control measures. The aim of the study was to assess the level of knowledge, attitude and practices relating to dengue fever in 200 consecutive inward patients treated as having dengue fever in two medical units of Teaching Hospital, Jaffna.

Methods: A cross-sectional descriptive comparative study was carried out among dengue patients in two medical units of Teaching Hospital, Jaffna from January 2017 to April 2017. Dengue patients were recruited by convenient sampling, interviewed with validated questionnaires to assess their knowledge about dengue fever. Data were analyzed using SPSS (version 21) analytical package.

Results: Awareness that dengue fever is transmitted by mosquitoes was shown by 97% of respondents. The Media was an important tool to gain information about dengue. Among students, schools played a key role in conveying health information. More than 90% of the study population exhibited health seeking behavior and the majority used paracetamol as a home remedy. Practices regarding preventive methods were predominantly for prevention of mosquito bites in the form of using nets (46%) and mosquito coils (34%), rather than elimination of breeding sites.

Conclusions: Significant association was found between knowledge about dengue fever and educational level. Although knowledge regarding mosquito control measures was 80%, this knowledge of preventive measures was not demonstrable in practice. A change in the approach of the health education program should be focused based on these findings for effective prevention.

Keywords: Dengue, Knowledge
**Introduction**

Dengue fever is recognized as one of the major vector borne diseases and causes significant morbidity in tropical countries. The incidence of dengue fever has grown dramatically around the world in recent decades mainly in tropics and sub tropics. The *Aedes aegypti* mosquito which is highly adapted to human habitations is the primary vector of dengue. Dengue viruses are primarily maintained in a human-to-mosquito-to-human cycle. There is no specific treatment for dengue fever. During the initial 5 months of 2017, 40264 suspected dengue cases have been reported to epidemiology unit from all over Sri Lanka. As there is no treatment for dengue fever, vector control is an important means of combating this disease.

**Methods**

**Study design**

A cross-sectional descriptive comparative study.

**Study area and setting**

Dengue patients admitted to two Medical Units of the Teaching Hospital Jaffna. The Teaching Hospital, Jaffna is the only tertiary care centre in northern Sri Lanka. The majority of patients with dengue fever from the Jaffna Peninsula are admitted to the general medical wards of the Teaching Hospital, Jaffna. The study cohort therefore was representative of the community and would reflect the knowledge, awareness, adherence and life-style practices of the community.

**Sample size**

200 patients were recruited from the two medical units of the Teaching Hospital in Jaffna.

**Sampling method**

The eligible respondents were selected by convenient sampling method.

**Study period**

The study was conducted for a period of 4 months from January 2017 to April 2017.

**Diagnosis of dengue fever**

Dengue fever was diagnosed among patients with clinical manifestations, complete blood count and dengue IgM antibody which was performed on or after day 5 of the febrile illness.

**Inclusion and exclusion criteria**

Those who were eligible for inclusion into this descriptive cross-sectional, qualitative phenomenological survey were a cohort of 200 adult male and female patients with dengue fever drawn from the medical units, Teaching Hospital, Jaffna. Patients with dengue fever who were willing to participate in the study were included whereas patients who were below 12 years or unable to give consent (eg: mentally incompetent) were excluded.
Development of Dengue Fact Questionnaire

The Dengue Fact Questionnaire was designed as a tool, using the existing literature, for practicing physicians to assess the knowledge and awareness among the dengue patients. The questionnaire was initially designed in English and then translated into Tamil. The questionnaire consisted of questions to assess the patients’ knowledge and awareness on dengue. The patients who met the inclusion criteria were interviewed to assess their knowledge, attitudes and practices related to dengue fever by trained post intern medical officers.

Data analysis

Data were entered in to a Microsoft Excel sheet and analyzed using SPSS (version 21) analytical package. Baseline results were presented as counts and percentages and as mean ± SD for continuous variables. A $P < 0.05$ was considered significant.

Results

Socio demographic characteristics

121 of the 200 study subjects were males of whom 58.5% were in the 12-30 year age group and 32% were in the 31-45 year age group. Most of people belonged to secondary school level (Table 1). 52% of participants were unemployed.

Knowledge of the vector

Of the study subjects, 97% were aware that dengue was spread by mosquito bites and 55% were able to name Aedes as the vector. The most common breeding places for Aedes mosquitoes recognized were water containers (tyres, coconut shell, tins, cans and lids) by 82.5% respondents followed by stagnant water reserves (rivers / ponds). The forest and trees were identified by 17.5% of the respondent as a source of mosquito breeding. 51.5% of respondents identified that Aedes mosquito bites during the day.

Knowledge of clinical features

Fever as a symptom of dengue was stated by 76 patients with 49 patients (24.5%) identifying two other classical symptoms and 75 patients could enumerate one other clinical feature of dengue (skin rash, bleeding tendency). Dengue fever was identified as a preventable condition by 76% of the respondents and 75% of respondent perceived that dengue fever is a serious illness. Half the respondents (50.5%) knew that dengue fever could recur.

Table 1. Selected demographic pattern among dengue patients

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<tr>
<td>Higher education</td>
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</table>
Knowledge of treatment
Of the 200 patients, 182 had been treated by a doctor during the febrile illness before admission to hospital. Only 13 patients were admitted without being seen by a doctor. Paracetamol was used by 65.5% of the population as a home remedy. Home remedies for dengue fever included the use of paracetamol to control the fever (131 respondents), and 9 patients thought that drinking water was effective to control fever. Both options were used by 25 patients for control of fever.

The majority of respondents (37%) mentioned that they knew about dengue from media (TV, radio and newspapers), while 36% patients obtained knowledge through health personnel and only 23% gained knowledge from relatives and friends.

Knowledge of preventive measures
Water was stored at home by 76 patients, of whom 40% frequently changed the stored water. The most common measures to prevent dengue fever were preventing mosquito bites by using mosquito nets (46%), mosquito coils (34%), insecticides (15.5%) and repellents (4.5%) rather than elimination of breeding sites.

There was a significant association noted between education level and knowledge regarding dengue vector and species identification, biting time, breeding places, treatability and dengue recurrence (P <0.05).

Discussion
Most respondents in the current study knew that dengue fever is transmitted by mosquitoes and the main source of information was the media. Media (TV and radio) emerged as the most important source of health information in this study. It emphasizes the fact that mass media, in particular television, can be used to disseminate more knowledge and awareness regarding a dengue epidemic. A hospital-based study done in the outpatient department (OPD) of Safdarjang hospital in India also showed similar findings in 2003.\(^3\) In addition, school was shown to play a pivotal role in conveying health information to school-going children.

Knowledge about mosquito bites is vital in preventing dengue spread, and 51.5% patients in the current study correctly identified that the biting time was during the day. Arunachchalam \textit{et al.}\(^4\) showed that greater knowledge about dengue and its transmission was associated with lower mosquito breeding and production. Despite this fact, mosquito coils and bed netting which are not very effective in prevention of dengue fever, are widely used only at nights. The most common practice of our study population in preventing mosquito bites was using mosquito coils and bed netting rather than elimination of breeding sites, which is important as it undermines effective dengue preventive strategies.

Fever was recognized as the most common symptom of dengue infection which is on par with another study done among university students in the University of Gujarat in 2015.\(^5\) Most of the study population (91%) in the current study sought treatment from a primary care physician at the early phase of febrile illness. This could be due to the endemic nature of the disease in countries like Sri Lanka. Health seeking behavior (HSB) appears to be satisfactory in contrast to a study done in America which showed that HSB for dengue was around 60%.\(^6\)
Nearly two thirds (65.5%) of the population used paracetamol as a home remedy in contrast to some popular studies done in southeast Asian region where most people sought traditional remedies to treat dengue.\(^7\)

Another important fact that could be easily overlooked is the knowledge regarding recurrence of the illness. Victims of dengue have a high chance of recurrence which could have serious consequences. Only half of the study population in the current study thought that dengue could recur. A study done in Bangladesh recently has also found that knowledge regarding dengue recurrence was poor.\(^8\)

We found a significant association between educational level and knowledge regarding dengue fever. In contrast, there was no significant association found between educational level and preventive practices. Similar findings were reported in another study done in Pakse district, Laos in 2006.\(^9\)

The results of the current study highlighted the fact that good knowledge about dengue does not reflect good practices of preventing the illness. Many people knew about preventive measures yet did not practice them. Knowledge therefore does not necessarily lead to effective practice. Prevention and control depend mainly on effective and sustained vector control measures. Schools play a major role in health education, particularly in countries like Sri Lanka which provides mandatory free education and achieved literacy rates comparable to the developed nations. This study showed that students gain significant information through their schools. A series of activities are scheduled to promote enhanced awareness about dengue – an epidemic prone viral disease – and educate children about the need for dengue prevention and control.\(^10\)

**Conclusion**

Most of the patients treated as dengue had adequate knowledge about the vector, clinical features, treatment and preventive measures. However, they lacked awareness to practice effective preventive measures to control dengue. Based on this study, it is suggested that a focused health education program could be effective in improving the practices regarding dengue prevention.

**Acknowledgements:** We acknowledge the services rendered by Mrs. Uma Sriskantharajah in providing technical support in preparing this manuscript.

**Conflicts of interest:** There are no conflicts of interest declared.

Ethical clearance was obtained from the Ethical Review Committee, Faculty of Medicine, University of Jaffna, Sri Lanka. Informed written consent was obtained from all patients admitted to the study.

**References**

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doi: https://doi.org/ 10.14260/jemds/2014/2011


Case Report

Challenges in diagnosis and management of infective endocarditis by *Stenotrophomonas maltophilia*

A case report of an unusual nosocomial infection

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Abstract

*Stenotrophomonas maltophilia* is a Gram-negative bacillus found as a free-living organism in most aquatic and humid environments including hospital drinking water, and often associated with nosocomial infections. It is an uncommon cause of infective endocarditis. In Sri Lanka there are no reported cases in literature.

Here we report a 46 years old patient who presented with fever following Percutaneous Trans Mitral Commissurotomy (PTMC). He was diagnosed as an acute mitral valve endocarditis based on echocardiograph findings and 2 blood cultures taken 6 hours apart which grew *Stenotrophomonas maltophilia*. The patient responded rapidly to targeted antibiotic treatment with piperacillin tazobactam and oral cotrimoxazole for 4 weeks and a further 2 weeks of cotrimoxazole. He remained well at the 3 month follow up.

There is limited information on the best choice of antibiotics and the ideal duration of treatment. Early diagnosis and identification of the organism, prompt treatment with appropriate antibiotics and close collaboration between the clinical and laboratory teams contributed towards the successful management of this case.

**Keywords:** *Stenotrophomonas maltophilia, endocarditis, PTMC, nosocomial infection*

Introduction

*Stenotrophomonas maltophilia* is a glucose non fermenting Gram negative bacillus, found as a free-living organism in most aquatic and humid environments, including hospital drinking water.¹ Although it is an uncommon pathogen in humans, nosocomial infections especially among immunocompromised patients are increasing.² Importance of this organism is emphasized due to...
its multiple antibiotic resistance.

*S. maltophilia* is an uncommon agent to cause infective endocarditis with less than fifty cases reported worldwide so far.\(^1\) It has got higher morbidity and mortality rates. There have been no previous cases reported in Sri Lanka.

**Case Report**

A 46 years old male was admitted to the hospital with high fever associated with chills and rigors for 2 days. He was diagnosed to have tight mitral valve stenosis and had undergone PTMC for the second time (two months following the first PTMC) about three days prior to the onset of fever. He did not give a history of dental procedures or injections of intravenous drugs.

On examination he was febrile. His pulse rate was 71/min and blood pressure was 112/78mmHg. There were no peripheral stigmata of infective endocarditis. His white blood cell count was 8.7\(\times\)\(10^3\) /\(\mu\)L with 68.4% of neutrophils. The CRP was 74 mg/L. He was started on intravenous meropenem on the suspicion of a nosocomial infection but with poor response.

Two blood cultures were taken six hours apart on the day following admission which became positive for a Gram negative non fermentative bacillus, identified as *S. maltophilia* by RapID\(^\text{TM}\) (Remel) kit with “implicit” accuracy (>99%). Antibiotic sensitivity was performed using the disk diffusion method (CLSI guidelines 2018). Both isolates showed the same antibiotic sensitivity pattern, sensitive to co-trimoxazole and intermediate to levofloxacin. There were large zones for piperacillin-tazobactam, and amikacin (25mm and 18mm respectively) and no zones for meropenem, gentamicin and netilmicin. Minimum inhibitory concentration (MIC) could not be performed due to lack of facilities.

This patient with diagnosed valvular heart disease was admitted with fever following PTMC and two blood cultures grew the same organism, raising the suspicion of infective endocarditis. Transthoracic and transesophageal echocardiographs were therefore done. Both showed a suspicious mass attached to chordae of mitral valve which was 5×3mm in size. There were no other foci of infection such as central lines to cause bacteraemia and therefore, infective endocarditis was considered the most probable diagnosis.

The patient was started on IV piperacillin tazobactam 4.5g 6 hourly and oral cotrimoxazole 1920mg 12 hourly as per antibiotic sensitivity pattern of the isolate. Due to the unavailability of IV cotrimoxazole, high dose oral cotrimoxazole was given.
The response to the treatment was prompt and extremely satisfactory. Fever came down to the baseline from the second day of initiating the antibiotic combination (Figure 1). Similar results have been previously reported. Subsequent transthoracic echocardiographs done at 2 week intervals showed remarkable reduction in vegetation size. The patient improved clinically and CRP came down to 23mg/L within five days of commencing the antibiotic regime. He was given this combination of antibiotics for 4 weeks with an additional 2 weeks of oral cotrimoxazole. On discharge after 4 weeks of IV-oral combination, the CRP was <6mg/L and vegetations were not detected. He was regularly followed up in the clinic with repeated full blood count and CRP for 3 months during which he showed full clinical recovery with normal investigation reports.

**Discussion**

*Stenotrophomonas maltophilia* is a nosocomial pathogen and infections due to this organism have been increased along with their importance in the hospital setting. The risk groups to develop this infection include patients with debilitated illnesses, patients with indwelling vascular catheters and patients who had undergone surgical procedures.

*S. maltophilia* is a rare organism to cause infective endocarditis. Less than 50 cases have been reported worldwide so far with involvement of both prosthetic valves and native valves. Prior valve replacement had been a common predisposing factor. With the loss of intact skin barrier during the invasive procedures, organisms contaminating the medical equipment may be the cause for endocarditis.

Infective endocarditis due to *S. maltophilia* is known to carry a higher rate of mortality and complications. Some patients require surgery irrespective of the longer duration of antimicrobial therapy. Cerebrovascular diseases, congestive cardiac failure and organic abscesses were the observed complications. No complications were noted in this patient.

Treatment of this infection is challenging due to the intrinsic antibiotic resistance of this organism and limited experience. According to the literature, despite in-vitro sensitive zones for certain antibiotics, in-vivo resistance can occur as in piperacillin tazobactam where MIC is required to determine the sensitivity. However, piperacillin tazobactam has been used effectively in infections caused by *S. maltophilia*. Trimethoprim-sulfamethoxazole is considered the first line antibiotic. Being bacteriostatic, addition of other antibiotics is required for synergistic effect. There is evidence of effective combination therapy with aminoglycosides, fluoroquinolones, penicillins and the third and fourth generation cephalosporins but both isolates in the present case were resistant to these antibiotics. Intolerance to trimethoprim-sulfamethoxazole with a long duration of therapy can cause difficulty in treatment. Along with the antibiotic therapy, surgical removal of infected indwelling foreign materials should be done if required.
Early diagnosis and identification of the organism, prompt treatment with sensitive antibiotics and the collaborative work and the dedication of the cardiologists and the microbiology team have contributed to the rapid clinical improvement and the ultimate cure of the patient.

References


Case Report

Sepsis with cerebral lupus…. or is it?
A case report on Cryptococcaemia

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Abstract

We present a patient with SLE, with multiple comorbidities, whose blood cultures yielded Cryptococcus neoformans. Blood cultures became positive only after twelve days of hospital admission. The patient died within two days of starting Amphotericin B. Cryptococci are an important cause of infection in SLE. Only about 10% to 30% of those with cryptococcal disease have the organism isolated by blood cultures. Due to its non-specific clinical presentation, cryptococcal infection in SLE can be misdiagnosed as psychosis due to steroids, cerebral lupus and infections due to other non-fungal pathogens. This may lead to inappropriate therapy and delays in administration of antifungal agents. Therefore, cryptococcal infection should be considered when SLE patients present with sepsis irrespective of the presence of features of meningism.

Keywords: Cryptococcus, Systemic Lupus Erythematosus, Fungal, Sepsis

Introduction

Patients with Systemic Lupus Erythematosus (SLE) are prone to infections and have a poorer prognosis when they have fungal infections.¹ Among fungi, Candida species and Cryptococcus neoformans have been found to be the commonest. Importantly, cryptococcal meningitis is the foremost cause of mortality (85.7%) among SLE patients due to invasive fungal infections.²
Case history

A 33-year-old female, a diagnosed patient with SLE and lupus nephritis for nearly 10 years, presented to the local hospital complaining of fever and generalized body swelling of three days duration. Her disease was in remission, and she was on maintenance doses of mycophenolate mofetil. She had been living in Middle East, where she had been supposedly treated for a cardiomyopathy and bone marrow suppression. Unfortunately, information was scarce regarding this episode.

On admission, she was conscious and rational, but had a rapidly progressive pneumonia, right lower limb cellulitis with features suggestive of sepsis.

Her initial full blood count was 8.1 x 10^3/mm^3 with 85% neutrophils, haemoglobin 7.4 g/dL and platelet count 90 x 10^3/mm^3. On admission her C-reactive protein was elevated to 114 mg/L, while the urine full report had 3+ albumin, 6-8 pus cells / hpf and 5 red cells / hpf. She was also found to have acute respiratory distress syndrome (ARDS) and pulmonary embolism. She had a low urine output, with a serum creatinine of 221 μmol/L (60-104 μmol/L). Her initial blood culture revealed a mixed growth of Acinetobacter species and coliforms of which the former was resistant to all antibiotics tested, including carbapenems. Colistin was not tested due to unavailability of E-strips or paper discs. The coliforms were sensitive to carbapenems. The endotracheal secretions failed to reveal a pathogen.

The patient was admitted to the intensive care unit (ICU) for further management. A central venous line was inserted for monitoring, and she was started on IV meropenem 1g 8 hourly, oral mycophenolate mofetil 500mg twice daily, along with pulsed methyl prednisolone and IV cyclophosphamide 600mg pulses.

Despite the above, the patient’s CRP levels remained high (127mg/L). Her neutrophil leukocytosis was also increasing, while high resolution CT scan of the chest revealed an organizing but ongoing pneumonia.

On the 12th day after admission, her blood culture (BacT/ALERT® bioMérieux, U.S.A) became positive after 24 hours of incubation for Gram positive, oval shaped yeast cells with budding. The same organism was isolated in the repeat blood culture taken the next day. The isolated organism was suspected to be capsulated, hence India ink staining was performed (Fig 1a). The blood culture bottle was then dispatched to the reference laboratory as the required technical skills and experience in interpretation of the stain was not available locally.
The patient was initially started on IV fluconazole 400mg daily but was changed to IV liposomal amphotericin B 3mg/kg daily after a presumptive diagnosis of cryptococcal infection was made. A lumbar puncture was not performed due to her unstable condition.

The isolates were sent to the Mycology Reference Laboratory at the Medical Research Institute for further identification. Both isolates were identified as *Cryptococcus neoformans* based on culture morphology, demonstration of a capsule, positive urease test, carbohydrate assimilation and API Candida® (Biomerieux) results.

The patient meanwhile developed pulmonary haemorrhages 17 days after admission. Two days afterwards, therapeutic plasma exchange was performed, but unfortunately, she died the same day.

The timeline of the patient’s clinical course is given below
Discussion

SLE has the probability of affecting all organs in the body, causing much morbidity and mortality. Even though survival has improved in recent years due to early diagnosis and better treatment options, patients still have a three-fold increase in all-cause premature mortality, compared with the general population. Among the causes with highest mortality, is renal disease and infection, causing eight and five times increased mortality respectively, compared with the general population.¹

Both disease activity and therapy in SLE increases the patient’s risk of infection, as steroids and immunosuppressive therapy are used increasingly in those with severe and active disease. It has been found that treatment with steroids and immunosuppressive drugs are risk factors for infection in SLE patients.³ These patients are prone to infections by Gram positive cocci, Gram negative bacilli, Candida albicans, Cryptococcus neoformans, Pneumocystis jirovecii and Mycobacterium tuberculosis among others. It has also been noted that these patients have a poorer prognosis when they have fungal infections. Among fungal pathogens, Candida species and C. neoformans have been found to be the commonest.⁴

Cryptococcal meningitis is the foremost cause of mortality (85.7%) in patients with SLE who have invasive fungal infections.⁵ The reasons for this are multifactorial and include its non-specific clinical presentation in the early stage of infection, misjudging the risk for cryptococcal meningitis and misdiagnosis as psychosis due to steroids, cerebral lupus and other non-fungal pathogens.² This leads to inappropriate therapy and delays in administration of antifungal agents.

Cryptococcal meningitis should be a consideration when patients with SLE present with headache, nausea, vomiting or fever. Despite antifungal therapy, the consequences of cryptococcal meningitis are disastrous, as mortality is around 40-50%, even with antifungal therapy.⁵

C. neoformans can be isolated from the blood, as in our patient, only in around 10% to 30% patients with cryptococcal disease.⁶ It should be noted that when this happens, in those with cryptococcal meningitis, it heralds a poor prognosis. Also, cryptococcaemia can induce a systemic inflammatory response, and sepsis is its most common manifestation. Therefore, early diagnosis and initiation of antifungal treatment promptly is essential. We presume our patient had concomitant cryptococcal meningitis, as around 82% of those with cryptococcaemia have been found to have same.⁶ Hence, the treatment regime for cryptococcaemia is essentially the same as for cryptococcal meningitis.⁷

According to guidelines published by the Infectious Disease Society of America (IDSA), treatment induction is with IV Amphotericin B deoxycholate combined with oral fluycytosine, (or fluconazole in low resource settings) where lipid formulations of Amphotericin B could be substituted in the second 2 weeks. This is followed by a consolidation phase, where oral fluconazole is prescribed for 8 weeks and maintenance therapy where oral fluconazole is given for 6–12 months.

This case highlights an instance where cryptococcal disease was diagnosed via blood cultures. Although in disease remission, our patient with SLE was at a higher risk for infectious complications, owing to being immunosuppressed. Although it was possible that she had central
nervous system infection, this could not be proven. Sepsis in this patient may have been due to the bacterial infection, or cryptococcaemia or both.

Many issues confronted the optimal management of this patient. Rapid diagnosis of cryptococcal infection using the cryptococcal antigen test was not possible at the local hospital. Sending samples to the reference laboratory situated nearly 230 km away at short notice was difficult. Lack of local expertise in identification of cryptococci, in particular, interpretation of the India ink stain was also problematic. Recommended treatment strategies could not be implemented immediately as obtaining the necessary antifungal drugs was difficult and precious time was lost in the process.

In conclusion, we present a patient with SLE, with multiple comorbidities, where cryptococcal infection was diagnosed via blood cultures. She eventually died due to the disease process, with infection no doubt playing a major role. This case highlights the importance of including cryptococcal infection in the differential diagnosis of sepsis in immunosuppressed patients.

References


Case Report

Severe falciparum malaria with hypophosphataemia and presumed hypophosphatemia induced rhabdomyolysis: a case report

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Abstract

Malaria is eliminated from Sri Lanka, yet cases contracted overseas are still encountered in the country. It is therefore important to be vigilant of possible complications of severe malaria. We report a 31-year-old sailor (Sri Lankan national) with a recent travel history to West Africa who presented with an acute febrile illness of seven days duration. He had high fever, myalgia, drowsiness as well as profuse watery diarrhea. Clinical examination revealed a febrile patient with GCS 15 with no features of meningism. He was icteric with right hypochondrial tenderness.

Investigations showed hemolysis. Microscopy and rapid diagnostic assays revealed Plasmodium falciparum infection. He was started on IV artesunate followed by artemisinin based combination therapy orally to which he responded. On day 4 he developed evidence of rhabdomyolysis with low serum phosphate and normal urine phosphate levels. Hypophosphatemia was considered to be the driving factor for rhabdomyolysis, and phosphate replacement was done for which the patient responded and was discharged on day 10 fully recovered.

Key words: Malaria, Plasmodium falciparum, Rhabdomyolysis, Hypophosphatemia

Introduction

Sri Lanka was certified as having eliminated malaria in 2016 by the WHO. However, imported cases of malaria are still seen in the country. Severe malaria caused by Plasmodium falciparum is a serious condition with several possible complications. Our case draws attention to a rare complication and its possible etiology.

Case Report:

A 31-year-old previously healthy male (Sri Lankan national) presented to the casualty unit of the National Hospital, Sri Lanka with an acute febrile illness of seven days
duration. He is a sailor by profession and gave a history of recent travel to Europe as well as to West Africa in the past few weeks, with return to Sri Lanka two weeks previously. He had high fever of 39.8 °C with associated chills and rigors. There was no periodicity of fever. There was associated myalgia and malaise. Two days prior to admission, the patient developed profuse watery diarrhea with frequency of 10 times per day. He complained of headache and was drowsy with GCS of 15. There was no photophobia or seizures. He also complained of mild right hypochondrial pain.

On examination, he was febrile with a temperature of 39.8 °C and was drowsy with GCS of 15/15. There was no neck stiffness and Kernig’s sign was negative. His vital signs were warm. Lungs were clear to auscultation. The abdomen was soft with mild hepatomegaly and right hypochondrial tenderness. There was no splenomegaly. The patient was catheterized on admission and was producing adequate normal colored urine.

Investigations revealed the following. Complete blood count WBC 9.24 x 10³/uL (neutrophil 68.8% and lymphocytes of 22.9%), HB 9.7 g/dl and platelets of 27,000/l. The clotting profile was normal. The AST and ALT were 129 U/L and 79 U/L respectively with evidence of hyperbilirubinemia at 93µmol/L with elevation of the indirect fraction. Haemolysis was further supported by an increased LDH at 2385 u/L.

The serum creatinine was 146 µmol/L, potassium 5.7 mmol/l and sodium 138 mmol/l. Serum ionized calcium was 1.18 mmol/L (1.12- 1.32mmol/L) and serum phosphate was 2.9 mg/dl (2.7-4.5 mg/dl). CBS was normal. Ultrasound scan of the abdomen showed mild hepatomegaly with a prominent spleen. Stool full report revealed 30-40 pus cells /HPF with no red cells and negative amoebae, ova or cysts. UFR was normal. Blood picture showed haemolysis with severe thrombocytopenia and neutrophil toxic changes. There were no schistocytes. Microscopy was carefully assessed for malaria due to travel history and the clinical presentation and showed numerous malarial parasites in ring stages of falciparum malaria, schizonts and gametocytes. Subsequent quantification by the anti malarial campaign demonstrated a parasite density of > 500000/µL. The malaria rapid diagnostic test also became positive for Plasmodium falciparum. Due to myalgia and acute kidney injury, CPK was done which was normal. Arterial blood gas analysis revealed metabolic acidosis with pH 7.31 with lactate of 6 mmol/L.

Severe falciparum malaria was diagnosed, and patient was started on intravenous artesunate 2.4 mg/kg 3 doses 12 hours apart (0, 12 hrs. and 24 hrs.) and daily thereafter for 3 days according to the national treatment policy for management of falciparum malaria. IV fluid replacement was done, and supportive transfusion was given. Parasite load gradually declined to 218000/ µL by the end of day 2 of treatment and 1000/ µL by end of day 3. The patient’s clinical condition was improving gradually with improved sensorium and settling fever and he was switched to a full course of oral Artemisinin based combination therapy. His creatinine rose to a maximum of 224 µmol/l on day 2. He maintained an adequate urine output and there was no indication for hemodialysis.
On day 4 in hospital, when the parasite count was almost undetectable, repeat CPK was found to be elevated at 18932 u/L. At this point serum ionized calcium was low (1.00mmol/l) and surprisingly, the serum phosphate was also found to be low at 2.0 mg/dl (2.7 – 4.5g/dl). Urinary phosphate remained normal despite hypophosphatemia. Over the ensuing days CPK rose to maximum of 115278 u/L. The creatinine did not rise further. At the height of rhabdomyolysis serum phosphate remained at the lower limit of normal. Usually it is expected to have hyperphosphatemia at the peak of rhabdomyolysis. Persistence of hypophosphatemia in the phase of severe rhabdomyolysis led to possibility of hypophosphatemia as the possible mechanism driving rhabdomyolysis, which led to careful replacement of phosphate. With phosphate replacement rhabdomyolysis gradually improved and settled. During this period patient received alkaline diuresis as a temporizing measure.

By day 10, the patient was fully recovered, and rhabdomyolysis had completely settled and he was discharged after a single dose of primaquine.

The timeline of the patient’s clinical course is given below

Discussion:

Severe falciparum malaria is associated with a myriad of serious complications such as cerebral malaria, hypoglycemia, acute renal failure and metabolic acidosis. Rhabdomyolysis is also a recognized yet uncommon complication of severe malaria. There are only handful of cases worldwide and only one reported case in Sri Lanka. Rhabdomyolysis in severe malaria is thought to be due to several possible mechanisms. P. falciparum induces myositis by macrophages and T cells. It is also postulated that sequestration of red cells may induce microcirculatory occlusion
leading to muscle damage. Another mechanism is muscle damage via high TNF-alpha levels in malaria.

Electrolyte abnormalities are common in severe malaria. Hypophosphatemia is a recognized manifestation. Several mechanisms are responsible for this. It is assumed that serum phosphate relates inversely with body temperature such that high fever is a cause of hypophosphatemia in malaria. However a lowered renal threshold for phosphate appears to be a contributing factor too. In our case, presence of normal urinary phosphate excretion in the presence of hypophosphatemia may point towards renal wasting.

Rhabdomyolysis in our patient occurred quite late in the illness when the parasitic burden was undetectable. Therefore, rhabdomyolysis due to high parasitic burden or sequestration of parasitized RBC seemed less likely. As a result, mechanisms other than ones described above were sought. There was no previous alcoholism or poor nutritional state to suggest chronic hypophosphatemia. Usually rhabdomyolysis should result in hyperphosphatemia. Interestingly, in our patient, the serum phosphate remained low despite rhabdomyolysis and remained low normal at the height of rhabdomyolysis. This paved the way to consider hypophosphatemia as the driving factor for rhabdomyolysis.

As a result, the decision was made to carefully replace phosphate, and oral phosphate was started. This was done with careful measurement of serum phosphate to avoid hyperphosphatemia. The patient was also started on alkaline diuresis as a temporizing measure. Interestingly, acute kidney injury did not occur despite very high CPK levels. With phosphate correction the rhabdomyolysis gradually responded with a decline in CPK levels and subsequent normalization. The patient was fully recovered by day 10 and discharged home with normal CPK.

This case report highlights the possibility of rhabdomyolysis as an uncommon complication of severe falciparum malaria as well as hypophosphatemia as a possible etiology for the problem.

Conflict of Interest: Authors declare no conflict of interest

Ethics: Informed and written consent was obtained from the patient

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