The Microbial Quality of in-use Multidose Chloroquine Vials

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An evaluation of the microbial quality of 301 partially used chloroquine vials from non medical wards of Muhimbili Medical Centre, a referral and teaching hospital in Dar es Salaam Tanzania was done. Findings obtained revealed that 26.5% and 76.4% had their contents and septa contaminated respectively, in spite of the fact that the preservative 1% benzoic acid passed the challenge test. Some of these microbial contaminants were identified as Staphylococcus epidermidis, Streptococcus faecalis, Pseudomonas aeruginosa, Kiebsiella spp and Proteus spp. The presence of these bacteria in the contents of these vials indicates that the in-use chloroquine vials may act as a source for acquisition of nosocomial infections by inpatients.

INTRODUCTION

Chloroquine is the drug of choice for prophylaxis and treatment of malaria, caused by *Plasmodium vivax, P. ovale, P. malaria* and susceptible strains of *P. falciparum* [1]. Chloroquine injection is often packed in ampoules or vials. While the contents of ampoules must be used all at once, multidose vial containers allow the withdrawal of single doses of the injection, with the minimal risk of contamination to the remainder [2].

The potential hazard arising from the in-use of microbial contaminated multidose vials has long been recognized [3]. For example insulin and several intravenous infusions have been shown to be vulnerable to such in-use contamination [4-8].

The contamination of multidose containers may arise from many causes including failure to sterilize the cap before drawing the dose, failure to sterilize needles or syringes, injection of unsterile air, or inrush of unsterile air as the needle is withdrawn [2]. This may lead to the introduction of bacteria so that the last doses contain millions of potentially pathogenic micro-organisms which could lead to nosocomial infections [9].

Since multidose chloroquine injections are often used for malaria chemotherapy at Muhimbili Medical Centre, Dar es Salaam, it was deemed of interest to document the extent to which microbial contamination of in-use multidose vials of chloroquine injection occurs as no similar study has been reported.

MATERIALS AND METHOD

Sample collection

Three hundred and one (301) partially used multidose vials of chloroquine which had been kept between 1 - 23 days after opening were collected from 18 non-medical wards of Muhimbili Medical Centre Hospital. Hospital staff were not alerted in advance to avoid the chance of over carefullness in the use of the vials.

Microbial isolation

To asses the microbial contamination of the rubber septum, a sterile cotton wool bud was moistened with sterile triptane soya broth (Oxoid) containing 3% Tween 80 (as preservative inactivator) and wiped over the rubber closure without touching the metal rim. The bud was placed in 20 ml of triptane soya broth, incubated at 35^{o}c for five days and examined for turbidity. To asses the vial contents for microbial contamination, the rubber septum was first swabbed with 90% ethanol, then 0.5 ml of the chloroquine was withdrawn aseptically within the laminar flow cabinet and cultured in 20 ml of triptane broth as described above.

Microbial isolation and identification

The cultures which showed turbidity after the incubation period were sequentially inoculated onto Blood agar, MacConkey, Sabouraud's and Nutrient Agar Oxoid) using a laptul of the broth. The Blood, MacConkey and Nutrient Agar plates were incubated at 37^{o}c for 18 hours, while the Sabouraud's agar plates were incubated at 25^{o}C for one week for bacterial and fungal isolation respectively. Species identification to genus level and sometimes to species level was done using the appropriate biochemical tests and selective medium.

Preservative test

A challenge test was performed on 3 unopened chloroquine vials using S. *aureus* NCTC 6571 as specified by the BP 1988 [10].

RESULTS

The rubber septa of 230 out of 301(76.4%) showed microbial contamination while the contents of 77 vials (25.6%) were contaminated. The contaminants isolated included potential nosocomial pathogens as seen in

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tables 1 and 2 respectively. It was also observed that no contaminants were isolated from in use chloroquine vials which had been kept for less than 5 days after opening.

Representative results of the challenge test using *Staph. aureus* NCTC 6571 in unopened chloroquine vials preserved with 1% benzoic acid, which were stored at 25°C showed a 10^3 fold reduction within 18 hours from 5.2 x 10^6 cfu to 4.8 x 10^3 cfus, with no recovery after 28 days.

TABLE 1: Microbial isolates from rubber septa.

Micro-organism	Frequency of Isolation	Percentage
E. coli	19	6.3
Staphylococcus epidermidis	42	13.95
Streptococcus faecalis	17	5.6
Micrococci spp	1	0.3
Bacillus spp	161	53.5
Klebsiella spp	25	8.3
Pseudomonas aeruginosa	5	1.7
Lactose Fermenters	8	2.7
Proteus spp	3	1
Moulds	8	2.7

TABLE 2: Microbial Isolates from vial contents

Microorganism	Frequency of Isolation	Percentage
Bacillus spp	28	9.3
Staphylococcus epidermidis	16	5.3
Klebsiella spp	13	4.3
Pseudomonas aeruginosa	5	1.7
Streptococcus faecalis	1	0.3
Proteus spp	9	3.0
Micrococcus spp	10	3.3
Non Lactose Fermenters	1	0.3
Penicillin notatum	1	0.3
Other Moulds	4	1.3

DISCUSSION

The study revealed that 26.5% and 76.4% of sampled in-use chloroquine vials kept for more than 4 days after opening, had their vial contents and rubber septum contaminated respectively. This was in spite of the fact that the preservative, 1% benzoic acid, in unopened vials passed the challenge test according to the B.P 1988 specifications.

The high incidence of contamination of injectables is consistent with reports by other workers, that higher rates of contaminations are invariably seen in sterile products, after opening and use in hospitals [9].

The presence of potential pathogenic micro-organisms isolated from the vial contents of the sampled chloroquine vials such as, *Pseudomonas aeruginosa, Staphylococcus epidermidis, Streptococcus faecalis, Kiebsiella* spp suggests that in use contaminated chloroquine vials may act as vehicles for transmission of nosocomial infections. The source of these contaminants could probably be skin sheddings or contaminated hands of hospital staff in the case of S. epidermidis, PS. aeruginosa and St faecalis, contaminated disinfectant solutions in the case of PS. aeruginosa and the air in the case of the various other bacteria and fungi isolated. High levels of aeromicrobial contamination have previously been documented in this hospital [11].

These findings, therefore suggest that multidose chloroquine vials should be used within 4 days after opening the multidose vials in a hospital situation. On average a vial of chloroquine takes 19 days to finish.

CONCLUSION

We conclude that multidose chloroquine vials may easily be contaminated with potential pathogens during use. The contaminated vials may act as sources of acquisition of nosocomial pathogens by in-patients.

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