In Vitro Antibacterial Activity of Germisol™ on Bacterial Strains Isolated from Dental Patients

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CHARACTERIZATION OF GERMISOL: Low toxicity. LD₉₀ for Germisol is 20 mL/kg weight, CI 95% 14 mL/kg to 25 mL/kg in NIH strain male mice given orally. In humans, this is equivalent to the ingestion of 700 mL by a 70-kg adult.

Non-irritant. Germisol does not produce either edema or erythema in rabbits, according to the Official Mexican Standard of the Mexican Health Ministry NOM-039-SSA-I-1993. This test was done in June 2000. Its germicide effects have been tested on several microorganisms at different dilutions and times, yielding satisfactory results (Table 1).

USE: 1. Germisol, antiseptic and germicide. It is used to disinfect water, vegetables, surgical instruments, injection areas, scratches, wounds, as well as for tonsillitis, throat and trachea irritations, insect bites, skin, genital and gastrointestinal infections, otitis, conjunctivitis, rhinitis.

2. Germisol Dental - broad-spectrum. It is used in dental extractions, endodontics, alveolitis, periodontal treatment, and buccopharyngal infection to eliminate dental bacteria plaque and avoid tartar and caries formation.

HYPOTHESIS: The antiseptic and germicide effect of Germisol is equal or greater than that of streptomycin.

MATERIAL AND METHODS: The population consisted of pathogenic bacteria of four species in culture media obtained from the dental plaque of patients (Table 2). From all the dental bacterial plaques, the most abundant species were obtained in two different seasons, 15 each in summer and autumn; 10 from hospitalized patients of the Social Security Hospital in Mexico City, 10 from volunteers of the same hospital, and 10 from patients of the Dental Clinic “Pirules” from the Autonomous Metropolitan University (UAM, initials in Spanish) in Mexico City. The sampled bacteria were: Staphylococcus aureus, Streptococcus pyogenes, Streptococcus salivarius, and Streptococcus sanguis.

GERMISOL™ is an antiseptic and germicide product that contains gentian violet and iodophenol salts and vegetal extracts, such as origan and thyme, as active principles. This product is currently used to treat various infections and in antiseptic procedures. We studied the germicide properties of Germisol on bacterial dental pathogens. The interest in the analysis and development of this product relies on the emergence of bacterial strains resistant to many of the antibiotics currently used.

Bacterial resistance and susceptibility to a specific antibacterial agent differ for each microorganism [1]; hence, the ideal bactericide is a drug to which bacteria are not resistant.

Resistance to antibacterial agents is a relevant public health problem, due to the increasing number of bacterial strains becoming resistant to conventional treatment. Great concern was raised by the evidence of elevated resistance attained by Escherichia coli [2]. When a suspension test with incremental additions of Klebsiella pneumonia was used, the effects of some antibacterial iodine products disappeared at the third incremental addition of this microorganism [3]. Of concern is the increase in Staphylococcus aureus resistance to oxacillin in hospitalized patients, with rates as high as 60% [4]. The global resistance to erythromycin and tetracycline of 573 Streptococcus pyogenes strains increased significantly from 1992 to 1994 (p < 0.05) [5]. In a recent study in a Mexican hospital, an outbreak of Klebsiella pneumoniae infection produced a mortality rate of 62% (13 of 21) in pediatric patients [6]. The purpose of studies detailed here was to compare Germisol and streptomycin, treatment of the following pathogenic bacteria found in dental plaque: Staphylococcus aureus, Streptococcus pyogenes, Streptococcus salivarius, and Streptococcus sanguis.

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Resistance and susceptibility test. Resistance and susceptibility were determined by the disc diffusion method of Bauer-Kirby [7]. Whatman number 1 filter paper discs of 6 mm in diameter were impregnated with 0.025 ml of Germisol (Batch 0198) or streptomycin (Batch 127193), placed in Mueller-Hinton agar plates and incubated for 24 hrs at 36°C. The inhibition halo was measured in millimeters. The criterion used to characterize bacterial behavior was: an inhibition halo < 13.2 mm indicated resistance and > 18.5 mm indicated susceptibility [1].

Statistical analysis. In a preliminary analysis, a strong interaction among the season of the year, bacteria, and antibacterial effect had been found; therefore, each of the two seasons was analyzed separately. The statistical model used consisted of two-split plots 2x2 factorial design [8], one for summer and the other for autumn. The experimental unit for the large plot is the agar medium in a Petri dish, and the sub experimental unit is the antibacterial agent.
The model is as follows:

$$Y_{ijkl} = M + B_j + C_l(j) + A_i + AB_{ij} + E_{ijk}$$

Where:

- $Y_{ijkl}$ is the measure of the inhibition halo produced by an antibacterial $i$, with bacteria $j$, with repetition $k$, in the agar culture medium $l$

WHOLE PLOT:
- $M =$ The general mean
- $B_j =$ Bacteria effect
- $C_l(j) =$ Agar culture medium effect, considered a random effect

SMALL PLOT:
- $A_i =$ Antibacterial effect
- $AB_{ij} =$ the interaction term
- $E_{ijk} =$ Error

$i = 1, 2$ (antibacterial), 1 for Germisol and 2 for streptomycin

$j = 1, 2, 3, 4$ (bacteria). 1 = S. aureus, 2 = S. pyogenes, 3 = S. salivarius, 4 = S. sanguis.

$l = 1, 2, 3, \ldots, c. 54$ for summer and 48 for autumn (Agar Culture Medium)

$k =$ repetition for each bacteria $j$

The hypothesis is operationalized as: “The mean diameter of the inhibitory halo for Germisol is larger than or equal to the mean diameter of the inhibitory halo for streptomycin for at least one bacteria$ ^j$”

RESULTS: All the effects in the model were highly significant ($p <0.001$) Tukey test was performed for the significant effects of the interaction. In the summer, the inhibitory halo for S. aureus was larger with streptomycin than with Germisol, 32.7 against 24.0 mm ($p < 0.05$), whereas, in autumn, the opposite was observed: 18.9 with streptomycin against 28.6 mm with Germisol ($p < 0.05$) (Fig. 1 and 2). In the summer, S. aureus and S. salivarius were susceptible to Germisol with mean inhibitory halos of 24 and 26 mm, respectively; for S. pyogenes and S. sanguis, the mean inhibitory halos were 15 and 17 respectively, not reaching the 18.5 mm, established as criterion for susceptibility. In autumn, S. aureus and S. pyogenes were susceptible to Germisol with 28.7 and 26.7 mm of mean inhibitory halos, whereas S. salivarius and S. sanguis were not (17.6 and 17.1 mm of mean inhibitory halo, respectively).

DISCUSSION: The experimental results obtained with Germisol on the microorganisms of the dental plaque revealed its high antimicrobial efficiency, since a 1:100 dilution had a 100% germicide effect on S. aureus in 30 seconds (Table 1). Comparison of the effects of Germisol against those of streptomycin (Fig. 1 and 2) on components of the bacterial dental plaque indicated a highly efficient antibacterial action for Germisol. This finding suggests that Germisol could be used instead of streptomycin, a broad-spectrum antibiotic against *Staphylococcus aureus* infections, avoiding the use of antibiotics to reduce the increasing acquired resistance [9]. An acceptable germicidal effect was also obtained for *Pseudomonas aeruginosa*, a bacterium found in infected burn wounds, that has become resistant to streptomycin [10] and has also been demonstrated to cause corneal infections between 1997 and 2000 [11]. The germicide effect of Germisol has also been tested on *Escherichia coli* (Table 1), finding a good germicide effect. This result justifies the use of Germisol to avoid the use of antibiotics that produce resistance in this enterobacteria [2]. Another satisfactory germicidal effect has been obtained on *Salmonella typhi* (Table 1). In the presence of Germisol at a 1:100 dilution during 1 min, less than 10 CFU/mL were grown. This bacterium has been shown to cause perforation of the ileum in Mexican patients [12]. This is another indication for the use of Germisol in typhoid fever. Other satisfactory germicide effects have been found on *Bordetella bronchiseptica* and *Candida albicans*, better for the latter (Table 1). In 234 women with cervicovaginitis due to *Candida* infection a 10% resistance to the antymycotics commonly employed has been reported [12]. A recent report [13] referred considerable limitations of the available therapy for the effective management of complicated vaginitis. This could be another clinical field in which to test
Germisol. The germicide effect of Germisol evidences its antiseptic ability and also suggests the possibility of avoiding induction of microbial resistance due to complete elimination of the infecting microorganism, since 99.99% of the microorganisms were eliminated; a 100% elimination rate may be obtained by increasing the exposition time to the antiseptic. The irritability test revealed neither edema nor erythema, and the LD<sub>50</sub> test yielded very low toxicity, making it an almost harmless product. This can be interpreted that the intake of Germisol against every infections practically is harmless. Germisol is an antiseptic, not an antibiotic; therefore, it does not induce microorganism resistance. Finally, we do not have a plausible explanation yet for the differential seasonal effects depicted by both germicides (Germisol and streptomycin).

CONCLUSIONS: The studied bacterial strains were not resistant to Germisol; hence, this antiseptic is a good alternative to use against oral pathogenic bacteria.

The bacterial species <i>S. aureus</i> and <i>S. pyogenes</i> were susceptible to Germisol during the autumn. <i>S. aureus</i> and <i>S. salivarius</i> were susceptible to Germisol during the summer.

Germisol is a better antibacterial agent than streptomycin during the autumn for <i>S. aureus</i>.

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REFERENCES


