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Diversity and Resistance of Bacteria Isolated from a Neonatal Intensive Care Unit (NICU) Surfaces and the Hands of Nurses in Jeddah-Saudi Arabia

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Abstract: Healthcare-Associated Infections (HAIs) are a serious problem for neonates when admitted to NICUs. The healthcare environment and healthcare workers' hands may be a source of transmission. Therefore, there is a need to find microbial sources and identify the contaminants to improve disinfection protocols to reduce HAIs. Out of the 169 samples collected on nutrient agar media from NICUs of a hospital in Saudi Arabia, 122 bacterial strains were isolated and some were identified as infection-causing bacteria. The samples collected from various surfaces, equipment, and nurses' hands were divided into two groups. One of which was collected from close proximities of patients to the source of infection and the second was collected from a distance. The tested inanimate environments included incubators, oxygen monitors, heart monitors, temperature monitors, intravenous, stethoscopes, tables, IV trolleys, weighing scales, windows, and sinks. The results showed that all tested equipment and surrounding surfaces were contaminated with various species of bacteria except the sinks. Out of 122 bacterial samples, 78 isolates were identified as cocci and 44 as bacilli. Also, 105 isolates were Gram-positive and 17 were Gram-negative. Using 16S rRNA gene sequencing techniques, 11 genera of bacteria namely, *Enterococcus*, *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *lelliottia*, and *Acinetobacter* were isolated and identified. The most isolated and widely spread bacteria was *S. epidermidis*, which was followed by *B. cereus*. Some of these strains were found resistant to several antibiotics when susceptibility tests were performed by using Vitek 2 with cards (AST-GP67, AST-N292, AST-N291). The present study showed some bacterial species pose threats to the health of hospitalized babies; hence it is important to improve disinfection protocols in neonatal ICU to ensure the safety of baby patients.

Keywords: Neonatal Intensive Care Units, Equipment, Hospital-Acquired Infections, Nosocomial Infections, Bacterial Contamination

Introduction

Neonates in Intensive Care Units (NICUs) undergone surgery or having congenital abnormalities are usually immunocompromised. They are most likely to be at the highest risk of nosocomial infections (Stover *et al.,* 2001; Urrea *et al.,* 2003; Couto *et al.,* 2007; Hewitt *et al.,* 2013). A significant number of studies showed Healthcare-Associated Infections (HAIs) in NICUs ranging between 8.7 to 74.3%, which has been 3 to 20 times higher in developing countries (Gadallah *et al.,* 2014; Kumar *et al.,* 2018). In a study made in the city of Abha in Saudi Arabia, NICU indicated 77 neonatal developed infections out of 401 at the rate of 13.7 infections per 1000 patients/day.

Moreover, nosocomial-infected neonates have three times more death risk as compared to infection-free neonates (Mahfouz *et al.*, 2010).

Bacteria cause health-associated infections in neonatal intensive care units (Joshi and Litake 2013; Khan *et al.*, 2017). The origin of infective bacteria is still not known they are reported from various surfaces of the neonatal intensive care units, which serve as their reservoirs and provide a favorable environment (Bokulich *et al.*, 2013). Earlier studies found that many bacterial species isolated from NICU environmental surfaces are associated with nosocomial infection in neonates (Kumar *et al.*, 2018), whereas, other studies reported some bacterial species Multidrug-Resistant (MDR) (Razzaghi *et al.*, 2016; Saleh *et al.*, 2018; Nazeri *et al.*, 2019).

This study aimed to survey and identify bacterial species found in inanimate environments (surfaces and equipment) and nurses' hands and to provide the patterns of their antimicrobial resistance in the Neonatal Intensive Care Unit (NICU) in a hospital in Jeddah, Saudi Arabia. The present study will fill the gaps and provide scientific data for improving infection control protocols and prevention measures in NICU.

Materials and Methods

Sources of Samples

Bacteria were isolated from common inanimate surfaces and the hands of nurses in the Intensive Care Unit (NICU) at three different times i.e., 8.00-9.00 AM, 9:00-10:00 AM, and 13:00 -14:00 during two weeks in March 2019. The samples include the high-touch areas of touch screens, buttons, and handles of the NICU environmental surfaces and medical devices.

Sample Collection

A total of 169 swab samples were collected from the following three zones:

- Zone 1: Incubator which is the closest thing to a patient neonate
- Zone 2: Neonate-associated equipment that is around each incubator
- Zone 3: Non-associated environmental surfaces, which are the farthest environment from the patients

The number of swab samples that were taken from the selected surface in each zone are shown in Table 1. The sampling protocol of Wang *et al*. (2017) was applied in this study. It should be mentioned that the timing and sites of sampling were withheld from the Health-Care Workers (HCWs) of the NICU.

Limitation

The present study had a limitation; the samples were collected from the incubator which housed a neonate for at least 48 h and zone 2 of all the chosen patients has the same equipment.

Isolation of Bacteria

All the collected samples were immediately cultured in 3 mL of Nutrient Broth (NB) and incubated. Thereafter, 100 mL of each cultured NB tube was sub-cultured on Nutrient Agar (NA), which was evenly spread over the surface of NA and then incubated. Based on morphological differences, the individual colonies growing on the plates were selected and streaked onto another plate of NA to obtain pure cultures before incubating. All the incubation stages were done under aerobic conditions overnight at 37°C. Finally, all the pure isolates were stored in 50% glycerol and stored at -20°C until they were identified and used for the experiments.

Identification of Bacteria

The standard microbiologic techniques were used to identify the isolates. These techniques were based on phenotypes of the cells, colony morphology, Gram staining, and Catalase test.

Molecular Identification

DNA Extraction

Based on morphological similarity, 62 isolates were identified depending on 16S rRNA gene sequences. Total genomic DNA was isolated according to Azcárate-Peril and Raya (2001) with some modifications. One ml of pure bacteria broth culture grown overnight was centrifuged at 10000 RPM for 5 min. After discarding the supernatant 200 µL TES buffer was added and mixed well by a vortex followed by the addition of 20 µL lysozyme (10 mg/mL) and 20 µL of proteinase K (10 mg/mL). After each addition, the mixture was mixed completely and incubated at 37°C for 1 h in a water bath. Two hundred and fifty microliters of chloroform: Isoamyl (24:1) was added and the mixture was stirred by fingers and centrifuged at 8000 rpm for 5 min. The aqueous phase was transferred to a new Eppendorf tube. DNA precipitation was carried out by adding an equal volume of isopropanol and overnight stored at -20°C. The next day, the solution was centrifuged at 10000 RPM for 5 min and the supernatant liquid was discarded. The pellet was dried at room temperature for 10 min and finally resuspended with 50 µL of distilled water before storing it at -20°C.

PCR Amplification of 16S rRNA Genes

Extracted DNA was used as a template to amplify bacterial 16S rRNA genes, which have regions that provide sequence-based information for identification. PCR amplification was performed with universal bacterial primers i.e., Primer 5'-GCGGCTGCTGGCACRKAGT-3' (511) and Forward primer 5'-AGAGTTTGATCCTGGCTCAG -3' (27) 16S rRNA.

The PCR reaction was performed in 50 µL containing 2 µL of 10 pmoL of each appropriate primer and 25 µL of Master mix (GoTaq® Green Master Mix, 2X, Promega). About 2 µL of DNA was added to PCRs tube. Injection water was added to adjust the final volume to 50 µL. Thirty-five thermal cycles were performed for PCR amplification. The PCR protocol included an initial denaturation at 94°C for 5 min, 35 cycles at 94°C for 30 sec, 58°C for 30 sec and 70°C for 1.30 min followed by an extended amplification at 70°C for 10 min.

Gel Electrophoresis

Analysis of PCR products was performed on 2% agarose gel electrophoresis in 1X TAE (Tris Acetate EDTA). The PCR products were compared with molecular size markers 100 bp DNA Ladder (Promega, USA). After the products have moved way down to the depth of 75% of the gel and they were visualized under UV light using a gel documentation system (DATHAN Scientific Co., Ltd., Korea).

PCR products were sent to Macrogen Company laboratories in Korea to determine the sequence and identify the isolated strains. Data were analyzed by using Molecular Evolutionary Genetics Analysis (MEGA) program and compared with sequences in the NCBI database with an acceptable range of similarity ratio between 98-100%.

Antimicrobial Susceptibility Testing

The susceptibility test was performed for the identified pathogenic bacteria using VITEK 2 system (BioMerieux, US). According to the manufacturer's instructions (Bazzi *et al.*, 2017), AST-GP67, AST-N291, and AST-N292 cards were utilized. Moreover, the density of the bacterial suspension was adjusted at 0.5-0.63 McFarland in 3.0 mL of 0.45% sterile saline. Automatically, the cards were filled, sealed, and loaded into the Vitek 2 automated reader-incubator. The AST-GP67 card used for gram-positive bacteria contained Benzylpenicillin, Clindamycin, Erythromycin, Gentamicin, Levofloxacin, Linezolid, Moxifloxacin, Nitrofurantoin, Oxacillin, Quinupristin, Ciprofloxacin, Rifampicin, Tetracyclin, Tigecycline, Trimethoprim/sulfamethoxazole, and Vancomycin. The AST-N291 card used for Gram-negative (*Enterobacteriaceae*) contained Amikacin, Amoxicillin/clavulanate, Ampicillin, Cefuroxime, Cefalotin, Cefoxitin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, Meropenem, Nitrofurantoin, Piperacillin/tazobactam, Tigecycline, and Trimethoprim/sulfamethoxazole. The AST-N292 card used for Gram-negative (Glucose non-fermenter) contained Amikacin, Ticarcillin/Clavulanic, Ampicillin/Sulbactam, Cefepime, Colistin, Ceftazidime, Cefuroxime, Aztreonam, Etrapenem, Ciprofloxacin, Tobramycin, Gentamicin, Imipenem, Meropenem, Piperacillin/tazobactam, Levofloxacin, Minocycline, Tigecycline, and Trimethoprim/sulfamethoxazole. The results were recorded according to the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2019).

Results

Types and Distribution of Isolated Bacteria

In the present study, a total of 122 pure isolates out of 169 swab samples were obtained from three zones including inanimate environments (surfaces and equipment) and nurses' hands at the NICU of a hospital in Jeddah city, the Kingdom of Saudi Arabia.

On the timeline, there was not much difference in the detection rate of bacteria between the three tested times. However, the detection rate in the morning periodt AM, 8:00-9:00 AM) was significantly higher than that in the evening (13:00-14:00), which was 36, 34, and 4.61%, respectively (Table 2). The results also indicated that the number of bacteria found in non-isolation rooms was 97 isolates, representing 79.5%, whereas the other 20.5% of bacteria were found in isolation rooms numbering 25 isolates. Table 2 also shows that a high incidence of bacteria was found in zone 2 with 60 samples having positive growth out of 84, representing 49% of the total environmental samples collected. This was followed by 26% of samples collected from zone 1 with positive bacterial growth, constituting 31 out of 43 samples. The least number of bacteria was found in zone 3, where 31 out of 42 isolates had positive growth with 25%.

Bacterial counts were also determined in all selected equipment and surfaces in zone 2 and 3. The results of zone 2 indicated that the highest number of bacteria were found from the tables (16 isolates) with a detection rate of 27% followed by heart monitors and stethoscopes (12 isolates) representing 20% of the collected samples. On the other hand, the minimal number of bacteria in this zone was recorded from temperature monitors (4 isolates) (Fig. 1). As for the surfaces in zone 3, a significantly largest number of bacteria was found in windows (10 isolates) followed by nurses' hands with a detection rate of 32 and 29% respectively. However, all the samples collected from the sinks did not show any bacterial growth (Fig. 2).

The species of identified bacteria found on high-touch surfaces of equipment, environmental surfaces, and nurses' hands in NICU are shown in Table 3. The results showed that bacterial isolates belonged to eleven genera, namely: *Enterococcus*, *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *lelliottia*, and *Acinetobacter* (Table 3). However, the maximum number of isolated bacteria belonged to *Staphylococcus epidermidis* followed by *Bacillus cereus*.

Zone 1 representing the incubators was contaminated with most species of bacteria (15 species) representing *S. haemolyticus*, *E. faecalis*, *S. epidermidis*, *and S. hominis*

subsp. Novobiosepticus, S. caprae, S. hominis, M. aloeverae, B. cereus, K. pneumoniae, S. capitis, E. coli, B. haynesii, K. oxytoca, E. hormaechei subsp. Xiangfangensis and *B. altitudinis*. The most frequently occurring bacterial species in incubators were *S. epidermidis* and *S. haemolyticus*. The least frequently occurring species belonged to *Enterobacteriaceae* members including *E. coli*, *K. pneumoniae*, and *K.oxytoca* (Table 3, Fig. 3).

As for the equipment in zone 2, multi-bacterial contamination was found on the heart monitor's surface (*S. haemolyticus, S. epmonitor's, S. hominis subsp. Novobiosepticus, B. subtilis sub sp., inaquosorum, B. pumilus, B. stratosphericus* and *E. hormaechei sub sp., xiangfangensis)*. On the oxygen monitor surface, identified bacteria were *S. haemolyticus, S. epidermidis, P. luteola, M. aloeverae, B. pumilus*, and *E. hormaechei subsp. Xiangfangensis*. Temperature monitors showed *B. cereus, S. capitis, P. stutzeri*, and *S. epidermidis*, IV sets were identified with *S. epidermidis, S. haemolyticus*, and *A. baumannii*. The stethoscopes were found to be contaminated with *S. haemolyticus, S. epidermidis, and S. hominis subsp. novobiosepticus, B. haynesii, S. capitis, B. cereus, M. aloeverae*, and *E. hormaechei subsp. xiangfangensis*), tables were contaminated with S. *haemolyticus, S. caprae, B. cereus, M. aloeverae, S. epidermidis, M. luteus, B. xiamenensis, and B. pumilus* (Table 3, Fig. 3).

Zone 3 includes IV trolleys, weighing scales, windows, sinks, and nurses' hands. Windows were found to be contaminated with the highest number of bacterial species in Zone 3 with *S. haemolyticus, B. licheniformis, B. haynesii, and E. hormaechei subsp. steigerwaltii, L. amnigena, C. braaki*i, *P. stutzeri* and *B. cereus*; IV Trolleys with *M. aloeverae, B. subtilis, S. epidermidis, S. warneri and E. hormaechei subsp. xiangfangensis*); weighing scales for neonates with *S. caprae, B. subtilis, S. epidermidis, B. cereus*, and *E. hormaechei subsp. xiangfangensis*) and nurses' hands were contaminated with *S. haemolyticus, S. epidermidis, B. cereus*, and *B. xiamenensis*). However, sinks were found to be free of bacterial contamination (Table 3, Fig. 3).

Most bacterial isolates were cocci, positive for the gram-reaction and catalase test with 64, 86, and 98% sensitivity respectively. Among all tested sites, the high incidence of contaminated samples came from the high-touch surfaces such as incubators (26%), tables near the patients (13%), heart monitors and stethoscopes (10%), and windows (8%). The most frequently isolated bacteria belonged to the genus *Staphylococcus* with a rate of 54% followed by *Bacillus* (19.6%). At the species level, *S. epidermidis* (31), *S. haemolyticus* (21), and *B. cereus* (9) were the three most common strains. Also, our results indicated that some of the tested sites of the three zones and hands of NICU nurses shared similar types of isolated bacteria including *S.haemolyticus, S. epidermidis, B. cereus*, and *B. xiamenensis*.

Antimicrobial Resistance Patterns

The antibiotic resistance patterns of isolated bacteria were determined by using Vitek 2 system. The tests were performed with cards (AST-N291, AST-GP67, AST-N292).

Table (4) shows test results of antibiotic resistance obtained by using the AST-N291 card. The antimicrobial resistance patterns of *the E. coli* sample indicated that the sample was sensitive to almost all antibiotics in the card, which, however, resisted XL and AM. Additionally, samples of *Klebsiella* spp. (*K. pneumoniae* and *K. oxytoca*) isolated from incubators were resistant to AM only (100%). The sample of *L. amnigena* showed resistance to XM, CE, and CF.

As for the AST-GP67 card, all the seven strains of *Staphylococcus epidermidis* were strongly resistant to Benzylpenicillin and Oxacillin, whereas 71.5% of the strains were resistant to E, GM, LE, CI, and TE. The strains of *S. haemolyticus* were resistant to E (100%), whereas half of these strains showed resistance patterns to BE, CM and OX. Fifty percent of *S. capitis* isolates obtained from the environmental samples were resistant to CM, E, GM, and OX, while all the strains were resistant to BE (100%). The patterns of antibiotic resistance for the *S. caprae* sample showed 100% resistance to most antibiotics including BE, CM, E, GM, LE, MX, NIT, OX, CI, and RI. However, *S. warneri* isolate was resistant to just BE and OX. *M. aloeverae* sample was sensitive to all antibiotics except for NIT and OX. However, *the M. luteus* sample was resistant to BE, Cl, and E (Table 5).

Table 1: Selected surface types in each zone and number of swab samples that were taken from equipment per patient. However, the samples of zone 3 are shared in each room

Zone nu.	Categories	No. of swap samples	Zone nu.	Categories	No. of swap samples
Z1	Incubators	3-5		Iv Trolley	1
Z2	Oxygen monitor	1-0		Weighing Scale	1
	Heart monitor	2-0	Z 3	Window	1
	Temperature monitor	1-0		Sink	1
	IV	1-0		Nurse'Hands	2
	Stethoscope	1-0			
	Table	1-0			

Table 2: List identified bacteria from the Neonatal Intensive Care Unit (NICU) and their occurrence at different sites of the unit

	Time			Zones No.		Room types		
	8-9 am	9-10 am	13-14 pm	Z1	Z2	Z3	Non-isolation	Isolation
No. of tested samples	54	61	54	43	84	42	13.6	33.0
No. of bacterial isolates	42	44	36	31	60	31	97.0	25.0
Detection rate % *	34	36	30	26	49	25	79.5	20.5

*Was calculated by dividing the number of bacterial isolates by the number of total pure isolates (122)

Table 3: List identified bacteria from the Neonatal intensive care unit and the occurrence of isolated bacteria at different sites of the unit with their accession number

		Occurrence in positive sample N[b]												
Bacteria	Total10 N (%)[a]	[c]1	2	3	4	5	6	7	8	9	10	11	12	Accession numbers
S. haemolyticus	21(18)	4	3	2	0	2	1	5	0	0	1	0	3	CP045187.1 NR_113345 EU_659857.1 NR_113345.1
S. epidermidis	31(27)	8	2	3	2	5	3	3	2	1	0	0	2	NR_113957.1 NR_113957 MN_938189.1 FJ_613568.1
S. caprae	5(4.3)	3	0	0	0	0	0	1	0	1	0	0	0	MN_198058.1
S. hominis	1(0.87)	1	0	0	0	0	0	0	0	0	0	0	0	NR_036956.1
*S. hominis**	3(2.6)	1	0	1	0	0	1	0	0	0	0	0	0	NR_041323.1
S. capitis	4(3.5)	1	0	0	1	0	2	0	0	0	0	0	0	NR_113348.1 NR_117006
S. warneri	1(0.87)	0	0	0	0	0	0	0	1	0	0	0	0	NR_025922.1
B. cereus	9(8)	2	0	0	1	0	1	1	0	1	1	0	2	EU_621383.1 KJ_752763.1 NR_074540.1 JF_280125 NR_115714.1 NR_113266.1
B. pumilus	3(2.6)	0	1	1	0	0	0	1	0	0	0	0	0	EU_311209.1 NR_112637.1 KU_230026.1
B. subtilis	3(2.6)	0	0	0	0	0	0	0	2	1	0	0	0	NR_112116.2
B. subtilis **	1(0.87)	0	0	1	0	0	0	0	0	0	0	0	0	NR_104873.1
B. haynesii	3 (2.6)	1	0	0	0	0	1	0	0	0	1	0	0	NR_157609.1
B. xiamenensis	2(1.7)	0	0	0	0	0	0	1	0	0	0	0	1	NR_148244.1
B. stratospheric	1(0.87)	0	0	1	0	0	0	0	0	0	0	0	0	NR_042336.1
B. licheniformis	1(0.87)	0	0	0	0	0	0	0	0	0	1	0	0	NR_118996.1
B. altitudinis	1(0.87)	1	0	0	0	0	0	0	0	0	0	0	0	NR_042337.1
K. pneumoniae	1(0.87)	1	0	0	0	0	0	0	0	0	0	0	0	KX_898813.1
K. oxytoca	1(0.87)	1	0	0	0	0	0	0	0	0	0	0	0	KX_396002.1
E. coli	1(0.87)	1	0	0	0	0	0	0	0	0	0	0	0	KF_646677.1
P. stutzeri	2(1.7)	0	0	0	1	0	0	0	0	0	1	0	0	NR_103934.2 NR_103934.1
P. luteola	1(0.87)	0	1	0	0	0	0	0	0	0	0	0	0	KX_395963.1
M. aloeverae	6 (5)	2	1	0	0	0	1	1	1	0	0	0	0	NR_134088.1
M. luteus	1(0.87)	0	0	0	0	0	0	1	0	0	0	0	0	LK_020769.1
E. faecalis	2(1.7)	2	0	0	0	0	0	0	0	0	0	0	0	GU417284.1 NR_113901.1
*E. hormaechei****	7(6)	1	1	2	0	0	1	0	1	1	0	0	0	NR_126208.1
*E. hormaechei*****	1(0.87)	0	0	0	0	0	0	0	0	0	1	0	0	HQ_265401.1
C. Braakii	1(0.87)	0	0	0	0	0	0	0	0	0	1	0	0	NR_028687.1
L. amnigena	1(0.87)	0	0	0	0	0	0	0	0	0	1	0	0	NR_024642.1
A. baumannii	1(0.87)	0	0	0	0	1	0	0	0	0	0	0	0	NR_117677.1
Total	122(100%)													

[c]1: Incubators 2: Oxygen Monitor 3: Heart Monitor 4: Temperature Monitor 5: Iv 6: Stethoscopes 7: Tables 8: Iv Trolleys 9: Weighing scales 10: Windows 11: Sinks 12: Nurses' hands

N (%)[a] = Total number of isolated bacteria (percentage of the strains among the total isolates which was collected from the unit)

N[b] = Number of bacterial species found on each tested site in the unit

* *Staphylococcus hominis subsp. Novobiosepticus*

** *Bacillus subtilis subsp. Inaquosorum*

*** *Enterobacter hormaechei subsp. Xiangfangensis*

**** *Enterobacter hormaechei subsp. Steigerwaltii*

Table 4: Antimicrobial susceptibility with AST-N291 card for pathogenic Gram-negative isolates (Enterobacteriaceae)

Type of bacteria N [a]	Types of Antimicrobials (% R) [b]																
	[C]AK	XL	AM	PM	XM	CF	CE	TZ	TX	CI	GM	IP	MP	NIT	PTC	TI	TSU
E. coli (1)	0	100	100	0	000	000	000	0	0	0	0	0	0	0	0	0	0
K.pneumoniae (1)	0	000	100	0	000	000	000	0	0	0	0	0	0	0	0	0	0
K. oxytoca (1)	0	000	100	0	000	000	000	0	0	0	0	0	0	0	0	0	0
L.amnigena (1)	0	000	000	0	100	100	100	0	0	0	0	0	0	0	0	0	0
K. oxytoca (1)	0	000	100	0	000	000	000	0	0	0	0	0	0	0	0	0	0
L.amnigena (1)	0	000	000	0	100	100	100	0	0	0	0	0	0	0	0	0	0
K. oxytoca (1)	0	000	100	0	000	000	000	0	0	0	0	0	0	0	0	0	0
L.amnigena (1)	0	000	000	0	100	100	100	0	0	0	0	0	0	0	0	0	0
K. oxytoca (1)	0	000	100	0	000	000	000	0	0	0	0	0	0	0	0	0	0
L.amnigena (1)	0	000	000	0	100	100	100	0	0	0	0	0	0	0	0	0	0
K. oxytoca (1)	0	000	100	0	000	000	000	0	0	0	0	0	0	0	0	0	0
L.amnigena (1)	0	000	000	0	100	100	100	0	0	0	0	0	0	0	0	0	0
K. oxytoca (1)	0	000	100	0	000	000	000	0	0	0	0	0	0	0	0	0	0
L.amnigena (1)	0	000	000	0	100	100	100	0	0	0	0	0	0	0	0	0	0

[c]AK: Amikacin XL: Amoxicillin/clavulanate AM: Ampicillin PM: Cefepime XM: Cefuroxime CE: Cefalotin CF: Cefoxitin TZ: Ceftazidime TX: Ceftriaxone CI: Ciprofloxacin GM: Gentamicin IP: Imipenem MP: Meropenem TI: Tigecycline NIT: Nitrofurantoin PTC: Piperacillin/tazobactam TSU: Trimethoprim/sulfamethoxazole N [a] = Number of isolates (% R) [b] = Percentage of antimicrobial resistant isolates

Table 5: Antimicrobial Susceptibility AST-GP67 card for pathogenic gram-positive isolates

Type of bacteria N [a]	Types of Antimicrobials (% R) [b]																
	[c]BE	CM	LE	LZ	MX	TE	E	GM	NIT	OX	Q	CI	RI	TE	TI	TS	VA
S. epidermidis (7)	100	140	71.5	0	57	71.5	71.5	71.5	14	100	14	71.5	14	71.5	0	0	0
S. haemolyticus (4)	500	500	0.0	0	25	0.0	100.0	0.0	0	50	0	0.0	25	0.0	0	0	0
S. *capitis* (2)	100	500	0.0	0	0	0.0	50.0	50.0	0	50	0	0.0	0	0.0	0	0	0
S. hominis (2)	100	500	50.0	0	0	0.0	50.0	0.0	0	100	0	50.0	0	0.0	0	0	0
S. warneri (1)	100	000	0.0	0	0	0.0	0.0	0.0	0	100	0	0.0	0	0.0	0	0	0
S. caprae (1)	100	100	100.0	0	100	0.0	100.0	100.0	100	100	0	100.0	100	0.0	0	0	0
E. faecalis (2)	000	-00	0.0	0	-	50.0	100.0		0	-	100	-	0	50.0	100	-	2
M. aloeverae (1)	000	000	0.0	0	0	0.0	0.0	0.0	100	100	0	0.0	0	0.0	0	0	0

[c]BE: Benzylpenicillin CM: Clindamycin LE: Levofloxacin LZ: Linezolid MX: Moxifloxacin TE: Tetracycl E: Erythromycin GM: Gentamicin NIT: Nitrofurantoin OX: Oxacillin Q: Quinupristin CI: Ciprofloxacin RI: Rifampicin TE: Tetracyclin TI: Tigecycline TS: Trimethoprim/Sulfamethoxazole VA: Vancomycin N [a] = Number of isolates. (% R)[b] = Percentage of antimicrobial resistant isolates

Table 6: Antimicrobial susceptibility with AST-N292 card for pathogenic Gram-negative isolates (Glucose non-fermenter)

Type of bacteria N [a]	Types of Antimicrobials (% R) [b]																	
	[c] AK	TC	AS	PM	CO	TZ	XM	AZ	ET	CI	TOB	GM	IP	MP	PTC	LE	MC	TI	TS
P. stutzeri (2)	0	100	-	000	-	000	-	000	-	0	0	0	0	0	0	0	0	0	50
P. luteola (1)	0	000	-	000	-	100	-	100	-	0	0	0	0	0	0	0	0	0	0
E. hormaechei subsp. Xiangfangensis (1)	-	000	-	100	-	100	100	100	0	0	0	0	0	0	-	0	0	0	0
E.hormaechei subsp. Steigerwaltii (1)	-	000	-	100	-	100	100	100	0	0	0	0	0	0	-	0	0	0	0
A.Baumannii (1)	-	000	0	000	0	000	100	-	-	0	0	0	0	0	0	0	0	0	0

[c]AK: Amikacin TC: Ticarcillin/Clavulanic AS: Ampicillin/Sulbactam PM: Cefepime CO: Colistin TZ: Ceftazidime XM: Cefuroxime AZ: Aztreonam ET: Etrapenem CI: Ciprofloxacin TOB: Tobramycin GM: Gentamicin IP: Imipenem MP: Meropenem LE: Levofloxacin TS: Trimethoprim/Sulfamethoxazole PTC: Piperacillin/tazobactam MC: Minocycline TI: Tigecycline N [a] = Number of isolates. (% R) [b] = Percentage of antimicrobial resistant isolates

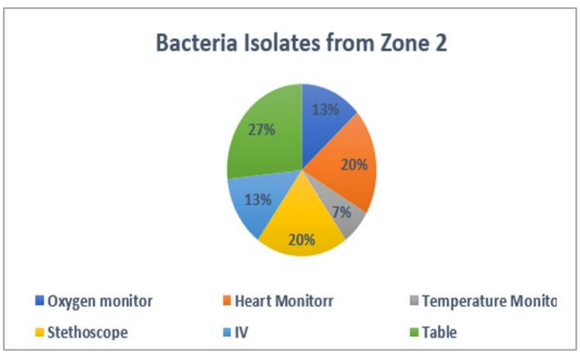

Fig. 1: Percentages of the bacteria isolated from different equipment in zone 2

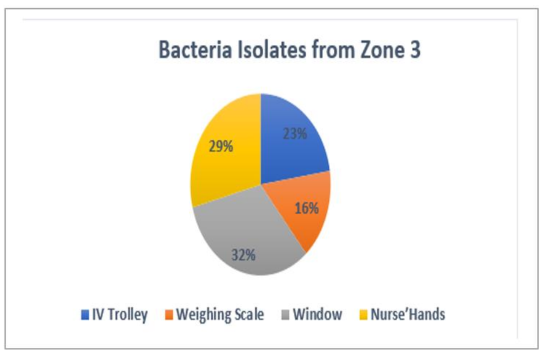

Fig. 2: Percentages of the bacteria isolated from different equipment and nurse' hands in zone 3

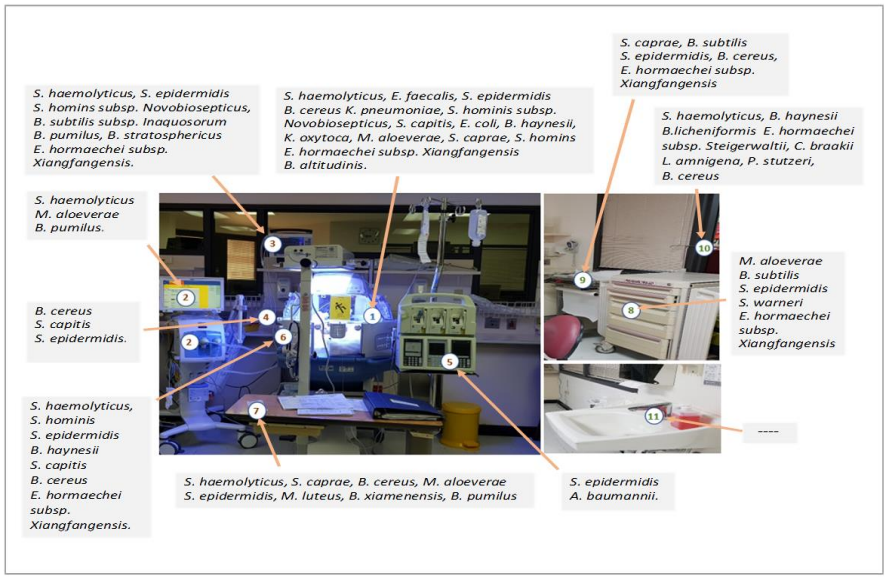

Fig. 3: Performance of distribution of the identified bacteria species in different sites in NICU, a hospital in Jeddah, Saudi Arabia. *1: Incubators 2: Oxygen Monitor 3: Heart Monitor 4: Temperature Monitor 5: Iv 6: Stethoscopes 7: Iv Trolleys 9: Weighing scales 10: Windows 11: Sinks 12: Nurses' hands

In the case of the AST-N292 card, the profile of antimicrobial resistance of two strains of *P. stutzeri*, isolated from temperature monitor and window, were resistant to TC (100%) and TS (50%) only. *P. luteola* sample showed resistance to TZ and AZ. Moreover, the results with *E. hormaechei subsp., xiangfangensis*, and *E. hormaechei subsp., steigerwaltii* samples showed the same patterns of antibiotics resistance, which was sensitive to all antibiotics except PM, TZ, XM, and AZ at the rate of 100%. *A. baumannii* isolate showed a particular resistance to only XM (Table 6).

Discussion

Surfaces and equipment in the NICU were found contaminated with a range of bacteria, which were presumed to be the sources of transmission leading to nosocomial infection in neonates (Kumar *et al.*, 2018). Previous studies have found that some of the bacteria are multidrug-resistant (Razzaghi *et al.*, 2016; Saleh *et al.*, 2018; Nazeri *et al*., 2019). During the present study, 169 samples collected from equipment, surfaces, and nurses' hands in the NICU were found infected with bacteria. The number of bacterial isolates obtained in the morning (08:00 10:00 h) was higher than those collected in the afternoon (13:00-14:00 h). Such differences are related to more people being in the NICU in the morning because it is usually considered a peak time for the visitors. The larger number of people may lead to an increase in the rate of contamination. This fact explains the results obtained from different types of rooms. We found that the number of bacterial isolates was much lower in isolation rooms than in non-isolation rooms. Entry into isolation rooms is usually allowed only for a limited number of people.

The isolated bacteria were predominately GP bacteria, with *Staphylococcus* being the most common (54% of isolates). This finding is consistent with Bokulich *et al.*, (2013), but it differs from a study by Okolo *et al.* (2016) who found *Klebsiella* was the most common bacteria.

At the species level, *Staphylococcus epidermidis* was isolated more frequently than other species. *S. epidermidis* is considered the most common bacteria to colonize healthy human epithelia (Otto, 2009). Consequently, a possible source in our study is the contact with individuals' skin, including that of health care workers, medical students, neonates, and parents. It may be considered a primary contributor which conforms with the observations made by Hewitt *et al.* (2013).

The present study estimated the distribution of bacterial contamination in the NICU on surfaces, equipment, and nurses' hands. Sites close to infants, including incubators, oxygen, heart and temperature monitors, IV, and stethoscopes, were contaminated with the following bacteria: *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *B. cereus*, *S. capitis*, *B. pumilus*, *E. hormaechei*, and *S. hominis subsp. novobiosepticus*. These results confirm previous reports from Conceição *et al.* (2012), Bokulich *et al.* (2013), and Hartz *et al*. (2015). The presence of infectious bacteria on different surfaces and equipment may be through direct contact with the hands of health care staff as they provide medical care for neonatal patients. We also found that incubators contained some *Enterobacteriaceae* including *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *E. hormaechei*. This finding

is also in agreement with a previous study by Aiello *et al.* (2003). These bacteria indicate fecal contamination in incubators possibly from the infants using them and are mostly associated with symptoms such as diarrhea.

Zone 3 represents the farthest area of the patient; our study indicated surfaces of drawers' handles, IV trolleys, and weight scales colonized by *M. aloevera*, *B. subtilis*, *S. epidermidis*, *S. warneri*, *S. caprae*, *B. cereus* and *E. hormaechei*, similar results were obtained by Hewittf *et al.* (2013). The presence of similarities to some of these bacteria with the members found spread on the devices around the patients in zone 2 increases the possibility of transmitting these bacteria by direct contact, whereas windows in NICU were not surveyed previously. Our results showed that windows were contaminated with *S. haemolyticus*, *B. licheniformis*, *B. haynesii*, E. hormaechei, *L. amnigena*, *C. braakii*, *P. stutzeri*, and *B. cereus*. Nonetheless, our findings differ from that of Hewitt *et al.* (2013) in not detecting any isolate from the sinks. The possible explanation is that the sinks work without a need to touch. Nurses' hands were considered a way of transmitting pathogens through direct contact as suggested by Khan *et al.* (2017). Hence, our results show agreement with Aiello *et al.* (2003) in finding *S. epidermidis* in the hands of NICU nurses.

Amongst the isolated bacteria, opportunistic pathogen species may cause nosocomial infections in humans e.g., *S. epidermidis* (Otto, 2009), *P. stutzeri* (Lalucat *et al.*, 2006), *S. hominis* (Jiang *et al.*, 2012) and *K. oxytoca* (Darby *et al.*, 2014). Particularly, *S. epidermidis* is considered one of the most common sources of infections on medical devices (Otto, 2009), whereas *S. capitis* and *S. hominis subsp. novobiosepticus* are problematic in NICUs and important nosocomial pathogens in neonates (Chaves *et al.*, 2005; Cameron *et al.*, 2015).

Many studies indicated that bacteria in NICU could develop resistance to many known multidrug resistance bacteria MDR (Chi *et al.*, 2012; Haque *et al.*, 2018). The antibiotic susceptibility pattern of pathogenic bacteria isolated was obtained by using the Vitek 2 system and the following results were observed. All isolated *Staphylococcus epidermidis* showed strong resistance of 100% to Benzylpenicillin and Oxacillin and a higher rate of resistance of 71.5% to Erythromycin, Gentamicin, Moxifloxacin, Ciprofloxacin, Tetracyclin. Similar results were obtained by Aiello *et al.* (2003) and Abd El Hafez *et al.* (2011). The susceptibility to Clindamycin, Linezolid, Nitrofurantoin, and Vancomycin was higher (100%) in El-Kersh *et al.* (2016) study. The differences in susceptibility patterns between this and other studies may be related to differences in the isolated strain and the use of infection control practices.

Our data of *Staphylococcus haemolyticus* isolates showed 50% resistance to Oxacillin. However, the susceptibility to this antibiotic was higher (90.3%) in the study made by Pereira *et al.* (2014). *Staphylococcus warneri* isolate result was like Aiello *et al.* (2003) study in its high resistance to Oxacillin and different in susceptibility results to the other tested antibiotics. This difference could be because of the increasing rate of inappropriate use of antibiotics. The *Enterococcus faecalis* isolates showed 100% susceptibility to Quinupristin, Tigecycline, and Vancomycin. These results agree with the Saudi study of El-Kersh *et al.* (2016). However, our results about Erythromycin were different from the findings of the isolates which were 50% resistant.

The antibiotic susceptibility of *Escherichia coli* isolates was resisted to Amoxicillin/clavulanate and Ampicillin. These results also showed agreement with studies such as Chmielarczyk *et al.* (2014) and Ballot *et al.* (2019). Moreover, *Acinetobacter baumannii* isolate was sensitive to all tested antibiotics and only resistant to Cefuroxime. However, these results were different from the study of Touati *et al.* (2009). *Klebsiella* spp. isolates showed full resistance to Ampicillin (Ballot *et al.*, 2019).

These results indicate that the isolated bacteria, especially multi-drug resistant bacteria, may possess threats to the health of newborn patients and increase the challenges to control HAIs. Therefore, high caution and prevention should be taken in the cleaning, disinfection, and routine screening of the NICU environment.

Conclusion

In conclusion, the present study identifies the hands of NICU nurses as well as environmental surfaces as a potential source of bacteria contaminations in the study area. Therefore, it is important to improve disinfection protocols and investigate the unit regularly for potential microbial contamination to decrease the likelihood of HAIs and ensure human safety.

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Author's Contributions

Roqaiah M. Al-Jabri: Experimental work, laboratory and data analyses, manuscript drafting.

Ahmed M. Al-Hejin, Rukaia Gashgari: Experimental design, development, data analyses, and final manuscript revision.

Mohamed Abu-Zeid: Contribution to the experimental works and data analysis.

Noor Mohammed Bataweel: Contribution to the experimental works and materials and equipment management.

Mohamed Morsi M. Ahmed: Experimental design and final manuscript revision.

Ethics

This article is originally from the author's works. The ethical research was approved by the Biomedical Ethics Research Unit of the hospital, Neonatal Intensive Care Unit Department (Reference No. 177-18). In addition, informed consent was obtained from all participating nurses in this study.

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