Evaluation of the *in vitro* bactericidal activity of a sodium hypochlorite solution sold in the Cameroonian markets on the germs responsible for nosocomial diseases in Cameroon

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Nosocomial infections contribute significantly to the increase of mortality in overall maternal, Neonatal and infant or new born baby in Cameroon while this can be avoided by good hygiene practices. The use of sodium hypochlorite remains a preventive hygiene solution that has already proved its effectiveness and that is mainly use in Cameroonian hospital but this is used diluted and it effectiveness effect has been never evaluated.

In order to standardize the use of a commercial sodium hypochlorite solution, we evaluated its action on the viability of the main bacteria (*Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus*) responsible for nosocomial infections in Cameroonian hospitals and its stability to light and darkness.

The results obtained showed that non-diluted sodium hypochlorite (2.4% a.c.) destroys all the bacteria species after 5 min of incubation. But diluted its bactericidal effect remains effective until dilution 1/10 (0.24% a.c.) and beyond this dilution its bactericidal effect decreases strongly and becomes dependent of the studied bacterial species, incubation penal method and type of preservation.

Key words: Nosocomial infection, Sodium hypochlorite, Bactericidal effect, Cameroon

1. Introduction

The hospital environment is a setting in which day-to-day care of various types is provided: consultations, vaccinations, medical analysis, surgical interventions, or any other medical care. Therefore, it is called to be a sterile place to compete for the provision of quality care to patients. Paradoxically, this environment has become, according to the World Health Organization (WHO), one of the environments, more and more conducive to the outbreak or even spread of so-called nosocomial infections of Health-care-associated infection (HAI) (1).

These infections are now the subject of particular and constant attention in hospital practice in general in both developed and developing countries (2) (3).

The World Health Organization estimated the burden of HAI and shows that it affects 5% to 15% of hospitalized patients in developing countries (4) and as many as 50% or more of patients in intensive care units (ICUs) (5). The magnitude of the problem remains underestimated or even unknown largely because nosocomial infections diagnosis is complex and surveillance activities to guide interventions require expertise and resources (6).

In Cameroon particularly, HAI prevalence is around 15% (7). Hence, nosocomial infections (HAI) contribute significantly to the increase in overall maternal, neonatal or infant mortality rates (8).

Generally, the agents that are usually involved in the HAI include many germs like *Streptococcus* spp, *Acinectobacter* spp., *coagulase negative Staphylococci*, *Staphylococcus aureus*, *Bacillus cereus*, *Legionella* and Enterobacteriaceae family members namely *Klebsiella pneumoniae*, *Escherichia coli* and others (9).

According to the Committee for the Control of Nosocomial Infections (CLIN), the use of sodium hypochlorite solution (bleach) reduces these infections by 75% (10). In most commonly encountered hospital facilities, the use of bleach is common but subject to caution. Concentrations of active chlorine are generally not standardized. The contact time is generally variable from one user to another. Also, incompatibilities between bleach and other surface care products remain unknown to most users (11). It is in this context that this work, in which we propose to determine the minimum concentration usable in hospital to destroy the germs responsible for nosocomial infection.

Material and methods

Strains origin and collection

Five bacterial isolates known to be responsible for HAI were obtained from Centre Pasteur du Cameroun, the Cameroon-Pasteur Institute International Network. Were they have been stored at -80°C after they have been isolated from patients that have been hospitalized in mains hospital of Yaoundé (the Political town of Cameroon) and suspected

for nosocomial infection. These isolates comprised gram negative bacteria - *Escherichia coli* - *Klebsiella pneumonia* - *Pseudomonas aeruginosa* and Gram positive bacteria - *Enterococcus faecalis* - *Staphylococcus aureus*.

Bleach collection

A commercial solution of sodium hypochlorite (La Croix, 2.4% active chlorine, Colgate-Palmolive, Cameroon) was used. It was bought at random in shops in the city of Douala. This brand was chosen because it is the most widespread and also because the disinfecting activity has been demonstrated (11).

Bleach stability evaluation

The stability of the active chlorine consisted in assaying the active chlorine in the commercial bleach solution immediately after purchase and during 35 days' storage.

The active chlorine content was first determined immediately after purchase. The contents of each bottle were then divided into two parts.

One part was preserved in the presence of light and another was shielded from light for two months. The active chlorine dosage in each of the samples was first performed daily for seven days and thereafter every seven days according to Ballereau and collaborators (12).

Briefly each sample was diluted 1 / 10th. Thereafter, 10 mL of this solution were introduced into an Erlenmeyer, and 1 g of potassium iodide (KI), 5 mL of acetic acid and 100 mL of distilled water were then successively added. The mixture was homogenized for two minutes and then titrated with a solution of 0.1 N sodium thiosulfate contained in a 50 mL burette. The starch paste served as an indicator of the presence of iodine during the assay. The test was repeated three times. At the end of each titration, the different equivalent volumes were noted. These volumes were used to calculate the percentages of active chlorine using the following formula:

$$\text{Concentration of active chlorine\%} = 0.355 \times n / d \times 10 \qquad (1)$$

With *d* corresponding to density of bleach and was estimated to 1.05, n representing the volume of thiosulphate in milliliters (+/- 0.01)

Bactericidal activity of bleach according to concentration and incubation time

We prepared a bacterial suspension ($\approx 1.5 \times 10^8$ CFU/mL of each bacterial species) from young colonies using Mac Farland's 0.5 scales. Seven dilutions (0; 1/5; 1/10; 1/15; 1/20; 1/50; 1/100) of the commercial hypochlorite solution (2.4% a.c.), corresponding respectively to 2.4%; 0.48%; 0.24%; 0.16%; 0.12%; 0.048%; 0.024% active chlorine, were carried out. Each of this dilution was mixed with 3ml of each bacterial species suspension, then seeded on of agarized Mueller-Hinton medium (Oxoid, Cambridge, UK) Petri dishes after incubation at different times (5, 10, 15, 20, 25, 30 min). Incubation was carried out of 37°C in dry incubator for 24 hours. Inoculums without any treatment served as a positive control during the evaluation.

Ethical considerations

The administration authorization was gotten from Centre Pasteur of Cameroon, to obtain the isolated germs in the hospital services.

Data analysis

Fisher's exact and Pearson chi square tests were used to estimate the difference of stability of active chlorine between dark and sunlight conservation using statistical software R version 2.15.3 (www.r-project.org). Two-sided *p* values of 0.05 or lower were considered statistically significant.

Results

Stability of active chlorine in bleach with time according to the conservation mode

The control of the initial active chlorine concentration gives a value of 2,455%. This value corresponds to what is indicated by the manufacturer.

The figure 1 shows the degradation kinetics of active chlorine as a function of time according to it mode of conservation. From this, it appears that, the concentration of active chlorine reminds almost stable during the first five days, irrespective of whether or not it is exposed to light (Figure 1). Beyond this period, it concentration begins to decrease irrespective of the mode of conservation. But this decrease seemed to be more accentuated for the sample exposed to light than that preserved in the absence of light.

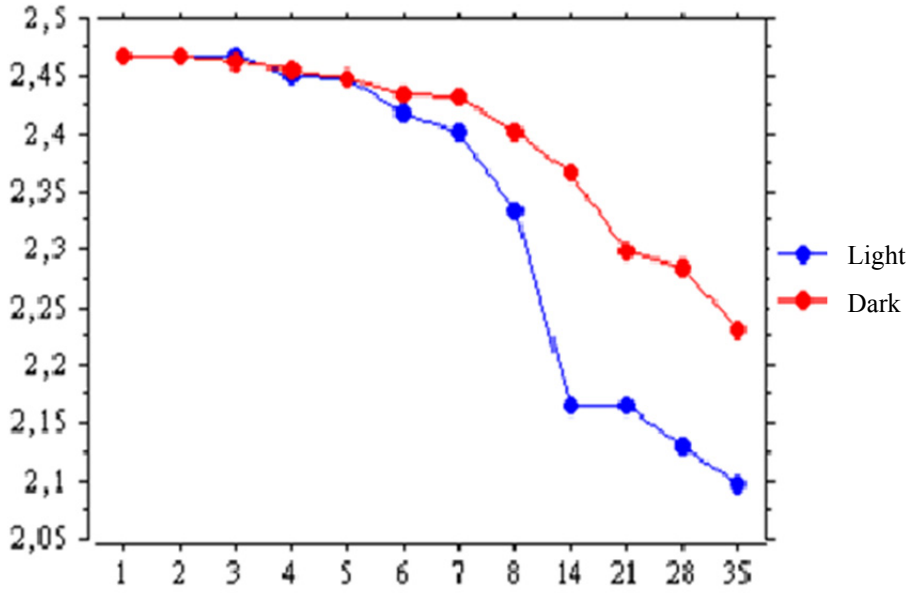

Figure 1: Kinetics degradation of active chlorine with time according to mode of conservation

Bactericidal activity of commercial bleach

Non-diluted bleach (2.4% c.a.) was bactericide on all the five main nosocomial bacteria. This bactericidal activity remains effective until 1/10e dilution (0.24%). Below this concentration, the bactericidal activity varies according to the germ (Table 1). The Gram-positive bacteria appeared to be more resistant to bleach action than the Gram-negative bacteria that began to be resistance only to 1/50e dilution (0.048%).

Table 1: Effects of the diluted sodium hypochlorite solution on its bactericidal activity

		Contact times					
Concentration in a.c. (%)	Bacteria	Control	T=5mn	T=10mn	T=15mn	T=20mn	T=30mn
	E. coli	+	-	-	-	-	-
	K. pneumonia	+	-	-	-	-	-
2,400	*P. aeruginosa*	+	-	-	-	-	-
	E. faecalis	+	-	-	-	-	-
	S. aureus	+	-	-	-	-	-
	E. coli	+	-	-	-	-	-
	K. pneumonia	+	-	-	-	-	-
0,480	*P. aeruginosa*	+	-	-	-	-	-
	E. faecalis	+	-	-	-	-	-
	S. aureus	+	-	-	-	-	-
	E. coli	+	-	-	-	-	-
	K. pneumonia	+	-	-	-	-	-
0,240	*P. aeruginosa*	+	-	-	-	-	-
	E. faecalis	+	-	-	-	-	-
	S. aureus	+	-	-	-	-	-
	E. coli	+	-	-	-	-	-
	K. pneumonia	+	-	-	-	-	-
0,160	*P. aeruginosa*	+	-	-	-	-	-
	E. faecalis	+	-	-	-	-	-
	S. aureus	+	+	+	+	+	+

	E. coli	+	-	-	-	-	-
0,120	*K. pneumonia*	+	-	-	-	-	-
	P. aeruginosa	+	-	-	-	-	-
	E. faecalis	+	+	+	+	+	+
	S. aureus	+	+	+	+	+	+
	E. coli	+	-	-	-	-	-
	K. pneumonia	+	+	+	+	+	+
0,048	*P. aeruginosa*	+	-	-	-	-	-
	E. faecalis	+	+	+	+	+	+
	S. aureus	+	+	+	+	+	+
	E. coli	+	+	+	+	+	+
	K. pneumonia	+	+	+	+	+	+
0,024	*P. aeruginosa*	+	+	+	+	+	+
	E. faecalis	+	+	+	+	+	+
	S. aureus	+	+	+	+	+	+

+ = Growth - = No growth; T = time; ◯ = Maximum bactericidal dilution of sodium hypochlorite

Discussion

Sodium hypochlorite solutions, which have been proved, to be bactericidal, viricidal and to dissolve protein, have been widely used as irritants in endodontics (13, 14). The concentration of sodium hypochlorite solutions used in endodontics may vary from 0.5-5.25% available chlorine (a.c.) (15). This assertion is in accordance with the commercial bleach solution used in this study in which the available chlorine (a.c.) concentration was 2.5%.

Our study showed that the a.c. of this commercial bleach solution was deteriorated with time but this deterioration was more accentuated with sunlight exposure. This result has also been obtained by Clarkson and collaborators (16). We think that the a.c. deterioration observe in dark and sunlight conservation of the bleach solution can be due to the average daily temperature of Douala which was 25°C as it have been showed that temperature of 24°C may decrease the a.c. concentration (15).

Interestingly the commercial bleach used in this study was bactericidal on all the bacteria species responsible of healthcare-associated infections (HAIs) at 2.4% a.c. after five minutes' incubation. This bactericidal effect remains effective in all bacteria until 1/10 dilution (0.24%). This result shows the importance to use this commercial bleach in undiluted condition when the objective is to destroy the bacteria responsible of HAIs. More interesting this bactericidal action remains effective for Gram negative bacteria species till 1/20 dilution (0.12% a.c.). This result showed that Sodium hypochlorite presents undeniable microbicidal action on causative agents of HAIs as shown elsewhere (17).

The Gram-positive bacteria species (*S. aureus and E. faecalis*) present resistance to sodium hypochlorite solution at active chlorine concentration below to 0.24%. This result might be due to difference in cell wall structure between gram positive and gram negative bacteria. In fact Gram-negative bacteria have high lipid content in their external membrane compared to gram positive bacteria due to absence of outer membrane. And it has been shown that mechanism of action of sodium hypochlorite causes lipid and fatty acid degradation (18).

Conclusion

The present study confirmed the *in vitro bactericidal* efficacy of 2.4% a.c. of commercial sodium hypochlorite solution and the decrease of it activity with time according to de dilution and to type of conservation. It gives for the first time a condition of use of this endodontic irrigating solution in Cameroon.

Conflict of Interest Statement The authors of this study have no conflict of interest to disclose.

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