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Research Article

Effect of N-acetyl Cysteine and Probiotic on Oropharyngeal Microbial Flora in Mechanically-ventilated Patients

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Abstract

Introduction: This study assessed the effect of four doses of N-acetylcysteine %20 and probiotics on the variety of the oropharyngeal microbial flora of intubated ICU patients.

Methods: In this study, 60 ICU patients under mechanical ventilation were randomly assigned to three groups and treated with N-acetylcysteine %20 mouth rinse, probiotic, or routine care (chlorhexidine). A culture sample was obtained from the oropharyngeal zone before and 24 hours after mouth rinse.

Results: The colonized bacterial count of the sample was not significantly different before intervention among the N-acetylcysteine, probiotic, and routine care groups (P>0.05).

Conclusion: The probiotics and N-acetylcysteine increased the variety of bacterial groups in the oropharyngeal zone. These florae can compete for nutritional items and growth conditions with pathogenic bacteria.

Keywords: N-Acetylcysteine, Probiotic, Oropharyngeal Microbial Flora.

Introduction

The intubated ICU patients struggle with critical situations, exposing them to unwanted common bacterial infections. One of the common sites of disease is the oropharyngeal normal flora observed in more than half of the patients. Pneumonia is one of the critical factors involved in ICU mortalities. The prevalence of pneumonia varies between % seven and %40, and the raw mortality rate of ventilator-associated pneumonia is more than %50 [1, 2]. Due to the lack of possibility of oral feeding of intubated patients, the salivary secretion is diminished, resulting in significantly decreased natural cleansing of the oropharynx by saliva. This culminates in a significant increase in pathogenic germs in the oropharynx besides the normal flora [3].

On the other hand, inadequate oral care may lead to chronic xerostomia and stomatitis, reduced salivary flow, creation of dental plaque, gingivitis, periodontitis, and accumulation of pathogenic bacteria in the oropharynx. These bacteria can induce local and general complications like stomatitis, dental caries, and periodontal infections, followed by systemic distribution of infections and bacteremia, and even the spread of diseases to joints and the heart [4-9]. The chlorhexidine mouth rinse is one of the standard methods used in Iranian hospitals and most ICUs for oral care. Using chlorhexidine jell or solution as a mouth rinse decreases the risk of affliction with ventilator-associated pneumonia in critically ill patients from %25 to about %19 [10-13]. Some complications of chlorhexidine prevent its long-term application. These include color change in teeth and mucosa, desquamation of mucosa and formation of salivary stones, mouth and xerostomia, and adverse systemic effects if swallowed [5]. N-

acetylcysteine (NAC) is a non-antibiotic drug with antibacterial products.

NAC possesses some characteristics that hinder the growth of gram-positive and gram-negative bacteria. It can have antibacterial effects against Pseudomonas aeruginosa and can be used in treating biofilm-associated chronic respiratory diseases. Some studies with promising findings have been performed on the efficacy of each of these agents [6]. On the other hand, probiotics have recently attracted attention in the health research domain. They are living microstructures that can benefit the host's health, provided they are taken sufficiently. Probiotics can inhibit the pathogenic microbes and affect the expression of their virulent genes [9]. They are also specific living microorganisms that, if taken by man or animal, exert valuable effects on the host's health by influencing the body's microbial flora. Most probiotics belong to a large group of the main microbial flora of the human intestine living there as fellow traveling harmless bacteria [10, 11]. The present belief about the valuable effects of probiotics is based on the fact that the intestinal microbial flora plays a protective role against various diseases. The major impact of probiotics is manifested by stabilizing the population of human intestinal microbial flora [12]. The continuous consumption of probiotics has been observed to affect the decreased incidence rate of various diseases, which is more evident in high-risk populations. The consumption of these products has not been associated with any apparent complications yet [13]. Considering what was mentioned above, this study compared the effects of four doses of NAC %20 and Kidi Lact probiotic with routine care (chlorhexidine) during 24 h with 6-hour intervals on the variety of oropharyngeal microbial flora of intubated ICU patients.

Methods

The population study in this single-blind randomized controlled clinical trial, conducted in 2021, consisted of ICU patients hospitalized at Valie Asr Hospital, Razi Hospital, and Imam Reza Hospital in Birjand, Northeast Iran. The inclusion criteria were intubated ICU patients and passage of more than 48 hours after intubation. Also, the exclusion criteria were: history of smoking, history of tracheostomy, change of respiratory status from spontaneous to mechanical ventilation and vice versa, extubation, vomiting, intake of IV NAC, intervention less than four times, and a history of oral surgery. Moreover, for the intervention group with Kidi Lact probiotic and Lactogum, in addition to the mentioned exclusion criteria, other exclusion criteria were immunodeficiency, cancers, WBC less than 4000, neutrophil less than 1000, and chemotherapy. Given the lack of any similar study in the literature review, the sample volume was estimated to be 20 subjects in each group based on the results of a pilot study on ten patients of the NAC intervention group using the mean and SD obtained before and after intervention. The patients were randomly assigned into three groups using a random numbers table. First, the secretions were suctioned sterilely, and the oropharynx was mechanically cleared. Then, an aseptic method prepared a primary culture sample of the patient's oropharynx in a mini-ball container. In the first group, irrigation or lavage was done once every six h using NAC %20 spray for 24 h in 4 doses. Ten puffs of mist, each 0.5 mL, were used for each irrigation or lavage of the oropharyngeal environment. In the second group, each Kidi Lact probiotic stub was dissolved in 2 cc of distilled water with 1 Lactogum tablet, and ten puffs of spray, each 0.5 mL, were applied to the oropharyngeal zone in four stages.

The third group received just the routine ICU care (using NSS and chlorhexidine). For all three groups, the second culture was collected in mini ball containers after 24 h using a sterile method. After submitting the culture sample with an aseptic technique and a single-blind coding, the model was cultured in Sheep Blood Agar and EMB Agar and incubated at 35ºC for 24 h. After 24 h, when the bacterial colonies and the sample culture appeared with the fieldoplatin standard loop (0.001 nichrome), the 1000 colony count index was considered, and the colony count was reported based on "manifested colony count x 1000". In the next stage, to identify the isolated stub (strain), specialized diagnostic work was performed for each stub (strain) according to the number of various manifested colonies. Imvic gallery biochemical and oxidase tests were carried out for gram-negative bacteria. For gram-positive bacteria, gram staining including catalase test, sensitivity to bacitracin, Optochin, and neobiotin and, if necessary, tubal mannitol test, coagulase tube, NaCl %6.5 tolerance, and escolin hydrolysis were completed. Finally, sensitivity tests to antibiotic discs were performed based on the request and choice of the researcher.

The requested discs included Gentamicin (GM), Piperacillin (PIP), Ampicillin/Sulbactam (SAM), Ceftriaxone (CRO), Tobramycin (TOB), Ciprofloxacin (CP), Cefazolin (CZ), Cefoxitin (FOX), Vancomycin (VA), Meropenem (MEN), Ceftazidime (CAZ), and Colistin (COL). The Committee of Ethics in Human Research approved the research proposal at the Khorasgan Branch of Islamic Azad University with code of ethics no.: IR.IAU.KHUISF.REC.1398.146. Informed written consent was obtained from each patient or their legal representative if they were unconscious. Besides, the research protocol was registered in the Iranian Registry of Clinical Trials with no. IRCT20170316033099N9. The gleaned data were analyzed with SPSS19 using descriptive and inferential statistics, including One-way ANOVA, Tukey post hoc test, Chi-square test (X2), Fisher's exact test, and paired t-test. Data without normal distribution were analyzed with non-parametric tests like the Wilcoxon, Kruskal-Wallis, and Mann-Whitney tests (P=0.05).

Results

A total of 60 patients were studied in this research (20 patients in the routine care group, 20 in the probiotic group, and 20 in the NAC group). The findings suggested no significant difference in the frequency distribution of gender, type of feeding tube, mean age, and mean Glasgow coma score among the three groups. Yet, there was a significant difference in moisturizing methods used for different groups (Table 1).

Table 1: Comparison of demographics of the groups under study

Group Variable	NAC F(%)	Probiotic F(%)	Routine care F(%)	Statistical Analysis
Gender				
Male	12 (60)	7 (35)	14 (70)	Value=5.253 P=0.072
Female	8 (40)	13 (65)	6 (30)	
Age				
Mean age	61.85±25.12	54.45±29.13	49.60±26.28	F=1.05 df=57 & 2 P=0.356
Glasgow coma score				
Mean Glasgow Coma score	8.60±2.77	8.15±2.92	8.85±2.51	F=0.334 P=0.718
OGT	0 (0)	2 (10)	0 (0)	Value=4.760 P=0.1
NGT	20 (100)	17 (85)	20 (100)	
None	0 (0)	1 (5)	0 (0)	

The results of the Wilcoxon test indicated that the colonized bacterial counts were the same before intervention in the NAC, probiotic, and routine care samples (P=1). Besides, there was no significant difference in colonized bacterial counts of the examples of groups after intervention (P=0.368) (Table 2). The data in Table 3 presents comparisons of sensitivity to various antibiotics among the different groups before intervention. The findings showed no significant difference in the frequency distribution of sensitivity to multiple antibiotics before intervention among the groups under study. Regarding the type of microbial flora after intervention among the probiotic and N-acetylcysteine groups, the gram-positive Bacillus stub (strain) and gram-positive Coccus stub (strain) were the most common stubs, respectively (Table 4).

Table 2: Comparison of mean colonized bacterial counts before and after intervention among the groups under study

	Before intervention X±SD	Median	After intervention X±SD	Median	Wilcoxon test
N-acetylcysteine	1000000	1000000	1000000	1000000	P<0.001
Probiotic	1000000	100000	1000000	1000000	P=0.31
Routine care	1000000	1000000	1000000	1000000	P<0.001
Kruskal-Wallis test	P=1, X^2=1.2		P=0.368, X^2=2.0		

Table 3: Comparison of frequency distribution of sensitivity to various antibiotics before intervention among the groups under study.

Group Variable	NAC F(%)	Probiotic F(%)	Routine care F(%)	Statistical analysis
Vancomycin (before intervention)				
Sensitive	0 (0)	0 (0)	0 (0)	Value=1.92 P=0.766
Semi-sensitive	1 (5)	2 (10)	0 (0)	
Resistant	19 (95)	18 (90)	20 (100)	
Vancomycin (after intervention)				
Sensitive	1 (5)	0 (0)	0 (0)	Value=3.77 P=0.322
Semi-sensitive	1 (5)	0 (0)	0 (0)	
Resistant	18 (90)	20 (100)	20 (100)	
Cefoxitin (FOX) (before intervention)				
Sensitive	1 (5)	0 (0)	0 (0)	Value=1.85 P=1
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	19 (95)	20 (100)	20 (100)	
Cefoxitin (FOX) (after intervention)				
Sensitive	1 (5)	0 (0)	0 (0)	Value=1.851 P=1
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	19 (95)	20 (100)	20 (100)	
Cefazolin (CZ) (before intervention)				
Sensitive	1 (5)	2 (10)	0 (0)	=1.92value =0.766p
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	19 (95)	18 (90)	20 (100)	
Cefazolin (CZ) (after intervention)				
Sensitive	1 (5)	2 (10)	0 (0)	=1.92value =0.766p
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	19 (95)	18 (90)	20 (100)	
Ciprofloxacin (CP) (before intervention)				
Sensitive	1 (5)	0 (0)	0 (0)	=1.85value =1p
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	19 (95)	20 (100)	20 (100)	
Ciprofloxacin (CP) (after intervention)				
Sensitive	1 (5)	0 (0)	0 (0)	=1.85value =1p
Semi-sensitive	18 (90)	15 (75)	20 (100)	
Resistant	19 (95)	20 (100)	20 (100)	
Tobramycin (TOB) (before intervention)				
Sensitive	1 (5)	5 (25)	0 (0)	=8.16value =0.322p
Semi-sensitive	1 (5)	0 (0)	0 (0)	
Resistant	18 (90)	15 (75)	20 (100)	
Tobramycin (TOB) (after intervention)				
Sensitive	1 (5)	5 (25)	0 (0)	=8.16value =0.322p
Semi-sensitive	1 (5)	0 (0)	0 (0)	
Resistant	18 (90)	15 (75)	20 (100)	
Piperacillin (PIP) (before intervention)				
Sensitive	1 (5)	3 (15)	0 (0)	=3.88value =0.31p
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	19 (95)	17 (85)	20 (100)	
Piperacillin (PIP) (after intervention)				
Sensitive	1 (5)	2 (10)	0 (0)	=1.92value =0.76p
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	19 (95)	18 (90)	20 (100)	
Meropenem (MEN) (before intervention)				
Sensitive	1 (5)	1 (5)	0 (0)	Value=1.27

Semi-sensitive	0 (0)	0 (0)	0 (0)	P=1
Resistant	19 (95)	19 (95)	20 (100)	
Meropenem (MEN) (after intervention)				
Sensitive	1 (5)	1 (5)	0 (0)	Value=3.116 P=0.76
Semi-sensitive	1 (5)	0 (0)	0 (0)	
Resistant	18 (90)	19 (95)	20 (100)	
Gentamicin (GM) (before intervention)				
Sensitive	1 (5)	1 (5)	0 (0)	Value=2.62 P=0.68
Semi-sensitive	1 (5)	1 (5)	0 (0)	
Resistant	18 (90)	18 (90)	20 (100)	
Gentamicin (GM) (after intervention)				
Sensitive	1 (5)	1 (5)	0 (0)	Value=3.16 P=0.76
Semi-sensitive	1 (5)	0 (0)	0 (0)	
Resistant	18 (90)	19 (95)	20 (100)	
Ampicillin/Sulbactam (SAM) (before intervention)				
Sensitive	1 (5)	3 (15)	0 (0)	Value=3.11 P=0.31
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	19 (95)	17 (85)	20 (100)	
Ampicillin/Sulbactam (SAM) (after intervention)				
Sensitive	0 (0)	3 (15)	0 (0)	Value=4.32 P=0.1
Semi-sensitive	0 (0)	0 (0)	0 (0)	
Resistant	20 (100)	17 (85)	20 (100)	

Table 4: Changes in frequency of type of bacteria before and after intervention among the groups under study.

Strains of flora	Before intervention F(%)	After intervention F(%)	After intervention F(%)
		The first most common stub (strain)	The second most common stub (strain)
N-acetylcysteine Group			
Acinetobacter	2 (10)	1 (5)	0 (0)
Kalbesila	17 (85)	16 (80)	0 (0)
Staphylococcus aureus	0 (0)	1 (5)	2 (11.1)
Staphylococcus epidermatis	0 (0)	1 (5)	0 (0)
Streptococcus	1 (5)	1 (5)	0 (0)
Gram positive *coccus*	0 (0)	0 (0)	16 (88.9)
Probiotic Group			
Acinetobacter	7 (35)	7 (5)	1 (5.3)
Kalbesila	11 (55)	12 (80)	0 (0)
Staphylococcus aureus	1 (5)	0 (0)	0 (0)
Serratia	1 (5)	1 (5)	0 (0)
Gram positive *Bacillus*	0 (0)	0 (0)	18 (94.7)
Gram positive *Coccus*	0 (0)	0 (0)	0 (0)
Routine Care Group			
Acinetobacter	2 (10)	2 (10)	0 (0)
Kalbesila	18 (90)	18 (90)	0 (0)
Staphylococcus aureus	0 (0)	0 (0)	0 (0)
Serratia	0 (0)	0 (0)	0 (0)
Gram positive *Bacillus*	0 (0)	0 (0)	0 (0)
Gram positive *Coccus*	0 (0)	0 (0)	0 (0)

Discussion

This clinical trial investigated changes in the type and count of oropharyngeal microbial flora of ICU patients under mechanical ventilation resulting from mouth rinse spray of N-acetylcysteine and probiotics. Generally speaking, the findings suggested that after intervention with N-acetylcysteine and probiotics, the dominant flora changed from one type to two or more types of bacterial

colonies. Hence, it may be concluded that using probiotics and NAC adds to the variety of oropharyngeal bacteria in ICU intubated patients under mechanical ventilation. This microbial flora can compete for nutritional items and conditions of growth with pathogenic bacteria. In this way, they exert their probable inhibitory effects on pathogenic bacteria. Various studies with different protocols have been carried out concerning the effects of spray mouth rinses on oral and oropharyngeal microbial flora in mechanically ventilated patients. The heterogeneity of the methodology of these studies has resulted in different and somehow contradictory outcomes. The results of the study by La Combe et al. (2018) on oropharyngeal bacterial colonization after rinsing with chlorhexidine in mechanically-ventilated patients demonstrated that a total of 48 pathogens, including streptococci (gram-positive cocci) and Enterobacteriaceae (gram-negative) have grown in the samples under study. Thus, the use of chlorhexidine mouth rinses not only did not decrease the pathogenic bacterial count but also increased their count with time [14]. Moreover, the study by Jafari et al. on ICU patients indicated that oral cleansing with a chlorhexidine solution swab twice a day did not confer any extra effect on preventing dental plaque formation during the first two days of hospitalization compared to NSS [15]. Consistent with our findings, some studies have been carried out concerning the effect of oral probiotics on ventilator-associated pneumonia, the result of which indicated its effect on diminishing the incidence of pneumonia [16, 17]. Besides, the results of the study by Zang et al. (2016) suggested that administering probiotic oral capsules for 14 days reduced the incidence of ventilator-associated pneumonia, lengthening its incidence [16]. In addition, another clinical trial demonstrated that the daily consumption of two *Lacto Bacillu*s-containing capsules by mechanically-ventilated patients for 14 days, i.e., the *Streptococcal* and *Bifidobacterium* strains,
significantly reduced ventilator-associated pneumonia, ICU stay, and hospital stay in these patients [17]. As mentioned before, various studies using different approaches have reported a wide range of varying results. Let's consider NAC's irritating effects on mucous secretions and its effects on relatively changing the gram-negative microbial flora into gram-positive cocci (observed in treated patients in the present study). It may be concluded that the long-term use of this drug will be effective in the long-term changing of oral microbial flora, preventing ventilator-associated infections. To confirm this finding, Zhao and Liu indicated that NAC inhibits pseudomonas aeruginosa [18]. Along with this finding, the systematic review by Blasi et al. revealed that using NAC is associated with appropriate antimicrobial effects, at least at the *in vitro* level [19]. Furthermore, probiotics were observed to change the dominant flora from *Kalbesila* into gram-positive bacilli. Thus, it appears that the long-term use of probiotics can exert some effects similar to NAC. The present study showed that chlorhexidine and NSS cannot induce any changes in the created microbial flora. This demands the necessity of conducting more research on antimicrobials and disinfectants that affect gram-negative bacteria. The use of antibiograms showed that the dominant bacterial flora present in these patients is highly resistant and that these 11 types of antibiotics induced no response to the colonial count of these bacteria. To confirm the findings of the present study regarding the effects observed with probiotic use, reference may be made to the studies by Van Ruissen et al. [20] and Mahmoodpoor et al. [17]. They showed in their study that oral probiotics are associated with changing the microbial flora of the environment into non-pathogenic bacteria, thereby reducing the incidence of ventilator-associated pneumonia. One of the limitations of the present study was the use of patients with a history of previous ICU hospitalization and mechanical ventilation. This may be associated with increased odds of the growth of resistant bacteria; hence, this may interfere with the results observed for the efficacy of any treatment options. Also, the small sample volume obtained with a convenient sampling method and the project's costs may jeopardize the generalizability of the results, especially if we consider the various types of grown bacteria. Another limitation was the length of observation (24 h), which necessitates longer observational periods (at least four days) given the patients' long hospital/ICU stay, making the sufficient assessment of bacterial colonization changes possible.

Conclusion

This study showed that NAC %20 mouth rinse spray induced a primary variety in gram-positive bacteria. The relative switch from specifically gram-negative microbial flora to gram-positive cocci indicated that the

long-term topical use of NAC that irritates the mucous secretions could also affect the changing of microbial flour, some factors involved in ventilator-associated pneumonia. Probiotic use most often resulted in the switch of the dominant microbial flora into *Kalbesila* with simultaneous program-positive positives. The long-term use of probiotics may change the dominant microbial flora and switch gram-positive bacteria. The routine use of probiotics in the nutritional diet and continual irrigation and lavage can hinder the growth of resistant bacteria, resulting in significant therapeutic outcomes. Also, NAC can be used instead of probiotics in patients whose immune system has been jeopardized. Besides, the study's results demonstrate that routine care, including chlorhexidine and NSS, causes resistant flora like *Kalbesila*, *Acinetobacter*, and other resistant gram-negative bacteria not to change on treatment. More studies should be done to change the disinfectants that affect these florae.

Moreover, it is likely asserted that probiotics and NAC are effective in creating changes in the dominant flora. This study expunged upon switching patients' oropharyngeal colonization into non-pathogenic strains with low virulence, so the infections may be treatable with conventional antibiotic therapy. Also, creating a competing environment for the use of food items prevents the resistance of resistant flora against treatment. Given the similarity between the primary and created flora counts after the intervention, the odds of treatment and efficacy of antibiotics are increased. All patients had resistant flora before intervention; after intervention, the colonies were changed and contained antibiotic-sensitive flora. Continuing therapy with any of these drugs may change the microbial flora from antibiotic-resistant to antibiotic-sensitive.

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Conflict of Interest Disclosures

The authors declare no conflict of interests.

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Authors' Contributions

A.C. and B.C. conceived of the presented idea. A.C. developed the theory and performed the computations. C.A. and A.B. verified the analytical methods. B.C. encouraged A to investigate [a specific aspect] and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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Ethical Statement

The Committee of Ethics in Human Research approved the research proposal at the Khorasgan Branch of Islamic Azad University with code of ethics no.: IR.IAU.KHUISF.REC.1398.146. Informed written consent was obtained from each patient or their legal representative if they were unconscious. Besides, the research protocol was registered in the Iranian Registry of Clinical Trials with no. IRCT20170316033099N9.

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