Original Research Article

Environmental contamination with multi-drug resistant organisms in a Nigerian neonatal intensive care unit. Impactful infection source

ABSTRACT

Background: Neonates admitted into neonatal intensive care unit (NICU) are at high risk of acquiring infections due to the immaturity of their immune systems and the barrage of intensive medical care. Multiple drug resistant organisms are mostly implicated in causing these infections, usually acquired through contaminated environment. This research was designed to assess the environmental contamination with multi-drug resistant organisms in neonatal intensive care unit.

Method: Environmental samples were collected from all the equipment in each of the 3 rooms in neonatal intensive care unit and cultured on 5% sheep blood agar and MacConkey agar plates. The isolates were identified with standard biochemical tests. Antibiotic susceptibility profile was done by modified Kirby Bauer disc diffusion method and interpreted according to the guidelines by Clinical and laboratory standard institute. Methicillin resistant *Staphylococcus aureus* was detected using cefoxitin disc while extended spectrum beta lactamases (ESBL) were confirmed with ESBL chromogenic agar.

Result: Out of 36 samples collected from different sites in the 3 rooms, 19(53%) yielded bacterial growth. A total of 28 (78%) bacterial isolates were detected, with majority 25(89%) isolated from room 1. *Staphylococcus aureus* 15(42%) was the predominant organisms isolated followed by *Klebsiella species* 5(14%) and non-Candida albicans spp 3(8%). Most of *Staphylococcus aureus* isolates were from samples collected from incubators. Out of 15 *Staphylococcus aureus* isolated, 11 (73%) were MRSA. Among the Gram negatives, all 5(100%) *Klebsiella species* and 3(60) *Acinetobacter* species were positive for extended spectrum lactase production. All the isolates were resistant to 3 or more classes of antibiotics. Imipenem and linezolid were the most sensitive antibiotics to the isolated organisms.

Conclusion: High degree of contamination of environment in NICU with multi drug resistant bacteria is worrisome because of the serious threat it poses. This calls for immediate holistic

interventional measures. Strict Compliance with infection prevention measures and more aggressive environmental cleaning is needed. Additionally, there should be regular infection surveillance in NICU.

Keywords: Contamination, Environmental, ESBL, Multi-drug resistant, Organisms, Neonate, ,MRSA,

INTRODUCTION

Hospital acquired infection (HAI) poses a public health threat in neonatal intensive care unit (NICU). The infection occurs in both developed and developing countries with significant burden on both the patient and public health as a whole. Most of the bacteria that cause HAI are resistant to multiple antibiotics with increasing trend, leading to, longer hospital stay, higher mortality serious emotional consequences, as well as appreciably increased overall costs.[1-3] Multi drug resistant (MDR) infections may also result in disabilities that may affect the quality of life in these neonates. Globally, more than one million neonatal deaths per year are due to HAIs [4] with the rate being higher in developing countries varying from 11.9 to 14.7%.[5] In developing countries, the burden of the neonatal death is more in Sub-Saharan Africa which Nigeria is part, accounting for 98% of neonatal deaths.[6]

As a result of immature immune systems, hospital procedures, administration of broad-spectrum antibiotics and frequent use of invasive devices, neonates admitted in neonatal intensive care unit (NICU) have an increased risk of acquiring infections from the bacteria colonizing the hospital environment[7]. Additionally, these patients are also exposed to multiple broad spectrum antibiotics therapy which normally alter their normal microbiota and also increase the risk of acquisition of MDR organisms. The sources of HAI could be the patient's own flora, other patients, health care workers, caregivers or contaminated hospital environment.[8] outbreak of hospital-acquired infection have been associated with hospital environment and medical equipment [9] with the frequency of outbreaks likely to be very high im low and middle income countries due to overcrowding, understaffing, sharing and reuse of equipment. [10] Bacteria, fungi and viruses can survive on the inanimate surfaces, equipment and indoor environment for variable durations. [11-12] Hands of health care workers (HCWs), and patients have been shown to be associated with outbreaks of HAI.[13]

Determining the level and profile of environmental contamination in NICU is necessary for better patient management and efficient control of HAI. The profile of the organisms contaminating NICU environment may change within a setting overtime and has been noted to vary between one country and the other.

The study therefore, aimed at determining the level of environmental contamination of multi-drug resistant organisms in neonatal intensive care unit. This data will provide information on the type of bacterial contamination, antibiotic resistance patterns of the isolates and probable sources of infection in NICU. In addition, this research will give an insight in the effectiveness of environmental cleaning in NICU.

MATERIALS AND METHODS

STUDY AREA AND SETTING

This study was a hospital based cross- sectional study conducted in Neonatal intensive care unit (NICU) at the University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla. UNTH Ituku-Ozalla is located in Enugu state which is the capital city of the state. The hospital has both neonatal and adult intensive care unit. The NICU has 3 rooms with 15 incubators and 20 cots. Room 1 was frequently used for admission and majority of the samples were collected from the room.

SAMPLE COLLECTION AND CULTURE

A total of 36 environmental samples were collected from surfaces of incubators, radiant warmers, trolley, ventilators, electric switches, pulse oximeter and weighing basin. Other sites included; digital weighing machines, mothers' beds, phototherapy machine, shelves, oxygen cylinder. sink and slippers The samples were collected from the predefined surfaces by swabbing with sterile swab pre-moistened with peptone water and immediately transported to the medical microbiology laboratory for analysis.

ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES

The collected samples were inoculated in peptone water and incubated at 37^0C overnight. Sub-cultures were performed on MacConkey agar, chocolate agar and blood agar plates (Oxoid Laboratories, Cambridge UK). Plates were incubated aerobically at 37^0C for 24 hours. Purity plating was done on mixed cultures by sub-culturing them unto blood agar plates and incubated at 37^0C for 24 hours. The organisms isolated were identified with their

cultural morphology and biochemical characteristics using standard biochemical tests. Confirmation of the organisms were done with API 20E, 20E and API- STAPH (Oxoid Laboratories, Cambridge UK)

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Muller Hinton agar plates using modified Kirby Bauer method disc diffusion. A maximum of 6 antibiotic discs were placed on each plate for each isolated strains of *Staphylococcus aureus, Klebsiella pneumoniae* and *Acinetobacter baumanii.* The cultured plates were incubated at 37^0C for 24 hours, and interpreted according to the Clinical and Laboratory Standard Institute guidelines. American type culture collection (ATCC) 25922 was used to quality control the susceptibility testing.

Detection of the Methicillin resistant *Staphylococcus aureus* (MRSA)

Methicillin resistant *Staphylococcus aureus* was detected using cefoxitin disc (30µg) and incubated at 35^0C for 18 hours. Diameter of ≤ 21mm was regarded as positive. *Staphylococcus aureus* ATCC 25923 was used as control.

Detection of extended-spectrum beta lactamase (ESBL)

ESBL producing organism was confirmed with ESBL chromogenic agar (Oxoid Laboratories, Cambridge UK). *E. coli* ATCC 700603 and *Staphylococcus aureus* ATCC 25923 were used as a positive and negative control respectively.

Statistical analysis

Statistical analyses were conducted in SPSS (version 22.0). Descriptive statistics of qualitative variables were expressed as frequency (N) with percentage (%). Inferential statistics were also used. $P \leq 0.05$ was considered significant.

Results

Out of 36 samples collected from different sites, bacterial growth was observed in 19 (53%) samples, with more than one organism isolated from 6 sites. A total of 28 (78%) bacterial isolates were retrieved from 36 samples. Majority 25(89%) of the pathogens were isolated in

room 1 while none were isolated in room 3. The sampling sites and their bacterial isolates are depicted in figure 1.

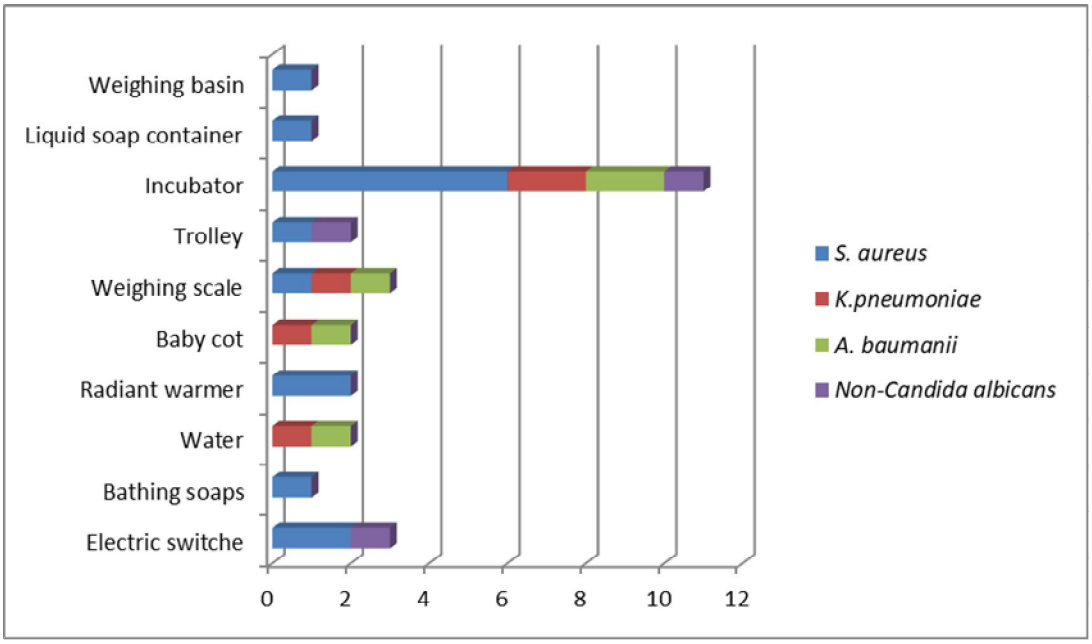

Figure 1: Organisms isolated from different sites

Four pathogens isolated were *Staphylococcus aureus* 15(42%), *Klebsiella species* 5(14%), *Acinetobacter species* 5(14%) and non-*Candida albicans spp* 3(8%). Figure 2 showed majority of *Staphylococcus aureus* isolates were isolated from incubators. Combinations of *Klebsiella species* and *Acinetobacter species* were isolated in the following; sites incubator: weighing scale, baby cot and bathing water. Among *S. aureus* isolates, 11 (73%) were MRSA and remaining were methicillin sensitive *Staphylococcus aureus* (MSSA).

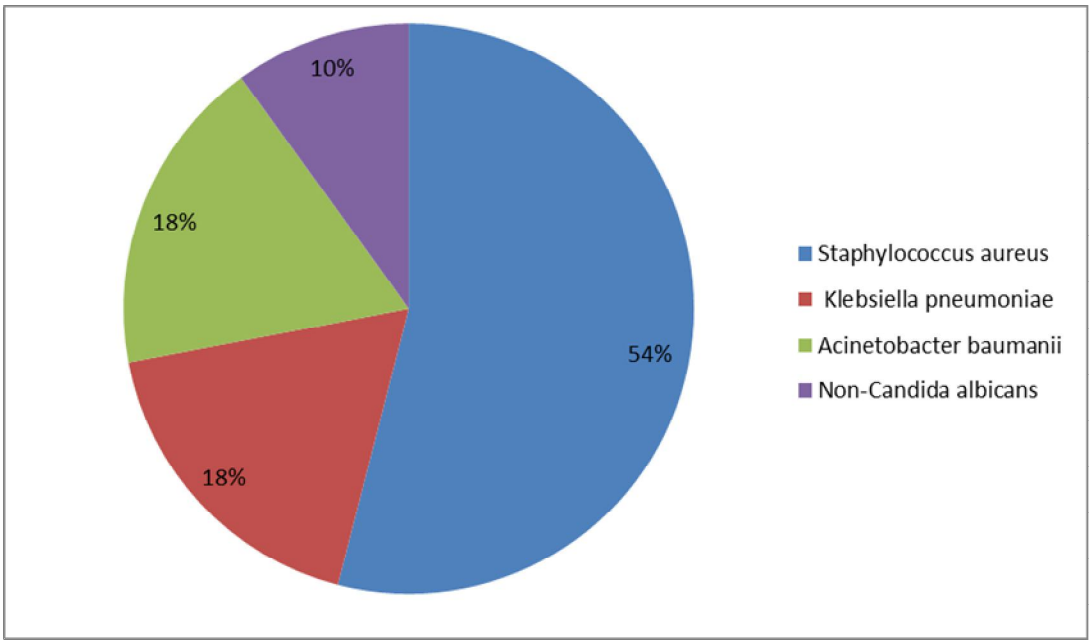

Figure 2: Distribution of the organisms

Among the Gram negatives, all 5(100%) *Klebsiella species* and 3(60) *Acinetobacter species* were positive for extended spectrum lactase production. Table 1 There is no statistical differences between the ESBL and MRSA. (P = 1.0)

Table 1: ESBL and MRSA production of status of the isolated pathogens

Pathogens	**ESBL**		**MRSA**		**Total**
	ESBL producers (%)	**ESBL non-producers (%)**	**MRSA Producer (%)**	**MRSA non-Producer(%)**	
Klebsiella pneumoniae	**5(100)**	**0(0)**	**-**	**-**	**5**
***Acinetobacter* species**	**3(60)**	**2(40)**	**-**	**-**	**5**
S. aureus	**-**	**-**	**11(73)**	**4(27)**	**15**
Total	**8(32)**	**2(8)**	**11(44)**	**4(16)**	**25**

The antibiotic resistance patterns of bacterial isolates are shown in Table 2. Multi-drug resistance was observed in all the bacteria isolated. The 5 *Klebsiella pneumoniae* isolated were susceptible to imipenem, meropenem, piperacillin/tazobactam and resistant to all other antibiotics tested. Two (40%) *Acinetobacter spp* were sensitive to imipenem with 1(20%) each sensitive to meropenem and piperacillin/tazobactam. Table 2 Majority of *Staphylococcus aureus* were susceptible to linezolid 13(87%).

Table 2: Antimicrobial susceptibility pattern of the isolates

Antibiotics	*Klebsiella pneumoniae* N= 5(%)		*Acinetobacter baumannii* N= 5(%)		*Staphylococcus aureus* *N*= 15(%)	
	S	R	*S*	*R*	S	R
Ceftazidime	0(0)	5(100)	0	5(100)	-	-
Nitrofurantoin	0(0)	5(100)	-	-	3(20)	12(80)
Gentamicin	0(0)	5(100)	0	5(100)	-	-
Imipenem	5(100)	0(0)	2(40)	3(60)	4(27)	11(73)
Meropenem	5(100)	0(0)	1(20)	4(80)	2(13)	13(87)
Cefotaxime	0(0)	5(100)	0	5(100)	-	-
Aztreonam	0(0)	5(100)	-	-	-	-
Amoxicillin-clavulanic acid	0(0)	5(100)	0	5(100)	1(7)	14(93)
Amikacin	0(0)	5(100)	1(20)	4(80)	-	-
Ampillicin	0(0)	5(100)	-	-	-	-
Piperacillin/tazobactam	5(100)	0(0)	0	5(100)	0(0)	15(100)

Ciprofloxacin	0(0)	5(100)	0	5(100)	5(33)	10(67)
Cefoxitin	0(0)	5(100)	-	-	4(27)	11(73)
Azithromycin	0	5(100)			4(27)	11(73)
Linezolid	-	-	-	-	13(87)	2(13)

S= Sensitivity, R= Resistance, N= number, %= Percentage, - = not tested

DISCUSSION

Environmental bacterial contamination of neonatal intensive care unit is one the major factor driving higher incidences of healthcare-associated infections and there is evidence that environmental disinfection reduces the incidence of HAI. [14] Healthcare-associated infections caused by multi drug resistant organisms in NICU are one of the causes of increased morbidity and mortality. The neonatal mortality rates vary between 20% to 80.[13]

The bacterial growth observed in this study demonstrates the considerable contamination of different areas of the unit. The rate of isolated in this study is comparable to the rate of 74.6% in a similar study done by Bhatta *o*.[15] Another study done by Yusuf et al[16] reported a rate of 68.8%. A much lower rate of 54.7%, was reported in a similar work done by Asinobi et al [17] The variations in rates of NICU bacterial contaminations recorded from different studies may be attributed to the number of sampling sites, neonatal admission rates, accessibility of NICU to visitors and poor compliance to infection control measures. Furthermore, neonates admitted in the NICU have life threatening infections necessitating strict monitoring by the healthcare workers, and frequent visits by parents and caregivers. This increase in the activities will surely lead to exchange of flora and touching of surfaces, resulting in high burden of environmental multi drug resistant bacterial contamination.[15] An observational study that was done by Risso *et al*,[18] identified "double touch" to be the commonest misbehaviour by nursing staff and consultants, thus promoting cross-transmission in the wards.

In our study findings, there were different potential pathogens isolated from various environmental surfaces. Majority of the isolated organisms in the study was *staphlococcus aureus*. This compared favorably with the study done by Asinobi et al,[17] where 56.6% of the isolates were *s. aureus*. Previous similar studies have also reported the predominance of *Staphylococcus aureus* in their different works.[19-21] This could easily be explained as it is the organism that is present on most surfaces especially the incubators and switches and also easily carried even on the hands of the NICU healthcare workers and caregivers as evidenced by the previous studies that reported MRSA rate of 19 to 30% infections in hands of health care workers. [22,23] *S. aureus* has the ability to survive on inanimate objects for several days due to presence of biofilms formation.[24]

Of important findings are the high rate of multi-drug resistant isolates recorded which was note-worthy. Multi-drug resistant bacteria are known to cause severe life threatening

infections which are difficulty to treat because of limited therapeutic options, leading to longer hospital stay and increased morbidity and mortality. The rate of MRSA (73%) recorded in this study was higher than the report from other similar studies.[15,17] MRSA is a known leading cause of nosocomial infections in developing countries [25,26] and has the potentials to survive longer in inanimate surfaces. (Kramer) [24] It was mainly isolated from incubators implicating human hands as important source of *S. aureus* in NICU. Incubators are considered frequently touched surfaces by HCW and parents, therefore the organisms might have been shed by the infected/colonized HCW or parents through cross transmission.

Other notorious pathogens reported in this work were the ESBL producing *Klebsiella spp* and *Acinetobacter spp*. ESBLs are genes that confer resistant to third generations cephalosporins and other classes of antibiotics. The genes are easily transferred horizontally to other species and this will hamper the control of infectious diseases caused by organisms that produces the resistant genes. ESBL producing *Klebsiella spp* and *Acinetobacter spp* are major Gram negative organisms that are known to cause severe infections in neonates, with increasing rise in the incidence and causes occasional outbreaks. Several researches have also reported ESBL producing organisms in a similar work.[15.17] Environmental contamination of NICU by ESBL organisms will no doubt lead to greater risk of life threatening systemic infections with resultant increase in mortality and morbidity in NICU.

The least isolated pathogen was non-candida species. *Candida species* is known as one of the most common cause of late onset septicemia in very-low-birth-weight infants.[27,28.] Other studies have also identified non-candida species in environmental samples and hands of HCWs; they reported that genotypes of the isolates from the two sources were the same.[29]

High percentage of MDR was observed among both Gram-negative and Gram-positive bacterial isolates. The *S. aureus* isolated was most sensitive to linezolid and resistant to commonly used antibiotics. This could be explained by the fact that linezolid is a relatively new antibiotic in the unit and so the organisms are still sensitive to it compared to the other antibiotics. Secondarily, as earlier stated, most of the *S. aureus* isolated were MRSA and these organisms are known to be resistant to all beta-lactam antibiotics. In a similar study that was done by Asinobi *et al*,[17] linezolid was not tested but they reported imipenem as the most sensitive antibiotics, contrasting the findings in this study. The high resistance of carbapenems noted in this study is not surprising. Apart from the fact there is high rate of MRSA which is expected to be resistant to carbapenems because of the beta- lactam ring

hydrolysis, carbapenems is used frequently in the NICU and this will no doubt contribute to the high resistant nature that was noted in this work.

The antibiotic susceptibility pattern in this study showed a growing resistance in the susceptibility of the organisms to the penicillin and first generation cephalosporin and similar findings have been reported in other studies.[15,16] This is a disturbing trend toward susceptibility to only third generation cephalosporin and the newer antibiotics like the linezolid.

Carbapenem is the most sensitive antibiotics in ESBL producing *Klebsiella pneumonia*.infection. Carbapenem is considered as the last therapeutic options in life threatening infections caused by extended-spectrum beta-lactamase producing Gram negative organisms[2]

NICU is an important place in the hospital where infection prevention and control measures should be practiced effectively. All the equipment and surfaces in the NICU environment is a potential reservoir of infection. The high rate of isolation of multi drug resistant bacteria confirms the impact of poor infection prevention and control measures including poor cleaning/disinfection and negligence of hand hygiene procedures by HCW and visitors. Decreasing the environmental reservoirs of these multi-resistant bacteria will undoubtedly break the chain of transmission of HAI and decrease the neonatal mortality rate. The findings of this study have provided information on the need to urgently institute strict infection prevention and control measures. IPC training should be done including provision of documented protocol of disinfection and cleaning in all the rooms in NICU.

CONCLUSION

These results identified the environmental surfaces as the probable sources of bacterial and fungal agents involved in HAIs in neonatal intensive care unit. Stricking, the high level of presence of multidrug resistant organisms calls for immediate holistic interventional measures. Strict Compliance with contact precautions and more aggressive environmental cleaning may decrease the cross- transmission.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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