fortune Journals

Research Article

FORTUNE JOURNAL OF HEALTH SCIENCES

ISSN: 2644-2906

Surveillance of Nosocomial Infections in Neonatal Intensive Care Units at A Tertiary Care Hospital in Bangladesh

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Abstract

Background: Neonatal Intensive Care Units (NICUs) play a vital role in the treatment of life-threatening conditions in newborns. The presence of bacterial contamination on instruments and surfaces within NICUs is a significant factor contributing to the occurrence of hospital-acquired infections.

Objective: This study aims to evaluate the bacterial contamination on instruments and surfaces that are frequently handled by healthcare staff and are often in contact with neonates in the NICU.

Methods: The study was carried out in the NICU of a tertiary care hospital in Bangladesh. A total of 185 samples were collected from various surfaces and instruments utilized in the NICU. The study involved the isolation, identification, and antibiotic susceptibility testing of the bacterial isolates. Blood culture isolates from NICU patients were compared with environmental isolates.

Results: Bacterial growth was identified in 121 of the 185 samples. The potential pathogens detected included *E. coli* (n=28), *Klebsiella pneumoniae* (n=27), and *Staphylococcus aureus* (n=18). Most *E. coli* and *Klebsiella pneumoniae* were found on incubators and mothers' beds, while the majority of *Staphylococcus aureus* isolates were obtained from air conditioning units. The majority of isolates showed susceptibility to imipenem, amikacin, and gentamicin.

Conclusion: A notable degree of bacterial contamination was found on objects and instruments in the NICU. *E. coli, Klebsiella pneumoniae*, and *Staphylococcus aureus* present a significant risk for nosocomial infections. The blood culture findings from neonates suggest a potential risk of hospital-acquired infections stemming from contaminated sources. For empirical treatment of suspected nosocomial infections in the NICU, imipenem, amikacin, and gentamicin may be considered effective options.

Keywords: Nosocomial infections, Bacterial contamination, Antibiotic resistance, NICU

Introduction

One of the most crucial elements of the healthcare system is the intensive care units (ICUs) which deliver vital care to patients suffering from life-threatening illnesses. Bacterial contamination within the unit significantly contributes to the elevated rates of infections associated with the ICU, leading to a rise in nosocomial infections, accounting for roughly 40% of ICU admissions [1]. Neonatal intensive care units (NICUs) specifically

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Citation: Sanjida Khondakar Setu, Abu Naser Ibne Sattar, Shaheda Anwar, Towfique Hasan Firoz, Sanjar Taufiq. Surveillance of Nosocomial Infections in Neonatal Intensive Care Units at A Tertiary Care Hospital in Bangladesh. Fortune Journal of Health Sciences, 8 (2025): 115-122.

Received: February 09, 2025
Accepted: February 14, 2025
Published: February 20, 2025

Setu SK., Fortune J Health Sci 2025
DOI:10.26502/fjhs.261

cater to low birth weight infants, who are particularly susceptible to infections due to their compromised immune systems [2,3]. Annually, over one million neonatal deaths are reported globally, with nosocomial infections accounting for 30–40% of these cases, especially in low-resource settings [4.5]. A major contributor to these infections is the bacterial contamination of equipment and surfaces within the ICU [6]. Contamination can arise from several sources, including cross-transmission, the spread of pathogens, and high occupancy levels. Additionally, therapeutic devices like stethoscopes and the clothing of healthcare personnel play a role in this problem [7,8]. Contaminated items and clinical samples from both healthcare workers and patients can exacerbate the risk of infection [9,10,11]. A major factor contributing to the transmission of pathogens is the failure of healthcare professionals to adhere to fundamental handwashing practices. This neglect heightens the likelihood of cross-contamination through interactions with patients or contaminated surfaces [12,13]. During medical procedures, bacteria are consistently released and can persist on human skin. Pathogens can also be found in the immediate environment of infected healthcare workers and patients [14,15,16]. Various factors, including the type of microorganism, the source of contamination, surface contamination, humidity, and the size of the inoculum, influence the spread of infections [17]. A diverse array of bacterial pathogens has been detected in ICU environments.

Clinically relevant potential pathogens encompass Staphylococcus aureus (including methicillin-resistant Staphylococcus aureus, or MRSA), Klebsiella species, Escherichia coli, Pseudomonas species, Acinetobacter species, and Enterococcus species [18].

There is an increasing incidence of antimicrobial resistance and the rise of multidrug-resistant (MDR) pathogens, such as MRSA, vancomycin-resistant Staphylococcus aureus (VRSA), extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae, and Acinetobacter species within the NICU, contributing to heightened morbidity and mortality rates. The distribution of organisms identified in the NICU setting evolves over time and can differ between hospitals. This study focused on assessing the bacterial contamination of frequently used items and instruments in the NICU. Limited research has been conducted on the impact of bacteriological contamination on NICU surfaces. Hospitals across Bangladesh rarely monitor contamination levels in NICU and there is a lack of data on such monitoring. This study aimed to evaluate the degree of bacterial contamination on instruments and objects that are often handled by healthcare personnel or that come into contact with neonates. Performing bacteriological analyses of environmental samples from the NICU may yield critical insights into the level of bacterial contamination and the antibiotic resistance profiles of the identified isolates.

Materials and Methods

Study place

The study was carried out in the Neonatal Intensive Care Unit (NICU) and the Newborn Special Care Unit at BSMMU in Dhaka, Bangladesh. Prior to initiating sampling and analysis, ethical approval was obtained from the committee of the Department of Microbiology & Immunology at BSMMU.

Sample Collection and Processing: For sample collection, the swabbing technique outlined by Cheesbrough was employed [19]. Sterile swab sticks, moistened with sterile water, were utilized to thoroughly swab the surfaces of various fomites. To ensure comprehensive coverage, the swab was rolled back and forth before being securely capped and labeled. The collected samples were subsequently sent to the laboratory for analysis. The swab samples were inoculated onto suitable media, including Blood Agar and MacConkey Agar, and incubated for 24 hours at 37°C. A total of 185 samples were gathered from surfaces such as air conditioners, sucker machines, incubators, stethoscopes, phototherapy beds, mothers' beds, respiratory support door handles, weighing machines, doctors' mobile phones, bedside lockers, BP machines, hands of healthcare workers, switchboards, sterilizer swabs from gallipots, water supply, CPAP machines, kidney trays, medicine trolleys, pulse oximeters, sinks, and baby cots. Most of these surfaces are in direct contact with healthcare professionals or neonates.

Organism isolation and antimicrobial susceptibility

Samples were promptly inoculated into peptone water and allowed to incubate overnight. Subsequent subcultures were conducted on MacConkey agar and Blood agar plates. These plates were incubated aerobically at a temperature of 37 °C for a duration of 24 to 48 hours. Organisms were identified based on morphology, culture characteristics, and biochemical reactions according to standard microbiological techniques. All the isolates were tested for antimicrobial susceptibility on Muller Hinton Agar (HI Media, India) by Kirby Bauer disc diffusion method, according to the Clinical Laboratory Standard Institute (CLSI) guidelines [20,21]. The following antibiotics were used for gram-negative bacteria: amoxicillin (10mg), amoxicillin-clavulanic acid, ciprofloxacin (5mg), ceftriaxone (30mg), cefoxitin(30mg), trimethoprim-sulphamethoxazole (1.25/23.75mg), amikacin (30mg), imipenem(10mg), azithromycin(30mg). For gram-positive bacteria, the following antibiotics were used: amoxicillin(10mg), ciprofloxacin (5µg), erythromycin (15mg), trimethoprim-sulphamethoxazole (1.25/23.75mg), cefoxitin(30µg), vancomycin(30mg) and clindamycin(2mg). All the antibiotic disks were commercially purchased from Biomaxima, Poland. *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were included as quality control

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strains of antimicrobial susceptibility testing. Colistin susceptibility was conducted by the Broth microdilution method according to the CLSI [20]. All the antibiotic disks were commercially purchased from Biomaxima, Poland. *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were included as quality control strains of antimicrobial susceptibility testing. Bacterial isolates that exhibited resistance to at least one agent across three or more antimicrobial categories were classified as multidrug-resistant (MDR). Cefoxitin (30µg) was used as a surrogate marker of *mecA* resistance in *Staphylococcus aureus*. *S aureus*, which showed a zone of inhibition ≤21 mm with cefoxitin on Mueller Hinton Agar after overnight incubation at 37ºC, was considered as MRSA [22]. Extended spectrum beta-lactamase (ESBL) detection among the Enterobacteriaceae strains were performed by the double disc synergy test [22]. Approximately 1–2 ml of blood is collected from each neonate. Blood culture samples are then incubated using the BacTAlert 3D automated blood culture system (Biomerieux, India®). One milliliter of blood is inoculated into BacT/ALERT PF Plus culture bottles (yellow color-coded) designed for pediatric use, ensuring all necessary precautions are taken, and the bottles are shaken thoroughly. After scanning the barcode on each bottle, they are placed into the instrument for incubation. The BacT/ALERT Microbial Detection System determines whether the culture bottles are positive or negative. A blood culture is deemed negative only after a full 7 days of incubation. The identification of the organism is confirmed using the automated Vitek 2 compact 60 system (BioMerieux India®) with Vitek 2 cards. The Vitek-2 ID and AST cards are recorded and inserted into the Vitek-2 Compact system. Antibacterial sensitivity testing is conducted, and the minimum inhibitory concentrations (MICs) are categorized into three clinical groups (susceptible, intermediate, and resistant) based on the interpretative criteria outlined in the CLSI guidelines [22].

Data analysis

Data were collected manually and entered and analyzed by using SPSS version 27 software. The statistical analysis used in the study was descriptive and did categorical data analysis. Frequency and percentage were examined for categorical independent variables. Results were presented through graphs and tables. P-value < 0.05 was considered statistically significant.

Ethical statement

Informed written consent was obtained from the parents or the legal guardians of the neonates to use their blood culture data and culture susceptibility reports.

Results and Observation

Out of 185 samples collected from various locations, bacterial growth was detected in 121 specimens, while 64 samples exhibited no bacterial growth. A total of 136 bacterial isolates were obtained from the 121 samples. Information regarding the sampling sites and bacterial isolates were shownd in Table 1. The most significant pathogens identified included *E. coli* (28 out of 136), *Klebsiella pneumoniae* (27 out of 136), and both *Pseudomonas aeruginosa* and *Staphylococcus aureus* (18 out of 136). The majority of *E. coli* and *Klebsiella pneumoniae* isolates were located in incubators, sucker machines, and mothers' beds, whereas most *Staphylococcus aureus* isolates were cultured from air conditioning units. Among the *Staphylococcus aureus* isolates, 27.8% (5 out of 18) were classified as MRSA (Methicillin-resistant *Staphylococcus aureus*), with the remainder being MSSA (Methicillin-sensitive *Staphylococcus aureus*). No Vancomycin-resistant *Staphylococcus aureus* (VRSA) was found. Additional bacterial isolates included *Acinetobacter baumannii*, coagulase-negative Staphylococci, *Enterococcus faecalis*, *Burkholderia cepacia*, Micrococcus species, diphtheroids, and aerobic spore bearers. The antibiotic resistance profiles of the bacterial isolates in Table 2. The majority of bacterial isolates demonstrated susceptibility to imipenem, gentamicin, and amikacin. A significant level of multidrug resistance was noted in *E. coli,* with 39% (11 out of 28) exhibiting resistance, and in *Klebsiella pneumoniae*, where 55.5% (15 out of 27) were resistant. Among the 18 *Staphylococcus aureus* isolates, 33.3% (6 out of 18) were classified as multidrug-resistant (MDR), as well as 55.5% (10 out of 18) of *Pseudomonas aeruginosa* isolates. The prevalence of MRSA among the isolates was 27.8% (5 out of 18). In the Gram-negative category, a substantial proportion of *E. coli* (71.4%, or 20 out of 28) and *Klebsiella pneumoniae* (59.25%, or 16 out of 27) were identified as producers of Extended-Spectrum Beta-Lactamase (ESBL), which was significantly higher compared to non-fermentative Gram-negative bacilli (p-value < 0.01). The specifics of ESBL production among the Gram-negative isolates are presented in Table 3. The predominant isolates detected in blood cultures were *Staphylococcus aureus* (n = 12) and *Klebsiella pneumoniae* (n = 6). Notably, seven *Staphylococcus aureus* isolates and three *Klebsiella pneumoniae* isolates from blood cultures exhibited antibiograms that were consistent with those of the environmental isolates.

Discussion

Bacterial contamination in the Intensive Care Unit (ICU) plays a crucial role in the rising rates of nosocomial infections, adversely impacting both patients and healthcare personnel [10,23]. In low-resource countries, mortality rates in Neonatal Intensive Care Units (NICUs) range from 11.9% to 14.7%, significantly higher than the 6.1% to 7.1% observed in high-resource settings [24,25]. The hospital environment, including the NICU, harbors various microbial agents. The prevalence of bacterial contamination

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DOI:10.26502/fjhs.261

Table 1: Bacteria isolated from NICU environmental surfaces

	E. coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Staphylococcus aureus*	CoNS	*Enterococcus faecalis*	*Berkholderia cepacia*
Air conditioner	3	4	2	3	1	4	-	-
Sucker machine	7	4	8	1	-	-	1	-
Incubator	7	1	2	-	-	5	1	-
Stethoscope	-	-	-	-	2	2	-	-
Phototherapy bed	2	2	-	-	-	-	-	-
Mothers' bed	4	4	-	2	2	-	-	-
Respiratory support	1	4	-	-	-	-	-	-
Door handles	-	-	-	-	4	2	-	-
Weighing machine	2	2	-	-	-	2	1	-
Doctors' mobile	-	-	-	2	-	3	2	-
Bedside locker	-	-	-	1	-	1	-	-
BP Machine	-	-	-	1	-	4	-	-
Hand of HCWs	-	1	-	1	6	-	-	1
Switch bord	-	-	-	-	2	1	-	-
Sterilizer	-	-	-	-	1	-	-	-
Swab from gallipot	-	1	-	2	-	-	-	-
Water supply	2	-	-	-	-	-	-	-
Cpap machine	-	1	1	-	-	-	-	-
Kidney tray	-	-	1	-	-	-	-	-
Medicine trally	-	-	-	-	-	-	-	--
Pulse oximeter	-	-	-	1	-	-	-	-
Sink	-	2	2	-	-	-	-	1
Baby cot	-	1	-	-	-	-	-	-
Total	28	27	18	14	18	24	5	2

Table2: Antibiotic resistance pattern of bacterial isolates

Antibiotic	*E. coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Berkholderia cepacia*
Ampicillin	28(100)	27(100)	-	-	-	-
Ciprofloxacin	9(32.14)	9(33.33)	8(44.44)	6(42.85)	6(33.33)	2(100)
Gentamicin	11(31.28)	13(48.14)	4(22.22)	3(21.42)	1(5.55)	0
Ceftazidime	23(82.14)	23(85.18)	8(44.44)	6(42.85)	-	1(50)
Ceftriaxone	22(78.57)	22(81.48)	-	-	-	-
Co-trimoxazole	10(35.71)	8(29.62)	-	-	5(27.77)	0
Amikacin	0	0	0	0	0	0
Imipenem	0	0	0	0	-	0
Cefoxitin	-	-	-	-	6(33.33)	-
Erythromycin	-	-	-	-	16(88.88)	-
Clindamycin	-	-	-	-	5(27.77)	-

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Table 3: ESBL producing status of the Gram-negative organism N=87

Organisms	ESBL producers n (%)	ESBL nonproducers n(%)	Total
E.coli	20(71.42)	8(28.58)	28
Klebsiella pneumoniae	16(59.25)	11(40.75)	27
Pseudomonas aeruginosa	04(22.22)	14(77.78)	18
Acinetobacter baumannii	04(28.57)	10(71.43)	14
Total	44(50.57)	43(49.43)	87

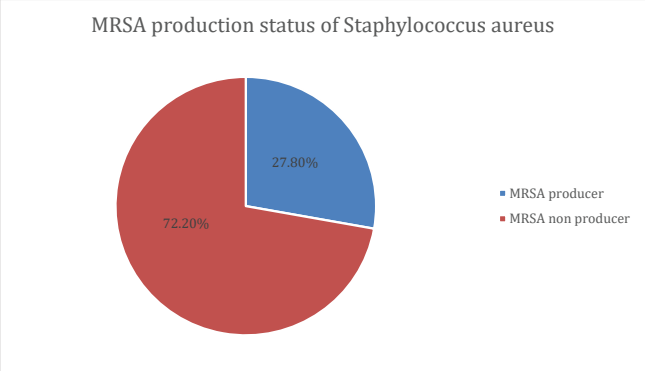

Figure 1: MRSA production status of *Staphylococcus aureus*

in the NICU is a key contributor to the surge in nosocomial infections. Notably, high levels of bacterial contamination were detected on frequently handled objects and instruments within the NICU. The overall contamination rate reached 73.51% (136 out of 185 samples), surpassing figures reported in other studies, which range from 59.2% to 67.8% [26,27]. Similar studies have reported contamination rates ranging from 59.2 to 67.8% J[18, 27]. Several factors may account for this elevated contamination rate, such as the admission of neonates with diverse clinical conditions, overcrowding, fecal contamination, unrestricted visitor access, understaffing, and inadequate adherence to infection control protocols. Extended stays in the NICU result in increased visits from mothers and healthcare workers, leading to heightened human activity that promotes the transfer of bacterial flora. Bacterial cultures indicated a diverse array of organisms, including opportunistic microbes and potential pathogens. Commonly identified potential pathogens included *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Various members of the Enterobacteriaceae family are capable of colonizing the NICU and contributing to neonatal infections [28]. Neonatal infections in resource-limited countries are most frequently associated with pathogens such as *Klebsiella species, E. coli, Pseudomonas spp, and Staphylococcus aureus* [29]. In our research, we discovered various potential pathogens present on different surfaces, including *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* The highest concentrations of *E. coli* and *Klebsiella pneumoniae* were found on the sucker machine, incubator, and mother's bed. *E. coli* is often linked to neonatal sepsis and is a prevalent cause of acute pyogenic meningitis in newborns. The presence of *E. coli* and *Klebsiella pneumoniae* on NICU surfaces heightens the risk of systemic infections, such as neonatal septicemia, pneumonia, and meningitis, especially in premature infants. This contamination is likely related to neonatal fecal matter. Among the *E. coli* and *Klebsiella pneumoniae* strains, 71.4% (20 out of 28) and 59.25% (16 out of 27) were found to be ESBL producers, respectively. The significant proportion of ESBL-producing bacteria restricts treatment options and may result in therapeutic failures.

The highest number of *Pseudomonas aeruginosa* isolates was obtained from the sucker machine, followed by the air conditioner, incubator, and sink. Additionally, *Staphylococcus aureus*, a well-known nosocomial pathogen, was isolated predominantly from the surfaces of healthcare workers (HCWs), with the highest counts also found on door handles and mother's beds. The hands of HCWs and visitors are common reservoirs for *Staphylococcus aureus* and Methicillin-resistant Staphylococcus aureus (MRSA) in healthcare settings. Another research has indicated that HCWs' hands contribute to 20 to 40% of infections due to cross-transmission in healthcare environments [30,31]. The presence of *Staphylococcus aureus* and MRSA on these surfaces raises the risk of transmission, potentially leading to serious conditions such as sepsis and pneumonia. Among the *Staphylococcus aureus* isolates, 27.8% were identified as MRSA. Managing neonatal infections caused by MRSA presents significant challenges, often leading to extended hospitalizations and prolonged treatment regimens. *Staphylococcus aureus* is identified as a major nosocomial pathogen, capable of surviving on non-living surfaces for several days [6]. The presence of opportunistic pathogens, such as *Acinetobacter baumannii*, coagulase-negative Staphylococci, *Enterococcus faecalis*, and *Burkholderia cepacia*, on NICU surfaces poses a considerable threat to vulnerable neonates, particularly those with low birth weight, premature infants, or those with congenital defects. In our research, we observed that the bacterial isolates demonstrated substantial resistance to frequently prescribed antibiotics, including ampicillin, ceftazidime, ceftriaxone,

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DOI:10.26502/fjhs.261

and ciprofloxacin. Similar findings have been reported in other studies [18, 32].

A notable proportion of multidrug-resistant (MDR) strains was identified among both Gram-negative and Gram-positive isolates, with over 50% of Gram-negative bacilli classified as MDR, raising concerns. Additionally, 27.8% of *Staphylococcus aureus* isolates (5 out of 18) were also found to be MDR. The high incidence of MDR among these bacterial pathogens may be linked to the use of advanced-generation antibiotics for empirical treatment and the prophylactic administration of antibiotics to high-risk mothers and neonates. It is crucial for clinicians to comprehend the antibiotic resistance trends of these pathogens, as this knowledge can inform the development of empirical antimicrobial treatment strategies for suspected nosocomial infections in the NICU. Such an approach could potentially shorten NICU stays and decrease neonatal mortality rates, ultimately fostering improved antimicrobial stewardship over time. Blood culture is one of the most common microbiological investigations ordered in the NICU. Data from blood cultures of NICU patients revealed that *Staphylococcus aureus* and *Klebsiella pneumoniae* were the two most common causes of neonatal sepsis. This study observed similarities in the antibiograms of the majority of *Staphylococcus aureus* and *Klebsiella pneumoniae* isolated from blood cultures and those found in the NICU environment. This finding likely indicates the nosocomial transmission of these pathogens, resulting in sepsis. Comparing antibiotic resistance patterns between environmental isolates and blood culture isolates in the NICU is an effective phenotypic method. This study has successfully achieved this comparison, unlike other studies.

Due to high bed occupancy in the NICU, the standard guidelines for cleaning and disinfection are often poorly implemented. This situation leads to increased bacterial colonization and subsequent spread within the Neonatal Intensive Care Unit (NICU). Maintaining sterility in the NICU environment is practically challenging due to the high rate of healthcare worker activities and the frequent use of equipment. Therefore, meticulous cleaning and disinfection protocols are essential to prevent the retention and spread of virulent microbial pathogens in the sensitive environment of the NICU. In this study, we have included most of the objects and instruments commonly touched by healthcare workers, as well as those that frequently come into contact with neonates. The findings of this study provide baseline information regarding the degree of contamination and the resistance patterns of environmental isolates. These findings are significant for enhancing the awareness of healthcare professionals in resource-limited countries regarding contamination in the NICU and its potential contribution to neonatal nosocomial infections. The study seeks to motivate scientists and researchers in these regions to delve deeper into neonatal nosocomial infections and to devise effective preventive strategies.

Limitations of the study

The association between potential pathogens found on objects and instruments in the NICU and instances of sepsis was determined solely through the antibiogram of these isolates. Genotypic analysis of the isolates was not conducted.

Conclusion

The findings of this study are essential for the infection control and prevention unit of the hospital providing insights into hygiene standards and the compliance of staff with infection control protocols. This research lays the groundwork for developing intervention strategies. The presence of clinically significant pathogens on commonly used objects and in critical areas raises serious concerns due to their clinical ramifications. The infection control and prevention unit of the hospital should implement regular monitoring, ensure thorough cleaning of instruments and other objects before and after use, and strictly follow basic standard infection control practices, particularly hand hygiene.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors Contributions

All authors contributed equally to this work.

Acknowledgements

Authors would like to thank the Department of Microbiology and Immunology and NICU unit of pediatrics Department, Bangabandhu Shiekh Mujib Medical University for their help and support.

Funding

This study did not receive any funding.

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Citation: Sanjida Khondakar Setu, Abu Naser Ibne Sattar, Shaheda Anwar, Towfique Hasan Firoz, Sanjar Taufiq. Surveillance of Nosocomial Infections in Neonatal Intensive Care Units at A Tertiary Care Hospital in Bangladesh. Fortune Journal of Health Sciences,. 8 (2025): 115-122.