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Incidence and Antibacterial Susceptibility Profile of Isolates from the Surfaces of Inanimate Objects in a Paediatric Unit of a Tertiary Hospital in Kano, Nigeria.Mohammed Aisha¹, Ali Muhammad Hadiza², Bala Kawu Hauwa¹.Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Bayero University Kano¹.

Department of Biological Sciences, Faculty of Sciences, Nigeria Police Academy, Kano.

Author for correspondence: amohammed.phb@buk.edu.ng/+234-803-589-7992.

<https://dx.doi.org/10.4314/sokjmls.v10i4.20>**Abstract**

Contaminated surfaces of inanimate objects of healthcare facilities have been identified as possible routes for transmission of Hospital Acquired Infections amongst patients, especially children. These infections have been attributed to the increase in morbidity and mortality as well as increased length of hospital stays. This study aimed to identify pathogens found on inanimate surfaces in a Paediatric ward of Aminu Kano Teaching Hospital, Kano, as well as the antibiotic susceptibility profile of the organisms. Seventy (70) swab samples were collected from different inanimate surfaces using sterile swab sticks. The samples were inoculated on MacConkey and Blood agar at 37 C for 18-24 hours. Gram staining and biochemical tests were used to identify the organisms. Antibiotic susceptibility profile was carried out using Kirby Bauer Disc Diffusion method. A total of 48 organisms, which include *S. aureus*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* were isolated. Gram positive organisms were 41.7% while the Gram negative were 58.3%. *S. aureus* was the most frequently isolated pathogen. Occurrence of isolates was 20.8% on children's lockers, bedside floor and beds while doorknobs had 16.8%. Teddy bears and stethoscopes had 10.4%. A 100% resistance to ciprofloxacin was observed in the *K. pneumoniae* isolates. Varying resistance from 40-45% against ceftazidime, cefuroxime and nitrofurantoin was observed in the *S. aureus* isolates. The Gram-negative pathogens showed resistance pattern ranging from 61.5% to 75% against Amoxicillin clavulanic acid, ceftazidime and ciprofloxacin. The study has shown that inanimate surfaces in the paediatric ward harbours pathogenic organisms. Effective hygiene control and disinfection should be implemented.

Keywords: Inanimate objects, Paediatric ward, Nosocomial infection, Antibiotics, bacteria

Introduction

Healthcare-associated infections (HAI) are infections that arise in the course of receiving medical related services in a healthcare center, which the patient did not present when they were admitted (WHO 2002, WHO 2016). Such an illness is thought to be HAI if it is evident or following 48 hours of admission in a healthcare facility and within 48-72 hours after the patient is sent home (Akeau, 2016). These infections can also be contracted by healthcare givers during the process of medical care delivery (WHO 2016). The spread of HAIs occurs mostly through contaminated hands of healthcare givers, their equipment and engaging with patients, improper sterilization of devices, and overall, the rising trend of microorganisms that are multidrug resistant (Guillaume, 2020).

There is a rising concern of HAIs in the twenty-first century because of many reasons. Some of which include the healthcare centers having a large number of sick patients a lot of whom are children whose immune system is developing; a high number of outpatients services; several medical treatments that by-pass the body's defense mechanism; hospital staff that mingle with patients allowing easy passage of pathogens; poor hygiene practices for clothes, beddings, equipment, sterilization and other preventive measures, and the constant administration of antibiotics in hospitals, that exert selection pressure leading to the emergence of multidrug resistant organisms (Revelas, 2012).

Microorganisms can infect inanimate objects in hospitals and serve as a channel for their propagation. Fomites, or inanimate items, are an important source of infectious disease transmission when they constantly encounter people or the pathogenic organisms' natural environment (Osterholm *et al.*, 2005). Surface bio-contamination of fomites has

been documented to have a crucial impact in the outbreak of nosocomial and community-acquired illnesses (Nwankiti *et al.*, 2012). It is not clear the extent of impact fomites reservoirs have on the overall incidence of hospital or environmentally acquired infections, despite the fact that fomites have a vital part in the transmission of many diseases (Agu *et al.*, 2024). Cell phones, clothing, doorknobs, and stethoscopes are among these inanimate items that are especially prone to contamination from the hospital environment by bacteria and are regarded to be plausible causes of these disorders. *E. coli*, *Klebsiella pneumoniae*, *Proteus* species, and coagulase-negative *Staphylococcus aureus*. The most frequently isolated organisms from inanimate surfaces in hospitals are *Pseudomonas aeruginosa*, *Acinetobacter* species, and *Staphylococcus* species (Sikora and Zahra, 2021; Murray, 2022).

Nosocomial infections have been reported to spread through the surface colonisation of numerous inanimate objects in a hospital setting by microbes. Dynamic attempts to control the spread of nosocomial infections by fomites are rarely implemented in Nigeria (Umar *et al.*, 2015)

Considering the present setting of our community and healthcare, there is an increasing worry pertaining the occurrence and dissemination of multidrug resistant organism on surfaces that are routinely touched such as door handles, lockers, and work surfaces. Children are especially at danger since they have a developing immune system hence contamination from surfaces could lead to severe diseases including sepsis, pneumonia and gastrointestinal infections. The absence of detailed information on the types of bacteria prevalent on such layers in paediatric units and their resistance pattern to antibiotics creates substantial hurdles in efforts to prevent infection. It is therefore essential to isolate, identify, and carry out antibacterial susceptibility studies of bacteria present on frequently touched surfaces in paediatric wards to understand the associated risks which would help to come up with sustainable measures to reduce the transmission of pathogenic agents. The aim of this study is to isolate, identify, and determine the antibacterial sensitivity profile of bacteria isolated from routinely utilised inanimate surfaces in a paediatric ward of Aminu Kano Teaching Hospital, Kano.

Materials and Methods

Study area

The research was conducted at the Paediatric Ward of Aminu Kano Teaching Hospital Kano. It is a referral Health institution which was commissioned in 1992

to serve hospitals and health centres in Kano State. The hospital has a bed space for 500 patients with various Departments including Pharmaceutical services, Nursing services, Anaesthesia, Catering services, Ophthalmology, Dental surgery, Radiology, Physiotherapy, Haematology and Blood Transfusion, Chemical Pathology, Medical Microbiology, Pathology, Paediatrics, Surgery, Medicine, Immunology, Radiology and Community Medicine. There are separate wards for in - patients, presenting different health categories. It has a good number of medical personnel including consultants and specialists in various fields of medicine.

Ethical Approval

Ethical approval was obtained from the Health Research and Ethics Committee of Aminu Kano Teaching Hospital

Sample collection

A total of Seventy (70) samples were collected from doorknobs, beds, bedside drawers, Stethoscope, room floors and teddy bears. A sterile swab stick was placed in sterile peptone water was used to obtain each sample by swabbing the fomites' surface. The samples had the proper labels and codes. After that, every sample was taken to the lab at Bayero University Kano's Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences.

Sample culture

The swab sticks were then incubated in peptone water for a period of about 8hrs then inoculated on MacConkey and Blood agar after which were then incubated for 18 hours at 37 C.

Bacteria identification

After incubation, the petri dishes were analysed for growth and morphological characteristics were observed. Gram staining and biochemical tests which include catalase, coagulase, citrate, urease, indole, sugar fermentation and Triple Sugar Iron Agar tests were carried out (Cheesbrough, 2000).

Gram Staining

A 24-hour culture of organism was heat fixed on a clean microscope slide. Crystal violet was added and allowed to stand for 30-60 seconds after which it was rinsed off with distilled water. Lugol's iodine was added and allowed to sit for 60 seconds after which it was rinsed off with distilled water. 95% ethanol which is the decolourizer used was poured for 20 seconds on the slides and rinsed off with water. Safranin was added for 60 seconds and then washed

off with water. The microscope slide was blotted and allowed to air dry after which it was viewed under $\times 100$ oil immersion microscope.

Catalase test

About 3mls of hydrogen peroxide solution was poured in a test tube. A wooden spatula was used to pick multiple colonies of the test organism and placed it inside the hydrogen peroxide solution. Formation of bubbles immediately signifies a positive reaction while absence of bubbles suggests a negative reaction.

Coagulase test

A colony of test organism was picked with an inoculating loop and emulsified on a clean slide free from grease on which a drop of distilled water had been placed. A loopful of suspended plasma was added and observed for coagulation. A control slide on which no plasma was added was used to identify possible granular appearance of the bacterium from real coagulation. Clumping signifies a positive reaction while lack of clumping suggests a negative reaction.

Indole test

The organisms were cultured in 10mls of tryptone water at 37 C for 24 hours. About 0.5ml of Kovacs reagent was added to the culture, shaken and inspected after one minute. A positive result was observed by the presence of a red layer in the medium while nonappearance of the layer signifies a negative result.

Citrate test

A Simmon citrate agar slant contained in bijou bottle was streaked with an inoculum of test organism. The slant was incubated at 37 C for 48 hours. A colour change from blue to green along the slant signified a positive reaction.

Urease test

A small amount of test organism culture was streaked on the urease test slant containing phenol red as indicator and incubated for 7 days at 37 C. A colour change from yellow to red or pink indicates a positive reaction while absence of colour change indicated a negative reaction.

Oxidase test

A fresh culture of isolate was smeared using a wire loop on a Whatman No 2-filter paper containing 3 drops of freshly prepared oxidase reagent. A positive reaction was indicated by the appearance of dark purple tint on the paper within 10 seconds of application of the culture.

Triple Sugar Iron Agar Test (TSIA)

A loopful of bacteria culture was streaked on the slant of a Triple Sugar Iron gel in a test tube and the butt stabbed. This was incubated for 24 hours at 37 C and subsequently observed for sugar fermentation which was signified by colour change in the butt and slant, also production of gas and hydrogen sulphide.

Antibacterial susceptibility testing

The antibiotics used were Ceftazidime (30 μ g), Cefuroxime (30 μ g), Gentamicin (10 μ g), Cefixime (5 μ g), Amoxicillin/Clavulanic acid (20/10 μ g), Nitrofurantoin (30 μ g) and Ciprofloxacin (5 μ g). The antibacterial sensitivity test was performed using the Kirby-Bauer Disc Diffusion method. A loopful of organism was picked from a pure culture and transferred into a test tube containing 5mls of normal saline. The mixture was gently homogenized to obtain a uniform suspension and standardized to obtain a turbidity equivalent to 0.5 McFarland standard. A sterile cotton swab was immersed into the suspension, excess liquid removed by gently rotating on the inner surface of the tube and used to inoculate the surface a Mueller Hinton Agar plate. Antibiotic discs were placed and incubated at 37 C for 18-24hours. After incubation, the diameter of zone of inhibition was measured and interpreted (CLSI, 2022).

Results

Percentage distribution of bacterial isolates based on Gram's reaction

The percentage distribution of bacterial isolates based on Gram's stain is shown in Figure 1. The only Gram-positive organisms were 20 (41.7%) while the Gram negative were 28(58.3%).

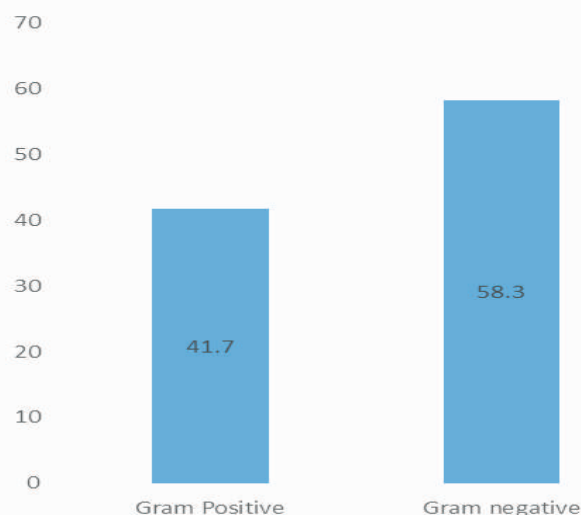


Figure 1: Percentage distribution of organisms based on their Gram' reaction

Distribution of isolates from different sampling sites

Table 1 shows the distribution of isolates from inanimate objects in the study location. The results showed that a total of 48 organisms were isolated which include *S. aureus* 20(42%), *P. aeruginosa* 13(27%), *E. coli* 12(25%), and *K. pneumoniae* 3(6%) respectively. The most isolated organism was *S. aureus*.

Table 1: Distribution of isolates from Inanimate objects

Organism	Frequency	(%)
<i>S. aureus</i>	20	41.7
<i>P. aeruginosa</i>	13	27
<i>E. coli</i>	12	25
<i>K. pneumoniae</i>	3	6.3
Total	48	100

Occurrence of isolates according to the type of inanimate object

The occurrence of the isolates from the different inanimate objects is shown in Table 2. The results showed that from the 48 isolated microorganisms, 8(16.8%) of the isolates were found on door handles, 10(20.8%) on children's locker, 5(10.4%) on teddy bears, 5(10.4%) on stethoscopes, 10(20.8%) on bedside floor and 10(20.8%) on beds. Equal numbers of isolates were found on children's lockers, bedside floors and beds. Also, same number of isolates were found on teddy bears and stethoscopes.

Table 2: Occurrence of isolates on different inanimate objects

Organism	Doorknobs	Children's locker	Teddy Bear	Stethoscope	Bedside Floor	Bed
	Frequency (%)					
<i>S. aureus</i>	5 (25)	2 (10)	2 (10)	5 (25)	0 (0)	6(30)
<i>P. aeruginosa</i>	1 (7.7)	4 (30.8)	1 (7.7)	0 (0)	3 (23.1)	4(30.8)
<i>E. coli</i>	2 (16.7)	2 (16.7)	2 16.6)	0 (0)	6 (50)	0 (0)
<i>K. pneumoniae</i>	0 (0)	2 (66.7)	0 (0)	0 (0)	1 (33.3)	0(0)
Total	8 (16.8)	10 (20.8)	5 (10.4)	5 (10.4)	10 (20.8)	10 (20.8)

Antibiotic Susceptibility Pattern of Isolates from Inanimate Objects

The antimicrobial susceptibility profile of the isolates from inanimate objects is shown in Table 3. The results showed that the *S. aureus* isolates were most sensitive to CIP 15(75%), CFX 14(70%), CN 13(65%), CXM 12(60%) and AMC 10(50%) and they were most resistant to CAZ 9(45%), CXM 8(40%) and NF 8(40%). The *P. aeruginosa* isolates were most sensitive to CXM 10(76.9%), NF 10(76.9%) and CN 9(69.2%) and most resistant to AMC 8(61.5%) and CAZ 6(46.2%). The *E. coli* isolates were most sensitive to CAZ 6(50%), CXM 6(50%), CFX 6(50%), AMC 6(50%) and NF 6(66.7%) while for the *K. pneumoniae* isolates, they were most sensitive to CXM 3(100%) and most resistant to CIP 3(100%).

Table 3: Antimicrobial Susceptibility Profile of isolates from inanimate objects

Organism	ASP	Antimicrobials with susceptibility Pattern N (%)						
		CAZ	CXM	CN	CFX	AMC	NF	CIP
<i>S. aureus</i> (n=20)	S	5(25)	12(60)	13(65)	14(70)	10(50)	6(30)	15(75)
	I	6(30)	0(0)	2(10)	2(10)	5(25)	6(30)	3(15)
	R	9(45)	8(40)	5(25)	6(30)	5(25)	8(40)	2(10)
<i>P. aeruginosa</i> (n=13)	S	2(15.3)	10(76.9)	9(69.2)	5(38.5)	3(23.1)	10(76.9)	5(38.5)
	I	5(38.5)	0(0)	1(7.8)	3(23.1)	2(15.4)	1(7.7)	3(23.1)
	R	6(46.2)	3(23.1)	3(23.1)	5(38.5)	8(61.5)	2(15.4)	5(38.5)
<i>E. coli</i> (n=12)	S	6(50)	6(50)	5(41.7)	6(50)	6(50)	8(66.7)	3(25)
	I	3(25)	0(0)	1(8.3)	1(8.3)	1(8.3)	0(0)	0(0)
	R	3(25)	6(50)	6(50)	5(41.7)	5(41.7)	4(38.3)	9(75)
<i>K. pneumoniae</i> (n=3)	S	1(33.3)	3(100)	2(66.7)	2(66.7)	2(66.7)	0(0)	0(0)
	I	0(0)	0(0)	1(33.3)	1(33.3)	1(33.3)	3(10)	0(0)
	R	2(66.7)	0(0)	0(0)	0(0)	0(0)	0(0)	3(100)

Key: ASP: Antimicrobial susceptibility pattern, R: Resistant, I: Intermediate, S: Susceptible, CIP: Ciprofloxacin, CN: Gentamicin, AMC: Amoxicillin-clavulanic acid, CXM: Cefuroxime, CAZ: Ceftazidime, CFX: Cefixime, NF: Nitrofurantoin

Discussion

Identifying pathogenic microorganisms that are frequently found on inanimate objects and their antibiotic susceptibility profile is a crucial step in curtailing Hospital Acquired Infections and the spread of antibiotic-resistant pathogens.

HAIs are a major problem to health workers because they cause hindrance in the effective management of healthcare delivery in hospitals globally. HAIs in Africa ranges from 5.7% to 45.8% (Sepideh *et al.*, 2011). Findings from this research work showed that from the 50 swab samples collected, 48 (68.6%) were positive for bacterial growth. A high incidence of bacterial pathogens has been reported (Saka *et al.*, 2016). The type of hospital, the patient demographic, the kind of inanimate objects, and the surveillance method employed to detect the nosocomial infection all affect the prevalence of nosocomial infections (Muhammad *et al.*, 2023). There have been reports of nosocomial infections occurring often (Abubakar *et al.*, 2022; Muhammad *et al.*, 2023).

Gram-negative bacteria were more commonly isolated than Gram positive bacteria according to this study. In contrast, other research have indicated that Gram-positive bacteria are more common than Gram-negative bacteria (Saka *et al.*, 2016; Agu *et al.*, 2024). This inconsistency could be because of differences in the time of sampling, the techniques used in sampling, culturing methods, and variations in hospital wards. The most predominant contaminant was *Staphylococcus aureus* accounting for 41.7% of the

organisms isolated. Similar results have been reported (Saka *et al.*, 2016; Muhammad *et al.*, 2023; Agu *et al.*, 2024). *S. aureus* is known to exist as part of the normal microbiota, found in the skin and mucous membranes, and is regularly shed into the healthcare facility by patients and healthcare provider. Also, *S. aureus* prefers inanimate objects and are generally resistant to drying, heat and sodium chloride which allows it to survive on inanimate surfaces.

In this study, four (4) organisms were isolated from different inanimate surfaces. The bacteria isolated and their incidence was *S. aureus* (41.7%), *P. aeruginosa* (27%), *K. pneumoniae* (6.3%) and *E. coli* (25%). *S. aureus*, *P. aeruginosa* and *Klebsiella* spp are the most frequent pathogens in Africa isolated from hospital formites (Yallew *et al.*, 2019). A review conducted in Africa revealed *Klebsiella* spp, *S. aureus*, *P. aeruginosa* and *E. coli* as the most prevalent bacteria in HAIs (Irek, 2018). These pathogens have high resistance to antibiotics and are more resistant to sterilization and disinfection treatments. Due to their qualities, they frequently have larger prevalence rates than others (Yallew *et al.*, 2019).

The most contaminated surfaces were the children's locker, bedside floor and beds with a contamination rate of 20.8% each. Another study reported doorknobs as the most contaminated surface (Agu *et al.*, 2024). *S. aureus* was isolated on all the surfaces sampled except the bedside floor. This could be due its transmission from sources such as skin, nostrils and boils of healthcare personnel and patients

(Madebo *et al.*, 2022). *E. coli* was most isolated from the bedside floor which could be due to inadequate hygiene practices, high traffic and cleaning challenges. Paediatric environments have more opportunities of faecal organisms like *E. coli* to enter and persist in the environment. The high occurrence of *E. coli* and *P. aeruginosa* in this study could be attributed to their ability to adapt, giving them the advantage of being able to survive in various conditions across the hospital environment (Azimi *et al.*, 2019). *K. pneumoniae* was the least isolated. A study carried out in Ilorin revealed a high occurrence of *K. pneumoniae* (Saka *et al.*, 2016).

In this study, 75% of *S. aureus* isolates were sensitive to Ciprofloxacin, Cefixime (70%), Gentamicin (65%) and Cefuroxime (60%). Also 45% resistance to Ceftazidime was observed. For the *P. aeruginosa* isolates, highest level of resistance was observed with Amoxicillin-Clavulanic acid (61.5%) while for the *E. coli* highest level of resistance was observed with Ciprofloxacin (75%). *K. pneumoniae* isolates showed 100% resistance to Ciprofloxacin. Other studies have shown a much higher level of resistance (Ayalew *et al.*, 2019; Breijyeh *et al.*, 2020; Recalde-Reyes *et al.*, 2021; Agu *et al.*, 2024). Antibiotics are apparently taken in enormous numbers in hospital and community settings every year, which, among other factors, greatly adds to the increasing incidence of multi-drug resistant (Kraemer *et al.*, 2019). Resistance to antibiotics may develop because of antibiotic misuse in man and animals, as well as wrong and substandard prescriptions made by unqualified healthcare workers and poor diagnostic facilities (Aminu *et al.*, 2020).

Conclusion

The findings of this study revealed that there are bacteria contaminants of on inanimate surfaces in the Paediatric Ward at Aminu Kano Teaching Hospital. The organisms isolated were *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. coli*. This reiterates the fact that hospital acquired infections persist inside our hospital surroundings as documented in prior studies and inanimate surfaces function as receptacle for harmful micro-organism. This is evident in the isolation of pathogenic bacteria from inanimate surfaces in this investigation, so indicating that inanimate objects in paediatric wards are potential routes via which nosocomial diseases can spread among the paediatric population. The type of pathogens from this study is of great concern because of the immaturity of the immune system of the paediatric population. Cases of infection with disease causing organisms could lead to extended hospital stay and higher cost of therapy.

Conflict of Interest

The authors attest that they all agree to the entire research and that there are no conflicting interests in this research.

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