Tropical Journal of Pharmaceutical Research, October 2009; 8 (5): 441-447

Available online at http://www.tjpr.org

Research Article

Microbiological Assessment of Commercially Available Quinine Syrup and Water for Injections in Dar Es Salaam, Tanzania

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Abstract

***Purpose**: To conduct microbiological assessment of commercially available quinine syrups and water for injection in Dar es Salaam, Tanzania.*

***Methods**: This was a cross-sectional study conducted in Dar es Salaam Region. Samples of quinine syrups (QNSs) and water for injection (WFI) of different batches were randomly purchased. Each QNS was inspected for label disclosure, and physicochemical properties were examined by the use of sense of organs and pH meter. Isolation and quantification of microbial contaminants from each sample was preceded by 24 - 48 h incubation at 37 ºC, and the microbial contaminants were expressed as colony forming unit per millilitre (cfu/ml). Microbiological identification of contaminants was performed by examination of colony morphologies and growth characteristics. Gram staining technique, as well as biochemical and serological tests were also conducted for further identification. Albino rabbits were used for the pyrogen test to determine the presence of microbial contamination in WFI.*

***Results:** Twenty-four samples of QNS underwent label disclosure, physical-chemical and microbiological assessments. All QNS samples complied with the guidelines and microbial limits as per United States Pharmacopoeia (USP). All batches of WFI were found to be microbiologically contaminated, revealing average microbial counts of 87, 94 and 100 cfu/ml, and this was buttressed by pyrogen test, with the animals showing temperature rise of 1.0, 2.2 and 2.4 ºC, respectively.*

***Conclusion:** The QNS products available in the Dar es Salaam market were of good microbial quality. However, WFI products were microbiologically contaminated. We recommend that regulatory authorities in Tanzania should diligently enforce regulatory control of the products to assure consumer safety.*

***Key words:** Microbiological quality, quinine syrups, water for injection, pyrogen test*

Received: 12 February 2009 *Revised accepted: 10 June 2009*

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INTRODUCTION

Malaria has been a leading source of mortality and morbidity for Tanzanian children particularly for under 5-year-old [1]. Consequently, adequate disease management in primary health care units is the basis for malaria control which is in line with national and global malaria control strategies [2-4]. On the other hand, accurate diagnosis and treatment cannot be overemphasized for effective malaria control. Quinine (QN) is a drug of choice for treatment of *Plasmodium falciparum* malaria. In addition to its anti-malarial activity, the drug also possesses antipyretic, analgesic and anti-inflammatory properties [5]. Quinine was previously superseded by chloroquine in terms of usage, but because of the rise of chloroquine resistance, its use has increased and currently is available on prescription only basis. It can be administered orally, intramuscularly, or intravenously. However, the use of intramuscular QN in children may cause abscess, and therefore, this route of administration is discouraged [6]. As a result of its characteristic bitter taste, QN is preferably used in children in the form of syrup [7] but poorly prepared quinine syrups (QNSs) are subject to microbial contaminations, which may ultimately contribute to secondary bacterial infections, especially as the immune system of children is immature.

Undoubtedly, the use of water in the pharmaceutical industry is indispensable, especially in pharmaceutical liquid preparations. Not only is water used as a component of various pharmaceutical formulations, but also for cleaning and rinsing of medical devices and appliances. One of the most important and delicate use of water is in the production of water for injection (WFI), which essentially is pure water (H_2O). Production of WFI or any other pharmaceutical products intended for parenteral application is widely recognized as a critical process, which requires strict adherence to good manufacturing practices (GMP) and quality control. Such pharmaceutical products must be free from pyrogens, which can originate from Gram-negative or Gram-positive bacteria, viruses and fungi, or from any other viable microorganisms [7-10]. This study, therefore, assessed the microbiological quality of commercially available QNSs and WFI available in the market in Dar es Salaam, the largest city in Tanzania.

EXPERIMENTAL

Study design and area

The study was conducted in Dar es Salaam Region, across all the three districts of the region, viz, Ilala, Kinondoni and Temeke. Eight different batches/brands of QNSs were purchased, and at least one batch of WFI was also randomly purchased from at least one pharmacy in each district.

Inspection of label disclosure and physicochemical analysis

All the samples were inspected for any physical irregularities/defects. Colour, odour, taste and appearance of the QNSs were assessed by the use of sense of appropriate organs. The pH of each sample was determined with a pH meter (Jenway-3505, UK). Label information such as batch number, expiry date, manufacturing date, direction for use and ingredient composition were recorded. WFIs were packed in sealed plastic containers.

Preparation of samples and assessment of microbial quality

The following selective and non-selective culture media were employed for quantification and isolation of the microbial contaminants: nutrient agar (NA, Oxoid, England), Saboraud's dextrose agar (SDA, Pronadisa, Spain) thiosulphate-citrate-bile-sucrose agar (TCBS) and MacConkey agar (MCA, Roth, German). One millilitre-aliquots of each sample of QNS was directly spread-plated onto the sterile NA and SDA, and incubated for

24 - 48 hours at 37 ºC. This temperature was selected because fungal dimorphic growth is not demonstrated above differential incubation temperature (35 ºC), thus facilitating the quantification of yeast cells rather than the mycelia forms that grow better at lower temperatures [11].

Prior to observation and recording of any microbial contamination, serial dilution was performed whenever deemed necessary by dispersing QNS in WFI. Quantification of viable microbial counts was conducted and expressed as colony forming unit per millilitre (cfu/ml). Therefore, WFI alone was also included as negative control. These procedures were conducted in duplicate and repeated twice, and the cfu/ml was expressed as a mean. Pure and single microbial colonies were sub-cultured onto solid and liquid media, incubated at 37 ºC for 24 hours and finally stored at 4 ºC until further use.

Microbiological identification of contaminants

Identification of the isolated microbial contaminants was preliminarily performed by macroscopic examination of the microbial morphology of the pure cultures of isolated microorganisms and other typical growth characteristics on non-selective, selective, and differential culture media, such as lactose fermentation on MCA, and TCBS, and haemolysis in blood agars, complemented with Gram staining technique. Gram-negative curved rods with distinctive darting motility, when observed, were immobilized by *Vibrio cholerae* O-group I antiserum (Difco, USA). Furthermore, microbial identity was confirmed by conventional microbiological (biochemical and physiological) tests [11-12].

Pyrogen test

Following the observation of microbial contaminations in WFIs that were employed as negative controls, pyrogen test was performed on them (WFIs). Four healthy albino rabbits of the same age weighing 2.1 - 2.7 kg were employed in each assay. Prior to conducting the pyrogen test, the animals were trained for 3 days consecutively by parenterally administering them with 10ml/kg of sterile saline solution. Using a digital thermometer (Mode ECT-1, accuracy $\pm$ 0.1 ºC), rectal temperature was taken thrice each at 0, 15, 60 and 360 minutes. The test commenced by first recording the rabbits' body baseline temperature and then each animal was intravenously administered with 10ml/kg of WFI. A rise of temperature by 0.5 ºC was considered positive [13]. Other criteria for pyrogen testing were applied in line with approved guidelines [9-10, 14-15].

Ethical issues

This study was conducted in partial fulfillment of the Bachelor of Pharmacy degree for one of the authors (FSF) and funded by the Ministry of Higher Education Science and Technology (Tanzania), and ethical clearance was given by the Muhimbili University of Health and Allied Sciences Ethical Committee.

Statistical analysis

The data obtained were analyzed using the Statistical Package for the Social Sciences software (SPSS+ 15.0, 1999, SPSS Inc., Chicago, IL, USA). Differences of means for cfu/ml and rabbits' body temperature rise for each test were compared using Student t-test and were considered significant at $p < 0.05$.

RESULTS

Twenty four samples of commercially available QNSs were subjected to label disclosure inspection, physical-chemical examination and microbiological assessment. The results are shown in Table 1. All the samples complied with the requirement for microbial limit (≤ 100 cfu/ml) [9,14]. Microbiological assessment of 3 batches of WFI revealed heavy microbial contaminations: 87, 100 and 94 cfu/ml for the first, second and third batches, respectively.

Microbiological tests revealed the presence of *Staphylococcus spp* in all the batches of WFIs,

Table 1: Description of test quinine syrups and results of assessment of pH and organoleptic properties

Syrup type	Man. date	Expiry date	Origin	Batchno.	Colour	Smell	pH
QB	Jul 2006	Jul 2008	Nairobi	6H28	Green	Fair	2.95
QB	Aug 2006	Aug 2008	Nairobi	6H39	Green	Fair	2.98
QB	Feb 2007	Feb 2009	Moshi	ONPS 7005	Orange	Fair	3.16
QB	Jan 2007	Jan 2010	Nairobi	48414	Color less	Fair	2.99
QB	Feb 2007	Jan 2009	Nairobi	7B168	Green	Fair	2.96
QB	Aug 2006	Jul 2009	Nairobi	6H29	Green	Fair	2.94
QB	Nov 2006	Oct 2008	DAR	6004	Green	Fair	3.32
QB	Mar 2007	Feb 2009	Moshi	QNPS 7011	Orange	Fair	3.25
QB	Feb 2007	Jan 2009	Nairobi	7B168	Green	Fair	2.81
QH	Oct 2006	Sept 2008	Kampala	4506	Light Pink	Fair	6.03
QB	Feb 2007	Jan 2009	Nairobi	7B170	Green	Fair	2.80
QB	Feb 2007	Jan 2009	Moshi	QNPS 7001	Orange	Fair	3.28
QB	Jan 2007	Dec 2009	Nairobi	7A126	Green	Fair	3.03
QB	Dec 2006	Nov 2008	DAR	6005	Green	Fair	3.34
QB	Jan 2007	Jan 2009	Moshi	QNPS 7002	Orange	Fair	3.28
QB	Jan 2007	Dec 2008	Nairobi	7B168	Green	Fair	2.78
QB	Feb 2007	Jan 2009	Nairobi	7B171	Green	Fair	2.82
QH	Sept 2006	Aug 2008	Kampala	4505	Light Pink	Fair	6.05
QB	Mar 2007	Feb 2009	Moshi	QNPS 7004	Orange	Fair	3.22
QB	Mar 2007	Feb 2010	Nairobi	7A129	Green	Fair	3.18
QB	Jan 2007	Dec 2009	Nairobi	7A127	Green	Fair	3.02
QB	Jan 2007	Dec 2010	DAR	6008	Green	Fair	3.36
QB	Feb 2007	Jan 2010	Moshi	QNPS 7012	Orange	Fair	3.29
QB	Nov 2006	Oct 2008	DAR	6003	Green	Fair	3.35

Key: *DAR = Dar es Salaam; Man = manufacture; QB = quinine bisulphate; QH = quinine hydrochloride*

while *Bacillus sublitis*, *E. coli* and *Vibrio spp*. were isolated only in some of the batches. ‘

The results of the pyrogen test, which was specifically performed to ascertain the presence of both pyrogenic substances and viable microbial contaminants in WFIs, are summarised in Table 2. The rabbit pyrogen test confirmed our observation of probable contamination of the WFI samples as all the animals showed a rise of temperature of as high as 4 ºC following parenteral administration with WFI. Mean temperature rise of 1, 2.4 and 2.2 ºC were recorded for the first, second and third batches of WFI, respectively (Table 2). Temperature rise in each case was significant ($p < 0.001$) in relation to the baseline temperature.

DISCUSSION

The present study has demonstrated that commercial QNSs in the Dar es Salaam market are of good microbiological quality. Out of the 24 assayed samples, none has revealed any microbial contamination upon being sub-cultured onto non-selective as well as selective culture media. As expected, low water activity, which characterises almost all syrup preparations, did not favor microbial proliferation in these products [15]. Furthermore, QNS provides an unfavourable environment for microbial growth, due to its acidity with pH ranging from 2.78 to 6.2 [9] (as

indicated in Table 1). This level of acidity does not permit a number of non-acidophilic microorganisms to survive. However,

Table 2: Pyrogen tests on water for injection (WFI) in Albino rabbits.

Batch	Rabbit	Weight (kg)	Recorded temperature				Result
			0 min	15 min	60 min	360 min	
1st	1	2.45	38.6	38.7	38.9	39.3	Positive
	2	2.50	38.7	39.7	39.8	39.9	Positive
	3	2.66	37.7	37.7	37.9	39.5	Positive
	4	2.12	38.1	38.2	38.6	38.8	Positive
2nd	1	2.45	38.2	38.3	38.5	38.9	Positive
	2	2.50	38.0	38.2	38.6	39.0	Positive
	3	2.66	37.6	38.6	39.5	40.6	Positive
	4	2.12	37.3	40.3	40.9	41.3	Positive
3rd	1	2.45	38.6	39.8	39.9	41.4	Positive
	2	2.50	38.2	38.9	39.8	40.7	Positive
	3	2.66	38.1	38.5	38.8	39.8	Positive
	4	2.12	37.7	38.0	38.7	39.7	Positive

acidophiles would strive for survival in this kind of environment. If this had happened, the microbial by-products would have altered the pH, thus creating a more favourable condition for growth of other kinds of microorganisms [16]. Absence of detectable microbial contamination is indicative of adherence to GMPs by both local and foreign manufacturers of the products.

A previous unpublished study by us showed a high level of microbial contaminants in extemporaneously prepared QNSs in hospital pharmacies in Tanzania. This motivated us to embark on the present study. Moreover, it was found that some of the QNSs bore labels indicating a shelf-life of 2 years or longer (see Table 1). However, it was observed in a previous study that such a long shelf-life in poor storage conditions may lead to decomposition of some ingredients in the formulation[10], resulting in pH change and ultimately, microbial contaminations.

The results for WFI were surprising since preparations intended for parenteral or ophthalmic administration should be strictly sterile though this may alter during use [8,10]. Additionally, WFI should be free from pyrogens. The fact that microbial contamination was found in the WFI batches indicates that the WFI products were indeed substandard. According to the Parenteral Drug Association, during in-process quality control procedures, a sampling error of 1cfu/10ml is an acceptable action limit, but if action limits are exceeded, then immediate action must be taken to rectify the problem [8]. However, sterility test, on its own, does not provide a guarantee as to the sterility of a batch, but it is an additional check, and continued compliance with the test does gives confidence as to the efficacy of a sterilization or aseptic process. Failure to conduct sterility test, despite its shortcomings, may have important legal and moral consequences [18].

The rabbit pyrogen test, based on the intravenous injection of sterile solutions, has been employed for many years and it is still valid for the quality control of parenteral preparations [14,19-20]. Regardless of the existing gap between the pyrogenicity in rabbits and the expected pyrogenicity in humans as a consequence of species differences, rabbit pyrogen test continues to have one major advantage over other pyrogen test systems in that it can best replicate and

show the production of fever in humans. Moreover, the test not only detects a variety of injectable substances that can trigger fever but also microbial endotoxins or endogenous pyrogenic cytokines [21]. In this study, the test has proved to be useful and accurate, because apart from pyrogens, microorganisms were also isolated from the WFIs. In accordance with the requirements and definitions of WFI [8-9,16], the findings of this study show the presence of microbial contamination in the WFI preparations tested, thus rendering them unfit for the intended purposes. One of the findings of great concern is the presence of viable and potentially pathogenic microorganisms, namely, *Staphylococcus aureus* and *E. coli* [22]. The microbial contamination may be attributable to poor adherence to GMP, quality control procedures and inadequate post-marketing surveillance of medicines and other medical appliances in Tanzania.

CONCLUSION

This study has revealed that QNSs that were commercially available in the Dar es Salaam market, Tanzania were microbiologically fit for use. However, WFI were found to be microbiologically contaminated. The isolation of potentially pathogenic opportunistic microorganisms such as *S. aureus* and *E. coli* calls for more stringent measures during the production of the products as well as effective post-marketing surveillance. We, therefore, recommend that the Tanzania Food and Drugs Authority should improve on its regulatory control of WFI products prior releasing them into the market in order to assure the safety of users.

ACKNOWLEDGEMENT

The authors express their gratitude to the Ministry of Science, Technology and Higher Education of Tanzania for providing financial aid.

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