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Poor Quality Sterile Water For Injection In Nigeria Drug Distribution Chain: A Threat To The Attainment Of Health Related Millennium Development Goals In Nigeria

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Running title: *Poor quality sterile water for injection in Nigeria drug distribution chain.*

ABSTRACT: Water is frequently used in pharmaceutical industries and practice. For reconstituting parenterals, water is expected to be devoid of physicochemical and heavy metals contaminants, and maintain absolute microbial sterility with bacterial endotoxins not exceeding 0.25 EU/ml or 0.25 IU/ml. Thus the five (5) most frequently utilised brands of Sterile Water for Injection (SWFI) [coded SWFI-1, SWFI-2, SWFI-3, SWFI-4 and SWFI-5] in, South-South, Nigeria were assessed to determine if the products met the physicochemical, heavy metal and microbiological quality criteria stipulated in official compendia. The SWFIs were assayed for microbial contamination, bacterial endotoxins (pyrogens), heavy metals and physicochemical contaminants using the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP) methods. Results indicated that almost all the physicochemical quality indices of the SWFIs were within acceptable limits. However, none of the brands passed the heavy metal content criteria; with respect to lead, manganese, cadmium and chromium. Since 80, 60, 40 and 60% of the samples, were respectively laced with these heavy metals above 0.1 mg/l. No viable bacteria colony (VBC) was detected, after culture on nutrient agar and incubation at 37 ± 1 ºC over a 48 hour period. The bacterial endotoxins levels ranged from 0.239 ± 0.001 – 1.259 ± 0.000 EU/ml, and revealed that only SWFI-4 (0.239 ± 0.001 EU/ml bacterial endotoxins content) passed the pyrogen test for Sterile Water for Injection. Taken together, none of the samples passed all the quality assurance criteria for wholesome Sterile Water for Injection. Thus each of the products is unfit for clinical use; as such use is likely to be associated with heavy metal toxicity and pyrexia. Besides assigning control number to these products, various national drug regulatory agencies should up-grade the regulatory control of the total quality of SWFIs, in order to guarantee the safety of users.
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INTRODUCTION

Water is the most widely used substance, raw material or starting material in the production, processing and formulation of pharmaceutical products[1]. It has unique chemical properties due to its polarity and hydrogen bonds. This means it is able to dissolve, absorb, adsorb or suspend many different compounds. These include contaminants that may represent hazards in themselves or that may be able to react with intended product substances, resulting in hazards to health[1]. To guarantee product stability and safety, pharmacopoeias have described minimum critical quality requirements for water intended for pharmaceutical use, otherwise called Water for Pharmaceutical Use (WFPU). The basic prescriptions indicated that the least grade of WFPU must meet the Drinking Water (DW) standard recommended by the WHO and/or other international, regional and national guidelines on DW standard.

The various grades of WFPU listed in official compendia include: Purified Water (PW), Highly Purified Water (HPW), Water for Injection (WFI) and Sterile Water for Injection (SWFI). Most importantly, control of the quality of SWFI throughout the production, storage, distribution and clinical utilisation processes, including pyrogen, microbiological and chemical quality, is a major concern. Therefore, the assurance of its quality to meet the on-demand expectation is very essential.

WFI is used for the preparation of injectable drugs, whereas PW can be used in the manufacture of tablets, capsules, creams and lotions. Water for reconstituting injectable drugs must meet more stringent quality requirements. It is the most frequently employed vehicle for sterile products.

Since water constitutes the medium of all natural body fluids[2].

Water for injection is usually drinking water purified by distillation or such other processes that guarantee complete elimination of chemicals and microorganisms. WFI contains no added substance and must pass the test for Total Organic Carbon (TOC) and Water Conductivity (WC). Furthermore, WFI is expected to meet the requirement of the test for bacterial endotoxin as well as all the requirements of the tests recommended under Sterile Purified Water (SPW)[3].

Sterile water for injection (SWFI) is prepared from WFI that is sterilised and suitably packaged. It is free from added substances. When examined in suitable conditions of visibility, it is clear and colourless. Each SWFI container should contain a sufficient quantity of water for injections to permit the nominal volume to be withdrawn[4].

The quality standard of SWFI is broadly described by certain physicochemical and microbiological quality indices of WFPU. The physicochemical parameters, *inter alia*, include physical appearance, conductivity, pH, TOC, inorganic anions, metal and heavy metal ions, oxidisable substances, *et c*. While the microbiological quality index, is captured by products' sterility status and absence/presence of bacterial endotoxins, otherwise known as pyrogens.

A firm and continuous assessment of these parameters became imperative, since a number of valid scientific reports have highlighted the various health risk associated with each SWFI quality index. For instance, patients with cardiovascular disease are pre-disposed to congestive heart failure and acute pulmonary disease when excessive amount of sodium is administered parenterally[5]. Among other effects, lead poisoning (arising from cumulative ingestion or injection of water containing more than 0.1 mgPb/L) has been linked with delayed physical and mental development; especially in children[6]. Also, it has been indicated that the intravenous introduction of exogenous pyrogens into patients triggered the production of endogenous pyrogens via the action of phagocytic leukocytes. Thence, the endogenous pyrogens act on the thermoregulatory centre through the action of cyclic adenosine-3',5'-monophosphate (cAMP) and prostaglandins; resulting in increased oxygen utilisation and heat conservation, which leads to fever[7]. Clinical use of non-sterile WFI (NSWFI) could lead to bacteraemia, especially with gram negative organisms. This condition may be associated with fever, hypotension and intravascular coagulation; and also accounts for the presence of endotoxins in the blood of patients with NSWFI-induced sepsis[8].

In view of the strict quality control requirements of SWFI, the current study was designed to evaluate the physicochemical and microbiological quality indices of the highly demanded brands of SWFI in the South-South Geopolitical Zone of Nigeria. The findings will serve as a veritable template to discuss the suitability or otherwise of the various SWFI for clinical use and equally define their safety. This becomes extremely important, since there is a significant dearth of information in this segment of pharmaceutical products (SWFI) quality assessment and safety.

MATERIALS AND METHODS

Sample collection

The most highly demanded brands of sterile water for injection were purchased from pharmacy shops in the South-South Geopolitical Zone of Nigeria. The samples were handled and stored as prescribed by the manufacturers. Each of the SWFI brand was assigned a code and the manufacturer's and label information recorded, as presented in Table 1.0.

Table 1: Sample codes and product description of the sampled SWFI

Sample code	Sample description	Country of origin	Batch number	NAFDAC* registration Number	Manufacture date	Expiry date
SWFI-1	Sterile Water for Injection B.P.	Nigeria	BN 55BJ03	04-6442	Oct., 2012	Sept., 2015
SWFI-2	Sterilised Water for Injections B.P.	India	27/94	NA#	May, 2012	May, 2018
SWFI-3	Sterilised Water for Injections U.S.P.	Syria	165 09 11	A4-2635	Sept., 2011	Sept., 2014
SWFI-4†	Sterilised Water for Injections B.P.	India	1T542130	A4-1871	Nov., 2012	Oct., 2015
SWFI-5	Sterilised Water for Injections B.P.	India	2T543038	A4-4141	Feb., 2013	Jan., 2016

*NAFDAC: National Agency for Food and Drug Administration and Control.

#NA: Not Available

†SWFI-4 and SWFI-5 were manufactured by the same pharmaceutical industry in India and distributed by different pharmaceutical industries in Nigeria.

Reagents and instruments

GenScript ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (Catalogue No.: L00350; Lot No.: C50091310) was procured through local chemical stores and was used as supplied. Agilent Technologies Atomic Absorption Spectrophotometer, *Jenway* 6405 UK Ultraviolet/Visible Spectrophotometer, *ESCO* Tech. Inc., USA, Laminar Flow Chamber; PHS – 25 pH Meter, China, pH Meter; New Life DG-9023A, England, Electric Oven; Memmert 100-800, Incubator; LD2X-40B, Autoclave; Metler Chemical Balance and XH-D, Jiangsu Kangjian, Votex Mixer were employed at the various stages of the quality assessment study.

pH determination

The pH meter was calibrated with standard buffer solutions of pH 4.0, 7.0 and 9.10. Then pH of the SWFI samples was obtained by inserting the pH probe into each sample and reading off the value after stabilisation of the sample bath. The measurement was done thrice for each brand and the mean ± standard error of mean (SEM) was deduced and recorded.

Acidity/alkalinity

A 0.05 ml portion of phenol red was added to 20 ml aliquot of each of the test sample. A yellow colouration which turns red on addition of 0.1 ml of 0.01 M NaOH (aq.) indicates acidity.

Alkalinity is indicated by a red solution which turns yellow on addition of 0.15 ml of 0.01 M HCl (aq.)[4].

Oxidisable substasnces

To 100 ml of each test sample was added 10 ml dilute H_2SO_4 and heated to boiling. Then 0.4 ml of 0.02 M $KMnO_4$ was added to the mixture and boiled for further 5 minutes. A fairly pink colour indicated minimal amount of oxidisable substances, which is acceptable[3].

Chloride

To 20 ml of each of the test sample was added 5 drops of concentrated nitric acid and 1 ml of AgCl (aq.) TS and mixed. The test sample mixture was compared with that of a 20 ml aliquot of standard chloride solution (0.5 mg/l) prepared with high purity water. Turbidity formed within 10 minutes not greater than that produced in similarly treated control indicated that chloride ion is within acceptable limit[4].

Nitrates

To 5 ml of each test sample in a test-tube immersed in ice water was added 0.4 ml of a 100 g/l solution of KCl, 0.1 ml of diphenylamine solution. A 5 ml nitrate-free sulphuric acid was added to the mixture drop wise with shaking. The tube was placed in a water bath maintained at 50 ºC. After 15 minutes, any blue colour in the solution is not more intense than that in a reference solution prepared at the same time in the same manner using a mixture of 4.5 ml of nitrate-free water and 0.5 ml of standard nitrate solution (2 mg/l NO_3^-) indicates acceptable amount of nitrate[4].

Sulphates

To 10 ml of test sample was added 0.1 ml of saturated $BaCl_2$ (aq.) solution; the absence of turbidity indicated the absence of sulphate ion[4].

Residue on evaporation

A 100 ml aliquot of each sample was placed in tarred crucible, evaporated to dryness over a water bath and dried in an oven at 100 – 105 ºC. The weight of the residue was deduced, and per cent residue on evaporation evaluated from the equation below.

% Residue on evaporation = {[Wt. of residue (g)]/100} * 100

Determination of metal and heavy metal content

The levels of calcium, potassium, magnesium, aluminium, zinc, sodium, cadmium, lead, manganese and chromium were determined using Atomic Absorption Spectrophotometer (AAS). Standard metal solutions were prepared for each metal and calibration curves for each metal was obtained from a linear plot of the absorbance of standards against concentration in parts per million (ppm). The absorbance due to each metal in the test samples was similarly determined, and the corresponding concentration of each metal ion was extrapolated from the calibration curves.

Test for sterility

A 1-in-10 dilution of each SWFI sample was prepared by adding 1 ml of each sample into a 9 ml aliquot of sterile normal saline. Then 1 ml portion of the 1-in-10 dilution was thoroughly mixed sterile 20 ml nutrient agar and poured into an labelled petri dish. The nutrient agar was allowed to solidify, before incubating it at 37 ºC for 24 hours. The number of discrete colonies were counted and expressed as bacterial cells per ml. A blank experiment was also carried out.

Bacterial endotoxin test

For each of the standards, samples and LAL reagent water, 0.1 ml was carefully dispensed into different endotoxin-free vials and labelled. Samples were mixed thoroughly for 30 seconds. A 0.1 ml aliquot of reconstituted LAL was added to each vial. The vials were capped and mixed well by swirling gently. The rack with all the vials was incubated at 37 ± 1 ºC using water bath for 8 minutes.

After incubation, 0.1 ml of reconstituted chromogenic substrate solution was added to each vial. It was mixed gently by swirling with a vortex mixer. The vials were incubated for 6 minutes at 37 ± 1 ºC using water bath. A 0.5 ml aliquot of reconstituted stop solution (colour-stabilizer #1) was added to each vial and vortex-mixed by swirling gently without shaking or inverting to avoid foaming. To the resulting mixture in each vial was added 0.5 ml of reconstituted colour-stabilizer #2 and mixed well. Finally, 0.5 ml of reconstituted colour-stabilizer #3 was added to each vial. Each vial was gently

swirled to mix well foe 3 seconds. The absorbance of the resulting solution in each vial was read at 545 nm, and the corresponding pyrogen concentration deduced. The LAL reagent water was used as blank.

Statistical analysis

Where applicable, results were expressed as Mean ± SEM. Means were compared for statistical significant difference by one-way analysis of variance (ANOVA) using Duncan post-hoc test with aid of GraphPad Prism® statistical software version 6.0. Observations were considered significant at $p < 0.05$ in all cases.

RESULTS

The result of the physicochemical quality indices of the sampled SWFI is presented in Table 2. While Table 3, indicated the levels of metals and heavy metals contained in the various brands of SWFI sampled.

Table 2: Physicochemical quality indices of the sampled SWFI

Physicochemical Parameter	SWFI-1	SWFI-2	SWFI-3	SWFI-4	SWFI-5	Reference standard
Physical appearance	Clear	Particles present	Clear	Clear	Clear	Clear
pH	6.87 ± 0.01	6.60 ± 0.00	6.86 ± 0.04	6.86 ± 0.03	6.70 ± 0.02	5.00 – 7.00
Dissolved CO_2	Clear	Clear	Clear	Clear	Clear	Clear
Chloride	No turbidity	No turbidity	No turbidity	No turbidity	No turbidity	Not > 0.50 $mgCl^-/l$
Nitrates	Slightly blue	Slightly blue	Slightly blue	Slightly blue	Slightly blue	Not > 2.00 $mgNO_3^-/l$
Oxidisable substances	Faintly pink	Faintly pink	Faintly pink	Faintly pink	Faintly pink	Fairly pink colouration
Sulphates	No turbidity	No turbidity	No turbidity	No turbidity	No turbidity	Absence of turbidity
Residue on evaporation (%w/v)	Nil	0.01	Nil	Nil	Nil	Not > 0.004% w/v

Table 3: Levels of trace metals and heavy metals (mg/l) in the sampled brands of SWFI

Metal ion (mg/l)	SWFI-1	SWFI-2	SWFI-3	SWFI-4	SWFI-5	Reference standard
Sodium	ND†	1.149 ± 0.001	0.010 ± 0.001	ND	0.017 ± 0.000	NA*
Calcium	ND	0.074 ± 0.002	0.012 ± 0.001	ND	ND	NA
Potassium	ND	0.480 ± 0.001	0.102 ± 0.002	0.028 ± 0.000	0.044±0.001	NA
Magnesium	0.004±0.001	0.058 ± 0.001	0.004 ± 0.000	0.006 ± 0.001	ND	NA
Aluminium	ND	0.940 ± 0.002	13.047± 0.001	3.457 ± 0.000	10.693±0.002	0.100 – 0.200 mg/l
Zinc	ND	ND	ND	0.006 ± 0.001	ND	3.000 mg/l maximum
Manganese	0.095±0.001	0.072 ± 0.000	0.149 ± 0.000	0.210 ± 0.001	0.180 ± 0.001	0.100 mg/l maximum
Lead	0.363±0.000	ND	0.152 ± 0.002	0.124 ± 0.001	0.323 ± 0.000	0.100 mg/l maximum
Cadmium	0.400±0.001	ND	0.146 ± 0.000	ND	0.046 ± 0.001	0.100 mg/l maximum
Chromium	0.018±0.001	0.538 ± 0.002	0.614 ± 0.001	0.406 ± 0.002	0.040 ± 0.001	0.100 mg/l maximum

†ND: Non-determinable
*NA: Not Available

The result of the sterility test was presented in Table 4. It revealed no form of microbial contamination. The levels of bacterial endotoxins (pyrogens) contained in the sampled SWFI were indicated in Table 5.

Table 4: Total viable bacteria count (colony forming unit per millilitre, cfu/ml) of the sampled brands of SWFI

SWFI sample	Total viable count (cfu/ml)	Reference standard
SWFI-1	0.00 ± 0.00	No microbial culture[†]
SWFI-2	0.00 ± 0.00	No microbial culture
SWFI-3	0.00 ± 0.00	No microbial culture
SWFI-4	0.00 ± 0.00	No microbial culture
SWFI-5	0.00 ± 0.00	No microbial culture

[†]That is, there should be NO evidence of MICROBIAL GROWTH after a sterile culture medium has been inoculated with aliquots of SWFI.

Table 5: Levels of bacterial endotoxins (EU/ml) present in the sampled brands of SWFI

SWFI sample	Endotoxin level (EU/ml)	Reference standard (EU/ml)
SWFI-1	0.514 ± 0.001	≤ 0.250
SWFI-2	1.259 ± 0.000	≤ 0.250
SWFI-3	0.304 ± 0.002	≤ 0.250
SWFI-4	0.239 ± 0.001	≤ 0.250
SWFI-5	0.382 ± 0.00	≤ 0.250

DISCUSSION

The sampled SWFIs, except SWFI-2, meet the physicochemical quality criteria for clinical use. However, SWFI-2 had 0.01% (w/v) residue on evaporation; which exceeded the upper limit of 0.004% (w/v) prescribed in the British Pharmacopoeia[4]. Under suitable visibility condition, particulate matter was observed in the product vials. This indicated poor filtration process or recontamination before packaging, which are among the resultant effects of non-adherence to Good Manufacturing Practice (GMP). Lead contents of 80% of the test samples were higher than 0.1 mg/l recommended by the British and European Pharmacopoeia[4]. In addition, 60, 40 and 60% of the samples, respectively failed the limits of manganese, cadmium and chromium contents for SWFI. Since these outcomes were observed across the samples, none of the SWFI passed the heavy metal quality criteria and could be regarded as unfit for clinical use.

Water intended for reconstituting or diluting parenteral injections or ophthalmic administration is expected to be strictly sterile and void of bacterial endotoxins[9]. The microbial examination of SWFIs suggested adequate microbial quality. Endotoxins estimation however indicated that all the samples, except SWFI-4, contained endotoxins/pyrogens above the pharmacopoeia limit of 0.25 EU/ml[3]. It has been established that the intravenous introduction of pyrogens triggers the production of endogenous pyrogens, which results in increased oxygen demand and heat conservation. This may be encountered in the clinical use of the SWFIs, thus leading to fever in patients. Sustained and prolonged fever could cause dehydration; which in turn, among other harmful effects, results to shrinking of red blood cells and tissue damage.

It must be emphasized that none of the samples passed all the prescribed quality criteria for SWFI. Thus all the brands were declared unfit for use, and constitute public health concerns.

In a similar study Mwambete *et al*.[9] reported heavy microbial contaminations (87 – 100 cfu/ml) in three brands of SWFIs distributed for clinical use across all the three districts of Dar Es Salaam, Tanzania. Further microbial tests revealed the presence of *Staphylococcus spp*. in all the brands of SWFIs, while *Bacillus sublitis*, *Escherichia coli* and *Vibrio spp*. were isolated in some of the brands. A rabbit pyrogen test confirmed the presence of bacterial endtoxins in the SWFIs, since the mean temperature

rise of 1 – 2.4 ºC among the sampled SWFIs were significant ($P < 0.001$) with respect to the base line temperature.

CONCLUSION

The result of this study and the Tanzanian report, suggested a probable high incidence of clinically unfit SWFIs in African drug distribution chains. Also, post-marketing surveillance of the various national agencies charged with the regulations of drug administration in this regard, is suspected to be grossly inadequate. Therefore, we recommend that these agencies should up-grade their regulatory control of SWFIs before and after release into the distribution chain in order to guarantee the safety of users.

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