SIMPLE ADDITIVES TO INCREASE THE ACTIVITY OF CHLORHEXIDINE DIGLUCONATE AGAINST URINARY PATHOGENS*

By W. E. S. HARPER, M.Sc., E.I.L.M.S., M.A.S.M.

Department of Microbiology Royal Perth (Rehab.) Hospital, Shenton Park, Western Australia.

Summary. The concentration of chlorhexidine digluconate required to kill Gram-negative bacteria which cause urinary tract infection associated with urethral catheterisation also damages the bladder mucosa. Increased bactericidal activity was obtained by the addition of a chelating agent (EDTA) and a buffer (Tris) enabling a low concentration of chlorhexidine (0·01 per cent) to be effective against *Escherichia coli* NCTC 86, *Pseudomonas aeruginosa* NCTC 7650 *Proteus mirabilis* NCTC 8309 and *Streptococcus faecalis* NCTC 8213. The type of fluid in which the bacteria were suspended affected the time required to achieve 99·9 per cent killing. The 0·01 per cent chlorhexidine solution with added EDTA and Tris did not cause severe erosive damage to the bladder mucosa of rats. The potential of this solution for use as a skin antiseptic prior to catheterisation and as a bladder instillation after catheterisation is discussed.

Key words: Urinary tract infection; Bladder irrigation; Antiseptics; Chlorhexidine digluconate.

Introduction

URINARY tract infection (UTI) is the most common nosocomial infection reported and the urethral catheter is the major single cause (Lindan, 1972). Spinal injury patients who are being intermittently catheterised are therefore exposed to an increased risk of acquiring a bladder infection. The instillation of an antimicrobial solution prior to withdrawal of the catheter has been shown to reduce the incidence of bladder infection caused by catheterisation (Beeuweeks & de Vries, 1956; Gillespie *et al.*, 1962; Pearman, 1971, 1977).

The selection of a bladder instillation solution for all species of bacteria commonly found in UTIs can prove to be difficult for a number of reasons. Recent studies of some bladder instillation solutions have been shown to have erosive side effects (Harper & Matz, 1976) and in the case of chlorhexidine there have been several reports of clinical haematuria (Paterson *et al.*, 1960; Macfadyen & Simmons, 1968; Pearman, 1971). Other solutions that have been used may only be effective against certain Gram-negative species which limits their overall cover for catheter induced infection.

This work was prompted by reports that EDTA could enhance the action of antiseptics (Brown & Richards, 1965; Caplin & Chapman, 1976; Goldschmidt *et al.*, 1972) and that Tris increased the activity of EDTA

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(Voss, 1967; Goldschmidt and Wyss, 1967). It was hoped that the addition of these agents would enable the concentration of chlorhexidine to be reduced so that it was less erosive and also overcome any resistance that has been previously reported by Stickler (1974).

As urine has a high concentration of Ca^{++} and Mg^{++} it could inhibit the action of EDTA when used in the bladder. Consequently pooled urine has been compared with other vehicles to evaluate the potential *in vivo* effectiveness of chlorhexidine in combination with EDTA and EDTA/Tris. The test organisms used were *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus faecalis*, and *Proteus mirabilis*.

Materials and Methods

Chemicals and Antiseptics

Chlorhexidine digluconate (1-6-di (4-chlorophenyldiguanido -Hexane) ICI, Australia EDTA (Ethylenediamine tetraacetic acid-disodium salt) BDH, Poole England. Tris (2-Amino-2 Hydroxymethyl-Propane-1,3-Diol) BDH, Poole England.

Media

Blood-Agar base No. 2 (Oxoid Ltd., London).
Nutrient Agar base No. 2 (Oxoid Ltd., London).
Nutrient broth (Dehydrated Oxoid Ltd., London).
Lubrol broth (Bergan & Hysted, 1971).
Minimal medium (Clowes & Hayes, 1968).

Bacterial Strains

E. coli	NCTC	86
Ps. aeruginosa	NCTC	6750
P. mirabilis	NCTC	8309
Str. faecalis	NCTC	8213

Pooled Urine

Urine was collected over 24 hours from five healthy males aged 25–50 years, pooled and mixed well prior to filtration through a 'Millipore' filter (0·22 microns). Sterility was tested by culturing 0·1 ml of pooled urine using standard methods. the males supplying the urine samples had no medications, were on an unrestricted diet and had a full urine analysis prior to the collection of the 24 hour samples. The pH of the pooled urine was 6·0–6·4.

Inoculum

All strains of bacteria were grown at 37°C in the vehicle used for testing on the day prior to experiments except distilled water and saline. One hundred mls of the vehicle were then inoculated with 0·1 ml of the over-night culture and shaken in the water bath for 2 hours prior to addition of the test mixtures.

Viable Counts

Viable counts were performed just prior to the addition of the test solutions and

thereafter at hourly intervals for six hours. This time was chosen because in hospitals bladder instillation solutions are usually changed every six hours during intermittent catheterisation procedures.

For each count three sets of 10-fold dilutions were made and o·1 ml of each dilution spread on to air dried nutrient agar plates and incubated for 24 hours at 37°C. All experiments were performed at least three times in each vehicle and the mean values recorded. When pooled urine was used each experiment was repeated on at least three separate urine collections. Lubrol broth was used as the diluent for viable counts on mixtures containing chlorhexidine. Similarly EDTA was neutralised with saline.

In the schematic figures the initial colony count is recorded as 10^{0} (usually 10^{7}–10^{8} orgs/ml) and subsequent values recorded as the log of the difference to the initial count. When the dilution gave a value of $> 10 - 7$ no organisms were detectable on the viable count plates.

Results

As expected *Ps. aeruginosa* grew in nutrient broth (NB), urine (UR) and minimal media (MM) but not in saline (SA) or distilled water (DW) (Fig. 1). When ETDA (500 mg/l) was added to each of the vehicles the viable count was reduced in MM and DW (Fig. 2). The addition of o·05M Tris alone reduced the viability of *Pseudomonas* slightly in all vehicles except in NB (Fig. 3). However when EDTA was used in conjucntion with Tris the killing effect was most marked in the case of DW and gradually decreased in effectiveness in the other vehicles (Fig. 4).

Chlorhexidine rapidly killed *Pseudomonas* in DW, SA, and MM. In NB the organism was reduced far less rapidly and in UR the organism started to re-grow after showing an initial reduction in viability (Fig. 5).

The experiments demonstrated that of all the vehicles used, pooled urine was the most inhibitory to the agents tested. Consequently in order
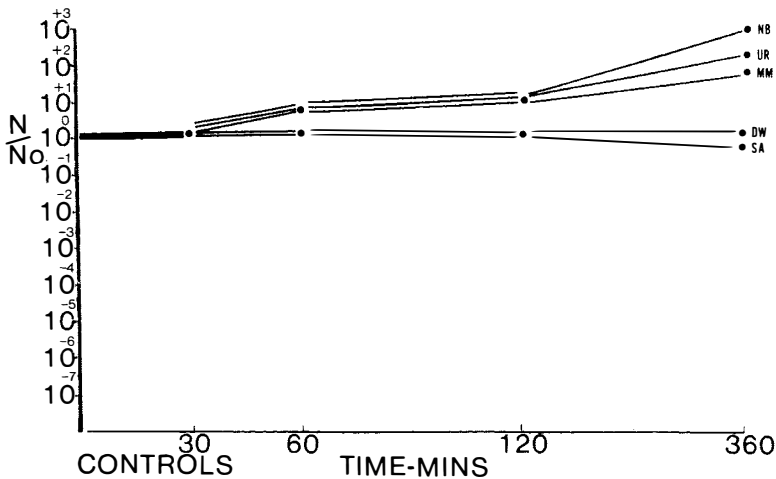

FIG. 1
The effect of instillation solutions on the viability of *Ps. aeruginosa* in different vehicles.
D.W. = Distilled water; S.A. = Saline; M.M. = Minimal medium; N.B. = Nutrient broth;
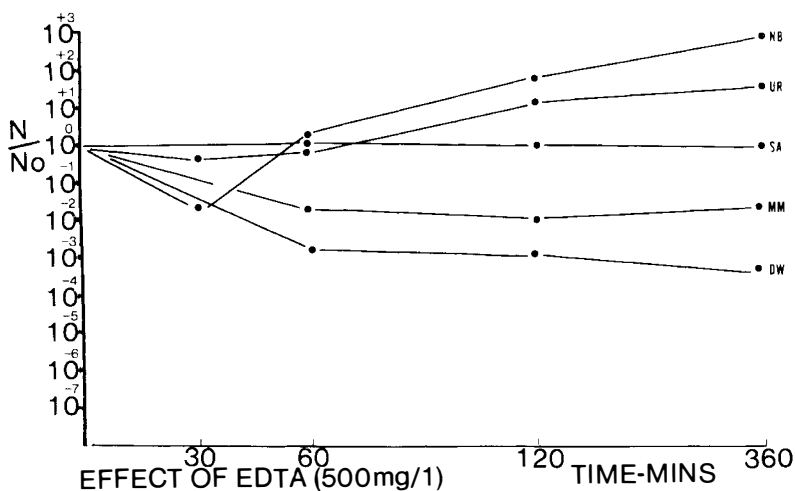U.R. = Pooled urine.

FIG. 2
The effect of instillation solutions on the viability of *Ps. aeruginosa* in different vehicles.
D.W. = Distilled water; S.A. = Saline; M.M. = Minimal medium; N.B. = Nutrient broth;
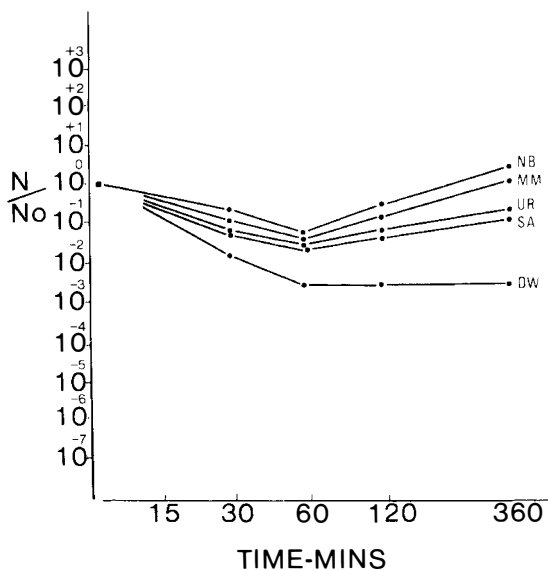U.R. = Pooled urine.

FIG. 3
Effect of Tris (0·05 m)
D.W. = Distilled water; S.A. = Saline; U.R. = Pooled urine; M.M. = Minimal medium;
N.B. = Nutrient broth.

to simulate some of the *in vivo* conditions, pooled urine was used in all further tests. In the presence of EDTA or Tris, the concentrations of chlorhexidine tested only improved its killing action slightly (Fig. 6).
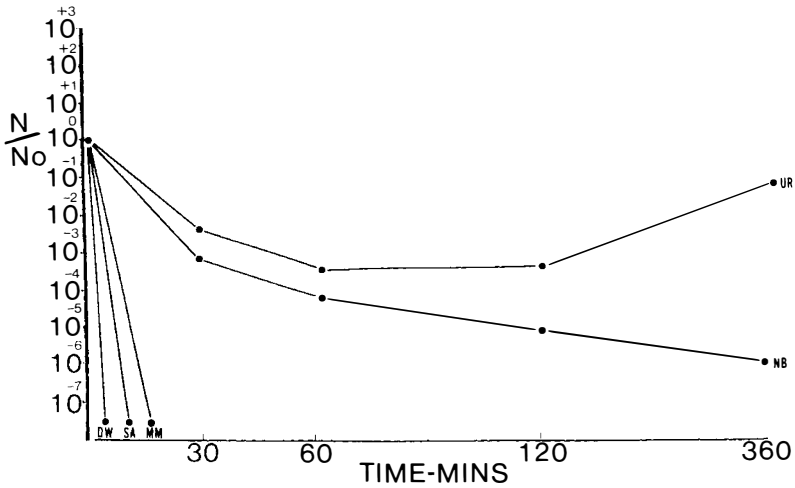
FIG. 4

Effect of different vehicles on the action of chlorhexidine (0·01%) against ● *Ps. aeruginosa.*
D.W. = Distilled water; S.A. = Saline; M.M. = Minimal medium; N.B. = Nutrient broth;
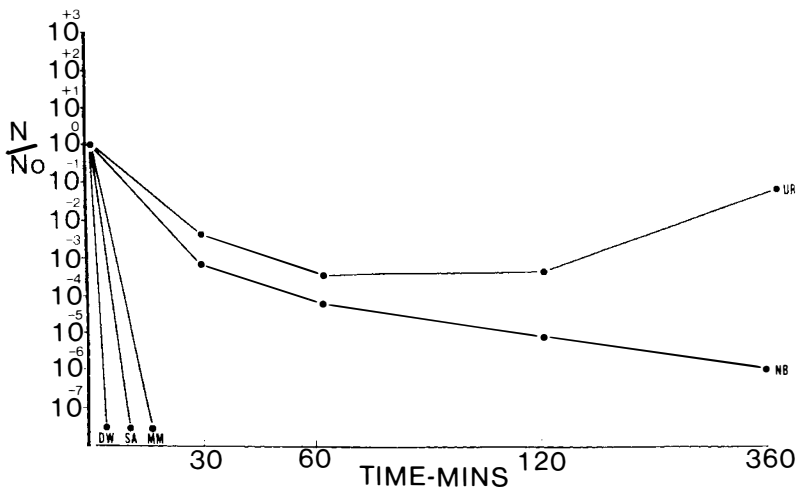U.R. = Pooled urine.

FIG. 5

Effect of different vehicles on the action of chlorhexidine (0·01%) against ● *Ps. aeruginosa.*
D.W. = Distilled water; S.A. = Saline; M.M. = Minimal medium; N.B. = Nutrient broth;
U.R. = Pooled urine.

However the full combination of EDTA/Tris and chlorhexidine resulted
in rapid killing of Pseudomonas even in the presence of pooled urine. (Fig.
6). Similar results were obtained when chlorhexidine/EDTA/Tris was
tested on *E. coli P. mirabilis* and *Strep. faecalis* (Fig. 7).

Discussion

The purpose of any *in vitro* testing of an antibacterial bladder instillation
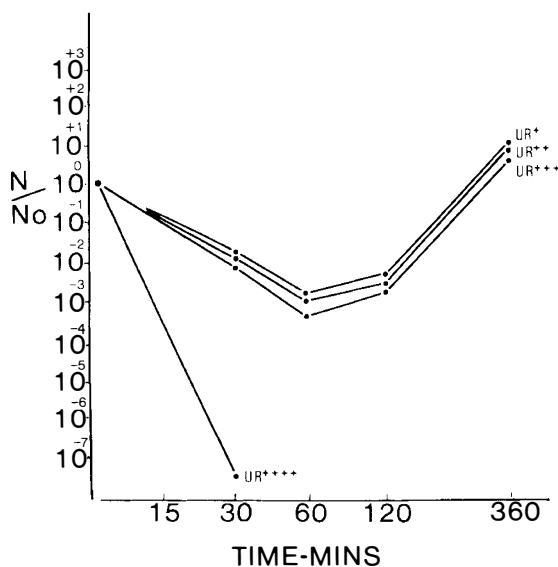
FIG. 6

UR$^+$ Chlorhexidine (0·01%) EDTA (500 mg).
UR^{++} Chlorhexidine (0·01%) Tris (0·05 M).
UR^{+++} Chlorhexidine (0·2%) EDTA (500 mg).
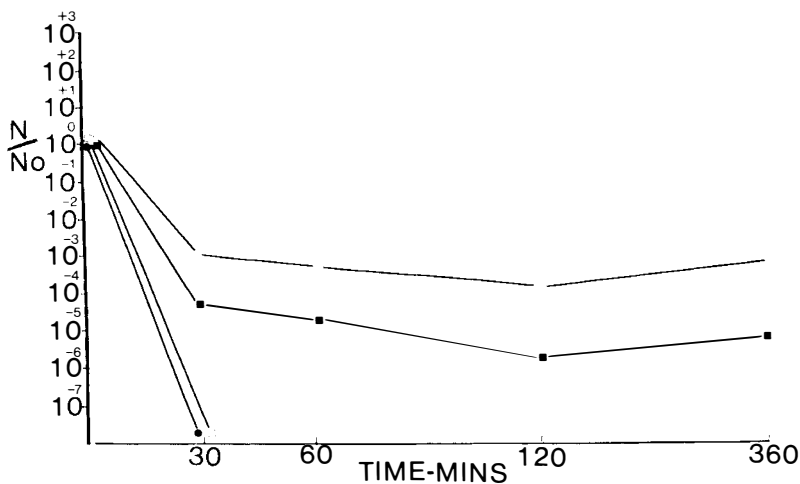UR^{-+-+} Chlorhexidine (0·1%) EDTA (500 mg) Tris (0·05 M).

FIG. 7

Effect of chlorhexidine (0·01%) EDTA (500 mg/l) Tris buffer (0·05 M) in pooled urine on □ *E. coli*, ● *Ps. aeruginosa*, ■ *P. mirabilis* and ○ *Strep. faecalis*.

solution is to assess its possible efficacy for application *in vivo*. For this reason pooled urine was investigated as a vehicle for testing the antimicrobial effect of a bladder rinse solution. In general it was found that the solutions tested were less effective in urine than in other vehicles. This indicates that the *in vitro* tests that have been performed in other vehicles are

probably not a true indication of the efficacy of chlorhexidine and could be relevant to other antimicrobial solutions used as bladder rinse solutions.

The addition of EDTA/Tris buffer to chlorhexidine appears to enhance the antiseptic action even in the presence of urine. As the composition of urine may vary with fluid intake and other factors, urine pooled from different individuals was used in order to minimise some of these variables. The replicate experiments with different batches of pooled urine gave similar results suggesting that these variables did not have a significant effect on the results. Other factors such as leucocytes, cellular and organic debris, found in infected urine which can reduce the activity of antiseptics should also be considered.

The strain of *Ps. aeruginosa* used appeared less sensitive to EDTA than was expected from previous reports on this species (Voss, 1967). This may be due to the different experimental conditions and/or the strain of bacteria used. Consequently, it is considered that the methods employed in this study are stringent for evaluating bladder instillation solutions. Although similar results were obtained with *E. coli* and *P. mirabilis* the solutions were less effective with *Strep. faecalis*. This may be due to the structure of the Gram-positive cell wall, for example the higher techoic acid content (Cutinelli, 1967). Although only one strain of each species has been tested in this present study, the results demonstrate the need to duplicate as far as possible *in vivo* conditions when performing *in vitro* tests on bladder instillation solutions. The conditions of the test procedure used are not an exact model of the intermittent catheterised bladder which has urine continuously flowing into it and can cause dilution of the antiseptic, further reducing its effect.

Using a physical model of the bladder such as that described by Greenwood and O'Grady (1975) with pooled urine as the medium might lead to further useful information as to the frequency and duration of instillation solutions required over a 6 hourly period.

Investigations of combinations of chelating agents for Gram-positive and Gram-negative bacteria should be further explored. Their use in alternated six hourly combinations could possibly give wider cover against the different species found in UTIs. Bathing in similar antiseptic mixtures by patients colonised with multiple antibiotic resistant bacteria may also prove useful. This procedure may be helpful in either decolonising the patients skin and/or help to control the spread of these organisms throughout the hospital environment.

Résumé

La concentration de chlorhexidine digluconate qui est nécessaire pour tuer les bactéries Gram-négatives, une cause de l'infection des voies urinaires qui est associée avec le cathétérisme urétral, a l'effet d'endommager la muqueuse vésicale aussi. Une augmentation de l'activité bactéricide a été obtenue par l'addition d'un agent de chélation (EDTA) et une solution tampon (TRIS), ce qui a permis une faible concentration de chlorhexidine (0·01%) d'agir contre *Escherichia coli* NCTC 86, *Pseudomonas aeruginosa* NCTC 6750, *Proteus mirabilis* NCTC 8309 et *Streptococcus faecalis* NCTC 8213. Le type de fluide dans lequel les bactéries ont été suspendues a affecté le temps nécessaire pour obtenir une extermination de 99·9%. La solution de 0·01% de chlorhexidine avec l'addition de EDTA et de TRIS n'a pas causé une érosion sévère de la muqueuse vésicale des rates. Nous avons considéré le potentiel de l'emploi de cette solution comme un antiseptique pour la peau avant le cathétérisme, et comme une instillation dans la vessie après le cathétérisme.

ZUSAMMENFASSUNG

Die Konzentration von Chlorhexidine Digluconate die gebraucht wird um die gramnegative Bakteria zu töten, die Ursache sind von der Urinary Tract Infektion und verbunden mit einer Schädigung der Blasenschleimhaut. Vergrösserte bactericidal Aktivität wurde errungen bei der Zufügung von einer chelating wirkender Kraft und einem Prellbock (tris) ermöglicht eine niedrige konzentration von Chlorhexidine (0·01%) wirksam zu sein gegen *Escherichia coli* NCTC 86, *Pseudomonas aeruginosa* NCTC 6750, *Proteus mirabilis* NCTC 8309 und *Streptococcus faecalis* NCTC 8213. Der Typ von Flüssigkeit in welcher die Bakteria schwebte, beeindrückte die Zeit die gebraucht wurde um 99·9% erfolgreich zu sein. Die 0·01% Chlorhexidine Auflösung mit Zusatz von edta und tris gründete keinen schweren zersetzenden Schaden an der Blasenschleimhaut bei Ratten. Die Leistungsfähigkeit dieser auf Lösung zur Benützung als ein antiseptisches Hautmittel vor der Catheterisation und als eine Blaseneintropfung nach der Catheterisation wird besprochen.

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