Role of environmental surfaces and hands of healthcare workers in perpetuating multi-drug-resistant pathogens in a neonatal intensive care unit

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Abstract
Neonates admitted to neonatal intensive care units are at a risk of developing healthcare-associated infections, leading to increased risk of mortality. This study aimed to identify organisms causing such late-onset infections in neonates and determine whether these isolates were genetically identical to those from the surrounding environmental surfaces and hands of healthcare workers (HCWs). A cross-sectional study was carried out over a period of 4 months in a university neonatal intensive care unit (NICU). Samples were collected from all neonates with symptoms of late-onset infections (n = 180). Fingerprint samples of 21 healthcare workers as well as 330 random environmental samples were also taken from the unit. Isolates from neonates, environment and fingerprints were subjected to protein electrophoresis followed by sequencing to detect genetic similarities. Almost half of neonatal samples were culture positive (91/180, 50.6%), out of which 72% of bacterial isolates (49/68) were multi-drug resistant. Klebsiella pneumoniae (32.6%) and Candida spp. (28.4%) were the commonest neonatal isolates. A cluster of two homologous Klebsiella pneumoniae strains was isolated from a neonate and an examining bed, while another homologous cluster was from a neonatal sample and a portal incubator. A third cluster was isolated from hands and three neonatal samples. This cluster (caused by Klebsiella pneumoniae strain NH54 chromosome) was found to perpetuate over the 4 months of the study. All three clusters were multi-drug-resistant Klebsiella pneumoniae. A homologous pair of each of Candida tropicalis and Candida glabrata was isolated from the blood of two neonates, and one neonatal and a crash cart sample, respectively. Overall, 8.8% (8/91) of neonatal samples were found to be homologous to other neonatal/environmental/hand isolates, denoting perpetuation of pathogens between neonates themselves and also other reservoirs of infections.

Conclusion: The hands of HCWs, crash carts and incubators are reservoirs of pathogens and can lead to nosocomial infections. Clusters of multi-drug-resistant Klebsiella pneumoniae and Candida spp. were the predominant neonatal pathogens in this NICU.

What is Known:
• The role of hands and the environment in transmission of infections to neonates is a subject of debate.
• Genetic sequencing provides solid evidence for detecting homologous strains.

What is New:
• K. pneumoniae was the most frequently isolated pathogen, and concomitant isolation was found in two cases from the neonatal surroundings (bed/incubator) and hands.
• Candida spp. with homology were also found in different neonates and environmental samples suggesting risk of transmission.

Keywords Neonates · Healthcare-associated infections · Protein electrophoresis · Sequencing · Dendogram

Abbreviations
C. glabrata · Candida glabrata
C. tropicalis · Candida tropicalis
CSF · Cerebrospinal fluid
HCAIs · Healthcare-associated infections
HCWs · Healthcare workers

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Neonates admitted to the neonatal intensive care units (NICUs) are at a risk of developing healthcare-associated infections (HCAIs). Reported rates of HCAIs per admission range from 6 to 50%, with 3–20-fold higher rates in developing versus developed countries [1]. HCAIs in the NICU cover the entire spectrum of organisms: bacterial, fungal, viral and rarely parasitic [2]. Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* spp. and *Acinetobacter* spp. have been established as predominant causes of serious neonatal infections in developing countries [3]. In contrast, the predominant organisms isolated from invasive neonatal infections in developed countries are Gram-positive cocci (coagulase-negative staphylococci and group B *Streptococcus*) [4]. HCAIs of neonates result from the interaction of several risk factors, such as prematurity, underlying diseases, immune system immaturity, exposure to broad-spectrum antibiotics, prolonged duration of NICU stay and the use of invasive medical devices (e.g. urinary catheters, central venous catheters, mechanical ventilators, umbilical catheters and parenteral alimentation systems) [5, 6]. Occasionally, infections are transmitted by members of the medical staff who harbour pathogenic bacteria on the skin [6, 7]. The NICU environmental surfaces harbour large numbers of bacteria and fungi associated with HCAIs in neonates. These genera contain many commensal species in healthy persons that do not necessarily represent pathogenic strains [1]. There is paucity of literature comparing the microbiological profile of organisms causing HCAIs with the environmental pathogens and those on the hands of medical staff. Pathogens causing neonatal sepsis can either transfer to, or originate from, environmental surfaces or the hands of medical staff. Genotyping of pathogens from environmental surfaces and the hands of medical staff allows tracing possible sources of infection that might lead to neonatal infections in a NICU. Therefore, this study aimed to identify the prevalent pathogens causing neonatal infections in a particular NICU, and also investigate whether the same pathogenic strains were perpetuating in NICU environmental surfaces and the hands of healthcare workers (HCWs).

### Data collection and sampling

Neonates with clinical signs of infection after 72 h of admission were included and were followed up until their hospital discharge/death. A written consent was taken from each neonate’s parent/guardian, and the research ethical approval was obtained from the HIPH. Consecutive inclusion of neonates was done until the required statistical sample size was reached. Samples including blood, cerebrospinal fluid (CSF), bronchoalveolar lavage (BAL) and urine were collected from 180 neonates who developed symptoms of...
infection after 72 h from admission (hypothermia or fever, poor reflexes, lethargy, respiratory distress, apnoea, bradycardia, convulsions, abdominal distension or bleeding).

The sites of environmental sampling were chosen based on previous literature describing these sites as ‘high-touch’ surfaces that are frequently contaminated by patients and doctors. In addition, these sites were recommended by the infection control consultant in the NICU, who stated that during their routine surveillance cultures from environmental surfaces, the chosen sites were the commonest to yield positive bacterial cultures (Supplement II). These sites are also reported in several studies to be reservoirs for pathogens. Sampling for solid samples was done using pre-moistened cotton swabs, which were then carried to the laboratory in brain heart infusion broth. Ten millilitres of each liquid sample was transported to the laboratory in a sterile container.

Fingerprints of dominant hands from the 21 HCWs (8 physicians and 13 nurses) working in the NICU were taken only once, after obtaining their verbal consent. Samples were taken weekly throughout the 4 months of the study. Weekly, cultures were done for the hands of 1–2 HCWs that were taking daily shifts this particular week.

**Laboratory processing**

All samples were cultured on blood and MacConkey’s agar plates and incubated aerobically at 37 °C. Isolates were identified by conventional microbiological tests [8]. Isolates that could not be identified by biochemical tests were subjected to matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF). All identified bacterial isolates of neonatal samples were subjected to antimicrobial susceptibility testing using the single-disc diffusion method described by the Clinical and Laboratory Standards Institute (CLSI) on Mueller–Hinton agar (Clinical Laboratory Standards Institute [CLSI], 2015). All isolates that were resistant at least one agent in three or more antimicrobial categories were defined as multi-drug-resistant (MDR) isolates, while those that were resistant to at least one agent in all but two or fewer antimicrobial categories were categorized as extensively drug resistant (XDR). All isolates from neonates, environment and fingerprints were stored in 15% glycerol broth at −80 °C for further use. In order to choose similar isolates for sequencing, protein electrophoresis for all neonatal, environmental and fingerprint isolates was done using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to select similar isolates based on their protein fragmentation pattern. Protein electrophoresis was done according to the methods of Laemmli (1970) by TriFast kit (Peqlab, VWR Company, USA) [9]. A gel documentation system (GelDoc-it, UVP, England) was applied for data analysis using the TotalLab analysis software, www.totallab.com (Ver.1.0.1) (Number, 2000) [10]. Isolates of each genus sharing similar protein bands were chosen and subjected to polymerase chain reaction (PCR) and sequencing. The total genome of each sample was extracted and purified through GeneJet Genomic DNA Purification Kit (Thermo Scientific, #K0721) according to the manufacturer’s protocol. PCR products (1350 bp of bacterial and 163 bp of fungal gene sequences obtained by PCR) were loaded on 1.5% (w/v) agarose gel, stained with ethidium bromide, separated by electrophoresis (75 V, 150 mA) and viewed on a UV plate. GeneJet PCR Purification Kit (Thermo Scientific, K0701) was used for DNA purification. ABI PRISM® 3100 Genetic Analyser was applied for sequencing PCR products using primers 27F and 1392R and performed by Macrogen Inc., Seaw, Korea. A gel documentation system (GelDoc-it, UVP, England) was applied for data analysis using the TotalLab analysis software, ww.totallab.com (Ver.1.0.1). Aligned sequences were analysed on the NCBI website (http://www.ncbi.nlm.nih.gov/website) using BLAST to confirm their identity. The genetic distances and multi-alignments were computed by the pairwise distance method using ClusteralW software analysis (www.ClusteralW.com). Nucleotide sequences were also compared with bacterial 91 isolate sequences available in GenBank. Data were fed to the computer and analysed using IBM SPSS software package version 20.0 (IBM Corp., Armonk, NY). Qualitative data were described using number and percent. The significance of the obtained results was judged at the 5% level.

**Results**

Clinical and descriptive data on neonates are shown in Supplement III. Positive neonatal cultures were found in 91/180 of neonatal samples (50.6%), and this was found to be significantly associated with ‘mortality’. The odds of death among neonates with positive cultures were 2.7-fold higher than those among neonates with negative ones (relative risk = 2.7, CI = 1.45–5.02, p = 0.002). The case fatality rate among neonates with positive and negative cultures was 49.4% and 28%, respectively. A total of 95 isolates were obtained from the 91 neonates with positive cultures. Gram-negative bacteria were the most prevalent microorganisms (47.4%) and comprised mainly *Klebsiella pneumoniae* (32.6%) and *Acinetobacter* (11.6%). *Candida* spp. was the second commonest group (28.4%), while Gram-positive bacteria constituted 24.2% of all isolates and comprised mainly methicillin-resistant *Staphylococcus aureus* (MRSA) (11.6% of all isolates) (Table 1). Forty-nine percent of the total neonatal isolates were MDR. All the isolates of *Acinetobacter* spp. were MDR, with 100% resistance to all of amoxicillin-clavulanic acid, ceftazidime, amikacin and imipenem while they were all sensitive to ofloxacin. As for *K. pneumoniae*, 71% of isolates showed an MDR pattern as follows: ceftazidime (77%),
ampicillin sulbactam and amoxicillin-clavulanic acid (74% for each), while 65% of isolates were resistant to cefotaxime and cefepime. Moreover, 19% and 9% of *K. pneumoniae* and *Acinetobacter* isolates, respectively, were resistant to colistin. Concerning gentamicin and cefoperazone, which comprise the routine prophylactic antimicrobial protocol for this NICU department, it was found that 32% of *K. pneumoniae*, 91% of *Acinetobacter* and 72% of MRSA isolates were resistant to gentamicin, while 9% of *Acinetobacter* were also resistant to cefoperazone (Supplement III).

Out of the total 50 HCWs in this NICU, unfortunately, only 21 agreed to participate in our study, and all of those agreed to give culture only once. The commonest isolated organism from the hands of both physicians and nurses was coagulase-negative *Staphylococcus aureus* (CoNS) (75% and 76.9%, respectively). MRSA and *Acinetobacter junii* were isolated from the hands of one physician each (12.5%), while the hand of one nurse harboured *K. pneumoniae* (7.7%) and 2 other nurses yielded *Bacillus* (15.3%) in their fingerprint cultures. There was no statistically significant difference in the distribution of bacteria from the hands of physicians and nurses.

### Environmental samples

A total of 330 environmental samples (315 surfaces and 15 liquid samples) were collected randomly from all the 9 rooms of the NICU (numbers and types of environmental samples are described in the Supplement file). Environmental samples were collected weekly throughout the 4 months of the study. These were collected with an average of 80–85 samples monthly. Total environmental samples (*n* = 330) were almost as double as the total neonatal samples collected (*n* = 180). The distribution of neonatal, hand print and environmental samples is clarified in Supplement III.

Out of the total 330 environmental samples; only 75 samples (22.7%) were culture positive. CoNS was the commonest species (12%) while the other pathogens were *Enterococcus faecalis*, *K. pneumoniae*, *Acinetobacter*, *Pseudomonas aeruginosa* and *Candida* spp. (1.8%, 1.5%, 1%, 1% and 0.6%, respectively). The most frequently pathogen-contaminated samples were from sinks, as 15/25 (60%) of the samples from sinks were contaminated by different microorganisms (*E. faecalis* 7.5%, *K. pneumoniae* 6.25%, *Acinetobacter* spp. 3.75% and *P. aeruginosa* 3.75%).

### Protein band phylogenetic tree

Protein electrophoresis showed that 90 samples had similar protein patterns. The protein phylogenetic tree (Fig. 1) shows different main clusters, which were then subdivided into several sub-clusters. The highly similar strains (which were more than 60% similar) coming from the same sub-cluster were chosen for sequencing (*n* = 22). These similarities were measured by the similarity index, at which closely related strains with high percentage of similarities were chosen. This figure shows, for example, that the 2 isolates located in lanes 20 and 14 were 78% similar. Also, lanes 62 and 63 had a 60% similarity index. Based on similarity patterns, 22 isolates (bacterial and *Candida* spp.) were chosen for genetic sequencing according to their locations in the protein phylogenetic tree. Figure 2 is a phylogenetic tree showing relationships among the 17 bacterial isolates based on their genetic similarities. All isolates were clustered into 3 main clusters. The first cluster grouped 3 *K. pneumoniae* isolates, while the second cluster grouped another two *K. pneumoniae* isolates, all four *Acinetobacter* isolates and two MRSA isolates. The third cluster grouped six *K. pneumoniae* isolates. *Candida* isolates clustered into 3 main clusters (Fig. 3).

### Phylogenetic tree after genetic sequencing (Table 2)

Table 2 shows 10 groups of the 22 pathogens that underwent sequencing. The first group showed two homologous strains of *Candida tropicalis* (accession number KY766068.1) which came from blood and CSF samples of two different neonates. Another two highly similar strains of *Candida glabrata* (XR_002648375) came from a crash cart and a CSF sample. All *Klebsiella* isolates in the groups from 3 to 7 were recovered from different sources and were all *K. pneumoniae*. Also groups

### Table 1 Distribution of 95 microbial isolates from 91 neonates in the NICU of Alexandria Children’s University Hospital, Egypt

| Organism                       | No | Percent |
|--------------------------------|----|---------|
| G+ve cocci                     | 23 | 24.2%   |
| Methicillin-resistant *S. aureus* | 11 | 11.6%   |
| *Enterococcus faecalis*        | 9  | 9.5%    |
| *Streptococcus pneumoniae*     | 3  | 3.1%    |
| G−ve bacilli                   | 45 | 47.4%   |
| *Klebsiella pneumoniae*        | 31 | 32.6%   |
| *Acinetobacter*[baumannii, junii] | 11 | 11.6%   |
| *Escherichia coli*             | 2  | 2.1%    |
| *Citrobacter koseri*           | 1  | 1.1%    |
| *Candida spp.*                 | 27 | 28.4%   |
| Total                          | 95 | 100%    |

Different microbial Kingdoms (bacteria/ fungi) are written in bold. Also, different bacterial morphologies(bacilli= rods/ cocci= spherical) and Gram stain results (gram-positive= violet colour on staining/ gram-negative= pink colour on staining) are expressed in bold.

Microbial genera and species are written in italic.
Fig. 1 Phylogenetic tree clusters after protein electrophoresis of all microbiological isolates recovered from the NICU of Alexandria Children’s University Hospital.
Fig. 2 Phylogenetic tree clusters after protein electrophoresis of all microbiological isolates recovered from the NICU of Alexandria Children’s University Hospital
were isolated in 28.4% of positive neonatal samples. In a ples and mainly comprised MRSA (11.6%). Candida spp. bacteria were prevalent in 24.2% of positive neonatal sam-

Regarding the microbial profile of the neonatal infections, which was undetectable by conventional culture methods. It might reflect a higher microbial load among neonates with viral infections), or might have had a lower microbial load with clinical signs of infection and yet a negative culture positive cultures and thus a higher mortality rate. Neonates might reflect a higher microbial load among neonates with association between positive neonatal cultures and mortality of death to 6.42 times compared to other causes [12]. The factor for neonatal deaths was sepsis, which raised the risk by Mohaddesi et al. reported that the most common risk (28.5% and 8.6%, respectively) [11]. Another study done nture results compared to those with negative culture results. This cluster was isolated over the 4 months of the study, suggesting its persistence and failure to eradicate it with the current environmental cleaning efforts done in the NICU. All neonatal strains of K. pneumoniae that were homologous to environmental strains or those from the hands of HCWs were MDR isolates as well.

Discussion

In the present study, the case fatality rate among neo-

3 and 4 showed that the same strain of K. pneumoniae (Klebsiella pneumoniae subsp. pneumoniae strain ATCC 43,816 KPPR1) was detected from different sources. There was also one common strain of K. pneumoniae (Klebsiella pneumoniae strain NH54 chromosome) isolated from groups 5, 6 and 7 despite originating from different sources. This cluster was isolated over the 4 months of the study, suggesting its persistence and failure to eradicate it with the current environmental cleaning efforts done in the NICU. All neonatal strains of K. pneumoniae that were homologous to environmental strains or those from the hands of HCWs were MDR isolates as well.

recent previous study in the same NICU, closely related results were reported, where the positive neonatal samples comprised mainly K. pneumoniae (29.2%), Candida albicans (20.8%), Acinetobacter baumannii and S. aureus (12.5% each) and P. aeruginosa (8.3%) [13]. This consistent predominance of K. pneumoniae, Acinetobacter spp. and Candida spp. in both studies emphasizes the importance of tracing the reservoirs of such pathogens in this particular NICU and the implementation of proper infection control measures. Similarly, Gadallah et al. reported Klebsiella spp. as the predominant pathogen isolated from HCAIs [14]. However, contrasting results were reported in other studies. In Egypt, a study by El-Din et al. reported that among their positive cultures of neonates in NICU, Gram-positive bacteria were predominant [15]. Also, a study in Nigeria reported a predominance of Gram-positive bacteria among their positive culture results [16]. Another study in China reported that S. aureus was the commonest microorganism isolated from their positive cases (37.5%) [17]. This difference may be due to variations in population characteristics and predisposing factors.

It was also noticed that 32% of K. pneumoniae were resistant to gentamicin and cefoperazone, which are routinely used in the protocol of this NICU department. Cefoperazone was also ineffective against 91% of Acinetobacter spp. isolates. The choice of these antibiotics should thus be reconsidered. Physicians, nurses and workers at the NICU can serve as reservoirs and vehicles for the spread of pathogens from different hospital wards to NICUs. HCWs’ hands are a source of transmission of HCA pathogens. Bacterial contamination is often acquired after direct contact with patients or body fluid secretions or indirectly after touching contaminated environmental surfaces.

In the current study, 21 HCWs (8 physicians and 13 nurses) had their fingerprints cultured. The following pathogenic microorganisms were isolated from the hands of 19% of HCWs: MRSA (12.5%), K. pneumoniae (7.7%) and A. junii (12.5%). Sixteen (76%) of HCWs had CoNS on their hands. Sepehri et al. reported lower rates, where nearly 40% of HCWs’ hands had bacterial isolation with Staphylococcus epidermidis, while contamination with HCAI pathogens was observed among 6% only of HCWs’ hands [18]. Contamination of the NICU environment plays an important role in the acquisition of HCA pathogens by both patients and HCWs. The rate of positive cultures of environmental surfaces in the current study was 23%. The most frequently pathogen-contaminated samples came from sinks, as 60% of the samples from sinks were contaminated by different microorganisms (E. faecalis 7.5%, K. pneumoniae 6.25%, Acinetobacter spp. 3.75% and P. aeruginosa 3.75%). In a study of Tarabay et al., sinks were related to a P. auroginosa infection outbreak in a NICU, where exposure occurred through bathing of neonates, or
healthcare staff using contaminated sinks for hand washing [19]. In the current study, 21.7% of crash cart samples were contaminated by *E. faecalis*, *K. pneumoniae* and *P. aeruginosa*. These carts are very important since they are used for neonatal drug preparations and are frequently touched by nurses. Incubators were contaminated (28.8%) with different microorganisms, which comprised mainly CoNS (84.75%), *E. faecalis* (7.5%), *K. pneumoniae* (6.25%), *Acinetobacter* spp. (3.75%) and MRSA (2.5%). In a study by Gray and Omar, bacteria were isolated from 30% of incubators in a NICU which was close to the current study [20]. The role of the environment in transmitting infections to neonates has been a subject of debate, with conflicting results between studies. In the present study, the same microorganisms were isolated from incubators, examining beds and crash carts, denoting the possible role of environment in transmitting these pathogens to/from neonates. Sequencing showed that 3 *Candida* isolates from different neonates were identified as *C. tropicalis*, two of which were homologous, suggesting transmission among neonates, either directly or indirectly. The second *Candida* cluster was composed of one environmental strain (isolated from a crash cart) and another homologous neonatal strain, denoting a possible source of infection including environmental surfaces. An isolate of *K. pneumoniae* from the CSF showed homology with another isolate from a portal incubator. Similarly, another *K. pneumoniae* strain was detected in both a neonatal blood sample and an examining bed. There was also another common strain of *K. pneumoniae* isolated from different sources (CSF and HCW fingerprinting). Similarly, Malik et al. reported that *K. pneumoniae* environmental strains were the source of nosocomial infections [21].

Table 2  Genetic sequencing for identification and comparison of 22 microbial isolates using NCBI nucleotide blast tool

| Group | Strain no | Strain sources | Date of isolation | Match of highest homology | Accession no |
|-------|-----------|----------------|-------------------|---------------------------|--------------|
| 1     | 17-14 N   | Neonate (blood) | 23 Jan            | *Candida tropicalis* strain MSY11 | KY766068.1  |
|       | 20-30 N   | Neonate (CSF)   |                   | *Candida tropicalis* strain VKSY2 | KU359154.1  |
|       | 14-4 N    | Neonate (blood) | 14 Jan            | *Candida tropicalis* strain MSY11 | KY766068.1  |
| 2     | 23-23E    | Crash cart      | 28 Jan            | *Candida glabrata*         | XR_002648375.1 |
|       | 19-27 N   | Neonate (CSF)   | 8 Feb             | *Candida glabrata*         | XR_002648375 |
| 3     | 44-16 N   | Neonate (CSF)   | 1 Feb             | *Klebsiella pneumoniae* subsp. *pneumoniae* strain ATCC 43,816 KPPR1 | CP009208.1 |
|       | 56-35E    | Portal incubator | 25 Feb            | *Klebsiella pneumoniae* subsp. *pneumoniae* strain ATCC 43,816 KPPR1 | CP009208.1 |
| 4     | 38-5 N    | Neonate (blood) | 15 Jan            | *Klebsiella pneumoniae* subsp. *pneumoniae* strain SCKP020079 chromosome | CP029384.1  |
| 5     | 36-2 N    | Neonate (blood) |                   | *Klebsiella pneumoniae* subsp. *pneumoniae* strain SCKP020046 chromosome | CP028783.1  |
|       | 55-27E    | Armstrong        | 4 Feb             | *Klebsiella pneumoniae* strain 616 chromosome | CP026495.1  |
|       | 52-7C     | Fingerprint of HCW | 4 Feb           | *Klebsiella pneumoniae* strain NH54 chromosome | CP024916.1  |
| 6     | 41-10 N   | Neonate (CSF)   | 17 Jan            | *Klebsiella pneumoniae* strain NH54 chromosome | CP024916.1  |
|       | 39-7 N    | Neonate (CSF)   |                   | *Klebsiella pneumoniae* strain CCUG 70,742 chromosome | CP15462615  |
| 7     | 62-65 N   | Neonate (CSF)   | 7 April           | *Klebsiella pneumoniae* strain NH54 chromosome | CP024916.1  |
|       | 63-70 N   | Neonate (blood) | 9 April           | *Klebsiella pneumoniae* strain NH54 chromosome | CP024916.1  |
| 8     | 2-6C      | Fingerprint of HCW | 4 Feb           | *Acinetobacter junii* strain WCHAJ59 chromosome | CP028800.1  |
|       | 5-39 N    | Neonate (BAL)   |                   | *Acinetobacter junii* strain F27 16S ribosomal RNA gene | MF681999.1  |
| 9     | 3-36 N    | Neonate (BAL)   |                   | *Acinetobacter baumannii* strain AFK_3 16S ribosomal RNA gene | MH357639.1  |
|       | 10-39E    | Crash cart      |                   | *Acinetobacter baumannii* Naval-17 clone 1,061,064,214,045 16S ribosomal RNA gene | JN669286.1  |
| 10    | 70-23 N   | Neonate (blood) |                   | *Staphylococcus aureus* strain CFSAN007896 chromosome | CP020467.1  |
|       | 72-12C    | Fingerprint of HCW |               | *Staphylococcus aureus* subsp. aureus 55/2053, complete genome | CP002388.1  |

Microbial strains with identical accession numbers (denoting homology with other strains) are written in bold

Microbial genera and species are written in italics

*N* neonatal sample, *E* environmental sample, *C* HCW fingerprinting
identify the source of A. baumannii outbreak after taking almost 300 environmental samples from different sites in the NICU [22].

In the present study, all homologous strains of K. pneumoniae (from all sources) were also multi-drug resistant. This denotes the perpetuation of such MDR pathogens between different reservoirs in the NICU, necessitating their tracing and eradication.

Despite the various spectra of pathogens isolated in the present study, it was noted that only Klebsiella spp. and Candida spp. showed homology between isolates from different sources. Their easy transmissibility between reservoirs in the NICU reflects high virulence and the need for more diligent infection control measures and antimicrobial stewardship.

Although the most highly contaminated environmental surface in this study were sinks (60% of them were contaminated), none of their strains was identical to any of those from neonates. On the other hand, environmental strains with shared homology to neonatal ones came from crash carts, examining beds and portal incubators. These sites therefore pose higher risk as surfaces of greater risk of infection transmission to neonates. We recommend that these sites should be addressed vigorously by the infection control team in this NICU with more frequent disinfection.

The results of this study were made available to the infection control team, who then adjusted their measures to be targeted towards environmental cleaning of the most contaminated surfaces in our study: incubators and crash carts. The level of environmental contamination (23%) was reported to the infection control specialists, who received a copy of our results for corrective actions. Physicians were also notified about the results of our study, and our first author (Marwa Elkady) gave HCWs (30 of them were available) two presentations (lectures) on the results of our study and stressed on the importance of proper hand hygiene. She also prepared wall-mounted posters in the entrance to the NICU as reminders of proper hand hygiene steps. The antibiotic resistance of neonatal pathogens to gentamicin, which is routinely used in the NICU, was also reported to the infection control team (91% of Acinetobacter spp., 32% of Klebsiella spp. 72% of and MRSA). A substitution with a more effective antibiotic was recommended.

**Limitations of the study**

The greatest limitation of our work was the inability to include all HCWs in hand cultures. Only 21/50 of HCWs agreed to participate, which reduced the chances for higher genotyping results. Also, from each participating HCW, cultures were performed only once and not repeated, reducing the likelihood of more homologous strains with other sources. Similarly, more environmental swab samples might have revealed more identical isolates shared between neonates, environment and HCWs. Differences in environmental surveillance cultures between studies might contribute to the discrepancy in the reported magnitude of the problem. The unequal number of environmental samples in our study should be reconsidered in future work, with more focus on the more highly contaminated surfaces. Another limitation of our work was that antifungal susceptibility testing was not done for Candida spp. isolates. Detailed data on neonatal causes of death and duration of survival was also a further limitation.

**Conclusion**

K. pneumoniae and Candida spp. were the commonest pathogens isolated from neonates in this NICU. The environment and hands of HCWs may play an important role in transmitting infection to neonates over an extended period of time, as evidenced by their genetic homology. Crash carts and incubators played a role in transmitting pathogens. The antimicrobial protocol of the NICU should continuously be adjusted to replace the less effective prophylactic antibiotics by more effective ones. The results of this study improved processes of infection control in this particular NICU.

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1007/s00431-021-04241-6.

**Authors’ contributions** Marwa Elkady was responsible for sample collection, microbiological processing and manuscript preparation. Wafaa Bakr and Eman Omran were responsible for manuscript preparation and data analysis. Hesham Ghazal was responsible for sample collection from neonates and facilitating work at the NICU with other HCWs.

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**Availability of data and material** All data will be provided by the researcher upon request.

**Code availability** N/A

**Declarations**

**Ethics approval** Approval from the Ethical Committee of the High Institute of Public Health, Alexandria University, was taken prior to the commencement of this study. The ethical consideration adheres to the Declaration of Helsinki.

**Conflict of interest** The authors declare no competing interests.
Consent to participate  Written informed consent from the parents/guardians of each neonate was taken prior to sample and data collection. Verbal consent was taken from each HCW prior to participation and fingertip sampling.

Consent for publication  Informed consent from the parents/guardians of each neonate was taken regarding the publication of this data.

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