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THE SURFACE SCREENING OF NEONATAL INTENSIVE CARE UNIT FOR MULTIDRUG RESISTANT GRAM-NEGATIVE BACTERIA

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ABSTRACT

Introduction: Contaminated surfaces within the hospital environment are potential reservoirs of health care associated multidrug resistant pathogens. **Methods:** The study was conducted in a Neonatal Intensive Care Unit, in Brazil. The Gram-negative bacteria were identified by MALDI-TOF MS. Antimicrobial susceptibility test was performed and screened for ESBL and AmpC production. **Results:** Of the 408 samples collected, 30 presented contamination by Gram-negative bacteria, including 19 multidrug resistant, in which 10 were ESBL producers and 19 were AmpC producers. **Conclusions:** High-touched surfaces of NICU were found to be clinical pathogenic bacteria contaminated, including multidrug resistant, ESBL and AmpC β-Lactamase producers.

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INTRODUCTION

Contaminated surfaces within the hospital environment are potential reservoirs of health care associated pathogens[1]. Gram-negative bacteria thrive in the nosocomial environment and contaminate numerous sites on surfaces and equipment[2]. The presence of these micro-organisms in the environment can serve as a source of cross infection in a hospital environment[3].

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Infection caused by Extended-Spectrum β-Lactamases (ESBL) positive Gram-negative bacteria have been emerged internationally. The problem is more alarming in developing countries, where there is limited antibiotic option, lack of surveillance networks, irrational drug use, and lack of appropriate diagnostics[4]. Similarly, Gram-negative bacteria producing AmpC β-Lactamase is an emerging problem related with health care associated bacteremia. Effective treatment of infection caused by AmpC producers has become a major challenge due to frequent resistance to various antimicrobials.

Thus, their detection is important from infection control point of view[5]. The aim of this study was determine the rate of contamination of inanimate surfaces by Gram-negative bacteria, analyzing resistance profile, as well as to determine the rate of the extended spectrum β-Lactamase (ESBL) and AmpC β-Lactamase production among isolates. The study was conducted in the Neonatal Intensive Care Unit (NICU) that has 20 beds, in the Hospital of the Federal University of Uberlândia, a university level tertiary care center, located in the city of Uberlândia, Minas Gerais, Brazil. The surface disinfection was made with INCIDIN® EXTRA N 0.5% that consists of 12.4% glucoprotamine, 15% alkyl dimethyl benzyl ammonium chloride, nonionic surfactant, solvent, complexing agent, anticorrosive agent and water. This disinfection is done by friction at least three times a day after change of shift (6:30 a.m., 12:30 p.m. and 6:30 p.m.). The environmental samples were collected in three periods (March, June and August of 2018), always on the same day of the week and respecting the same time (two hours before disinfection of the unit), covering the following surfaces: baby incubators, heated cribs, monitors table, respirator monitor, infusion pump, vital signs monitor, soap dish, towel paper holder, cabinet drawer, switch, medicine storage refrigerator's door, NICU access doors, door handle, medication preparation area, faucet spout and bath sink drains, totaling 136 points and 408 samples. The collections were made on surfaces of delimited areas. A sterilized swab was moistened in 3mL of 0.9% saline solution, and subjected to pressure and friction throughout the area delimited, for 20 seconds, starting the procedure horizontally, then wiping vertically and for end, in the diagonal direction[6]. Finally, the swab with the sample was placed in the tube with 0.9% saline solution, identified and transportation to the Laboratory for microbiological procedures. The species were identified by MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization-Time of Flight)[7].

The antimicrobial susceptibility test was performed using the Disk Diffusion technique proposed by Kirby and Bauer modified[8]. The following antimicrobials were tested: Amoxicillin-Clavulanate (30µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Cefoxitin (30µg), Piperacillin-Tazobactam (110µg), Ceftazidime (30µg), Cefepime (30µg), Meropenem (10µg), Aztreonam (30µg), Trimethoprim-Sulfamethoxazole (25µg), Ceftriaxone (30µg), Ampicillin-Sulbactam (20µg). Strains of the American Type Culture Collection (ATCC) of *Escherichia coli* ATCC 25922 (β-Lactamase negative) and *Pseudomonas aeruginosa* ATCC 15442 (broad spectrum of resistance to various commercial antimicrobials) were used. The definition of Multidrug resistant (MDR) samples is those that have resistance to 3 or more classes of antimicrobials[8]. The Minimal Inhibitory Concentration (MIC) of Colistin was determined using the reference broth microdilution method recommended by the Clinical and Laboratory Standards Institute M100[8]. Colistin resistance was defined as MIC ≥4µg/L. Additionally, the MIC of seven antimicrobials was determined for *Stenotrophomonas maltophilia*. The antimicrobials tested included Ticarcillin + Clavulanate (TIM), Ceftazidime (CAZ), Minocycline (MI), Levofloxacin (LVX), Chloramphenicol and Trimethoprim +Sulfamethoxazole (SXT). Intermediately-resistant isolates were considered to be resistant. The quality control for MIC was performed using *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213. Detection of ESBL production was done by combined disc method using cefotaxime (30µg), cefotaxime+clavulanic acid (30µg/ 10µg), ceftazidime (30µg) and ceftazidime+clavulanic acid (30µg/10µg). Inhibition zone ≥5mm increase in either antimicrobial tested in combination with clavulanic acid versus its zone when tested alone confirmed ESBL production[8]. Detection of β-Lactamase AmpC was done by using cefoxitin (30µg) and cefoxitin+cloxacillin disks (230µg). A difference of ≥4mm in the inhibition zones of cefoxitin+cloxacillin and cefoxitin disks was an indication of AmpC production[9].

Table 1. Phenotype, ESBL and AmpC β-Lactamase production of Gram-negative bacteria in the Neonatal Intensive Care Unit Environment from March to August 2018

Bacterial Species	Surfaces	n	Phenotype	ESBL Positive	AmpC Positive	Total	
						n	%
Pantoea aerogenes	Baby Incubators	1	MDR	0	1	13	43.4
		4	N-MDR	0	4		
	Monitor Table	1	N-MDR	0	1		
	Bath Sink Drains	2	MDR	2	0		
	Faucet Spout	2	MDR	1	0		
	NICU Access Door	2	MDR	2	0		
	Soap Dish	1	N-MDR	0	1		
Escherichia coli	Baby Incubators	1	N-MDR	0	1	6	20
	Monitor Table	1	N-MDR	0	1		
		1	MDR	1	0		
	Faucet Spout	1	MDR	1	1		
	Respirator Monitor	1	MDR	0	1		
		1	N-MDR	0	1		
Pantoea agglomerans	Baby Incubators	2	MDR	1	2	4	13.4
		1	N-MDR	0	1		
	Towel Paper Holder	1	MDR	1	0		
Serratia marcescens	Bath Sink Drains	1	MDR	0	1	3	10
	NICU Access Door	1	MDR	0	1		
	Door Handle	1	MDR	0	0		
Serratia liquefaciens	Baby Incubators	1	MDR	0	1	1	3.3
Stenotrophomonas maltophilia	Faucet Spout	1	MDR	0	0	1	3.3
Klebsiella pneumoniae	Baby Incubators	1	N-MDR	0	1	1	3.3
Klebsiella oxytoca	Bath Sink Drains	1	MDR	1	0	1	3.3
TOTAL		30		10	19	30	100

Legend: MDR: Multidrug-Resistant organism; N-MDR: Not Multidrug-Resistant organism.

Approved by the Committee of Ethics in Research with Human Beings of the Federal University of Uberlandia, with number 2.678.162/2018 and it is in keeping with the Helsinki Declaration. Of the 408 samples collected, 30 (7.3%) was

isolated Gram-negative bacteria, including 13 (43.4%) *Pantoea aerogenes*, 6 (20%) *Escherichia coli*, 4 (13.4%) *Pantoea agglomerans*, 3 (10%) *Serratia marcescens,* 1 (3.3%) *Serratia liquefaciens,* 1 (3.3%) *Stenotrophomonas maltophilia,* 1 (3.3%) *Klebsiella pneumoniae* and 1 (3.3%) *Klebsiella oxytoca*. There was a higher number of contaminant isolates in the baby incubator (n=11; 36.6%), faucet spout used to hygiene professional hands (n=4; 13.3%) and bath sink drains used for neonatal baths (n=4; 13.3%) (Table 1). Overall, 19 (63,3%) MDR environmental isolates were identified, preserved and rechecked for different antibiotics (13 antibiotics), screened for Extended Spectrum β-Lactamase production and AmpC β-Lactamase production. Among these isolates, 10 (33.3%) were ESBL producers and 19 (63.3%) were AmpC producers. Of the positive isolates at screening for ESBL, 5 (16.6%) were *Pantoea aerogenes* followed by 2 (6.7%) *Escherichia coli*, 2 (6.7%) *Pantoea agglomerans* and 1 (3.3%) *Klebsiella oxytoca*. Of the positive isolates at screening for AmpC, 7 (23.3%) were *Pantoea aerogenes* followed by 5 (16.7%) *Escherichia coli*, 3 (10%) *Pantoea agglomerans,* 3 (10%) *Serratia* spp. and1(3.3%) *Klebsiella pneumoniae*. A higher percentage of resistance was found for Cefoxitin (90%), Amoxicillin + Clavulanic Acid (86.7%) and Colistin (56.7%) (Table 2).

Table 2. Antibiotic susceptibility patterns of Gram-negative isolated from the Environment

Antimicrobial	Resistance	%
Cefoxitin (30µg)	27	90
Amoxicillin-Clavulanate (30µg)	26	86.7
Colistin (MIC ≥4µg/mL)	17	56.7
Ceftriaxone (30µg)	14	46.7
Gentamicin (10µg)	13	43.3
Amikacin (30µg)	11	36.7
Aztreonam (30µg)	11	36.7
Ampicillin-Sulbactam (20µg)	11	36.7
Ceftazidime (30µg)	10	33.3
Cefepime (30µg)	10	33.3
Trimethoprim-Sulfamethoxazole (25µg)	6	20
Ciprofloxacin (5µg)	5	16.7
Meropenem (10µg)	3	10
Piperacillin-Tazobactam (110µg)	2	6.7

There are several evidences that microbial contamination of the clinical environment may contribute for transmission of pathogens to intensive care unit patients. Transmission of pathogens can occur even after cleaning and disinfecting the hospital environment[2]. The results of the present study showed that 7.3% of the environmental surfaces sampled were contaminated by some kind of Gram-negative bacteria. *Pantoea aerogenes* was the most frequent pathogen detected in 13 (43.4%) environmental samples. In 10 (58.8%) of the 17 *Pantoea* isolates, MDR was observed. Study observed the evaluated *Pantoea* species over 3 years (2015-2017) and showed that the samples circulated within the hospital environment, being this reservoir, and rapidly acquired resistance to important antimicrobials such as carbapenens[10]. Other Gram-negative pathogens were detected in environment in this study including *E. coli*, *P. agglomerans, S. marcescens, S. liquefaciens, S. maltophilia, K. pneumoniae* and *K. oxytoca*. In totally, 23 (76.7%) of the isolated samples were obtained from dry surfaces and 7 (23.3%) of humid environments. Study shows that these pathogens can survive from 1.5 hours to 16 months on dry inanimate surfaces[1].Water or water-related equipment can serve as a waterborne pathogen reservoir in the hospital environment. Previous studies also have associated a water source with acquisition of Gram-negative pathogens because of the bacterium's ability to form a biofilm in moist environments[2]. These pathogens can cause nosocomial infections and outbreaks in severely immunocompromised or critically ill patients, particularly in NICU[11]. Through a research carried out in the unit's electronic system, it was observed that, unlike the other Gram-negative pathogens isolated in the unit environment, *S. maltophilia* was the only pathogen that, for the first time in 20 years of study, was isolated causing infection and colonization in the neonates of the studied NICU. Also found in the environment, the situation became alarming especially after two neonates had died. It is an environmental opportunistic pathogen that can also colonize niches in hospitals and clean rooms. Besides that, *S. maltophilia* has emerged as an important nosocomial pathogen among pediatric patients. It should be noted that in many reported outbreaks there is the undeniable participation of an environmental source[12]. It was observed in this research, that 63.3% of the environmental isolates were MDR. Studies have suggested that environmental contamination plays an important role in the nosocomial transmission of MDR micro-organisms[2].

The persistence of these micro-organisms in the environment can serve as a source of transmission and dissemination in the hospital environment. Many pathogens isolated from the environment presented a considerable level of resistance to antimicrobial agents. It is suggested that this is due to the frequent use of antimicrobial, especially in developing countries and Critical Units[3]. In the present study, of the 30 isolates of the environment, 10 (33.3%) were ESBL and 19 (63.3%) AmpC producers. A study conducted in 2019 with hospitalized patients in Tehran and Ilam, Iran, showed that 23 (35.4%) of the 65 *Escherichia coli* isolates were ESBL producers and 6 (9.2%) were AmpC producers[13]. Extended-spectrum β-Lactamase (ESBL) or AmpC β-Lactamase (AmpC) producing Enterobacteriaceae has been increasingly implicated in health care and community associated bacteremia[5]. Our data showed that 17 (56.7%) of the environmental isolates were resistant to Colistin. It is considered the antimicrobial of last resource because, although it has side effects, including nephrotoxicity and ototoxicity, it is widely active against Gram-negative bacteria[14]. Several resistance mechanisms, including Extended Spectrum β-Lactamases (ESBL), such as the AmpC gene, are reported to be responsible for carbapenem and Colistin resistance. Isolation of Colistin-resistant bacteria in many countries underscores the need to develop new strategies for the treatment of Gram-negative bacteria, including MDR samples[15].

The rate of contamination of inanimate surfaces in this research was low (7.3%), demonstrating the effectiveness of the substance used as well as the method of use of the product. Study[6] conducted in 2012 evaluated the efficacy of Incidin (containing glucoprotamine) over the current standard, Deconex (containing aldehyde) in a high-risk clinical setting that is the hematology transplant unit sector at the University Hospital of Basel in Switzerland. Of the 1,540 samples of surfaces disinfected with Incidin, 185 (12%) showed bacteria grown. In conclusion, high-touched surfaces of NICU were found to be clinical pathogenic bacteria contaminated, including multidrug resistant, ESBL and AmpC β-Lactamase producers. Overall, the role of the inanimate environment

derives from continued problems and researchers should be aware to discover this rote of transmission in cross infection acquisition of multidrug resistant organisms.

Conflicts of Interest: The authors declare that there is no conflict of interest.

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