

Original Article

Bacterial pathogens and their antibiotic susceptibility pattern in Intensive Care Units of the University of Maiduguri Teaching Hospital, Nigeria

ABSTRACT

Background: Nosocomial infections are among leading causes of morbidity and mortality and are associated with increased antibiotic resistance in Intensive Care Units (ICUs). This prospective study was conducted between March and October 2014 in the ICUs of the University of Maiduguri Teaching Hospital to assess the rate, types, and antibiotic susceptibility pattern of airborne and surface-borne bacterial contamination before and after fumigation.

Materials and Methods: Sixty samples were collected and investigated from fomites by surface swabbing and open plate air exposure in the ICUs before and after fumigation using standard microbiological methods.

Results: Out of the sixty samples investigated, 38 (63.3%) yielded positive bacterial growth. Twenty-six (68%) were before fumigation of the ICU and 31.5% ($n = 12$) from postfumigation culture. Coagulase-negative *Staphylococci* spp. (CoNS) accounted for 39.4% ($n = 15$) of the positive cultures, *Bacillus* spp. 15% ($n = 9$), *Klebsiella pneumoniae* 13.2% ($n = 5$), *Escherichia coli* 10.5% ($n = 4$), *Klebsiella oxytoca* 7.9% ($n = 3$), and *Streptococcus pyogenes* 5.3% ($n = 2$). The antibiotic susceptibility test results of the isolates revealed that CoNS were resistant to amoxicillin, ampicillin-cloxacillin, and cefuroxime. *K. pneumoniae* was also resistance to chloramphenicol, aminoglycosides, and penicillins, whereas *E. coli* showed resistance to fluoroquinolones, particularly pefloxacin and ofloxacin. Conversely, *Bacillus* spp., *K. oxytoca*, and *S. pyogenes* were susceptible to all test antibiotics.

Conclusion: The high level of bacterial contamination of equipment and inanimate objects in the ICUs and the presence of multidrug resistant bacteria calls for prompt and a holistic infection control interventions.

Keywords: Bacterial contamination, fomites, Intensive Care Unit, nosocomial infection

INTRODUCTION

Patients in Intensive Care Units (ICUs) are a significant subgroup of all hospitalized patients, accounting for about 25% of all hospital infections.^[1] The prevalence of ICU-acquired infections is significantly higher in developing countries than in industrialized countries, varying between 4.4% and 88.9%.^[2] Furthermore, device-associated infection rates in developing countries, especially ventilator-associated pneumonia followed by central venous catheter-related bloodstream infections occur at a higher frequency than in European countries and the USA.^[3]

There has been a recent interest in the use of chemical fumigation in health-care facilities because of concerns

about the role of the environment as a cause of healthcare-associated infections (HAIs) and a perception that surface cleaning and disinfection methods are ineffective.^[4]

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How to cite this article: Barma MM, Nasir IA, Babayo A. Bacterial pathogens and their antibiotic susceptibility pattern in Intensive Care Units of the University of Maiduguri Teaching Hospital, Nigeria. *J Med Trop* 2017;19:16-20.

Access this article online	
Website: www.jmedtropics.org	Quick Response Code 
DOI: 10.4103/2276-7096.207587	

Methicillin-resistant *Staphylococcus aureus* (MRSA) and other Gram-positive bacteria have become an increasingly common problem in health-care environments.^[5] A more recent concern is the upswing in the incidence of infections caused by *Clostridium difficile*.^[6] This organism is now considered to be the most important cause of diarrheal HAI.^[7] *Acinetobacter baumannii* is yet another microorganism involved in HAIs that has been linked to environmental contamination. Of course, for each infectious agent, there are a variety of potential pathways of exposure and different routes of exposure. It is commonly assumed that the most important risk factor for HAI is direct contact spread (principally via caregiver's hands).^[4,7]

Researchers have explored using chlorine dioxide, hydrogen peroxide vapor (HPV), super-oxidized water, or ozone for terminal disinfection of hospitals contaminated with mold and bacteria.^[8] In the past, paraformaldehyde has been used to decontaminate biological safety cabinets and entire buildings. Fumigants such as chlorine dioxide and HPV are two agents most frequently examined to decontaminate hospitals, animal research facilities, or similar environments.^[4,8]

Fumigants are being considered as an adjunct to conventional environmental disinfection due to their composition as a gas or vapor, allowing them to easily penetrate hard to reach areas.^[8] Although many of these fumigants will kill microorganisms, there are still concerns for the safety of patients and workers who may be inadvertently exposed to these toxicants.

Boyce *et al.* conducted a before-after intervention study of the effectiveness of HPV in the control of *C. difficile*.^[9] During the intervention period, rooms that had previously housed *C. difficile* patients were fumigated using the Bioquell system. Each room took approximately 3–4 h to disinfect. The average incidence rates of *C. difficile* infection dropped from 2.28 per 1000 patient days during the preintervention phase to 1.28 ($P = 0.047$) during the intervention phase. Andersen *et al.* used the Sterinis system (Gloster Stante Europe, Toulouse, France) to generate a 30–60 ppm concentration of hydrogen peroxide as a “dry fume.”^[10] This process involved closing the room door but not sealing the room, and the cycle time was reported to be 4–5 h. They used spore strips containing 2.5×10^6 spores of *Bacillus atrophaeus* as the test agent, and the results were reported as pass-fail. There was a 100% spore inactivation in the 48 surgical suite tests and an 87% inactivation (127 of 148 spore strips) in the other rooms tested.^[10]

Bacteria are also transmitted between ICU patients by respiratory droplets (from infected airway secretions)

and via other inanimate objects or fomites in the ICU environment.^[11,12] About 20% of ICU patients will develop nosocomial infections, often caused by resistant bacteria and many more become colonized by resistant bacteria.^[13,14]

In the ICUs, sanitation protocols are stricter than in other areas of the hospital, yet many patients treated are infected with nosocomial superbugs, often due to an underlying severe disease conditions. These nosocomial infections remain among the leading causes of death in hospitals of most countries. For example, they are a significant cause of morbidity and mortality in the USA; 1.7 million infections resulting in 99,000 deaths were reported in 2002.^[14]

A study carried out in Jos, Nigeria, estimated the prevalence of nosocomial infection in the ICU as 6.0%, and a disproportionate 20% occur in critically ill patients. The study also determined the contributory role of fomites in the spread of nosocomial infections. Overall 69 (99%) cultures yielded growth; *S. aureus* (44.3%), coagulase negative *Staphylococci* (CoNS) (74.3%), and *Bacillus* spp. (90.0%). Out of the *S. aureus* isolated, 7 (22.6%) were MRSA. These were isolates from fan switches, X-ray viewing boxes, dwarf partition walls, ventilators, tables, floors, and hand towels.^[15]

The distribution of bacterial pathogens in the ICU in another study conducted by Okon *et al.* in Maiduguri Teaching Hospital reported 38.4% cases of nosocomial bacteria among study sites that included the operating theater, the ICU, Special Care Baby Unit, dialysis center, and the Central Sterile Service Department. Out of the 267 samples collected and analysed, 70.0% ($n = 186$) were positive for bacterial growth; 72.1% CoNS, *Proteus* spp. (8.6%), *Pseudomonas aeruginosa* (6.8%), and *coliforms* (2.2%).^[16]

In view of the contributory role of the ICU environment to infection transmission, we conducted this study to determine the rate, types, and antibiotic susceptibility pattern of airborne and surface-borne bacterial contaminants before and after fumigation at the University of Maiduguri Teaching Hospital (UMTH).

MATERIALS AND METHODS

Study area

The study was conducted between March to October 2014 in ICUs of the UMTH, Maiduguri. The ICUs are multidisciplinary and care for all critically ill patients. Maiduguri, the capital city of Borno State, Nigeria, located in Northeast Nigeria shares borders with neighboring countries such as Niger Republic, Chad, and Cameroon. Within Nigeria, Maiduguri shares borders with other States such as Adamawa, Yobe,

and Gombe and has Sahel savannah vegetation. The annual average temperature of Maiduguri ranges from 19.1°C to 34.7°C and average annual precipitation is 562 mm.

Sampling

Sixty samples from the ICU work environment were purposively collected. These included open-plate air exposure culture and fomites swabbing (door handles, table surfaces, gowns, machines/equipment uses, and windows/walls). Forty-five samples were collected before fumigation (prefumigation culture) of the ICU, and 15 samples were collected after fumigation (postfumigation). Based on available information from the ICUs managerial staff, fumigation was last conducted on January 19, 2014, before sampling. Subsequently, post fumigation sampling was conducted 24 h of fumigation.

Methods

Labeled sterile swab sticks moistened with sterile physiological saline were used to swab the fomites in the ICU until the swabs were visibly dirty. All samples collected were transported immediately to the microbiology laboratory where they were inoculated in pairs onto dried blood agar and MacConkey agar plates. Open-plate air exposure was also done with blood agar and MacConkey agar plates. Plates were both aerobically and anaerobically incubated at 37°C for 24 h. Bacteria were identified using standard microbiological methods as described by Cowman and Steel^[17] and Lenette et al.^[18] while the antibiotic susceptibility pattern was determined using Kirby-Bauer disc diffusion method.^[19]

Ethical approval

This study was approved by the ethical research committee of the UMTH, Maiduguri, Nigeria.

RESULTS

Out of the sixty samples collected, 63.3% ($n = 38$) yielded positive bacterial growth. About 68% ($n = 26$) before fumigation of the ICU and 31.5% ($n = 12$) from post fumigation culture, whereas 36.6% ($n = 22$) yielded no bacterial growth [Table 1]. Fifteen distinct isolates of CoNS spp. from fomites and open-plate air exposure accounted for 39.4% ($n = 15$) of the positive cultures, followed by *Bacillus* spp. Fifteen percent ($n = 9$, respectively, predominating the positive cultures being isolates from both prefumigation and postfumigation cultures. *Klebsiella pneumoniae* accounted for about 13.2% ($n = 5$), *Escherichia coli* 10.5% ($n = 4$), *Klebsiella oxytoca* 7.9% ($n = 3$), and *Streptococcus pyogenes* 5.3% ($n = 2$).

The frequency distribution of isolated bacterial according to the site of sampling showed 8 (21.1%) were from air

Table 1: Frequency distribution of isolates from fomites and open plate cultures

Isolates	Prefumigation ($n=45$)	Postfumigation ($n=15$)	Frequency (%)
CoNS	7 (18.2)	8 (21.1)	15 (39.4)
<i>Bacillus</i> spp.	5 (13.2)	4 (10.5)	9 (23.7)
<i>Klebsiella pneumoniae</i>	5 (13.2)	-	5 (13.2)
<i>Klebsiella oxytoca</i>	3 (7.9)	-	3 (7.9)
<i>Escherichia coli</i>	4 (10.5)	-	4 (10.5)
<i>Streptococcus pyogenes</i>	2 (5.3)	-	2 (5.3)
Total frequency	26 (68.4)	12 (31.5)	38 (100)

exposure of the ICU by open plate method. The other fomites contaminants isolated by swabbing method included 12 (31.5%) from bed surfaces, 9 (23.7%) from ICU equipment/machines, 5 (13.2%) from table surfaces, 2 (5.3%) from windows and walls of the ICU, and 1 (2.6%) organism each from door handles and gowns accounting for the least number of bacterial isolates [Table 2].

The antimicrobial susceptibility profile of the bacterial isolates revealed that CoNS was most resistant to amoxicillin, ampicillin-cloxacillin, and cefuroxime. *K. pneumoniae* was resistant to chloramphenicol, all aminoglycosides, and penicillins used, whereas *E. coli* was resistance to pefloxacin and ofloxacin. Conversely, *Bacillus* spp., *K. oxytoca*, and *S. pyogenes* isolated was susceptibility to all test antibiotics [Table 3].

DISCUSSION

This study has shown a high prevalence of the ICU environment in Maiduguri. Although the prevalence reduced postfumigation, contamination was not completely eliminated. The isolated bacteria were CoNS and *Bacillus* spp. The highest contamination involved bed surfaces and equipment and contamination was particularly with CoNS (39.4%). There were also multidrug resistant isolates such as *K. Oxytoca*, *E. coli*, and *S. pyogenes* which were resistant to co-trimoxazole, amoxicillin, and augmentin.

The frequency of CoNS is in conformity with the previous study which reported CoNS as the major causative agents of nosocomial infections in the ICU particularly.^[20,21] CoNS infections are associated with increased morbidity particularly neonatal sepsis and neurodevelopmental impairment.^[20-22]

The frequency of ICU bacteria contamination reported from this study (63.3%) is higher than those previously by Hassan et al.^[23] The differences may be due to disparity in study location sample size and hygienic conditions of the

Table 2: Frequency distribution of fomites surface and airborne contaminants isolated from Intensive Care Units

Sites	CoNS	<i>Bacillus</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella oxytoca</i>	<i>Escherichia coli</i>	<i>Streptococcus pyogenes</i>	Frequency (%)
Open plate	2	3	1	-	-	2	8 (21.1)
Bed surfaces	4	3	1	2	2	-	12 (31.5)
Equipment	3	2	1	2	1	-	9 (23.7)
Table surfaces	3	1	1	-	-	-	5 (13.2)
Windows/walls	2	-	-	-	-	-	2 (5.3)
Door handles	1	-	-	-	-	-	1 (2.6)
Gowns	-	-	1	-	-	-	1 (2.6)
Total (%)	15 (39.4)	9 (23.7)	5 (13.2)	3 (7.9)	4 (10.5)	2 (5.3)	38 (100)

Table 3: Antibiogram profile of bacterial isolates from fomites and open plate cultures

Antimicrobial agent (µg)	CoNS		<i>Bacillus spp</i>		<i>Klebsiella pneumoniae</i>		<i>Klebsiella oxytoca</i>		<i>Escherichia coli</i>		<i>Streptococcus pyogenes</i>	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ciprofloxacin (10)	14 (93)	1 (17)	9 (100)	0 (0.0)	4 (80)	1 (20)	2 (67)	1 (33)	3 (75)	1 (25)	2 (100)	0 (0.0)
Streptomycin (30)	12 (80)	3 (20)	9 (100)	0 (0.0)	2 (40)	3 (60)	3 (100)	0 (0.0)	3 (75)	1 (25)	1 (50)	1 (50)
Co-trimoxazole (30)	11 (73)	4 (27)	9 (100)	0 (0.0)	3 (60)	2 (40)	0 (0.0)	3 (100)	0 (0.0)	4 (100)	0 (0.0)	2 (100)
Chloramphenicol (30)	NT	NT	NT	NT	2 (40)	3 (60)	2 (67)	1 (33)	2 (50)	2 (50)	NT	NT
Amoxicillin (30)	4 (27)	11 (73)	8 (89)	1 (11)	2 (40)	3 (60)	0 (0.0)	3 (100)	0 (0.0)	4 (100)	0 (0.0)	2 (100)
Ampicillin-cloxacillin (30)	3 (20)	12 (80)	8 (89)	1 (11)	NT	NT	NT	NT	NT	NT	0 (0.0)	0 (0.0)
Gentamycin (10)	12 (80)	3 (20)	9 (100)	0 (0.0)	2 (40)	3 (60)	0 (0.0)	3 (100)	3 (75)	1 (25)	1 (50)	1 (50)
Augmentin (30)	NT	NT	NT	NT	2 (40)	3 (60)	0 (0.0)	3 (100)	0 (0.0)	4 (100)	0 (0.0)	2 (100)
Erythromycin (10)	13 (87)	2 (13)	9 (100)	0 (0.0)	NT	NT	0 (0.0)	3 (100)	NT	NT	1 (50)	1 (50)
Pefloxacin (30)	14 (93)	1 (7)	9 (100)	0 (0.0)	5 (100)	0 (0.0)	2 (67)	1 (33)	1 (25)	3 (75)	2 (100)	0 (0.0)
Sparfloxacin (10)	NT	NT	NT	NT	4 (80)	1 (20)	2 (67)	1 (33)	2 (50)	2 (50)	NT	NT
Ofloxacin (10)	NT	NT	NT	NT	4 (80)	1 (20)	2 (67)	1 (33)	1 (25)	3 (75)	NT	NT
Ceftriaxone (25)	12 (80)	3 (20)	9 (100)	0 (0.0)	NT	NT	NT	NT	NT	NT	1 (50)	1 (50)
Cefuroxime (20)	5 (33)	7 (67)	8 (89)	1 (11)	NT	NT	NT	NT	NT	NT	0 (0.0)	2 (100)

S - Susceptible, R - Resistant, NT - Not tested

study area. Airborne contamination rate of 21% ($n = 8$) obtained from both prefumigation and postfumigation open plate cultures gave the bacteriological status of air circulating within the ICU and reliability of chemicals used for disinfection and fumigation. This value was relatively higher to the 14.1% contamination rate reported by Okon *et al.*^[16]

The isolation of pathogens from equipment and other fomites indicates that they could be a source of infection to ICU patients. Previous data provided that the ICU is regularly disinfected on daily basis despite the relative presence of these isolates recorded. The source and possible cause of contaminant include normal skin flora of medical personnel, patients, and invasive device and equipment. *Bacillus spp.* was found to be common with surface and airborne pathogen after CoNS. This is no surprise as *Bacillus subtilis* is evolving to be a persistent pathogen on inanimate objects.^[24]

The antibiotic susceptibility profile of isolates from this study revealed that CoNS was significantly resistant to amoxicillin (73%), ampicillin-cloxacillin (80%), and cefuroxime (67%). CoNS can persist as an important hospital and community pathogen. These bacteria have become a major concern to the medical

community due to the fact that they have an extraordinary ability to adapt rapidly to antibiotic stress.^[25] Because of the widespread use of penicillin in both communities and hospital, penicillin-resistant CoNS became rampant.

K. pneumoniae was also resistance to chloramphenicol, aminoglycosides, the penicillins, whereas *E. coli* showed resistance to some of the fluoroquinolones, particularly pefloxacin and ofloxacin. The first steps that contribute to the increasing incidence of these antibiotic resistant bacteria are the selection of resistant mutant strains from the patient's own flora during antibiotic treatment or the transfer between bacteria of mobile genetic determinants of antibiotic resistance.^[26] Subsequently, resistant strains spread among patients and environments in hospital. It is believed that these infections originated from either patients, healthcare givers, and visitors or inefficient disinfection. The driving force of antibiotic resistance is the widespread use of antibacterial drugs that begins in the community.

Limitation of study

This study would have given better information with regard to direct effects of the bacteria isolated from the ICUs on

ICU patients if patients admitted into these units were also screened for possible contraction of similar pathogens present before and after fumigation.

CONCLUSION

The high level of bacterial contamination of equipment and inanimate objects in the ICUs and presence of multidrug resistant bacteria calls for prompt and holistic interventional measures because fumigation only minimized pathogen load in ICUs

Acknowledgment

We greatly appreciate Mrs. Habiba Jimeta Balla, Dr. Adamu Sadiq Abubakar, Dr. Kenneth Okon, and Abdulkarim Sahabi of UMTH, for their technical inputs and proofreading the final manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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