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 four months in a University neonatal intensive care unit. 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 four homologous *Klebsiella pneumoniae* strains was isolated from two neonates as well as an examining bed and a portal incubator. Another cluster was isolated from hands and three neonatal samples. Both 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:* Hands of healthcare providers as well as surfaces are reservoirs of multi-drug resistant pathogens in the neonatal intensive care unit.

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
- Antibiotic resistance is a growing concern for all physicians

What Is New

- *Klebsiella pneumoniae* was the most commonly perpetuating pathogen among neonates, environment and hands.
- All homologous *Klebsiella* strains were multi-drug resistant
- Proven role of environment and hands in transmitting pathogens to neonates. Inter-neonatal transmission of pathogens also occurs.

Introduction

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 a 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 (eg, 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 surveillance isolates and those on hands of medical staff. Therefore, it is necessary to evaluate risk factors of HCAIs in NICU and correlate these with strains from the environment and hands of healthcare workers (HCWs).

Subjects And Methods

This cross-sectional study was conducted in ElShatby Paediatric Hospital- Alexandria University and the Microbiology Laboratory of the High Institute of Public Health (HIPH)- Alexandria University, over a period of four months (from the 1st of January – end of April 2019). The NICU comprised of 9 main rooms (5 of them were for admitted neonates, 2 of which were for critical and isolated cases and 3 were for stable cases). The total number of incubators was 80 (16 incubators/ room on average). It is the policy of the NICU department to routinely prescribe Gentamicin, Cefoperazone as well as Fluconazole to neonates upon admission for prophylaxis until the results of cultures appear. In case of microbiologically proven infections, physicians prescribe 3 types of antibiotics according to culture results. The sample size for neonates was calculated based on a previous study which reported that the prevalence of LOS was 5.9% among neonates in Egypt (2017) [5]. The minimal required sample size was found to be 82 neonates, with a power of 80%, precision 5%, and level of significance of 95% (α=0.05). The number of neonates in the study was increased to 180 neonates to compensate for expected negative culture results.

Data collection and sampling

Neonates with clinical signs of infection after 72hs 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 46 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 72hs from admission (hypothermia or fever, poor reflexes, lethargy, respiratory distress, apnea, bradycardia, convulsions, abdominal distension or bleeding). A total of 330 environmental samples (315 surfaces and 15 liquid samples) were collected randomly from all the 9 rooms of the NICU (**Supplement**). Pre-moistened cotton swabs of solid samples were carried to the laboratory in brain heart infusion broth, and 10 ml of liquid samples were transported to the laboratory in sterile containers. In addition, fingerprints of dominant hands from the 21 HCWs (8 physicians and 13 nurses) working in the NICU were taken, after obtaining their verbal consent.

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 single disc diffusion method described by Clinical and Laboratory Standards Institute (CLSI) on Mueller Hinton agar (Clinical Laboratory Standards Institute [CLSI], 2015). All isolates that were resistant to 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 68 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 fingerprints isolates was done using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to select similar isolates based on their protein frgmentation pattern. Protein electrophoresis was done according to the methodsof Lammli (1970) by TriFast kit (Peqlab, VWR company, USA) [9]. Gel documentation system (Geldoc-it, UVP, England), was applied for data analysis using Totallab analysis software, ww.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 manufacturer's protocol. PCR products (1350-bp of bacterial and 163bp 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 UV plate. Gene JET PCR Purification Kit (Thermo Scientific, K0701) was used for DNA purification. ABI PRISM® 3100 Genetic Analyzer was applied for sequencing PCR products using primers 27F and 1392R and performed by Macrogen In. Seaw, Korea. Gel documentation system (Geldoc-it, UVP, England), was applied for data analysis using Totallab analysis software, ww.totallab.com, (Ver.1.0.1). Aligned sequences were analyzed on NCBI website (http://www.ncbi.nlm.nih.gov/webcite) using BLAST to confirm their identity. The genetic distances and Multi-alignments were computed by Pairwise Distance method using ClusteralW software analysis (www.ClusteralW.com). Nucleotide sequences were also compared with bacterial 91 isolates sequences available in the GenBank. Data were fed to the computer and analysed using IBM

SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. Significance of the obtained results was judged at the 5% level.

Results

Positive neonatal cultures were found in 91/180 of neonatal samples (50.6%) and this was found to be significantly associated with “morality”. The odds of death among neonates with positive cultures was 2.7 folds higher than those 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 of *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 were comprised mainly of methicillin resistant *Staphylococcus aureus* (MRSA) (11.6% of all isolates) (table1). 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 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 Gentamycin and Cefoperazone, which are among the 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 Gentamycin, while 9% of *Acinetobacter* were also resistant to Cefoperazone (**Supplement**). 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 hands of physicians and nurses. Out of the total 330 environmental samples; only 75 samples (22.7%) were culture positive. CoNS was the commonest species (12%) while other pathogens were *Enterococcus faecalis*, *K. pneumonaie*, *Acinetobacter*, *P. 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 bands phylogenetic tree

Protein electrophoresis showed that 90 samples had similar protein patterns. The protein phylogenetic tree (figure 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. These similarities were measured by the genetic 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 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 with the samples 14,17 and 20 arising from the same sub-cluster (figure 3).

Phylogenetic tree after genetic sequencing (table 2)

Table 2 shows 10 groups of 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 3 and 4 showed that the same strain of *K.pneumoniae (Klebsiella pneumoniae* subsp. *pneumoniae* strain ATCC 43816 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. As regards *Acinetobacter* spp*., A. junii* was isolated from fingerprints of HCWs and a neonatal sample in group 8, while the same strain of *A. baumanni* was isolated from a neonate as well as an environmental sample (crash cart) in group 9.

All neonatal strains of *K. pneumoniae* that were homologous to environmental strains or those from hands of HCWs, were MDR isolates as well.

Discussion

In the present study, the Odds of death among neonates with positive cultures was 2.7 folds higher than those with a negative cultures (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. In a similar study, it was reported that the case fatality rate increased in neonates who had signs of infection with positive culture results compared to those with negative culture results (28.5% and 8.6%, respectively) [11]. Another study done by Mohaddesi et al, reported that the most common risk factor for neonatal deaths was sepsis, which raised the risk of death to 6.42 times compared to other causes [12]. The association between positive neonatal cultures and mortality might reflect a higher microbial load among neonates with positive cultures and thus a higher mortality rate. Neonates with clinical signs of infection and yet a negative culture might have had other non-culturable causes of sepsis (e.g: viral infections), or might have had a lower microbial load which was undetectable by conventional culture methods. Regarding the microbial profile of the neonatal infections, gram negative bacteria were the most prevalent microorganisms (47.4%) and were comprised mainly of *K.pneumoniae* (32.6%) and *Acinetobacter* spp.

(11.6%). Gram positive bacteria were prevalent in 24.2% of positive neonatal 182 samples, and were mainly comprised of MRSA (11.6%). *Candida* spp. were isolated in 28.4% of positive neonatal samples. In a recent previous study in the same NICU, closely related results were reported, where the positive neonatal samples comprised mainly of *K. pneumoniae* (29.2%), *C. albicans* (20.8%), *A. 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 Hassan 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 Gentamycin and Cefoperazone which are routinely used in the protocol of this NICU department were not effective against *K. pneumoniae*, *Acinetobacter* and MRSA isolates and their use should 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, 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 *S.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 carts 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 of CoNS (84.75%), *E. faecalis* (7.5%), *K. pneumoniae* (6.25%), *Acinetobacter* spp. (3.75%) and MRSA (2.5%). In a study by Gray et al., 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, weight balance and crash carts denoting possible role of environment in transmitting these pathogens to 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 *C. glabrata* 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 HCWs finger printing). Similarly, Malik et al reported that *K. pneumoniae* environmental strains were the source of nosocomial infections [21]. In contrast, a study by Gramatniece et al, reported that they failed to 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, and necessitating their tracing and eradication.

Despite the various spectrum 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), however, none of their strains were 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.

Limitations of the study

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.

Abbreviations

Candida glabrata (*C. glabrata*); *Candida tropicalis* (*C. tropicalis*); cerebrospinal fluid (CSF); healthcare-associated infections (HCAIs); healthcare workers (HCWs); *Klebsiella pneumoniae (K. pneumoniae)*; methicillin-resistant *Staphylococcus aureus* (MRSA); multi-drug resistant (MDR); neonatal intensive care unit (NICU)

Declarations

- **Funding**: This study was self-funded by the researchers with no external funding or grants.

- **Conflicts of interest/Competing interests**: The authors have no relevant financial or non-financial interests to disclose.

- **Availability of data and material**: All data will be provided by the researcher upon request
-**Code availability**: N/A
- **Authors' contributions**: Marwa Elkady was responsible for sample collection and microbiological processing as well as 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.

- **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

- **Consent to participate**: Written informed consents from the parents/guardians of each neonate were taken prior to sample and data collection. Verbal consent was taken from each HCW prior to participation and fingertip sampling.

- **Consent for publication**: Informed consents from the parents/guardians of each neonate were taken regarding publication of this data.

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Tables

Table (1): Distribution of 95 microbial isolates from 91 neonates in the NICU of Elshatby University Hospital, Alexandria

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

Table (2) Identification of 22 microbial isolates using NCBI nucleotide blast tool

Group	Strain no	Strain source	Match of highest homology	Accession no.
1	17-14N	Neonate (blood)	*Candida tropicalis* strain MSY11	KY766068.1
	20-30N	Neonate (CSF)	*Candida tropicalis* strain VKSY2	KU359154.1
	14-4N	Neonate (blood)	*Candida tropicalis* strain MSY11	KY766068.1
2	23-23E	Crash cart	*Candida glabrata*	XR_002648375.1
	19-27N	Neonate (CSF)	*Candida glabrata*	XR_002648375.
3	44-16N	Neonate (CSF)	*Klebsiella pneumoniae* subsp. pneumoniae strain ATCC 43816 KPPR1	CP009208.1
	56-35E	Portal incubator	*Klebsiella pneumoniae* subsp. pneumoniae strain ATCC 43816 KPPR1	CP009208.1
4	38-5N	Neonate (blood)	*Klebsiella pneumoniae* subsp. pneumoniae strain SCKP020079 chromosome	CP029384.1
	54-17E	Examining bed	*Klebsiella pneumoniae* subsp. pneumoniae strain SCKP020079 chromosome	CP029384.1
5	36-2N	Neonate (blood)	*Klebsiella pneumoniae* subsp. pneumoniae strain SCKP020046 chromosome	CP028783.1
	55-27E	Surface	*Klebsiella pneumoniae* strain 616 chromosome	CP026495.1
	52-7C	Fingerprint of HCW	*Klebsiella pneumoniae* strain NH54 chromosome	CP024916.1
6	41-10N	Neonate (CSF)	*Klebsiella pneumoniae* strain NH54 chromosome	CP024916.1
	39-7N	Neonate (CSF)	*Klebsiella pneumoniae* strain CCUG 70742 chromosome	CP15462615
7	62-65N	Neonate(CSF)	*Klebsiella pneumoniae* strain NH54 chromosome	CP024916.1
	63-70N	Neonate (blood)	*Klebsiella pneumoniae* strain NH54 chromosome	CP024916.1
8	2-6C	Fingerprint of HCW	*Acinetobacter junii* strain WCHAJ59 chromosome	CP028800.1
	5-39N	Neonate (BAL)	*Acinetobacter junii* strain F27 16S ribosomal RNA gene,	MF681999.1

9	3-36N	Neonate (BAL)	*Acinetobacter baumannii* strain AFK_3 16S ribosomal RNA gene	MH357639.1
	10-39E	Crash cart	*Acinetobacter baumannii* Naval-17 clone 1061064214045 16S ribosomal RNA gene	JN669286.1
10	70-23N	Neonate (blood)	*Staphylococcus aureus* strain CFSAN007896 chromosome	CP020467.1
	72-12C	Fingerprint of HCW	*Staphylococcus aureus* subsp. aureus 55/2053, complete genome	CP002388.1

N = neonatal sample E = environmental sample C=HCW

Figures

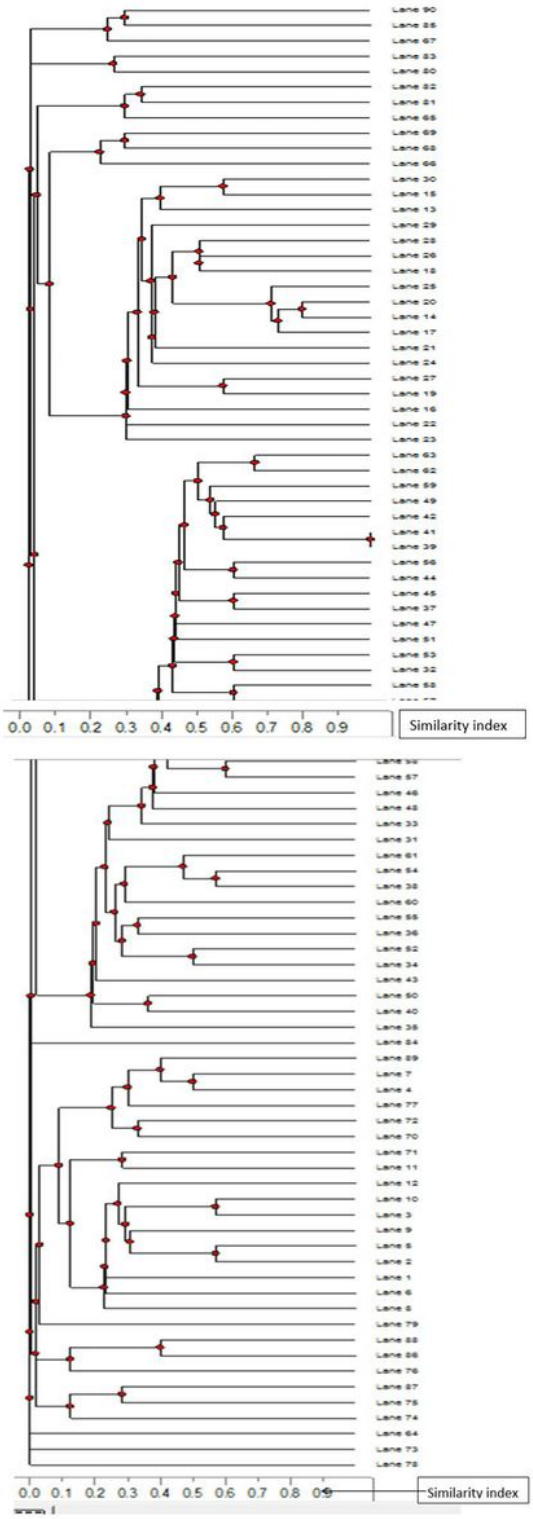

Figure 1

Phylogenetic tree clusters after protein electrophoresis of all microbiological isolates recovered from the NICU of Elshatby University Hospital

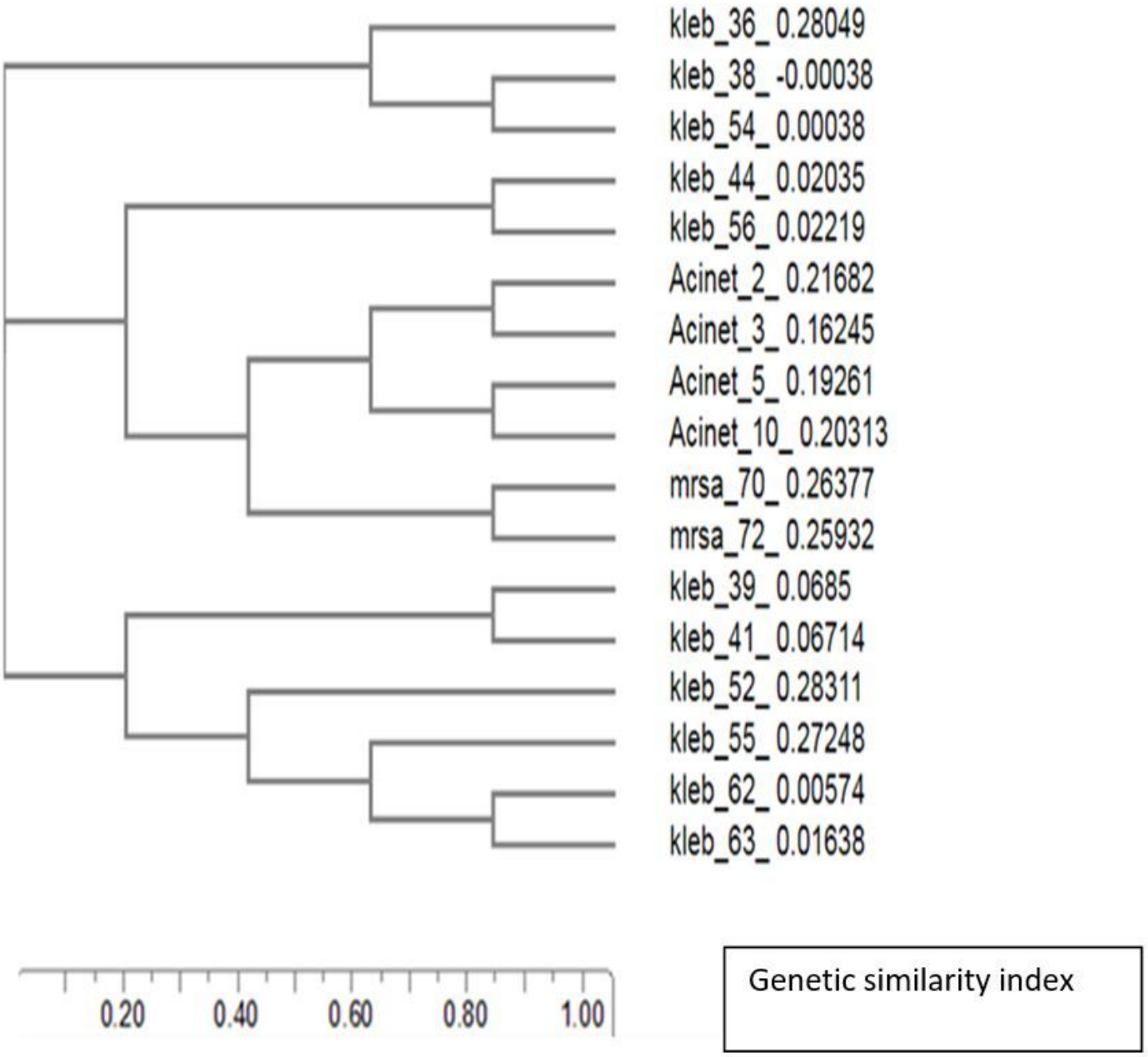

Figure 2

Phylogenetic tree of bacterial isolates based on sequencing data of 16S rRNA using ClusteralW software analysis

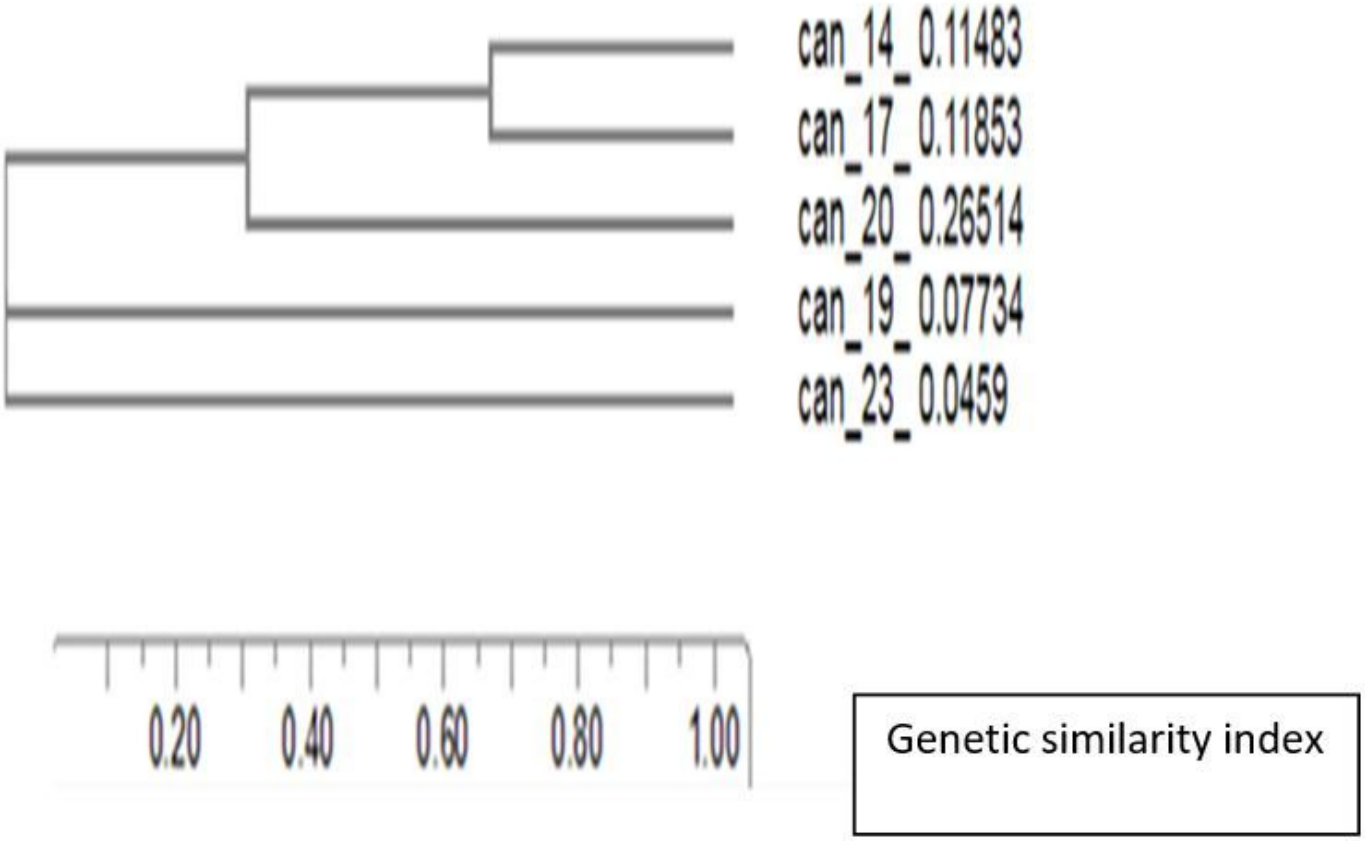

Figure 3

Phylogenetic tree of Candida isolates based on sequencing data using ClusteralW software analysis

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementIIINICU.doc