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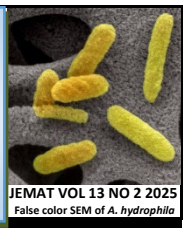

Antibacterial Activity of Disinfectants Against *Staphylococcus aureus* Isolated from Healthcare Equipment in Some Kano Metropolitan Hospitals

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Abstract

This study investigated the antibacterial activity of common hospital disinfectants, Dettol, Hypo, and Izal, against *Staphylococcus aureus* isolated from healthcare equipment in selected hospitals within Kano Metropolis, Nigeria. A total of 288 samples were collected from the hospital environment, including bed sheets, bed rails, toilet door handles, and Nurses' used gloves across Imam Wali General Hospital, Muhammad Abdullahi Wase Teaching Hospital, and Murtala Muhammad Specialist Hospital. Bacterial isolation and identification were conducted using standard microbiological methods, including culture on Mannitol Salt Agar and biochemical tests. Results showed that *S. aureus* was the predominant isolate (30.06%), followed by *E. coli* (27.27%), *Klebsiella pneumoniae* (22.02%), and *Pseudomonas aeruginosa* (8.39%). The antibacterial efficacy of the disinfectants was assessed using the disc diffusion method at varying concentrations (100%, 50%, 25%, and 12.5%). Statistical analysis (ANOVA, $p < 0.05$) revealed significant differences among the disinfectants. Hypo (sodium hypochlorite) demonstrated the highest mean zone of inhibition across all concentrations (21.26 mm at 100%), followed by Izal (18.06 mm) and Dettol (17.58 mm), while ethanol (control) exhibited the least activity (11.83 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results further confirmed Hypo's superior efficacy (MIC = 0.08 mL; MBC = 0.05 mL). These findings highlight Hypo as the most effective disinfectant against *S. aureus* isolated from hospital surfaces, likely due to its strong oxidative mechanism via hypochlorous acid production. The study underscores the importance of using highly effective disinfectants like Hypo in infection control protocols to reduce the risk of nosocomial infections and limit the spread of antimicrobial-resistant pathogens in healthcare facilities.

INTRODUCTION

A disinfectant is a chemical that kills or stops microorganisms from growing on surfaces that don't move. Disinfection doesn't always kill all microorganisms, especially bacterial spores that are resistant to it. Sterilization, on the other hand, is an extreme physical or chemical process that kills all types of life. Disinfectants are different from other types of antimicrobial agents, like antibiotics, which kill microorganisms inside the body, and antiseptics, which kill microorganisms on living tissue. Biocides and disinfectants are also not the same. Biocides are meant to kill all living things, not just microbes. Disinfectants, on the other hand, work by breaking down the cell wall of microbes or messing with their metabolism. It is also a way to get rid of germs, and it can be defined as the process of using physical or chemical methods to lower the number of harmful microorganisms on a surface [1]. Disinfectants can also kill germs on the skin and mucous membranes. In the past, the word "disinfectant" meant "to kill microbes" [2]. One way to compare disinfectants is to see how well they work against a disinfectant that is already known and give them a score based on that.

The "Phenol coefficient" is the name of the rating system that goes along with phenol. The disinfectant being tested is compared to phenol on a standard microbe, which is usually Salmonella typhi or *Staphylococcus aureus*. If a disinfectant works better than phenol, its coefficient is greater than 1. A coefficient of less than 1 means that something is less effective [2]. *Staphylococcus aureus* is a gram-positive bacterium that belongs to the Bacillus genus based on ribosomal RNA

sequences and grows in both aerobic and anaerobic environments, forming grape-like clusters. In humans, its habitats encompass the nasal membranes and the skin of warm-blooded animals, where it can induce a spectrum of infections, ranging from mild conditions like skin infections and food poisoning to severe illnesses such as pneumonia, sepsis, osteomyelitis, and infectious endocarditis. The organism produces toxins, and one of the toxins makes antibiotics less effective. *Staphylococcus aureus* is resistant to methicillin and many other antibiotics, including very strong beta-lactam drugs. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified as the primary etiological agent of nosocomial infections globally since the 1970s [3–6]. The strain MRSA is implicated as a contributing organism in the emergence of antibiotic resistance. It is well known in medicine that *Staphylococcus aureus* is resistant to antimicrobials. This is because the species has shown that it can evolve and become resistant, which makes it harder to treat with antibiotics [7].

Strains of methicillin-resistant *S. aureus* (MRSA) have acquired a gene that makes them resistant to almost all beta-lactam antibiotics. It is also common for MRSA that is linked to hospitals to be resistant to other antibiotics. These germs are very dangerous in hospitals, and it can be hard to find good treatment for them. Some places also have a lot of community-associated MRSA strains, which come from outside of hospitals. These organisms have generally been easier to treat, but some have moved into hospitals and are becoming more resistant to drugs other than beta-lactams. Sometimes, animals get MRSA from people. They may not show any symptoms, or they may get infections that take advantage of their weakened immune systems. The majority of MRSA identified in dogs and cats appears to be lineages linked to humans.

Colonization of dogs and cats is often temporary and usually happens at low levels. However, these organisms can be passed back to people, and pets may help keep MRSA alive in a home or facility. MRSA can also be a problem in places like veterinary hospitals, where the rates of carriage can be higher, especially when pets, horses, or other animals are sick [8–10]. However, emerging evidence shows that some *S. aureus* strains exhibit reduced susceptibility to commonly used disinfectants, potentially undermining infection control efforts [11]. Studying both the molecular profile and disinfectant susceptibility patterns of *S. aureus* can help identify high-risk strains with enhanced survival capabilities. Such insights are essential for refining disinfection protocols, preventing healthcare-associated infections, and curbing the spread of resistant clones within hospital environments [12–14]. Ultimately, this dual focus contributes to evidence-based policy formulation and strengthens antimicrobial stewardship programs.

Antimicrobial resistance is a major global health challenge, and hospitals serve as key players in combating this growing threat [15]. To mitigate the spread of resistant pathogens, healthcare facilities must adopt stringent infection control strategies, including rigorous hand hygiene practices, sterilization protocols, and robust surveillance systems to monitor resistance trends [16,17,12]. Antimicrobial resistance stands as one of the most critical challenges to global public health [11, 13-17].

MATERIALS AND METHODS

Study site

The samples used for the study were obtained from hospital bed sheets, bed rails, toilet door handles and nurses' used hand gloves from Imam Wali General Hospital, Muhammad Abdullahi Wase Teaching Hospital, and Murtala Muhammad Specialist Hospital Kano, while the isolation and bacterial susceptibility to disinfectants was conducted at the microbiology laboratory of Murtala Muhammad Specialist Hospital Kano.

Ethical approval for the study

The ethical clearance of this study was obtained from the ethical committee of the Kano state ministry of health with reference number **NHREC/17/03/2018**

Samples collection

Two hundred eighty-eight (288) samples were collected from patients' bed sheets, bed rails, toilet door handles and Nurses used hand gloves using a sterile swab stick and transported to the laboratory using conventional microbiological procedures then processed for bacterial isolation and identification according to methods described [18].

Bacterial isolation and identification

Isolation of bacteria was conducted according to the method of [19] where the samples transported to the laboratory are inoculated on different agar plates containing Mannitol salt agar (MSA) and nutrient agar to incubate at 37 ºC for 24 hours to observe for microbial growth. After incubation, the colonies formed were Gram-stained, followed by subsequent biochemical reactions like catalase, coagulase, oxidase, citrate, indole, and urease to confirm for the presence of the bacteria.

Disc preparation and disinfectant dilutions

Whatman number one filter paper was used for disc preparation to be used for the antimicrobial
disc diffusion method, where 6 millimeter discs are formed using a paper punch. The discs were sterilized by dry heat at 160 °C for one h and allowed to cool in a sterile, covered dish. Liquid disinfectants used for cleaning the hospital facilities, which are Dettol (Reckitt Benckiser brand), Hypo (Tolaram Africa brand), and Izal (Bellshaw brand) were prepared by dilution with sterile water using different dilution concentrations [20].

Table 1. Antimicrobial (disinfectant) disc concentrations.

Disinfectant (%)	Volume of stock (mL)	Ethanol disinfectant (control)	Dettol	Hypo	Izal
100	10	7000 ug/disc	480ug/disc	350 ug/disc	400 ug/disc
50	5.0	3500 ug/disc	240 ug/disc	175 ug/disc	200 ug/disc
25	2.5	1750 ug/disc	120 ug/disc	87.5 ug/disc	100 ug/disc
12.5	1.25	875 ug/disc	60 ug/disc	43.75 ug/disc	50 ug/disc

RESULTS NAD DSICUSSION

The bacterial culture results indicated that of the 286 total samples, 35 (12.23%) exhibited no microbial growth, while the remaining samples displayed varying degrees of growth on both nutrient agar (NA) and mannitol salt agar (MSA). The hand gloves used by the nurses (NHG) had the most contamination, with 62 (21.67%) colonies on NA and 34 (11.88%) on MSA.

The results probably indicate that insufficient glove-changing protocols could be a significant reason that facilitates the transmission of microorganisms among healthcare personnel and patients. This aligns with prior results that demonstrate the evidence that gloves can serve as potential reservoirs for hospital pathogens if not routinely replaced or sanitized [21]. Similar studies have found the same levels of contamination. For example, Visalachy et al. [22] found that 53.3% of healthcare workers' hand surfaces and gloves harbored numerous multidrug-resistant bacteria. This shows how important it is to use gloves correctly to stop the spread of infections in hospitals [22]. Abdullah and Mahmood [23] also found that *Staphylococcus aureus* often contaminated hospital surfaces and instruments when grown on MSA. They suggested that the contamination does increase the risk of cross-infection in clinical settings [23]. The results obtained in this study indicate that the increased growth observed on nutrient agar in this study correlates with the extensive nutritional support that nutrient agar offers to both Gram-positive and Gram-negative bacteria, whereas the selective growth on MSA primarily signifies the presence of Staphylococcus species.

In a comparable study conducted in Ethiopia, Firesbhat et al. [24] discovered that over 60% of high-touch hospital surfaces tested positive for cultures on MSA, primarily *S. aureus* and *S. epidermidis*. This underscores the critical importance of surface disinfection and personal hygiene in clinical settings. In another study, similar bacterial loads on mobile phone devices utilized by healthcare workers were documented, which underscores that sources of contamination beyond gloves may similarly facilitate hospital-acquired infections [25].

Table 2. Microbial growth observed.

Samples	Number grown on NA (%)	Number grown on MSA (%)
PBR	32 (11.18)	18 (6.29)
PBS	47 (16.43)	21 (7.34)
NHG	62 (21.67))	34 (11.88)
TDH	24 (8.39)	13 (4.54)
Total	165 (57.69)	86 (30.06)

NA = nutrient agar
MSA = mannitol salt agar

Staphylococcus aureus made up 30.06% of all isolates, making it the most common contaminant on hospital surfaces (**Table 3**). This species' high prevalence is linked to it being a natural part of the normal skin flora. In addition, it can live for a long time on dry, nutrient-poor surfaces. It is likely that patients and healthcare workers coming into contact with each other are probably the source of the bacterial spread throughout the hospital environment [26]. This premise is also reported by Odoyo et al. (2023), who found that *S. aureus* was the most common bacterial isolate from hospital wards in Kenya. They noted that it is tough and can form biofilms on surfaces that are often touched [27]. Another study shows that *S. aureus* was still the main surface contaminant in both intensive care and general hospital units, suggesting how prevalent this species is in spreading disease in hospitals. *E. coli* (27.27%), *K. pneumoniae* (22.02%), and *P. aeruginosa* (8.39%) were isolated less often, which shows that they are not as good at living in dry places.

Lordelo et al. [28] also pointed out that although *K. pneumoniae* and *P. aeruginosa* can adhere to surfaces through the formation of biofilms, their survivability is poorer than *S. aureus*. These results provide evidence that inadequate glove hygiene and poor surface cleaning and disinfection are the major sources of microbial contamination in the hospital environment. It is still important to have strict infection control policies, such as changing gloves often, teaching people how to wash their hands, and doing microbiological surveillance on a regular basis to stop cross-contamination.

Susceptibility patterns to disinfectants

From the susceptibility patterns of the isolated bacteria to disinfectants in **Table 4** below, it shows that Hypo is the most effective disinfectant for hospital isolated bacteria due to its strong oxidative power, which damages bacterial cell components through hypochlorous acid (HOCl), leading to cell death. It works by oxidizing fatty acids and amino acids, disrupting cell membranes and essential proteins, and reducing the viability of bacteria on surfaces. This broad-spectrum germicidal action, combined with its low cost, makes it a preferred choice in healthcare for disinfecting surfaces and preventing infections.

Table 4. Susceptibility patterns of the isolated bacteria to disinfectants.

Disinfectants	Disc concentrations			
Ethanol (control)	7000 ug/disc	3500 ug/disc	1750 ug/disc	875 ug/disc
susceptibilities	08S, 48I, 30R	2S, 48I, 36R	08I, 78R	86R
Dettol	480 ug/disc	240 ug/disc	120 ug/disc	60 ug/disc
susceptibilities	72S, 4I, 10R	41S, 38I, 07R	06S, 48I, 32R	10S, 07I, 69R
Hypo	350 ug/disc	175 ug/disc	87.5 ug/disc	43.75 ug/disc
susceptibilities	76S, 10R	82S, 04R	02S, 81I, 03R	27S, 25I, 34R
Izal susceptibilities	400 ug/disc	200 ug/disc	100 ug/disc	50 ug/disc
	76S, 1I, 09R	68S, 14I, 04R	79I, 07R	15S, 71R

S = Susceptible (≥ 16mm)
I = Intermediate (11-15mm)
R = Resistant (≤ 10mm)

Table 3. Cultural characteristics and Biochemical reactions observed.

Test	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Eschericia coli*
Cultural characteristics on nutrient agar	Forms rough circular colonies with shiny yellow colour	Forms smooth greenish colonies with grape-like odour	Forms white crystalline mucoid colonies	Forms smooth white and thick colonies
Subculture	Forms golden yellow colonies on MSA and B-haemolysis on blood agar	Forms white colonies on mackonkey	Forms white mucoid colonies on mackonkey	Forms red small colonies on mackonkey
Gram staining	+	-	-	-
Shape	Spherical cells under microscope, some in clusters and few are scattered	Rod-like cells occurring in clusters	Large rod-like cells which are scattered	Small rod-like cells which are scattered
Catalase	+	+	+	+
Coagulase	+	-	-	-
Oxidase	-	+	-	-
Citrate	+	-	+	-
Indole	-	-	-	+
Urease	-	-	+	-
Total	86 (30.06%)	24 (8.39%)	63 (22.02%)	78 (27.27%)

The antimicrobial efficacy tests shown in **Tables 5–9** show that the four disinfectants exhibit different effects on the bacterium. Hypo had the biggest mean inhibition zone (21.26 mm) at 100% stock concentration (**Table 5**). Izal (18.06 mm) and Dettol (17.58 mm) followed next, and ethanol (11.83 mm) exhibited the weakest effect. A similar trend at lower concentrations was observed (**Tables 6–8**), in which the inhibitory potency lessens in direct proportion to the disinfectant concentration. This confirmed that the activity was concentration-dependent. One-way ANOVA statistical analysis showed that there was a big difference between all the treatments ($p < 0.05$).

Based on the Tukey post hoc test, Hypo was found to work much better than Dettol, Izal, and Ethanol, while ethanol worked much worse than all the others. The MIC and MBC data (**Table 9**) support these results. Hypo demonstrated the lowest MIC (0.08 mL) and MBC (0.05 mL), indicating that it is the most effective at killing the bacterium. Similar results by prior research also show that sodium hypochlorite exhibits better efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in contrast to the phenolic-based disinfectants such as Dettol and Izal [2]. It is known that chlorine-based disinfectants are the best antiseptics for hospitals [1].

Table 5. Mean zones of inhibition at 100% stock disinfectants.

Disinfectant	Concentration (ug/disc)	Mean zones of inhibition (mm)	Standard Deviation (mm)
Ethanol (control)	7000	11.83	3.31
Dettol	480	17.58	4.90
Hypo	350	21.26	4.64
Izal	400	18.06	5.14

Table 6. Mean zones of inhibition at 50% stock disinfectants.

Disinfectant	Concentration (ug/disc)	Mean zones of inhibition (mm)	Standard Deviation (mm)
Ethanol (control)	3500	12.28	3.30
Dettol	240	15.79	3.78
Hypo	175	17.79	2.70
Izal	200	16.56	2.90

Table 7. Mean zones of inhibition at 25% stock disinfectants.

Disinfectant	Concentration (ug/disc)	Mean zones of inhibition (mm)	Standard Deviation (mm)
Ethanol (control)	1750	9.10	1.90
Dettol	120	12.48	3.38
Hypo	87.5	14.45	1.28
Izal	100	12.71	2.06

Table 8. Mean zones of inhibition at 12.5% stock disinfectants.

Disinfectant	Concentration (ug/disc)	Mean zones of inhibition (mm)	Standard Deviation (mm)
Ethanol (control)	875	7.12	1.28
Dettol	60	7.83	1.66
Hypo	43.75	10.02	1.28
Izal	25	8.80	1.15

Table 9. MIC and MBC values for disinfectants used.

Disinfectant dilutions	Ethanol (mL)	Dettol (mL)	Hypo (mL)	Izal (mL)
MIC	1.25	0.3125	0.08	0.156
MBC	10	10	05	2.5

CONCLUSION

The statistical analysis supports the notion that the disinfectant Hypo is the most efficacious disinfectant at all tested concentrations against the hospital isolates. Sodium hypochlorite exhibits superior antimicrobial activity, and the chemical, being the active ingredient in this product, is a well-known broad-spectrum biocide. The mechanism of sodium hypochlorite cell killing is through its strong action of oxidizing parts of cells, denaturing proteins, making enzymes stop working, and the abrupt cessation of DNA and RNA synthesis. This stops microbes from replicating and transcribing. It also damages the structure of the cell membrane, which causes the contents of the cell to leak out and eventually kill the cell. These mechanisms work together to make Hypo very effective at killing bacteria, which is why it is so good for disinfecting hospital surfaces where getting rid of germs quickly is very important. This discovery corresponds to previous findings that demonstrated the enhanced disinfectant efficacy of chlorine-based compounds over phenolic and alcohol-based agents in clinical environments.

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