



Research Article

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**Evaluation of the bactericidal efficacy of different dilutions of tincture of iodine on three bacterial reference strains**

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**ABSTRACT**

The aim of this study is to evaluate the bactericidal efficacy of a disinfectant (iodine) on three bacterial reference strains which are *Pseudomonas aeruginosa* (CIPA22); *Escherichia coli* (CIP 55.30) and *Staphylococcus aureus* (CECT 59), according to the standard (AFNOR NF T72\_150, 1995). The results showed that the Gram-negative *Pseudomonas aeruginosa* strains (CIPA22) and *Escherichia coli* (CIP 55.30) are more resistant to iodine. Furthermore, *Staphylococcus aureus*(CECT 59) is the most sensitive gram-positive strain to this disinfectant. No bactericidal dose has been observed in the dilutions used except for dilution (1/2) of *Staphylococcus aureus*(CECT 59). The results of this study were used to classify the disinfectant according to its activity, its vis-à-vis efficiency of Gram negative and positive strains and its bactericidal dose.

**Keywords:** Iodine, *Pseudomonas aeruginosa* (CIPA22), *Staphylococcus aureus* (CECT 59), *Escherichia coli* (CIP 55.30), efficiency.

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**INTRODUCTION**

Surgical procedures and even more invasive medical procedures, including gastrointestinal endoscopies are performed each year all over the world. Each procedure involves contact by a medical device or surgical instrument with a patient's sterile tissue or mucous membranes. A major risk of all such procedures is the introduction of pathogens that can lead to infection. Failure to properly disinfected or sterilized equipment carries not only risk associated with breach of host barriers but also risk for person-to-person transmission (hepatitis B virus) and transmission of environmental pathogens (*Pseudomonas aeruginosa*). Disinfection and sterilization are essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients. Because sterilization of all patient-care items is not necessary, health-care policies must identify, primarily on the basis of the items' intended use, whether cleaning, disinfection, or sterilization is indicated [1].

Since the latter part of the nineteenth century, much has been written extolling the alleged virtues of iodine as an antiseptic. This has been largely due to the fact that it fulfills a function that many bactericides do not and cannot fulfill. Iodine has been used in various forms as an antiseptic for the skin, wounds, and mucous surfaces of the body; for the sterilization of the air and inanimate objects such as catgut and surgical instruments, as a prophylactic and therapeutic agent in diseases caused by bacteria, viruses and fungi; for the disinfection of drinking water and swimming pool water; and for the sanitization of eating utensils[2]; [3]; [4].

The aim of this study is to evaluate the bactericidal efficacy of a disinfectant (iodine) on three reference strains *Pseudomonas aeruginosa* (CIPA22); *Escherichia coli* (CIP 55.30) and *Staphylococcus aureus* (CECT 59), according to the standard [5].

## EXPERIMENTAL SECTION

### 2.1. Material

Three reference strains are used to evaluate the bactericidal efficacy of a disinfectant (Bleach). The bacterial strains are *E. Coli* of Institut Pasteur Collection (CIP 55.30), *Pseudomonas aeruginosa* of Institut Pasteur Collection (CIP A22) and *Staphylococcus aureus* Type Culture Collection Spanish (CECT 59).

### 2.2. Methods

#### 2.2.1. Evaluation of the efficiency of the disinfectant

The method used is that recommended by the standard AFNOR NF T72\_150, 1995 [5]; requiring the use of a neutralizer. It includes several steps:

#### 2.2.2. Preparation of bacterial cultures

Culture of the bacterial strains is performed on specific media for each bacterium, then incubated at 37 ° C for 24h.

#### 2.2.3. Preparation of the bacterial suspension

It requires the transfer under aseptic conditions of a bacterial colony in a sterile tube containing 10 mL of diluent. The tube is then stirred until the obtention of an homogenous bacterial suspension.

#### 2.2.4. The suspension adjustment by spectrophotometer

Initial suspension of each bacterium is adjusted using a spectrophotometer at 620 nm to obtain the following values:

- (0,2 – 0,3) for the Gram negative bacteria
- (0,3-0,4) for the Gram positive bacteria

The value of the suspension should contain  $1-3 \times 10^8$  bacteria / mL.

#### 2.2.5. Preparation of dilutions from the initial suspension

Dilutions were prepared as follows:

**Table 1: Preparation of bacterial dilutions**

N°	1	2	3	4	5	6
Dilution	[1/10]	→[1/10]	→ [1/10]	→ [1/10]	→ [1/5]	→ [1/20]
Suspension in diluting (ml)	0,4 en 3,6	0,4 en 3,6	0,4 en 3,6	0,4 en 3,6	0,8 en 3,2	0,2 en 3,8
Bacterial concentrations					$1-3 \times 10^4$	$2-6 \times 10^3$ $1-3 \times 10^2$

#### 2.2.6. Seeding

A (1 ml) of each dilution ( $10^2$ ,  $10^6$ ) is seeded on a nutrient agar and incubated for 24h at 37 ° C.

#### 2.2.7. Preparation of neutralizing

The neutralizer is preparing for a single and double concentration for each disinfectant where each one has its own disinfectant neutralizer.

#### 2.2.8. Preparation of disinfectant dilutions

Dilutions are prepared in concentration C / 0,9 and are made with sterile distilled water. The various dilutions tested are 1/2, 1/4, 1/8, 1/16.

#### 1.2.9. Test the efficiency of disinfectant

Zero point one (0.1 mL) of the initial suspension ( $1-3 \times 10^8$  bacteria / ml) are added in 4 tubes containing different dilutions of the disinfectant (1/2, 1/4, 1/8, 1/16). The content is then stirred and maintained for 5 minutes at 20 ° C. After 5 minutes period, 0.25 ml of the composition (initial suspension + disinfectant) are added in 8 tubes containing 2,25 mL of neutralizer (2 tubes for each dilution).

All tubes are stirred and maintained for 10 minutes at 20 ° C. One (1 mL) from each tube is then seeded (in depth) on nutrient agar and incubated for 48h at 37 ° C. Only Petri dishes containing between 15 and 300 colonies are considered.

## RESULTS AND DISCUSSION

## 3.1. Bactericidal activity of tincture of iodine

The figure 1 shows the bactericidal activity of tincture of iodine at different dilutions on three reference strains.

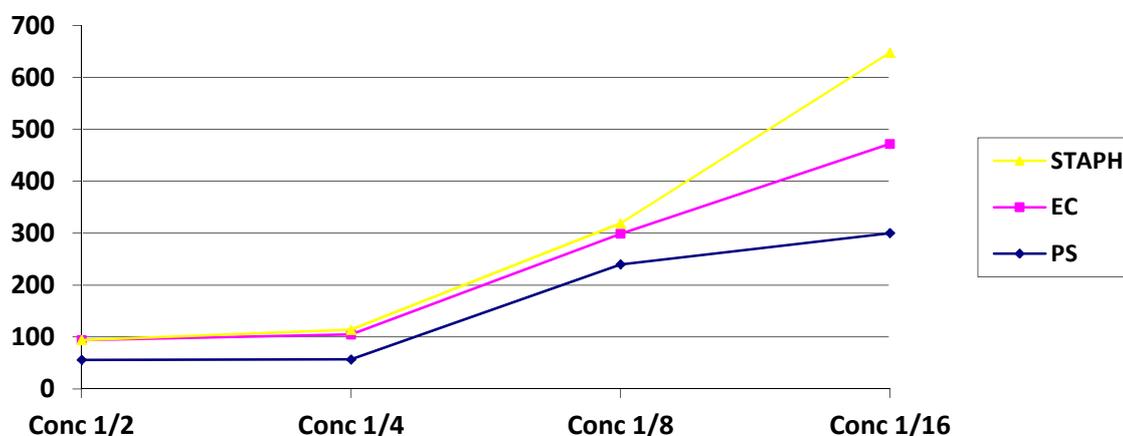


Figure 1: Bacterial growth curve under the effect of iodine

According to the figure 1, the three strains are resistant to iodine activity. However *Pseudomonas aeruginosa* (CIPA22) is the more resistant one followed by *Escherichia coli* (CIP 55.30) and finally *Staphylococcus aureus* (CECT 59) is the most sensitive gram-positive strain to this disinfectant.

The results indicate that the action of iodine depends on the bacterial species and especially dilutions used. Indeed, from 1/4 and 1/8 dilutions sanitizer efficiency tends to decrease. The results of this study are in discordance with several reports that reported intrinsic microbial contamination of antiseptic formulations of povidone-iodine and poloxamer-iodine caused a reappraisal of the chemistry and use of iodophors. Free iodine (I<sub>2</sub>) contributes to the bactericidal activity of iodophors and dilutions of iodophors demonstrate more rapid bactericidal action than does a full-strength povidone-iodine solution. The reason for the observation that dilution increases bactericidal activity is unclear, but dilution of povidone-iodine might weaken the iodine linkage to the carrier polymer with an accompanying increase of free iodine in solution. Therefore, iodophors must be diluted according to the manufacturer's directions to achieve antimicrobial activity. Published reports on the in vitro antimicrobial efficacy of iodophors demonstrate that iodophors are bactericidal, mycobactericidal, and virucidal but can require prolonged contact times to kill certain fungi and bacterial spores. Three brands of povidone-iodine solution have demonstrated more rapid kill (seconds to minutes) of *S. aureus* and *M. chelonae* at a 1:100 dilution than did the stock solution [6]. The virucidal activity of 75–150 ppm available iodine was demonstrated against seven viruses. Other investigators have questioned the efficacy of iodophors against poliovirus in the presence of organic matter and rotavirus SA-11 in distilled or tapwater. Manufacturers' data demonstrate that commercial iodophors are not sporicidal, but they are tuberculocidal, fungicidal, virucidal, and bactericidal at their recommended use-dilution [1].

## 3.2. Determination of log reduction of the disinfectant

According to the AFNOR [5], for a disinfectant or bactericidal concentration must be reduced to a minimum of  $10^5$  which means that  $\log(N \times 10^6) - \log n > 5$ , where "N" represents the number of colonies of the bacterial suspension ( $10^6$ ) and "n" is the number of colonies obtained for each dilution. Table 2 represents the log reductions of bacterial strains tested.

Table 2: Logarithmic reduction of the three reference strains

Concentration du neutralisant Bacteria	Concentrations			
	1/2	1/4	1/8	1/16
<i>Pseudomonas aeruginosa</i> (CIPA22)	3,72	3,72	3,09	3
<i>Escherichia coli</i> (CIP 55.30)	3,89	3,79	3,70	3,24
<i>Staphylococcus aureus</i> (CECT 59)	5,47	4,52	4,17	3,23

Table 2 shows that there are no logarithmic reductions for both *Pseudomonas aeruginosa*(CIPA22) and *Escherichia coli* (CIP 55.30) when the disinfectant is diluted. The only logarithmic reduction is observed for *Staphylococcus aureus* (CECT 59) (5.47) in the concentration (1 / 2). The efficacy of the tested disinfectant in our study may have been influenced by several factors. It has been found that the pH of the Iodine solutions are more effective virucides and bactericides and poorer cysticides at alkaline pH levels (> pH 7). To use iodine most effectively as a

disinfectant, the pH should be near neutral to mildly alkaline to allow adequate levels of both iodine and hypiodous acid [7]. Studies have shown a significant impact on iodine disinfection capability by temperature. One study showed CT's to provide 2-log inactivation of the *E. Coli* bacteria were 2-9 times higher in colder waters (2-5 °C) than in warmer waters of 20-25 °C [8]; [9].

### CONCLUSION

In this study we have found that the Gram-negative *Pseudomonas aeruginosa* strains (CIPA22) and *Escherichia coli* (CIP 55.30) are more resistant to iodine. On the other hand, *Staphylococcus aureus*(CECT 59) is the most sensitive gram-positive strain to this disinfectant.

Furthermore, no bactericidal dose has been observed in used dilutions except for the dilution ( $\frac{1}{2}$ ) of *Staphylococcus aureus*(CECT 59).

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