Original article

In vitro susceptibility of staphylococci to chlorhexidine and antibiotics

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Abstract: The study was performed to monitor the susceptibility of clinical isolates of staphylococci to chlorhexidine and antibacterial drugs *in vitro*. Forty-eight strains of staphylococci were isolated from Tikur Anbessa Hospital patients. All isolates were susceptible to <0.01% chlorhexidine out of which 90% were inhibited at <0.001%. Tetracycline, penicillin, and chloramphenicol were the least effective drugs with 69%, 56%, and 37% of the isolates resistant, respectively. Resistance to any of the other antibiotics tested was less than 30%. Fifty six percent of the isolates were resistant to two or more drugs. The study showed that there was no association between the antibiotic and disinfectant susceptibility. Both susceptible and resistant strains to the antibiotics tested were equally susceptible to low concentrations of chlorhexidine (P>0.05). The study also demonstrated that chlorhexidine is still an effective antiseptic/disinfectant to suppress or prevent staphylococcal infections. The high frequency of drug resistant strains could be an indication of the extensive use of antibacterial drugs in this hospital. Continued surveillance and antibiotic policy are needed in order to minimize the emergence and spread of resistant pathogenic bacteria. [*Ethiop. J. Health Dev.* 1999;13(3):223-227]

Introduction
Bacterial infections can be controlled by local administration of disinfectants such as chlorhexidine. Because it is mild and relatively nontoxic to human tissues, chlorhexidine has gained a wide range of application both as an antiseptic and as a disinfectant in medical practice (1). Although it is widely used, staphylococci resistant to chlorhexidine with positive cross resistance to other antiseptics and antibiotics have been documented (2-4). 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.

Staphylococci, especially *Staphylococcus aureus,* are major causes of community and hospital acquired infections. The use of antimicrobial agents has significantly reduced staphylococcal infections. However pathogenic taphylococci have become increasingly resistant to the commonly used antimicrobial agents worldwide. In Ethiopia, previous studies have shown the importance of *Staphylococcus aureus* in diseases and the emergence of multiple drug resistant strains (5-7).

The purpose of this study was to monitor the susceptibility of staphylococci to chlorhexidine and commonly used antibiotics and determine if there is any positive resistance link between the disinfectant and antibiotics.

Methods
Specimens: A variety of clinical specimens submitted to the Bacteriology Laboratory from adult inpatients of Tikur Anbessa Hospital were processed between January 1996 and March 1997. Out of

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a total of 503 specimens processed, 48 specimens (30 pus, 16 blood, one urine and one throat swab) which were positive for staphylococci were tested.

Isolation and Identification of bacteria: Appropriate culture media were used for isolation of microrganisms (Oxoid, Basingstoke, Hampshire, UK). Specimens were inoculated onto blood agar and incubated at 37ºC for 18-24 h. The bacterial isolates were identified by cultural and biochemical methods following standard procedures (8). *S. aureus* was differentiated from other staphylococci by coagulase test.

Chlorhexidine susceptibility testing: The susceptibility of bacterial isolates to chlorhexidine was determined by agar dilution method following standard procedures (2, 9). Chlorhexidine gluconate supplied as a 20%w/v stock solution was serially diluted with sterile distilled water. Chlorhexidine at a final concentration of 1, 0.1, 0.01, 0.001, and 0.0001%(w/v) was used for each test organism. One ml of diluted disinfectant was mixed to nine ml of molten Meuller-Hinton agar, transferred into 50 mm diameter petri dish. The agar was solidified at room temperature and briefly dried at 37^0C before inoculation with the test organism. Bacterial colonies were removed from an overnight incubated agar plate suspended in sterile saline solution to the proper density. The concentration was estimated by measuring the optical density. The density of the suspension was adjusted to approximately 10^7 to 10^8 CFU/ml by comparing to a McFarland 0.5 $BaSO_4$ standard. One ml of the bacterial suspension was spread onto the agar plate. As a control, bacterial suspension was also plated on agar plate without disinfectant. All agar plates were incubated at 37ºC for 18-24 h. The minimum dilution of chlorhexidine that inhibited growth was considered as the minimum inhibitory concentration (MIC).

Antibiotic Susceptibility testing: The susceptibility of staphylococci to different antibiotics was determined by standard agar disk diffusion method (10). Bacterial suspension adjusted to McFarland 0.5 standard (as above) was spread onto Meuller-Hinton agar plate. Bacterial isolates were tested using the following antibiotic disks (Oxoid): Penicillin (10 ug), carbenicillin (100 ug), cephalothin (30 ug), chloramphenicol (30 ug), gentamicin (10 ug), kanamycin (30 ug), tetracycline (30 ug), methicillin (5 ug), eryhtromycin (15 ug) and trimethoprim-sulphamethoxazole (25 ug). For quality control, reference strain (*Staphylococcus aureus* ATTC #29213) was used..

Statistical analysis: Student t-test was used to compare the mean minimum inhibitory concentration of chlorhexidine required for antibiotic sensitive strains with that of antibiotic resistant strains.

Results

A total of 48 strains of staphylococci were isolated from 30 pus, 16 blood, one urine and one throat swab specimens. Thirty-one strains were *Staphylococcus aureus* and 17 strains were coagulase negative staphylococci (CNS).

As Table 1 shows, all staphylococci were susceptible to $\leq$ 0.01% chlorhexidine. About 22% (7/31) of *S. aureus* and 35% (6/17) of CNS were inhibited by the lowest concentration of the disinfectant used, i.e., 0.0001%.

Table 1: **Staphylococci strains inhibited with specific MIC of Chlorhexidine.**

Bacteria spp (no)		% inhibition to MIC* chlorhexidine				
		0.0001	0.001	0.01	0.1	1
S. aureus	(31)	22.6	90.3	100	-	-
CNS**	(17)	35.3	94.1	100	-	-

* MIC: Minimum inhibitory concentration
** CNS: Coagulase negative staphylococci

As shown in Table 2, penicillin, tetracycline and chloramphenicol were the least effective antibiotics out of which 37% to 76% of isolates were resistant. Only 12.5% (6/48) of the isolates were susceptible to all antibiotics tested. Over 87% (42/48) of the isolates were resistant to one or more drugs. Over 60% (29/48) were resistant to two or more drugs. Since we were not able to test all isolates of staphylococci for methicillin and erythromycim, the results were interpreted separately. About 19% (6/32) of the isolates tested to methicillin were found resistant. All

methicillin resistant strains were simultaneously resistant to four or more drugs.   Table 2: **Staphylococcal isolates resistant to selected antibiotics.**

Bacteria (no)	Number (%) of strains resistant to							
	Pen*	Car	Ceph	Chl	Gen	Kan	Tet	Tsm
S. aureus	20	7	2	11	11	7	19	9
(31)	(64.5)	(22.6)	(6.4)	(35.5)	(35.3)	(22.6)	(61.2)	(29)
CNS**	7	4	0	7	0	1	13	5
(17)	(41.1)	(23.5)	(0)	(41.1)	(0)	(5.9)	(76.5)	(29.4)
Total	27	11	2	18	11	8	33	14
(48)	(56.2)	(22.9)	(4.2)	(37.5)	(22.9)	(16.7)	(68.7)	(29.2)

* Pen - penicillin G   Car - Carbenicillin   Ceph - Cephalothin
   Chl - Chloramphenicol   Gen - Gentamycin   Kan - Kanamycin
   Tet - Tetracycline   Tsm - Trimethoprim-sulphamethoxazole
** Coagulase negative staphylococci

Out of 22 strains tested to erythromycin, 4(18%) were resistant.

   Comparative analysis was made in order to see whether there was any positive cross resistance link between the antibiotic and disinfectant resistant strains. However, no difference in MIC of chlorhexidine was observed between bacteria which were single or multi-drug resistant to the antibiotics tested ($p > 0.05$). There were six strains sensitive to all antibiotics and their mean MIC of chlorhexidine was similar to that of the antibiotic resistant strains ($p > 0.05$, Table 3).

Table 3: **The mean MIC of chlorhexidine for antibiotic sensitive and resistant staphylococci.**

	No(%)	%(X_{geom})* MIC Chlorhexidine
Sensitive to all drugs	6(12.5)	0.00032
Resistant to 1 drug	18(37.5)	0.00088
Resistant to 2 drugs	7(14.6)	0.00072
Resistant to 3 drugs	7(14.6)	0.00027
Resistant to 4 drugs	5(10.4)	0.0016
Resistant to 5 or more	5(10.4)	0.00063

*:X_{geo} Geometric mean of minimum inhibitory concentration (MIC)

Discussion

   Chlorhexidine gluconate (CHG) is widely used for the management of wound infection, periodontal infection and skin disinfection before surgery (11, 12), and for disinfection of heat and moisture sensitive surgical instruments (13). The concentration of chlorhexidine has been found to be variable depending on the nature of application and the manufacturer's recommendation. The use of an appropriate concentration of disinfectant may inhibit most of the nosocomial strains and thus will prevent infection.

   Chlorhexidine is a bactericidal antiseptic that disrupts cell membrane functions. The mechanism of resistance of bacteria to chlorhexidine has not been well established. Some studies have shown the positive links between resistance to antibiotics and disinfectants (3, 14). In *S. aureus* strains, a single plasmid has been found to be responsible for both disinfectants and antibiotics (2).

   The susceptibility of bacterial isolates to chlorhexidine has not been studied previously in Tikur Anbessa Hospital or elsewhere in the country. Comparison was, therefore, not possible. In this study, staphylococci both susceptible and resistant to the antibiotics tested were equally sensitive to $\leq 0.01\%$ w/v chlorhexidine which was lower than the MIC used *in vivo* (0.02- 0.05%).   Such observation confirms that there was no positive cross resistance between chlorhexidine and antibiotics. Our results were in agreement with Al-Masaudi et al (15), Cookson et al (16) and Yasuda

et al (17).   However others have reported an increase in resistance to chlorhexidine or other disinfectants in drug resistant strains including methicillin resistant *S. aureus* (MRSA, 18).

Since chlorhexidine was equally effective for both sensitive and multiple antibiotic resistant staphylococci, hospital acquired infection can still be minimized by application of this antiseptic. However, our preliminary study showed that significant number of gram-negative isolates were not inhibited by the recommended concentration of chlorhexidine (unpublished observation).   Thus, there may be a need to use either higher concentration of chlorhexidine or in combination with other disinfectants.

Antimicrobial resistance is increasing and pathogenic staphylococci have multiple drug resistance to commonly used antimicrobial agents.   If one considers the susceptibility of staphylococci (eventhough the number of isolates was small), the resistance rates show an increasing trend compared to previous studies in this hospital (5, 6) or elsewhere in the country (7, 19-21).   The low sensitivity rates of the isolates to penicillin and tetracycline (32-44%) may be an indication of the widespread use of these antibiotics.   *Staphylococcus aureus* is the most frequent cause of nosocomial infections caused by gram positive bacteria.   Although the strains were highly susceptible to cephalothin, the detection of high rates of resistance to gentamicin is cause for concern.   This could be serious because gentamicin is one of the few drugs used for the treatment of serious bacterial infections in this hospital.

The cause of high resistance rates to easily available and commonly used antimicorbial agents in this study can not be determined from the available information. Since the strains were isolated from in-patients, selective pressure and spread of drug resistance plasmids might be the most important factors.

In conclusion, chlorhexidine is still an effective antiseptic to suppress or prevent staphylococcal infections in the hospital. However, there may be a need to monitor the efficacy of the commonly used disinfectants to other bacteria in order to reduce the risk of infection by resistant microorganisms. The increasing rates of drug resistance among bacteria could be a serious threat to the successful antimicrobial therapy. While appreciating the limitation of this study due to the small number of strains tested, there is compelling evidence for the existence of mutiple antimicrobial resistant strains in this hospital.   A continuous surveillance of antimicrobial susceptibility patterns and antibiotic policies are needed in order to minimise the emergence and spread of resistant pathogenic bacteria.

Acknowledgements

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