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IN VITRO SUSCEPTIBILITY OF GRAM-NEGATIVE BACTERIAL ISOLATES TO CHLORHEXIDINE GLUCONATE

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ABSTRACT

Objective: To investigate the susceptibility of clinical isolates of gram-negative bacteria to chlorhexidine gluconate.

Design: Prospective laboratory study.

Setting: Tikur Anbessa Hospital, Addis Ababa, Ethiopia.

Subjects: Clinical specimens from 443 hospital patients.

Main outcome measures: Significant number of gram negative bacteria were not inhibited by chlorhexidine gluconate (0.02-0.05%) used for antiseptics.

Results: Four hundred and forty three strains of gram-negative bacteria were isolated from Tikur Anbessa Hospital patients. *Escherichia coli* (31.6%) and *Klebsiella pneumoniae* (23%) were the most frequently isolated bacteria followed by *Proteus* species (13.3%), *Pseudomonas* species (9.2%), and *Citrobacter* species (6.1%). Each organism was tested to chlorhexidine gluconate (CHG), minimum inhibitory concentration (MIC) ranging from 0.0001% to 1% w/v. All *Salmonella* species and *E. coli* were inhibited by CHG, MIC \leq 0.01%. Twenty nine per cent of *Acinetobacter*, 28% of *K. pneumoniae* and *Enterobacter* species and 19-25% of *Pseudomonas*, *Proteus* and *Providencia* species were only inhibited at high concentrations of CHG (\geq 0.1%).

Conclusion: Our results showed that a significant number of the gram-negative bacterial isolates were not inhibited by CHG at the concentration used for disinfection of wounds or instruments (MIC 0.02-0.05% w/v). It is therefore important to select appropriate concentration of this disinfectant and rationally use it for disinfection and hospital hygiene. Continuing follow up and surveillance is also needed to detect resistant bacteria to chlorhexidine or other disinfectants in time.

INTRODUCTION

Chlorhexidine gluconate (CHG) is a cationic biguanide and a good bactericidal agent(1). Because it is mild and relatively non-toxic to human tissues, CHG has gained a wide range of application both as an antiseptic and disinfectant in medical practice. In the 1960's, different species of bacteria resistant to CHG were found(2,3). Since then a higher frequency of chlorhexidine resistant strains has been reported(4-9). Studies have also shown the possible positive resistance links between the antiseptic and antibiotics suggesting common mechanisms for the development of resistance(10).

There is increasing concern regarding the efficacy of many disinfectants on the market. It is possible that a significant proportion of laboratory or hospital acquired infections may be partly due to the use of ineffective or low concentrations of disinfectants. Antiseptics like CHG are extensively used in Tikur Anbessa Hospital,

Addis Ababa. Despite their wide use, the susceptibility of microorganisms to the disinfectants has not been studied. We investigated the efficacy of CHG to the clinical isolates *in vitro* and the results are reported herein.

MATERIALS AND METHODS

Specimens: Clinical specimens were collected from hospitalised patients of Tikur Anbessa Hospital, Addis Ababa between 1996 and 1997. These included blood, pus, urine, sputum, cerebrospinal fluid (CSF), body discharges and fluids.

Isolation and identification of bacteria: Appropriate culture media were from Oxoid dehydrated products (Oxoid Basingstoke, Hampshire, UK). Specimens were inoculated onto both blood and MacConkey agar and incubated at 37°C for 18-24 hours. The bacterial isolates were identified by biochemical methods following standard procedure(11).

Susceptibility testing: The susceptibility of bacterial isolates to CHG was determined by agar dilution method

following standard procedure(12,13). Briefly, the procedure was as follows: stock solution of CHG (20% w/v) was serially diluted with sterile distilled water. Chlorhexidine at a concentration of 1%, 0.1%, 0.01%, 0.001%, and 0.0001% was used for each test organism. One millilitre of diluted CHG was mixed with nine millilitre of molten Mueller-Hinton agar in 50-mm diameter petri dish. The agar was solidified at room temperature and briefly dried at 37°C before inoculation with the test organism. Bacterial cells were suspended in sterile saline solution and the concentration was estimated by measuring the optical density (OD) at a wavelength of 540 nm. Between 10^6 to 10^7 cells per ml (OD=0.7) were spread onto the agar plate. As a control, bacteria were also inoculated on agar plate without CHG. All cultured agar plates were incubated at 37°C for 18-24 hours.

RESULTS

A total of 443 strains representing eight genera of the family *Enterobacteriaceae* and one genus of the family *Pseudomonadaceae* were isolated from various clinical specimens collected from different wards of Tikur Anbessa Hospital, Addis Ababa, Ethiopia, between 1996 and 1997. As shown in Table 1, *Escherichia coli* and *Klebsiella pneumoniae* were the most frequent isolates and comprised 31.6% and 23 % respectively. Fifty nine (13.3%) were *Proteus* species, while 41 (9.2%) were *Pseudomonas* species. Others were isolated at a frequency of 2.7 to 6.1 %.

Table 1

Frequency of bacterial isolates from Tikur Anbessa Hospital

Bacterial species	Number (%)	
<i>Escherichia coli</i>	140	(31.6)
<i>Klebsiella pneumoniae</i>	102	(23)
<i>Proteus</i> spp	59	(13.3)
<i>Pseudomonas</i> spp	41	(9.2)
<i>Citrobacter</i> spp	27	(6.1)
<i>Enterobacter</i> spp	21	(4.7)
<i>Acinetobacter</i> spp	21	(4.7)
<i>Providencia</i> spp	20	(4.5)
<i>Salmonella</i> spp	12	(2.7)
Total	443	(100)

All bacterial isolates were tested for susceptibility to 0.0001% to 1% CHG. As shown in Table 2, 85% of all gram-negative bacterial isolates were inhibited by \leq 0.01% CHG, while 15% were inhibited by \leq 0.1%. *E. coli* and *Salmonella* species were most susceptible and were inhibited by \leq 0.01% CHG. Twenty nine per cent

Table 2

Percent of test organisms inhibited with specific MIC of chlorhexidine gluconate

Bacteria spp	(No.)	% of inhibition to CHG, MIC (%w/v)				
		0.0001	0.001	0.01	0.1	1
<i>E. coli</i>	(140)	20.7	82.1	100	-	-
<i>K pneumoniae</i>	(102)	2	28.5	71.6	97.1	100
<i>Proteus</i> spp	(59)	-	30.5	81.3	98.3	100
<i>Pseudomonas</i> spp	(41)	-	21.9	78	100	-
<i>Citrobacter</i> spp	(27)	14.8	55.5	88.8	100	-
<i>Enterobacter</i> spp	(21)	9.5	52.4	71.4	100	-
<i>Acinetobacter</i> spp	(21)	4.8	13.8	70.9	100	-
<i>Providencia</i> spp	(20)	5	40	75	100	-
<i>Salmonella</i> spp	(12)	-	50	100	-	-
Total	(443)	8.8	49	84.9	99.1	100

of *Acinetobacter* species, 28% of *K. pneumoniae* and *Enterobacter* species, and 19-25% of *Pseudomonas*, *Proteus* and *Providencia* species were only inhibited at high concentrations of CHG ($\geq 0.1\%$). Only four strains (three *K. pneumoniae* and one *Proteus* species) required the maximum MIC of CHG used in this study (1%).

DISCUSSION

Chlorhexidine gluconate (CHG) is widely used for the management of wound infections, periodontal infections and skin disinfection before surgery(14,15) and for disinfection of heat and moisture sensitive surgical instruments (16). The concentration of CHG has been found to be variable depending on the nature of application and the manufacturer's recommendation.

The susceptibility of bacterial isolates to CHG has not been studied in Tikur Anbessa Hospital or elsewhere in the country; comparison was therefore not possible. In this study, about 85% of all gram-negative bacterial isolates were susceptible to chlorhexidine gluconate, at a concentration which was lower than the minimum inhibitory concentrations used for skin and wound disinfection (0.02- 0.05%). However, different species of bacteria showed a heterogeneous susceptibility to CHG MIC distribution and a significant proportion of those isolates were only inhibited at MIC $\geq 0.1\%$. This suggests that they could be resistant to the concentration of chlorhexidine used in the disinfection process in the hospital. The higher frequency of resistant gram-negative bacteria to CHG has also been reported elsewhere(6,10,17-19).

The mechanism of resistance to chlorhexidine has not been well established. Some studies have suggested a correlation between resistance to antibiotics and disinfectants(10) or between disinfectants(20). A single plasmid has been found to be responsible for both disinfectants and antibiotics(12). In another study, outer membrane proteins of 45 kDa in *Serratia marcescens* and 50 kDa in *Pseudomonas cepacia* have been found exclusively in chlorhexidine resistant strains(21). Although multiple antibiotic resistant bacteria have been reported previously and in our study (unpublished observation), whether there are any positive cross resistance links between the antiseptic and the antibiotics remains to be established.

The use of appropriate concentrations of chlorhexidine may inhibit most of the nosocomial strains and thus prevent infection. Studies have shown that the use of two to four CHG for disinfection of heat sensitive surgical equipment and preoperative surgical scrub(16), inhibits postoperative infections. The application of 0.01 to 0.12% CHG oral rinses could reduce the incidence of dental plaque (22-23) or nosocomial respiratory infection(24). In order to minimise the emergence of resistant bacteria, the use

of combined antiseptics such as 0.5% CHG in 70% isopropanol(15) or with a combination of phenoxyethanol(25), has been reported to be effective.

In conclusion, our study showed that many of the gram-negative hospital isolates were still susceptible to chlorhexidine gluconate. Since a significant number of strains of some species were found resistant, the concentration of CHG that is recommended for skin antiseptics may not always be effective. A continuous monitoring of the efficacy of the commonly used disinfectant(s) is therefore imperative in order to minimise the risk of infection by resistant micro-organisms.

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