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ORIGINAL ARTICLE

Impact of alcohol gel on hand bacteria in healthcare professionals

Impacto del gel de alcohol en las bacterias de las manos en los profesionales sanitarios

Impacto do álcool gel nas bactérias das mãos de profissionais de saúde

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ABSTRACT

Background and objectives: Healthcare-Associated Infections are a problem reported by hospitals worldwide, increasing patient morbidity and mortality, prolonging hospitalization, and increasing health care costs. The hands of health professionals are still the main source of infections, making hand hygiene extremely important for spreading infection control. The objective of this study was to analyze the presence of bacteria on the hands of health professionals after hygiene with alcohol gel in a Neonatal Unit and describe the resistance of microorganisms to antimicrobials. **Methods:** Hand samples were collected using the modified glove-juice method on both occasions, before and after hand hygiene with alcohol gel. Bacteria were identified by MALDI-TOF and susceptibility tests according to Clinical and Laboratory Standards Institute document M100-E29. **Results:** A total of 214 samples were obtained, of which 104 (48.6%) showed bacterial growth before hand hygiene and 52 (24.3%) after hand hygiene with alcohol gel. There were 217 isolates from the cultures, of which coagulase-negative *Staphylococcus* was the most frequent with 41 (27.2%) and 24 (36.4%) positive cultures, respectively before and after hand hygiene. The second most frequent microorganism was *Klebsiella pneumoniae* with 32 (21.2%) and 16 (24.2%), respectively before and after hand hygiene. Multidrug resistance to antimicrobials was detected in 58.1% of gram-positive bacteria and in 34.3% of gram-negative bacteria. **Conclusion:** A decrease was observed, but not an elimination of the microbial load after hand hygiene with alcohol gel, demonstrating the need for improvements in hand hygiene.

Keywords: *Hand Hygiene. Health Personnel. Neonatal Intensive Care Units. Drug Resistance, Microbial.*

RESUMO

Justificativa e objetivos: As Infecções Relacionadas à Assistência à Saúde são um problema relatado por hospitais em todo o mundo, aumentando a morbimortalidade dos pacientes, prolongando a hospitalização e aumentando os custos dos cuidados de saúde. As mãos dos profissionais de saúde ainda são a principal fonte de infecções, tor-

nando a higienização das mãos extremamente importante para a disseminação do controle de infecções. O objetivo deste estudo foi analisar a presença de bactérias nas mãos de profissionais de saúde após higienização com álcool gel em uma Unidade Neonatal e descrever a resistência dos microrganismos aos antimicrobianos. **Métodos:** Amostras de mãos foram coletadas pelo método luva-suco modificado em ambas as ocasiões, antes e após a higienização das mãos com álcool gel. As bactérias foram identificadas por MALDI-TOF e testes de suscetibilidade de acordo com o documento M100-E29 do Clinical and Laboratory Standards Institute. **Resultados:** Obteve-se um total de 214 amostras, das quais 104 (48,6%) apresentaram crescimento bacteriano antes da higienização das mãos e 52 (24,3%) após a higienização das mãos com álcool gel. Foram 217 isolados das culturas, sendo Staphylococcus coagulase-negativo o mais frequente com 41 (27,2%) e 24 (36,4%) culturas positivas, respectivamente antes e após a higienização das mãos. O segundo microrganismo mais frequente foi Klebsiella pneumoniae com 32 (21,2%) e 16 (24,2%), respectivamente antes e após a higienização das mãos. A multirresistência aos antimicrobianos foi detectada em 58,1% das bactérias gram-positivas e em 34,3% das bactérias gram-negativas. **Conclusão:** Observou-se diminuição, mas não eliminação da carga microbiana após higienização das mãos com álcool gel, demonstrando a necessidade de melhorias na higienização das mãos.

Palavras-chave: *Higiene das Mãos. Pessoal de Saúde. Unidades de Terapia Intensiva Neonatal. Resistência a Medicamentos, Microbiana.*

RESUMEN

Justificación y objetivos: Las Infecciones Asociadas a la Atención de la Salud son un problema reportado por los hospitales a nivel mundial, aumentando la morbimortalidad de los pacientes, prolongando la hospitalización y aumentando los costos de la atención médica. Las manos de los profesionales de la salud siguen siendo la principal fuente de infecciones, por lo que la higiene de manos es extremadamente importante para el control de infecciones. El objetivo de este estudio fue analizar la presencia de bacterias en las manos de los profesionales de la salud después de la higiene con alcohol en gel en una Unidad Neonatal y describir la resistencia de los microorganismos a los antimicrobianos. **Métodos:** Se recogieron muestras de manos mediante el método guante-jugo modificado en ambas ocasiones, antes y después de la higiene de manos con alcohol en gel. Las bacterias se identificaron mediante MALDI--TOF y pruebas de susceptibilidad de acuerdo con el documento M100-E29 del Clinical and Laboratory Standards Institute. **Resultados:** Se obtuvieron un total de 214 muestras, de las cuales 104 (48,6%) presentaron crecimiento bacteriano antes de la higiene de manos y 52 (24,3%) después de la higiene de manos con alcohol en gel. Hubo 217 aislamientos de los cultivos, de los cuales el Staphylococcus coagulasa negativo fue el más frecuente con 41 (27,2%) y 24 (36,4%) cultivos positivos, respectivamente antes y después de la higiene de manos. El segundo microorganismo más frecuente fue Klebsiella pneumoniae con 32 (21,2%) y 16 (24,2%), respectivamente antes y después de la higiene de manos. Se detectó multirresistencia a los antimicrobianos en el 58,1% de las bacterias grampositivas y en el 34,3% de las bacterias gramnegativas. **Conclusión:** Se observó una disminución, pero no una eliminación de la carga microbiana después de la higiene de manos con alcohol en gel, lo que demuestra la necesidad de mejoras en la higiene de manos.

Palabras clave: *Higiene de manos. Personal sanitario. Unidades de Cuidados Intensivos Neonatales. Farmacorresistencia Microbiana.*

INTRODUCTION

Healthcare-associated Infections (HAI) are responsible for higher morbidity and mortality, longer length of hospital stay and greater health care costs.[1] Reducing HAI rates has become a challenge to be overcome by hospitals worldwide.

Neonatal Intensive Care Units (NICU) have had high HAI rates, especially because babies have different characteristics, with immature immune systems and low birth weight. In addition, in most cases, they depend on parenteral nutrition and often undergo invasive procedures, such as central venous catheters and orotracheal intubation, as well as broad-spectrum antimicrobial therapy.[2,3]

The reduction of the HAI rates is possible through early identification of risk factors, for instance through health surveillance, staff training, antimicrobial therapy management and especially, correct hand hygiene.[3,4] The hands of healthcare professionals are one of the main vehicles for the transmission of microorganisms between professionals and patients, especially because they are in frequent contact with the potentially contaminated surfaces.[4,5] Furthermore, a premature newborn is handled about 200 times in 24 hours, which increases the risk of infection.[6]

Bacteria present on hands include *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus* spp., *Pseudomonas aeruginosa, Klebsiella* spp., *Enterobacter* spp., *Pantoea* spp. and yeast belonging to the *Candida* genus. Some of these microorganisms are resistant to antimicrobials. For example, *S. aureus* and *S. epidermidis* were found to be resistant to oxacillin/methicillin, *Enterococcus* to vancomycin and *Enterobacteriaceae* and *P. aeruginosa*, to carbapenems.[1,7]

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Conventional hand hygiene (soap and water) is poorly performed among health professionals, especially those working in hospitals.[6,8,9] This is due to a gap in practice, perception and knowledge about the importance of hand hygiene and its impact on the occurrence of HAI. The difficulty in accessing places to perform this procedure or lack thereof, the prolonged time required for hand hygiene, as well as dermatological issues, such as dryness and/or hypersensitivity caused by the substances used in the cleaning process induce many professionals to not adopt the conventional appropriate cleaning processes.[8]

An alternative to conventional hand hygiene is hand hygiene with alcohol hand-rub (at concentration 60-80%, depending on the type of alcohol used),[7,8] which promotes hygiene without loss of effectiveness in removing and/or inactivating microorganisms. The use of this product is indicated when hands are not visibly dirty, it requires less time for hygiene, is easy to obtain and available, and can be arranged on the bedside, with less occurrence of hypersensitivity. Among the many products on the market for hand hygiene, alcohol gel is well accepted by health professionals.[10]

The aim of this study is to analyze the bacteria on the hands of healthcare professionals of a Neonatal Unit before and after using alcohol gel, and evaluate the susceptibility of microorganisms to antimicrobials.

METHODS

The study was carried out in the Neonatal Unit of the Hospital University of Uberlândia. The Neonatal Unit contains a total of 42 beds, 20 for intensive care, 16 for semi-intensive care and six for intermediate care in the "Kangaroo Care" model.

The Neonatal Unit has a multidisciplinary team composed of approximately 120 professionals who work directly with patients, including nurses, nursing technicians, physicians and physiotherapists.

Sample Collection

Three hand washing samples were collected during three work shifts (morning, afternoon and night), on three occasions; the first in April, the second in June and the third in September 2018, representing different seasonal periods.

Collections were performed according to the glove-juice modified technique.[11] Briefly, the research participant put both hands at the same time in a polypropylene bag containing 10 mL of BHI broth (*Brain Heart Infusion*, HIMEDIA®, India), bathing them in the broth for one minute. Then, the bag was sealed and the sample identified as "Before". Subsequently, the participant dried his/her hands with a sterile surgical dressing and performed hand hygiene with alcohol gel, according to the institution's protocol. After complete drying of the alcohol, about one minute, a second collection was performed in another bag identified as "After".

The alcohol gel brand was the same as the one available at the Neonatal Unit (Hydrated Alcohol Gel 70% v/v, Rioquímica®, Brazil).

Culture and bacterial identification

The samples (BHI in bags) were first incubated in a bacteriological incubator at 35 ± 2 °C for 18-24 hours. Subsequently, aliquots (10 µL) were taken and analyzed by Gram staining, seeded in 5% Sheep Blood Agar (HIMEDIA®, India), Mannitol-Salted Agar (HIMEDIA®, India) and Eosin-Methylene Blue Agar (HIMEDIA®, India), and incubated at 35 ± 2 °C for 18-24 hours. Microorganisms were identified by the analytical technique of mass spectrometry: Matrix Associated Laser Desorption-Ionization - Time of Flight (MALDI-TOF), which separates atoms or molecules according to their mass/charge ratio. MALDI-TOF has been recognized as a fast and reliable tool to identify bacteria, once mass spectra can be viewed as species-specific fingerprints, allowing for accurate identification of purified strains at the genus and species level.[12,13]

Antimicrobial susceptibility testing

The antibacterial susceptibility profile was determined by agar disk diffusion method, according to Clinical and Laboratory Standards Institute document M100-E29.[14]

Gram-negative bacteria were tested for the following (DME®, Brazil): amikacin (30 µg); amoxicillin and clavulanic acid (20/10 µg); ampicillin and sulbactam (10/10 µg), cefepime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ciprofloxacin (05 µg), gentamycin (10 µg), meropenem (10 µg), piperacillin and tazobactam (100/10 µg), sulfazotrim (25 µg), ceftazidime (30 µg), aztreonam (30 µg). Susceptibility of *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* to antimicrobials was assessed by the Minimum Inhibitory Concentration (MIC), as recommended by CLSI.[14]

Gram-positive bacteria (DME®, Brazil) were tested for the following: tetracycline (30 µg), ciprofloxacin (05 µg), chloramphenicol (30 µg), erythromycin (15 µg), clindamycin (02 µg), penicillin (10 µg), gentamycin (10 µg); sulfazotrim (25 µg), cefoxitin (30 µg) (also checking resistance to oxacillin), and vancomycin (30 µg) (only for *Enterococcus* spp.).

Microorganisms resistant to three or more classes of the tested antimicrobials were considered Multidrug-Resistant (MDR).[15]

Statistical analyses

Results were tabulated and expressed as relative and absolute frequencies. McNemar's test was performed to assess the effect of alcohol on hand bacteria. The level of statistical significance was set at P <0.05. The IBM SPSS Statistics for Windows, version 21.0 was used.

Study ethics

This study was approved by the Research Ethics Committee of the Federal University of Uberlândia, under no. CAAE 82191417.6.0000.5152. This research was conducted according to ethical standards required by Resolutions 466/2012 - 510/2016 - 580/2018 of the Brazilian Ministry of Health.

RESULTS

A total of 214 samples were collected, comprising before and after hand hygiene, with 48.6% (n = 104) displaying bacterial growth before hand hygiene with alcohol gel, decreasing to 24.3% after hand hygiene with alcohol gel. Table 1 displays the results in each collection period.

The study identified 217 microorganisms; 151 in the "Before" samples, and 66 in the "After" samples, as exhibited in Figures 1 and 2, respectively. Coagulase negative *Staphylococcus* (CoNS) was the most frequent, followed *by Klebsiella pneumoniae*.

Table 1. Results of the microbiological analyses of health professional hands from the Neonatal Unit, before and after hand hygiene with alcohol gel, by collection period.

Year	Professionals n	Positive cultures for bacteria				Reduction after Hand Hygiene (%)	P value
		Before*		After**			
		n	%	n	%		
First Collection (April)	80	37	46.2	20	25	45.9	< 0.001
Second Collection (June)	63	37	58.7	20	31.7	45.9	< 0.001
Third Collection (September)	71	30	42.3	12	16.9	60	< 0.001
TOTAL	107	104	48.6	52	24.3	50	< 0.001

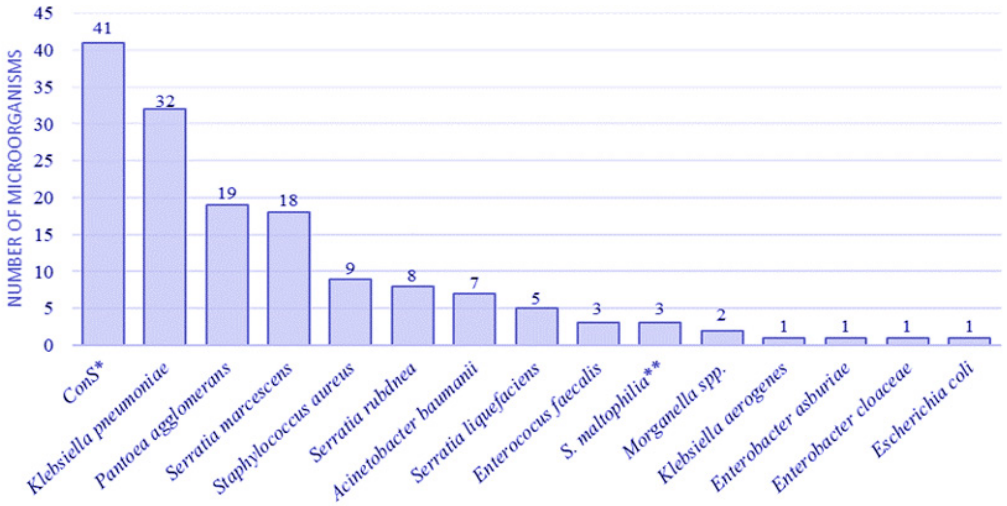

Figure 1. Frequency of microorganisms isolated from healthcare professional hands before hand hygiene in the studied Neonatal Unit. (Note: *= coagulase-negative Staphylococcus; **= Stenotrophomonas maltophilia).

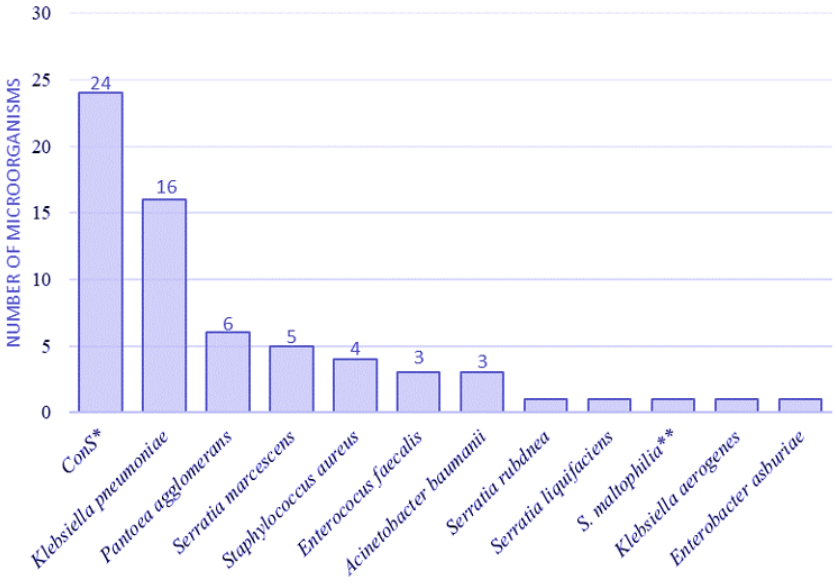

Figure 2. Frequency of microorganisms isolated from healthcare professional hands after hand hygiene in the studied Neonatal Unit (Note: *= coagulase-negative Staphylococcus; **= Stenotrophomonas maltophilia).

Tables 2 and 3 show the results obtained in the antimicrobial susceptibility testing of isolates after alcohol gel hygiene. In total, 18 (58.1%) gram-positive bacteria and 12 (34.3%) gram-negative bacteria were MDR. Furthermore, four (50%) of *Staphylococcus aureus* were MRSA (Methicillin-resistant *Staphylococcus aureus*).

Only one *A. baumanii* isolate was resistant to ampicillin and sulbactam, while one *S. maltophilia* was resistant only to ceftazidime, and one *Enterococcus* spp. to vancomycin.

Table 2. Antimicrobial resistance of gram-positive bacteria isolated from healthcare professional hands after hand hygiene in the studied Neonatal Unit.

Antimicrobial	Genus / Species					
	CoNS (n=24)		*Staphylococcus* aureus (n=4)		*Enterococcus faecalis* (n=3)	
	n	%	n	%	n	%
Ciprofloxacin	5	20.8	0	0.0	3	100.0
Clindamycin	7	29.2	2	50.0	*	*
Chloramphenicol	8	33.3	2	50.0	0	0.0
Erythromycin	12	50.0	3	75.0	3	100.0
Gentamicin	8	33.3	2	50.0	*	*
Oxacillin	9	37.5	2	50.0	*	*
Penicillin	21	87.5	3	75.0	0	0.0
Sulfazotrim	8	33.3	4	100.0	*	*
Tetracycline	7	29.2	3	75.0	1	33.3
MDR	14	58.3	3	75.0	1	33.3

*MDR= Multidrug Resistant; CoNS= coagulase negative Staphylococcus; *= cutoff points not defined by CLSI for bacterial species / genus. Oxacillin resistance determined by resistance to Cefoxitin.*

Table 3. Antimicrobial resistance of gram-negative bacteria isolated from healthcare professional hands after hand hygiene in the studied Neonatal Unit.

Antimicrobial	Genus / Species							
	P. agglomerans (n=6)		*K. pneumoniae* (n=16)		*Serratia* spp. (n=7)		*Other (n=2)*	
	n	%	n	%	n	%	n	%
Amikacin	2	33.3	1	6.3	1	14.3	0	0.0
Amox/Clav Ac.	4	66.7	5	31.3	4	57.1	2	100.0
Amp/Sulbactam	2	33.3	3	18.8	2	28.6	2	100.0
Aztreonan	3	50.0	1	6.3	1	14.3	0	0.0
Cefepime	4	66.7	1	6.3	1	14.3	0	0.0
Cefoxitin	6	100.0	15	93.8	7	100.0	2	100.0
Ceftazidime	3	50.0	1	6.3	0	0.0	0	0.0
Ceftriaxone	3	50.0	2	12.5	2	28.6	0	0.0
Ciprofloxacin	3	50.0	3	18,8	1	14.3	1	50.0
Gentamicin	3	50.0	0	0.0	1	14.3	0	0.0
Meropenem	2	33.3	1	6.3	0	0.0	0	0.0
Pipe/Tazo	0	0.0	1	6.3	0	0.0	1	50,0
Sulfazotrim	1	16.7	1	6.3	1	14.3	0	0.0
MDR	5	83.3	2	12.5	3	42.9	2	100,0

Amox/Clav Ac= Amoxicillin and Clavulanic Acid; Amp/Sulbactam = Ampicillin and Sulbactam; Pipe/Tazo = Piperacillin and Tazobactam; MDR = Multidrug Resistant; Others = Klebsiella aerogenes (1) and Enterobacter asburiae (1) P. agglomerans = Pantoea agglomerans; K. pneumoniae = Klebsiella pneumoniae

DISCUSSION

The Centers for Disease Control and Prevention (CDC) have recognized the importance of hand hygiene in reducing HAI rates since 1974.[9] Studies indicate that this practice is still underestimated by healthcare professionals worldwide, with adoption rates ranging from 30% to 60%.[8,9]

Studies assessing the presence of microorganisms on healthcare professionals' hands during their work shifts have reported different results. In India,[16] 42.7% of healthcare professionals' hands displayed bacterial growth, while in Italy,[17] only 5.41% healthcare professionals' hands showed positive culture for bacteria. In another study[18] was demonstrated that the hands of 97% of professionals in the United States exhibited bacterial growth. In Greece,[19] all professionals participating in the study displayed contaminated hands. These differences found in results of many studies may be due to the sample collection method (fingerprint, glove-juice or contact

plates), and especially, what each author considers as a positive sample. Herein, in our study all bacterial growth was considered positive, since premature newborns are extremely vulnerable to infections.[20]

In the present study, professionals were asked to clean their hands with alcohol gel to check for reduction of microbial load on hands after the hygiene process. The results indicate that 48.6% of professionals still had their hands contaminated to some degree with bacteria. Coagulase-negative *Staphylococcus* were the most frequent isolates, as described in the literature.[19,20] These microorganisms compose the skin microbiome, but are also opportunistic agents of infection in neonates, as demonstrated by Sanderson et al., in which 71% of cases of bloodstream infection in neonates were caused by CoNS.[21] In a study reporting the incidence of nosocomial infections in the same Neonatal Unit of this study, CoNS and *Staphylococcus aureus* were the most frequently causative agents of infections in neonates, representing 36.5% and 23.6% of total infections, respectively.[22]

Gram-negative bacteria are less common on hands compared to gram-positive cocci, but they are important infection agents in neonates. In the present study, *Klebsiella pneumoniae* was the most common gram-negative bacteria. Chen and colleagues reported *Klebsiella pneumoniae* as responsible for 16% of neonatal infections, followed by *Staphylococcus aureus* (12.3%).[3] In Egypt, the most frequent microorganism was also *Klebsiella pneumoniae*, present in 41.6% of infections, followed by CoNS, present in 22.8% of cases.[23]

A total of 24.3% of the samples from professional hands analyzed after hand hygiene with alcohol gel did not show any reduction in microbial load. Studies evaluating alcoholic preparations (gel, foam, liquid) demonstrate effectiveness in reducing microbial loads by up to 99.9%.[8,24]

In a study conducted in India, 35% of healthcare professionals displayed bacterial growth on their hands, becoming non-detected after hygiene with alcohol-based hand rub.[25] Similar results were reported in another study in which only 5% of hands showed bacterial growth after hand hygiene with alcohol-based handrub.[26,27]

The discrepancies between the literature and the present study may be related to the fact that in studies evaluating the effectiveness of alcoholic preparations, participants were trained so everyone could perform the proper hand hygiene technique. The effectiveness of these preparations has been proven under these conditions.[25] In this study, participants were instructed to perform hand hygiene with alcohol gel as usual, thus evidencing a possible deficiency in the way they usually perform hand hygiene, since part of participants (24.3%) exhibited bacterial growth after the hand hygiene procedure.

Concerning MDR bacteria, 58.1% of gram-positive bacteria and 34.3% of gram-negative bacteria were considered as multidrug-resistant in this study, while 50% of isolated *Staphylococcus aureus* were MRSA. Several studies have demonstrated that MDR bacteria are found on the hands of healthcare professionals, including MRSA, ranging from 1.3% to 1.6%. However, when evaluating only *Staphylococcus* spp., in general, 39% are categorized as MDR. Regarding gram-negative bacteria, frequencies range between 3.2% to 56% concerning the presence of MDR on health professionals' hands.[16,17,19] For example, in China, 7.3% of positive samples were reported when assessing the presence of gram-negative bacilli from the hands of health professionals in an intensive care unit, among which *Klebsiella pneumoniae* was the most common, and 50% were MDR.[4]

Working in Intensive Care Units increases the risk of carrying potential microorganisms that cause HAIs in neonates, including MDR[22]. Concerning NICUs, hand hygiene habits become extremely important, as most patients are premature and extremely vulnerable to infections, remain in the hospital for long periods and receive various essential life-sustaining procedures.[20]

The presence of bacteria on hands before hand hygiene indicates which pathogens may be spreading in the unit, and a key point is noted after hygiene, since hands should be cleaned before contact with any medical devices and patients, providing safe care according to World Health Organization guidelines. Successful hand hygiene must take into account two factors, namely the quality of the product used in the process and quality/effectiveness of the performed hygiene.[10]

This study has limitations, including the technique used for collection ("modified glove-juice"), as well as hand drying with towels, which may have reduced the microbial load on hands. In addition, the non-evaluation of the hand hygiene technique performed by the assessed professionals was to ensure that professionals whose samples tested positive after the hygiene procedure resulted from inadequate technique, amount of product or even insufficient hygiene time, which may be evaluated in future studies.

The persistence of these bacteria even after hand hygiene seems to demonstrate a failure in the hygiene process, either due to the applied methodology or the alcohol gel time of action. The most frequent microorganisms found before and after hand hygiene, were Coagulase Negative *Staphylococcus*, *Klebsiella pneumoniae* and *Pantoea agglomerans*. Multidrug resistance to antimicrobials was evaluated, revealing that most of gram-positive (58.1%) and many gram-negative (34.3%) bacteria are multidrug-resistant.

This study revealed the need for improvements concerning the hand hygiene process performed by healthcare professionals in the studied unit in order to reduce the microbial load on hands. Further studies may be conducted in an attempt to better determine the most important variables involved in this process, such as the alcohol gel amount, hygiene process and product efficiency (alcohol content, composition and gel alcohol presentation, among others). Thus, efforts are required to improve both hygiene adherence and quality, which can be demonstrated and proven by epidemiological studies involving *in vitro* experiments.

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All authors have approved the final version to be published and are responsible for all aspects of the work, including ensuring its accuracy and integrity.