Original Article

Chlorhexidine Wipes to Reduce Multidrug-Resistant Gram-negative Bacterial Colonization and Healthcare-associated Infections among Medical Inpatients: A Cluster-Randomized Trial

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Objective: To evaluate efficacy of chlorhexidine gluconate (CHG) to decolonize multidrug-resistant (MDR) gram-negative bacilli (GNB) bacteria, and to reduce healthcare associated infections (HAIs) in general medical inpatients.

Materials and Methods: A 1-year, cluster-randomized study was conducted in a university hospital-based general medical unit. Eligible patients were randomized by study ward for routine daily and extra wiping with non-rinsed CHG-cloths (CHG group, n=145) or rinsed, non-medicated soap bath (control group, n=145), consecutively to the end of study. Study nurses received training and audits per CHG protocol. In all participants, axillae, groins, and perianal area were sampled to detect GNB colonization, on day 4 to 7 and day 11 to 14 of admission, by surveillance culture. All were followed for incidence rates of HAIs to day 14 of the study, or study exclusion.

Results: MDR GNB colonization were significantly lower in CHG group than those of control group, both day 4 to 7 (15.9% versus 43.4%, respectively, p<0.01), and day 11 to 14 of admission (20.6% versus 65.4%, respectively, p<0.01). The incidence rates of overall HAIs did not differ between groups (5.80 versus 7.10 episodes per 1,000 patient-day, respectively, p=0.84). Three patients developed minor skin irritation in CHG group.

Discussion: To the investigators' knowledge, the present study is the first to demonstrate significant CHG reduction of MDR GNB colonization among medical patients in non-critical care unit. Use of non-rinsed CHG bath, personnel training, and audits, may maintain adequate skin concentration of CHG, and lower risk of cross-transmission. To effectively reduce HAIs, combined CHG bath and bundle of care may be required.

Conclusion: Non-rinsed chlorhexidine baths are safe, well-tolerated, and effective to reduce MDR gram-negative bacterial colonization among general medical inpatients, and possible to lower risk of subsequent HAIs and cross-transmission, by day 14 of admission.

Keywords: Multidrug-resistant gram-negative bacilli, Chlorhexidine bath, Decolonization, Healthcare associated infections, General medical inpatients

Received 12 October 2020 | Revised 29 December 2020 | Accepted 6 January 2021

J Med Assoc Thai 2021;104(4):629-36

Website: http://www.jmatonline.com

Gram-negative bacilli (GNB) bacteria, particularly multidrug-resistant (MDR) strains, have been predominant etiologic agents of healthcare associated infections (HAIs) in Thailand. Between 67.0% and 70.2% of HAIs in Thailand were caused by GNB bacteria, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* and, emerging carbapenem-resistant *Enterobacteriaceae* (CRE)[1-4]. Colonization with MDR bacteria is a potential source of MDR cross-transmission to other patients sharing the units, and a risk factor of subsequent infections[5-8].

Chlorhexidine gluconate (CHG), a cationic biguanide, destroying various organisms by disrupting cell membranes, is widely used as a safe, well-tolerated, broad spectrum antiseptic[9]. Full body bathing and oral care with CHG were studied in decolonization

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How to cite this article:

Rongrungruang Y, Sovachinda R, Ngampetch S, Tangkoskul T, Khamphimabood C, Nuangpud P, et al. Chlorhexidine Wipes to Reduce Multidrug-Resistant Gram-negative Bacterial Colonization and Healthcare-associated Infections among Medical Inpatients: A Cluster-Randomized Trial. J Med Assoc Thai 2021;104:629-36.

doi.org/10.35755/jmedassocthai.2021.04.12040

of MDR bacteria, to reduce risk of infections, and as a source control, to limit MDR spread[10,11]. CHG bath have been found to reduce bacterial colonization and HAIs with mixed results across studies[12-15]. Several CHG studies reported benefits in the settings of intensive care unit (ICU), gram-positive bacteria, particularly vancomycin-resistant Enterococci (VRE), and methicillin resistant *Staphylococcus aureus* (MRSA)[16,17], and pre-operative surgical patients[10]. Effects of CHG on gram-negative bacterial decolonization, and medical inpatients remains unclear. The investigators hypothesized that appropriate use of CHG would be effective across different patient units and causative agents. Therefore, the primary and the secondary objectives of the present study were to evaluate the efficacy of CHG to reduce MDR GNB colonization and incidence rates of HAIs, respectively, in general medical units.

Materials and Methods

The investigators conducted the single-centered, open-label, cluster-randomized study in two 20-bed general medicine units for female patients, in a university-affiliated, tertiary-care hospital, Bangkok, Thailand, between January 2019 and January 2020. The authors enrolled patients who were 18 years or older, on day 4 to 7 of admission, and who were bathed routinely by nurses. The patients with known allergy to CHG, pre-existing skin conditions intolerance to bathing, expected admission less than 4 days, or incomplete study data, were excluded. The eligible patients were randomized into CHG and control groups, 1:1 ratio, by the units admitted. In the CHG group, unit nurses received training to wipe the whole body of the participants with non-rinsed, 2% CHG-impregnated washcloths (Ironpad®, Pose Healthcare, Bangkok, Thailand), per CHG bath protocol described elsewhere[18]. Full body CHG bath were given routinely once daily, and repeated following tepid sponge for fever. Extra CHG wiping locally on groins, perineum, and perianal area were added following defecation or soiling. No other cleansing or moisturizing agents were allowed. The CHG log charts were at bedside of participants, for record and visual reminders. The investigators monitored compliance to CHG bathing throughout the study. The participants in the control group received the similar schedules of rinsed, routine, and extra, non-medicated soap bath by the unit nurses. All participants in both groups were scheduled for culture at two timepoints for GNB colonization, as the primary outcomes, first, between day 4 to 7, and second, day 11 to 14 of admission. No culture between day 1 to 3 was collected, given the rates of MDR colonization were highest between day 3 to 14, based on a prior study in the same center[19]. The samples were obtained from three sites, the axillae, groins, and perianal areas, transported in Cary-Blair media to the research laboratory of the Division of Infectious Diseases and Tropical Medicine, Department of Medicine, for isolation, identification, and susceptibility testing. Targeted MDR GNB were isolated using Mac-Conkey agar supplemented with ceftriaxone. Antimicrobial susceptibility tests were performed according to the 2019 Clinical and Laboratory Standards[20]. MDR GNB is defined as GNB non-susceptible to three antimicrobial classes or more. The culture sites, and day of collection, were selected, as the high yield for GNB, based on the result of the previous study in the investigators' institute[19]. All participants were bathed, on routine surveillance for HAIs rates, as the secondary outcomes, and followed to day 14 of study, death, discharged from hospital, or transferred. All types of HAIs including common device and non-device-associated respiratory tract, urinary tract, blood stream infections, and other sites, were determined in incidence rates, by using the 2019 surveillance definition of the U.S. Center of Disease Control National Health Safety Network[21]. The study was approved by the Institutional Review Board (COA No. Si652/2018). Written informed consents were obtained from all participants or legal representatives prior to patient enrollment and study initiation.

Sample size estimation and statistical analysis

Based on a previous study in the investigators' institute[17], overall bacterial colonization rates were 26.9% and 13.1% of the ICU patients in non-medicated soap group and CHG group, respectively. To provide 80% power to detect a statistically significant difference, with 2-sided type I error of 5%, and 20% missing data included, a sample size total of 290 patients, with 145 patients per group, was required. Unpaired t test or Mann-Whitney test was used to compare continuous variables. Chi-square or Fisher's exact test was used to compare categorical variables. The IBM SPSS Statistics software, version 20.0 (IBM Corp., Armonk, NY, USA) was used for data analysis.

Results

Study participants

By study protocol and enrollment, as shown in Figure 1, 348 patients were assessed for eligibility.

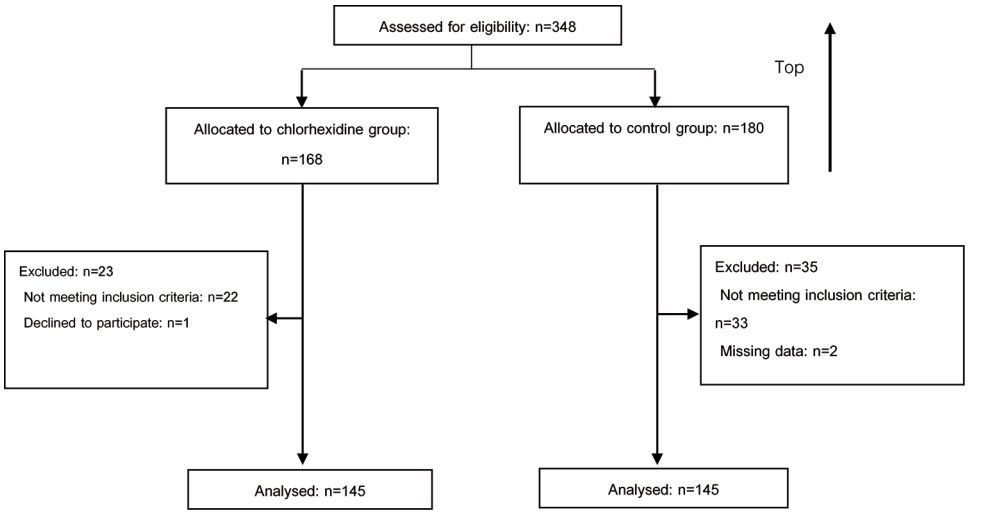

Figure 1. Flow chart of the study protocol and enrollment.

Table 1. Baseline characteristics of the patients in the study (n=290)

Characteristics	Chlorhexidine group (n=145); n (%)	Control group (n=145); n (%)	p-value
Age (years); mean±SD	64.3±19.2	63.2±20.3	0.63
Duration of admission (days); median (range)	11 (4 to 69)	10.5 (4 to 76)	0.48
Preexisting conditions			
Diabetes mellitus	55 (37.9)	45 (31.0)	0.22
Chronic kidney disease	24 (16.6)	23 (15.9)	0.87
Chronic kidney disease on long-term dialysis	11 (7.6)	10 (6.9)	0.82
Chronic lung disease	13 (9.0)	16 (11.0)	0.56
Heart disease	40 (27.6)	43 (29.7)	0.70
Cirrhosis	6 (4.1)	5 (3.4)	0.76
Immunosuppressive treatment	19 (13.1)	16 (11.0)	0.59
Malignancy	23 (15.9)	18 (12.4)	0.40
Cerebrovascular disease	18 (12.4)	19 (13.1)	0.86
Cause of admission			
Pneumonia	21 (14.5)	15 (10.3)	0.29
Urinary tract infection	15 (10.3)	15 (10.3)	1.00
Septic shock/bacteremia	30 (20.7)	23 (15.9)	0.29
Acute coronary syndrome	3 (2.1)	2 (1.4)	0.65
Heart failure	17 (11.7)	26 (17.9)	0.14

SD=standard deviation

Fifty-eight patients were excluded for error sampling between day 4 to 7, 22 in the CHG group and 33 in the control group that were not initially admitted in the study units, two that had missing data in control group, and one patients in the CHG group that declined to participate. Overall, 290 participants, including 145 in the CHG group and 145 in the control group, were included in the analysis.

The baseline characteristics of all participants are shown in Table 1. Mean age (±SD), and median (range) length of stay did not significantly differ between groups, 64.3±19.2 versus 63.2±20.3 years, and 11 (4 to 69) versus 10.5 (4 to 76) days, in CHG and control groups, respectively. The preexisting conditions and causes of admission were comparable both groups.

Table 2. Types of GNB bacterial isolates, GNB colonization rates, and overall HAI rates, by admission day 4 to 7

Category	Chlorhexidine group (n=145); n (%)	Control group (n=145); n (%)	p-value
Types of bacterial isolates			
Susceptible *Pseudomonas aeruginosa*	3 (2.1)	24 (16.6)	<0.01*
Susceptible *Acinetobacter baumannii*	0 (0.0)	17 (11.7)	<0.01*
ESBL-producing *Escherichia coli*	16 (11.0)	45 (31.0)	<0.01*
CR-*Escherichia coli*	1 (0.7)	3 (2.1)	0.62
ESBL-producing *Klebsiella pneumoniae*	4 (2.8)	15 (10.3)	0.01*
CR-*Klebsiella pneumoniae*	3 (2.1)	7 (4.8)	0.20
CR-*Pseudomonas aeruginosa*	2 (1.4)	2 (1.4)	1.00
CR-*Acinetobacter baumannii*	4 (2.8)	13 (9.0)	0.02*
ESBL-producing *Enterobacter* spp.	0 (0.0)	1 (0.7)	0.32
Nonfermentative gram-negative bacilli	0 (0.0)	3 (2.1)	0.25
Overall colonization rates	26 (17.9)	86 (59.3)	<0.01*
MDR colonization rates	23 (15.9)	63 (43.4)	<0.01*
Overall HAI rates (episodes per 1,000 patient-day)	3.22	4.72	0.50

ESBL=extended-spectrum beta-lactamase; CR=carbapenem-resistant; HAI=healthcare-associated infection

* Statistically significance

Table 3. Types of GNB bacterial isolates, GNB colonization rates, and overall HAI rates, by admission day 11 to 14

Category	Chlorhexidine group (n=68); n (%)	Control group (n=52); n (%)	p-value
Types of bacterial isolates, no of patients (%)			
Susceptible *Pseudomonas aeruginosa*	1 (1.5)	9 (17.3)	<0.01*
Susceptible *Acinetobacter baumannii*	0 (0.0)	4 (7.7)	0.03*
ESBL-producing *Escherichia coli*	5 (7.4)	15 (28.8)	<0.01*
ESBL-producing *Klebsiella pneumoniae*	8 (11.8)	14 (26.9)	0.03*
CR-*Klebsiella pneumoniae*	4 (5.9)	11 (21.2)	0.01*
CR-*Pseudomonas aeruginosa*	1 (1.5)	1 (1.5)	1.00
CR-*Acinetobacter baumannii*	2 (2.9)	10 (19.2)	<0.01*
Overall colonization rates	15 (22.1)	39 (75)	<0.01*
MDR colonization rates	14 (20.6)	34 (65.4)	<0.01*
Overall HAI rates (episodes per 1,000 patient-day)	5.80	7.10	0.84

ESBL=extended-spectrum beta-lactamase; CR=carbapenem-resistant; HAI=healthcare-associated infection

* Statistically significance

Study outcomes

The overall and the MDR GNB colonization rates on day 4 to 7 were lower in the CHG group than those of the control group with statistical significance (17.9% versus 59.3%, respectively; risk reduction [RR] 0.30; 95% CI 0.21 to 0.44, p<0.01 and 15.9% versus 43.4%, respectively; RR 0.37; 95% CI 0.24 to 0.55, p<0.01), as shown in Table 2.

On day 11 to 14, 170 patients were dead, discharged, or transferred. There were 120 patients in both groups available for the analysis, the overall and the MDR GNB colonization rates were lower in the CHG group than those of the control group with statistical significance, (22.1% versus 75%, respectively; RR 0.29; 95% CI 0.18 to 0.47, p<0.01, and 20.6% versus 65.4%, respectively; RR 0.31; 95% CI 0.19 to 0.52, p<0.01), as shown in Table 3.

Within day 7 and day 14 of admission, CHG groups had a trend towards lower incidence rates of overall HAIs than those of control group, without significant difference (day 7; 3.22 versus 4.72 episodes per 1,000 patient-day, in CHG and control group, respectively, RR 0.63; 95% CI 0.21 to 1.87, p=0.50, day 14; 5.80 versus 7.10 episodes per 1,000

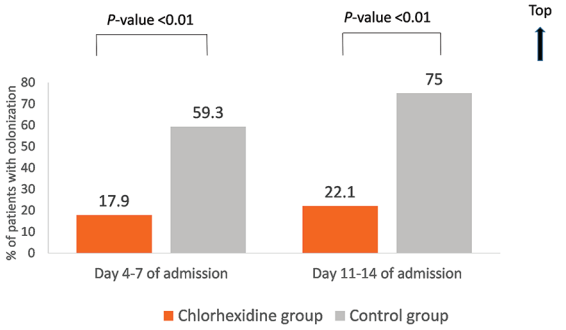

Figure 2. Colonization rates of overall gram-negative bacteria between groups, by day of collection (% of total patients).

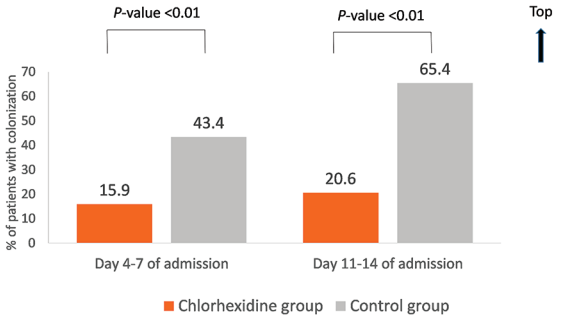

Figure 3. Colonization rates of multidrug-resistant gram-negative bacteria between groups, by day of collection (% of total patients).

patient-day, in CHG and control group, respectively, RR 0.83; 95% CI 0.37 to 1.83, p=0.84.), as shown in Table 2 and 3.

As shown in Figure 2 and 3, colonization rates of overall and MDR gram-negative bacteria, continued to increase over time of admission in both groups, as seen in prior study(20). Significant difference on rates of increase between groups were all demonstrated ($p<0.01$).

Adverse reactions

The adverse skin reactions in the present study were minor irritation without dermatitis (n=1), and weather-related skin discomfort and stickiness (n=2), all in CHG group. Neither allergic nor major adverse reaction was present.

Discussion

Effects of CHG bath to reduce colonization and HAIs in ICU patients have varied considerably among published trials[22]. Randomized-control and cluster-randomized trials of CHG cleansing among ICU patients demonstrated reduction of MDR gram-positive colonization and HAIs, including VRE and MRSA[16]. For reduction of VRE and MRSA HAIs in non-critical care hospitalized patients, were inconclusive[23,24]. Recent, large scale, CHG bath plus MRSA-targeted mupirocin study for non-critical care inpatients did not significantly reduce overall and MDR blood stream infections, but in the subset of patients with medical devices[11]. Based on prior studies, other settings associated with favorable results from CHG bath, include patients in bone marrow or transplant units, and pre-operative elective surgical patients[10,16]. These suggest CHG bath may benefit the group of patients with high risk of HAIs. The data on CHG decolonization of GNB have been limited. Recent, large scale, meta-analysis, and systematic review of CHG cleansing among ICU patients demonstrated significant and trend towards lower colonization rates of *Acinetobacter* species and other gram-negative bacteria, respectively, without significant reduction of HAIs[25-27].

In addition, factors associated with CHG effects include microbial susceptibility to CHG and skin concentration of CHG. Decolonization effects is highly associated with bacterial susceptibility to CHG[9]. Gram-positive bacteria effectively decolonized by CHG, has lower minimal inhibitory concentration (MIC) to CHG than those of gram-negative bacteria. CHG exerts sustained activity up to 24 hours by binding to skin protein[9]. Rinsing with water following CHG, or frequent soiling with body fluids and excretion may inactivate CHG and shorten activity[28]. Use of non-rinsed, additional bath or high concentration CHG bath, and CHG training and audits for health care personnel are likely to maintain higher level of CHG and antimicrobial effects than rinsed, CHG bath[28]. Therefore, once-daily, and additional bathing with CHG after soiling, may be adequate to reduce bacterial colonization, including gram-negative bacteria, also those with multidrug-resistance. Given that gram-negative HAIs are predominant in Thailand, the investigators hypothesized that non-rinsed, wiping with CHG plus additional bath to maintain skin concentration of CHG, may be effective for gram-negative decolonization among non-critical care patients with high risk of HAIs and cross-transmission of MDR GNB. Limited well-designed study on gram-negative decolonization among non-critical care inpatients, had been available. To the investigators' knowledge, the present study is the first to demonstrate the effects of CHG in the significant reduction of overall and MDR GNB colonization, particularly, ESBL-producing

Escherichia coli and ESBL-producing *Klebsiella pneumoniae* with statistically significance among medical inpatients within day 14 of admission.

Global concern on drug resistance have been focused on the overuse of antimicrobial agents. The use of CHG bath had been also associated with bacterial isolates with elevated MIC to CHG, or resistance to antimicrobial agents. Mechanism of resistance particularly in gram-negative bacteria, is proposed to be associated with efflux-pump[29]. *A. baumannii* with increased MIC, although no increase in clinical isolates with extreme drug-resistance, were reported in a hospital with widely CHG use[30]. There was no randomized trial identifying emergence of resistance associated with use of CHG bath[10]. For antimicrobial susceptibility monitoring, there was no alarming antimicrobial resistance on routine laboratory surveillance in the study units. However, use of CHG bath in non-critical care unit, is not routinely advised[31]. Use of CHG is preferably selected for patient unit with high risk of HAIs or MDR cross-transmission, particularly GNB bacteria, with adequate concentration, avoiding unintended dilution or neutralization of CHG, and regular audits of use.

The incidence of HAIs did not differ significantly between the groups, however, there was a trend towards lower rates in the CHG group than those in the control group within day 14 of admission. The present study was not powered to detect the difference. Interventions to reduce HAIs other than decolonization include hand hygiene compliance, urinary tract, respiratory tract, and other device-associated HAI bundles of cares and data on antimicrobial use may need to be controlled across study arms.

Based on clinical trials and practices worldwide, as well as the present study, CHG demonstrates safety, tolerability, and efficacy. The strength of the study included cluster-randomization, protocol training for nurses, enhanced compliance with visual reminder, and audits. The limitations were single-centered, and open-label intervention. For female participants in the present study, gender impact has been inconclusive for overall HAIs, albeit urinary tract infections, across the studies in Thailand[2-4]. MIC to CHG in the present study was not determined, given unclear clinical benefit in the long-term implementation of CHG.

Conclusion

Routine daily and additional bathing with non-rinsed, chlorhexidine wash cloths, plus compliance monitoring, are safe, well tolerated, and effective to reduce colonization with MDR gram-negative bacteria among adult patients in non-critical care units with high risk of subsequent infections and cross-transmission. Further study is warranted to determine the role of chlorhexidine to reduce HAIs.

What is already known on this topic?

CHG cleansing have a propensity to reduce MDR bacterial colonization and HAIs, particularly those caused by gram-positive bacteria, selected patient populations such as patients with critical illness, or medical devices, bone marrow, or transplant patients, and pre-operative elective surgical patients.

What this study adds?

Appropriate chlorhexidine cleansing, to maintain concentration, effectively decolonize overall and MDR gram-negative bacteria among adult patients in non-critical care units with high risk of subsequent infections and cross-transmission within day 14 of admission. Chlorhexidine effects on HAI and beyond day 14 remain unclear.

Acknowledgement

The investigators acknowledge registered and practical nurse team member of the medical units for chlorhexidine and non-medicated soap bathing, and Khemajira Karaketklang for statistical analysis of the data. Study chlorhexidine products were supplied and manufactured by Pose HealthCare, Co. Ltd.

Funding disclosure

The present study was funded in research grant by the National Innovation Agency (NIA), via Pose HealthCare, Co. Ltd.

Conflicts of interest

The authors declare no conflict of interest.

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