

Antibacterial Activity of Isometamidium (Veridium[®])

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Abstract

Isometamidium has been used for several years in the chemoprophylaxis of *Trypanosoma spp.* Antibacterial activity of veridium a new generic isometamidium was studied using *E. coli*, *B. cereus* and *S. aureus* micro-organisms.

The bacteria were cultured in Muller Hinton (MH) agar. Different serial dilutions of veridium 10,000 - 42 mg/ml were dispensed into wells with 10 mm diameter previously prepared on the agar. Cultured media were then incubated at 37EC for 24 hours. Zones of inhibition from four wells were then noted for each serial concentration and means calculated.

Veridium inhibited the growth of *E. coli*, *B. cereus* and *S. aureus*. The minimum inhibitory concentrations (MIC) for these organisms were 338, 100 and 40 mg/ml respectively.

This study indicates that veridium has antibacterial activity even following several dilutions. Sterile preparations of veridium by itself upon reconstitution contains 20,000 mg/ml of the drug and cannot therefore introduce any infection at the site of injection.

Introduction

Isometamidium has been used for many years as a prophylactic drug against *Trypanosoma spp.* It also has curative effect. The drug has been used to enhance livestock health and production in the tropics. Nearly all the trypanocidal drugs used in the African continent are either packed in sachets or tablets which are then reconstituted with distilled water or water for injection. These preparations are by themselves sterile since they are packed and sterilized by gamma radiation. Other trypanocidal drugs like diminazene and ethidium bromide also packed in similar sachets are known to have antimicrobial activity (Ondari *et al*, unpublished data, Nanede *et al*, 1970, Mitema and Wambia unpublished data). No previous reports have indicated any antibacterial effect on isometamidium. The present study was undertaken to investigate *in vitro* antibacterial activity of veridium on *B. cereus*, *E. coli* and *S. aureus* isolates. Veridium is normally reconstituted to make a 2% solution (20,000 mg/ml) before administration.

Materials and Methods

Bacterial isolates

Three bacterial organisms - *B. cereus var. mycoides* ATCC 11778, *E. coli var. 517* and *S. aureus* were used as test organisms. All these organisms have been adapted in our laboratory for more than ten years even though the first two are reference organisms.

Isometamidium assay

Isometamidium (Veridium (R), Sanofi SSNA, Libourne France) was used for titration. One gramme (1 g) of veridium was dissolved in 50 ml of sterile distilled water to make a 2% solution (20,000 mg/ml) in a volumetric flask. This concentration was further diluted with sterile distilled water in sterile tubes to give several other serial concentrations of 10,000 mg/ml, 5,000 mg/ml, 2,500 mg/ml, 675 mg/ml, 337.5 mg/ml, 168.75 mg/ml, 84.38 mg/ml and 42.19 mg/ml.

Physico-chemical properties

The time taken by one gram of veridium sachet to dissolve completely was noted. pH of the solution was also noted after dissolution.

Bacterial Cultures

The organisms were cultured in Muller Hinton (MH) agar (Difco Laboratories, Detroit, MI). The media was prepared as per manufacturer's instructions and autoclaved at a pressure of 15 PSI (Pounds per square inch), temperature 121EC. The spores (single colony) of *B. cereus var. mycoides* ATCC 11778, (10^6 - 10^7 CFU/ml) were incubated at 37EC in MH broth for 18 hours on a roller drum to ensure uniform distribution.

The molten agar was poured on sterile petri dishes and on solidifying, 1 ml of the 18 hour broth culture of *B. cereus* was inoculated onto the agar and 500 ml sterile MH broth added. This was emulsified onto the agar surface completely, allowed to dissolve for 15-30 minutes and wells of 10 mm diameter dug onto the agar. *E. coli* and *S. aureus* micro-organisms of 10^6 - 10^7 CFU/ml were treated the same way.

Different dilutions of veridium (10,000-40 mg/ml) were dispensed into the wells. Each dilution concentration was dispensed in four wells. The dispensed dilution of veridium (400 ml) was allowed to dissolve at room temperature for 30-60 minutes and the plates were incubated at 37EC for 24 hours.

Zones of diameter due to bacterial growth inhibition by veridium were measured with a divider and graduated ruler in millimeters. A mean of two diameter readings for each well was taken. Mean diameter readings for each concentration from the four wells was then noted.

Minimum inhibitory concentrations (MICs), defined as the minimum concentration of veridium to inhibit visible bacterial growth, was noted from the bacterial culture plates for each of the three bacteria.

Results

Physico-chemical properties

Veridium dissolved completely within 10 minutes to a dark red solution at room temperature.

Bacterial growth inhibition

Serial dilutions of veridium inhibited growth of *B. cereus*, *E. coli* and *S. aureus*. The Minimum Inhibitory Concentrations for *B. cereus*, *E. coli* and *S. aureus* were 100 mg/ml, 338 mg/ml and 40 mg/ml respectively. Figures 1 show linear response of various serial dilutions of veridium to the three bacteria. *S. aureus* was the most sensitive bacteria to veridium. In table 1 zones of inhibition (mm) with corresponding concentrations of veridium for the three bacteria are shown.

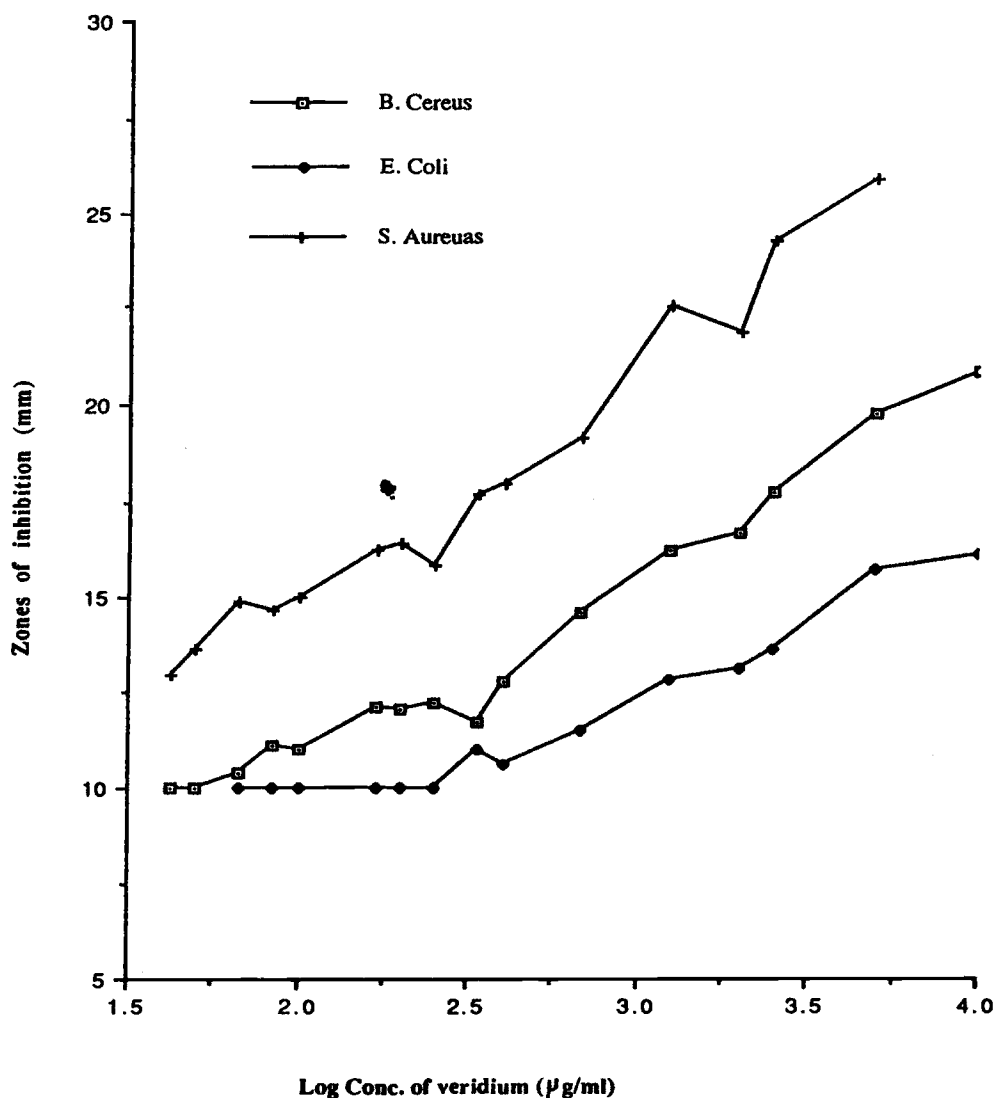


Fig. 1: Antibacterial activity of veridium on *B. cereus*, *S. aureus* and *E. coli* cultures at different serial dilutions.

Con (µg/ml)	Con (log 10)	<i>B. cereus</i>	<i>E. coli</i>	<i>S. aureus</i>
10,000	4.000	20.83	16.13	-
5,000	3.699	19.75	15.75	25.88
2,500	3.398	17.75	13.63	24.25
,000	3.301	16.67	13.13	21.88
1,250	3.097	16.25	12.88	22.50
675	2.829	14.63	11.50	19.17
400	2.602	12.83	10.63	18.00
337.5	2.528	11.75	11.00	17.67
250	2.398	12.25	10.00	15.83
200	2.301	12.10	10.00	16.38
168.75	2.227	12.13	10.00	16.25
100	2.000	11.00	10.00	15.00
84.375	1.926	11.13	10.00	14.67
66.67	1.824	10.38	10.00	14.88
60.00	1.699	10.00	-	13.63
42.1875	1.625	10.00	-	13.00

Discussion

Isometamidium has been used for several years in the chemoprophylaxis of *Trypanosoma spp* in cattle. In this investigation the compound was completely dissolved within ten minutes.

Antibacterial activity of isometamidium has not been reported in literature. In this study the MICs for *E. coli*, *B. cereus* and *S. aureus* were 338 µg/ml, 100 µg/ml and 40 µg/ml respectively at dilutions of 1:64, 1:200 and 1:512. Inhibition of the growth of these organisms from their cultures indicates that veridium has antibacterial activity and kills gram-positive and gram-negative bacteria in the body especially at the injection site where a sustained release is deposited. Other antitrypanocidal drugs reported to have antibacterial activities include diminazene (Nanede *et al.*, 1970, Mitema and Wambia unpublished data) and ethidium bromide (Ondari *et al.*, unpublished data). Structurally, isometamidium is a phenatridine compound containing diamidine and a homidium. MIC's for diminazene and homidium have not been investigated. The mechanism of antibacterial action was not studied in our investigation. However, it is known that most trypanocides inhibit DNA

multiplication of *Trypanosoma spp*. Isometamidium, however, is known to increase formation of RNA granules.

This study concludes that veridium has antibacterial activity for both gram positive and gram negative organisms. The sensitivity was observed more in gram positive organisms (*S. aureus*). It is also noted that constitution of gamma radiated veridium powder with water for injection is unlikely to cause any form of abscess at the site of injection. The normal strength used in the field is usually 2% (20,000 µg/ml) which would be a higher concentration and even have more antibacterial effect at the site of injection.

References

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