



Short Communication

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Quality of hydro-alcoholic products used in Senegal: pilot study

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ABSTRACT

Antisepsis and disinfection have always played an important role in the fight against infectious diseases. The use of these products has been effective in breaking the chain of transmission of microorganisms. Today, with the advent of COVID-19, the main recommendations are, among other things, physical distance and the use of antiseptic products, including hydro-alcoholic products. In Senegal, with poor regulations on the acquisition and distribution of antiseptics and disinfectants, this situation has led to a proliferation of antiseptics and disinfectants on the national market. This work aimed to study the quality of hydroalcoholic products found in the trade. We evaluated ten samples of hydro-alcohol products collected during the month of March 2020. Physical and microbiological controls were performed at the National Drug Control Laboratory. The alcohol content of the samples ranged from 63 to 85% and were consistent with WHO recommendations except for samples E3 and E5. The pH values varied between 4.02 and 6.64 and the densities of the hydro-alcoholic gel samples ranged from 0.84 to 0.92 g/cm³; E2, E4, E5, E6 samples had densities greater than 0.89 g/cm³. The samples of hydro-alcoholic products all conformed to the sterility test and no microbiological contamination was observed. Antimicrobial activities of the hydro-alcoholic samples tested ranged from 58.3 to 100% with two samples showing no activity (E2 and E5). In view of the results of this study, it would be relevant to expand and deepen investigations by a significant increase in the number of samples and by carrying out as complete an analysis as possible.

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Keywords: Hydro-alcoholic products; quality control; hand hygiene.

INTRODUCTION

Antisepsis and disinfection have always played an important role in the fight against infectious diseases. The use of antiseptic and disinfectant products has been effective in breaking the chain of transmission of microorganisms such as viruses, bacteria and

fungi (Chabot Daval, 2010). During the 2009 influenza A (H1N1) pandemic, public health measures had to be put in place to prevent and limit the transmission of the virus in the population (Afssaps, 2010). One of the main measures to fight this scourge was the use of antiseptics and disinfectants (Travkine, 2012).

Today, with the COVID-19 pandemic, the main recommendations include physical distance and the use of hydro-alcoholic products (Goetz, 2004; Meunier et al., 2012).

As a result of this pandemic, various forms of hydro-alcoholic products from a multitude of manufacturers have been put on the market as a consequence of the lack of a deficient regulation concerning the acquisition and distribution of antiseptics and disinfectants. In Senegal, no studies have been conducted to evaluate the efficacy of hydro-alcoholic products. The aim of this work was to study the quality of hydroalcoholic products on the physico-chemical and antimicrobial activities level found in the trade.

MATERIALS AND METHODS

Framework for the study

Samples of hydro-alcoholic products were collected in different health structures (Table 1). The physicochemical and microbiological controls were carried out at the National Drug Control Laboratory.

Materials

- The material used for physical and chemical analyses consists of conventional laboratory glassware and consumables. It also includes equipment such as: magnetic agitator, alcoholmeter, pH-meter (Metrohm, Switzerland) and a densitometer (05 DENSI 75 Germany).

- For microbiological analyses, the equipment included:

- Culture media: Mueller Hinton (MH), Thiogluconate Broth (BT); Consumables (petri dishes, discs, swabs, test tubes, vials, pipettes, tweezers, wash bottle, rack, test tubes and an oven).
- Reagents: sterile distilled water, sterile physiological water;
- Reference strain: *Staphylococcus aureus* ATCC 29293.

Physico-chemical control

Physical-chemical control consisted of determining the alcoholic degree (proportion of ethanol, contained in a hydroalcoholic product

at a temperature of 20 °C) using an alcoholmeter and making dilutions using the Gay Lussac table for hydro-alcoholic gels, determining pH and density.

Sterility test of hydroalcoholic products

For this test, we put 1 ml of hydro-alcoholic product sample in a liquid culture medium (Thiogluconate broth) that was incubated at 37 °C for 18 to 24 hours. The presence of turbidity visible to the naked eye in a sample of hydroalcoholic product incubated after 18 to 24 hours in thiogluconate broth is considered non-sterile.

Antimicrobial activity test of hydro-alcoholic products

This method consists in determining the antimicrobial activity of hydro-alcoholic products by comparing the number of colony-forming units (CFU) appearing on inoculated media in the presence of disinfectant and control media containing no disinfectant.

Preparation of the inoculum 0.5 Mac Farland

Starting from a young (18-24h) culture of the reference strain (*Staphylococcus aureus* ATCC 29293) 2 to 3 colonies are taken which are introduced into a test tube containing sterile physiological water. The density of the solution is measured with a Mac Farland densitometer and adjusted to a value of 0.5 on the Mac Farland scale.

Contamination of sterile gloves

A sterile glove is contaminated with 1 ml of the initially prepared 0.5 Mac Farland inoculum. The glove surface is rubbed and waited 30 seconds to ensure that the contamination is effective. We add 3 ml of hydro-alcoholic products and then rub our hands for 30 seconds to allow good product-germ contact.

Seeding of Thiogluconate Broth

Using a swab, a sample is taken from the contaminated gloves with added disinfectant and introduced into the Thiogluconate broth medium and incubated at 37 °C in the oven for 18 to 24 hours. Then two further samples were taken from the contaminated gloves without the addition of

disinfectant and the uncontaminated gloves which are used as positive and negative controls.

A total of five tubes of Thiogluconate broth were prepared for each sample of hydro-alcoholic products and distributed as follows:

- Tube 1: broth + sterile glove (negative control) ;
- Tube 2: broth + contaminated sterile glove (positive control);
- Tube 3: BT broth alone (sterility control medium) ;
- Tube 4: broth + 3ml of hydro-alcoholic product (gel sterility control);
- Tube 5: broth + contaminated sterile glove + 3 ml of hydro-alcoholic product (activity test).

Seeding of MH agar (Mueller Hinton)

A dilution series is carried out with the BT medium after 18 to 24 hours incubation by taking 10 µl of BT which are introduced in 1000 µl of physiological water. A series of

three dilutions is carried out from the solution obtained by taking 10 µl of the most concentrated diluted solution each time and introducing 1000 µl of physiological water until the working solution used to inoculate the MH agar for bacterial enumeration is obtained. This inoculation is done using a calibrated 10 µl handle. Incubation was done at 37 °C in an oven for 24 hours. The enumeration is performed by counting the colonies on the petri dish. The percentage antibacterial activity is given by the following formula:

N (CFU/ml) = number of colonies counted.

$$A = \frac{(N \text{ positive control} - N \text{ sample})}{N \text{ positive control}} \times 100$$

A = activity (%)

N positive control = number of positive control colonies

N sample = number of colonies in the sample.

Table 1: Lots of hydro-alcoholic products collected.

Sample	Name	Manufacturer
E1	Hydroalcoholic solution	CHN Aristide Le Dantec Hospital Pharmacy
E2	Hydro-alcoholic gel	A pharmacy in the department of Dakar
E3	Hydro-alcoholic gel	A pharmacy in the department of Dakar
E4	Hydro-alcoholic gel	A pharmacy in the department of Dakar
E5	Hydro-alcoholic gel	A pharmacy in the department of Dakar
E6	Hydro-alcoholic gel	A pharmacy in the department of Dakar
E7	Hydro-alcoholic gel	CHN Aristide Le Dantec Hospital Pharmacy
E8	Hydro-alcoholic gel	CHN Aristide Le Dantec Hospital Pharmacy
E9	Hydro-alcoholic gel	CHN de Fann Hospital Pharmacy
E10	Hydro-alcoholic gel	A pharmacy in the department of Saint Louis

RESULTS

The alcohol contents of the samples of hydroalcoholic products varied between 63 and 85% (Table 2).

The pH of the samples ranged from 4.02 to 6.64 (Table 2), with only the E2 sample having an acidic pH value.

The densities of the hydroalcoholic gel samples ranged from 0.84 to 0.92 g/cm³; samples E2, E4, E5, E6 had densities higher than 0.89 g/cm³ (Table 2).

Concerning the sterility of the hydro-alcoholic products, all the samples were in conformity with and no contamination of bacterial origin was observed (Figure 1).

The antimicrobial activities of the hydro-alcoholic product samples tested ranged from 58.3 to 100% with two samples showing no activity (E2 and E5). The hydro-alcoholic solution showed 100% higher activity than the hydro-alcoholic gels (Figure 2).

Table 2: Results of the physico-chemical parameters (Alcoholic degree pH and density) of the samples of hydroalcoholic products.

Samples	Alcoholic degree (%)	pH	Density (g/cm ³)	Standard
E1	83	6,64	-	➤ Alcoholic degree (%) 75 – 80% (WHO Standard)
E2	63	4,02	0,91*	
E3	84*	6,45	0,85	➤ pH (6,0 - 7,0)
E4	63	6,4	0,91*	
E5	85*	6,2	0,92*	➤ Density (g/cm³) (0,85-0,89)
E6	68	6,27	0,90*	
E7	75	6,5	0,84	
E8	72	6,7	0,89	
E9	73	6,45	0,88	
E10	67	6,02	0,89	

*not in conformity

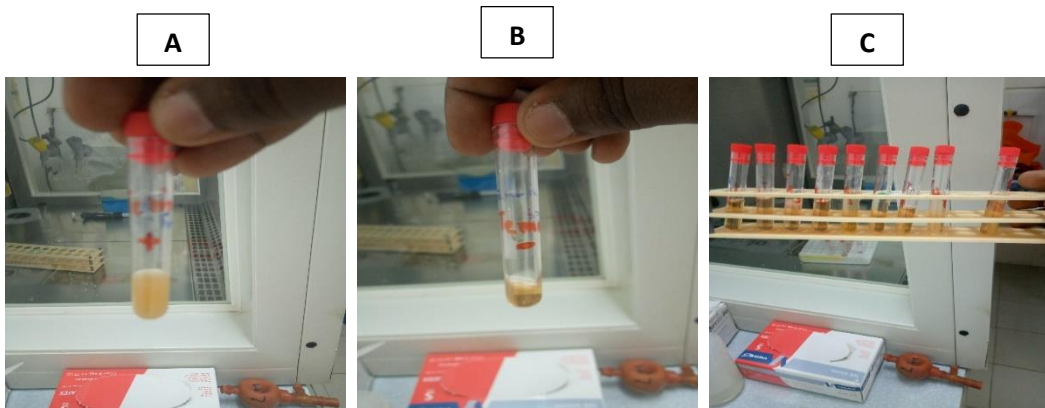


Figure 1: Sterility test results for hydro-alcoholic products. (A: positive control; B: negative control; C: samples).

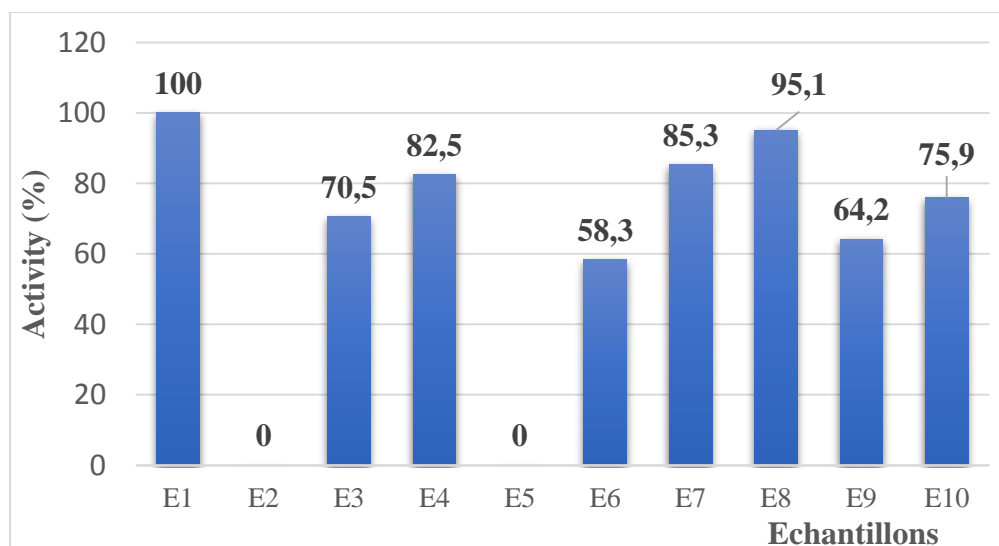


Figure 2: Results of the antimicrobial activity test of hydroalcoholic products.

DISCUSSION

The alcoholic degree is an important parameter in the evaluation of the quality of hydroalcoholic products. Indeed, ethanol is capable of inhibiting the growth of microorganisms even at low concentrations by acting on the wall, membrane and envelope of microorganisms (Rouzic et al., 2011). The activity of alcohol is concentration-dependent and it has been shown that hydroalcoholic products containing 60-80% ethanol is the most effective (WHO, 2005; Lejeune, 2008). In addition, both under- and overdosing can affect the antimicrobial activity of hydroalcoholic products. Indeed, it has been proven that anhydrous alcohol (100%) has no antimicrobial activity (Samake, 2012). The majority of samples showed an antimicrobial activity higher than the 50% recommended by AFNOR. However, samples E2 and E5 had not shown any antimicrobial activity. Also, lot E6 and E9 presented average antimicrobial activities which are respectively 58.3 and 64.2%. The study of the correlation between alcohol content and antimicrobial activity showed that the hydro-alcoholic products with an alcohol content between 60 and 80% also showed the best activities except for E2. This

sample was in conformity with the alcohol content but not in conformity with the pH and the density, which seems to show the importance of the latter two parameters on the quality and efficacy of the hydro-alcoholic products. On the other hand, the E5 sample should no antimicrobial activity, which seems to support the hypothesis that there is an upper limit of alcohol content compatible with antimicrobial activity.

Indeed, alcohol acts by denaturing microbial proteins. This denaturation would be facilitated by the presence of water, and that is why alcohol solutions containing 60 to 80% alcohol have better antimicrobial activity than absolute alcohol and alcohols at very high concentrations that are less effective on germs (WHO, 2005; WHO, 2009).

Conclusion

This preliminary work revealed that hydro-alcoholic products with alcoholic degrees of between 60 and 80% also recorded the best antimicrobial activity, and the hydro-alcoholic solution was more effective than hydro-alcoholic gels.

In the light of the results of this study, it would be appropriate to broaden and deepen the

investigations by significantly increasing the number of samples and by carrying out as complete an analysis as possible. This should make it possible to obtain much more exhaustive data on the quality of hydro-alcoholic products consumed in Senegal.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SA performed the various experiments and wrote the manuscript. AD and MD participated in the development of the paper. AM corrected the version of the paper. PMD; MMF; AD; MF and DF supervised the research. All authors read, improved the paper and accepted its final version.

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