African Journal of
Biochemistry and Molecular Biology Research

ISSN : 3434-6120

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJBMBR.v1i1.3353

PREVALENCE AND ANTIBIOTIC RESISTANCE OF STAPHYLOCOCCUS SPECIES ON TOILET SEATS IN FEDERAL UNIVERSITY WUKARI, TARABA STATE, NORTH-EASTHERN NIGERIA

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Jun 12, 2024	Jun 25, 2024	Jul 10, 2024	Jul 15, 2024

Abstract

Staphylococcus species are commensal bacteria and a major human pathogen that causes a wide variety of clinical manifestations. The most challenging feature of the Staphylococcus species bacterium is its rapid dissemination to humans and through toilet seats. The aim of this research is to assess for the presence of Staphylococcus species. A total of 20 toilet seat swab samples were obtained. The isolates were identified using biochemical techniques and were confirmed using microgen identification kits. A total of 16 (80%) isolates were identified as Staphylococcus species and 14 (87.5%) were identified as Staphylococcus aureus and 2(12.5%) as Staphylococcus epidermis. The antibiotic susceptibility test was carried out using Kirby Bauer antibiotic disk method. The prevalence of Staphylococcus species was 87.5% and 12.5% of Staphylococcus aureus and Staphylococcus epidermidis respectively. Most of the isolates were resistant to Ceftazidime, Erythromycin, Gentamicin, Levofloxacin, and Azithromycin due to the over use of the drug making most organism develop mechanism of resistance and acquiring resistance against them. Rifampin was more effective to Staphylococcus aureus in this research. The multiple antibiotic indices indicates that 87.5% of 87.5%, 12.5% of

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Staphylococcus aureus and Staphylococcus epidermidis and 12.5% Staphylococcus epidermidis had multiple antibiotic index greater 0.20. The presence of multi -drug resistant Staphylococcus species in this research emphasizes the need to formulate hygiene measures to prevent possible dissemination of Staphylococcus species and other transmissible pathogens to students and staff in the university.

Keywords: Pathogen, Opportunistic, Antibiotic, Resistant, Surface

INTRODUCTION

Microorganisms are present everywhere and play a significant role in the ecology. They can exist freely or as parasites in these conditions. Sometimes they exist as temporary contaminants in hands or fomites, where they pose a serious health danger by spreading infections that can be acquired in the community or in hospitals (Jabłonska-Trypuc *et al*., 2022). Some bacteria have been discovered to have a connection with environmental surfaces like door handles, toilet surfaces, stair case etc (Odigie *et al*., 2017).

Infections and/or diseases gotten by contact with environmental surfaces are common cold and sores, conjunctivitis, giardiasis, diarrhoea, impetigo, meningitis, pneumonia etc. These diseases are caused by a myriad of bacterial organisms (Krautkramer *et al.,* 2021). *Staphylococcus aureus* a common micro-organism is one of the major and leading cause of human bacterial infections due to its wide range of habitat. It became a public health concern when scientist discovered it is easily transmitted either from inanimate objects or animals (Tong *et al*., 2015).

Staphylococcus species are generally opportunistic pathogens or commensals resident on host skin and mucosae in animals and humans. Staphylococci from carriage sites can spread and be transmitted into the environment where they are able to survive for a long time (Kozajda *et al*., 2019). Staphylococci that are commensals may act as pathogens if they succeed in entering the host by several mechanisms, such as skin trauma, inoculation, device implantation, both in immunocompromised patients, and in all those showing an altered microbiota (Sabate *et al*., 2017).

The bacterium has a variety of habitat including environmental surfaces like classroom and toilet seats, door handles, tissue and blood stream, in nasal nares of domestic animals like cats, dogs and on human body surfaces as a part of normal microflora (Otto, 2014).

Staphylococcus aureus is a significant human pathogen that causes a wide variety of clinical infections. It is the leading cause of endocarditis as wells as skin and soft tissue, osteoarticular, pleuropulmonary and device related infection (Peton and Le Loir, 2014).

Infections with *Staphylococcus aureus* is frequent in both community-acquired settings and hospital-acquired settings. *Staphylococcus aureus* colonizes about 30% of the population of people (Tong *et al.,* 2015). Little is known about the reservoirs or contexts through which community members are exposed to *S. aureus*, with the exception of breakouts in high-risk settings. A variety of virulence factors that the Gram-positive bacteria possesses help the host become infected (Sharma *et al*., 2017). *Staphylococcus aureus* expresses a variety of virulence factors that promote tissue adhesion and aid in the establishment of infection, tissue invasion and evasion from host immune response (Kobayashi *et al*., 2015).

The organism's capacity to develop resistance to several antibiotic classes is well documented. Beta-lactam antibiotics are the first line treatment for staphylococcal infections. For many years, vancomycin was the antibiotic of choice, but the advent of resistance put a question mark on its usefulness. The emergence of antibiotic resistance has emerged as a major global problem, and *S. aureus* has developed resistance due to the selection pressure anti-microbials place on it. The resistance is mediated by chromosomes or plasmids, and transduction, transformation, and conjugation are blamed for it (Sultan *et al.,* 2018).

Therefore, the aim of the research is assessed toilet seats in Federal University Wukari and the outcome is expected to contribute significantly about information on prevalence and antibiotic resistance of *Staphylococcus* species on toilet seats.

MATERIAL AND METHODS

Study Area

The study area was Federal University Wukari. Founded in 2011, the Federal University Wukari is a public higher education institution located in Wukari with the population range of 50,000-249,999 inhabitants. Federal University, Wukari also provides several academic and non-academic facilities and services to students including a library, as well as administrative services. *Staphylococcus* species is also skin-surface commensal

microorganisms making it possible for it to be transmitted easily with the help of fomites like toilet seats.

Collection of Samples

Samples were obtained from toilet seats of Federal University Wukari campus. Toilet seats were swabbed using a sterile, cotton-tipped applicator (swab stick) moistened with normal saline. Each swab stick was labelled properly according to the rooms they were gotten from. The swab sticks were then transported to the Microbiology laboratory aseptically for identification and microbial analysis within 1-2 hours of sampling.

Isolation and Identification of *Staphylococcus* species

The sample on the swab stick was inoculated into prepared sterile bacteriological peptone water
and incubated at 37°C for 24 hours for enrichment after which the turbid broth was sub cultured on differential media such as Mannitol salt agar. Discrete colonies were further sub cultured onto fresh prepared plates of the mannitol salt agar plates to obtain pure
cultures. Colonies with distinct morphological traits were identified by Gram staining technique and some biochemical tests such as catalase, oxidase, coagulase, citrate utilisation, haemolysis, indole, motility, methyl red, nitrate reduction, urease, Voges Proskauer, hydrogen sulphide, mannitol, and DNase. The isolates were further confirmed using Microgen STAPH identification kits from Microgen Bioproducts Ltd. The identified isolates were inoculated on freshly prepared nutrient agar for antibiotic sensitivity test.

Standardization of the Isolates (McFarland Standard)

McFarland Standards was used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. The McFarland Standard was mixed on a vortex mixture prior to examination. A test suspension was prepared by obtaining a fresh, pure culture of the test organism and inoculated in a suitable broth. In the presence of good lighting, the turbidity of test suspension was visually compared with that of the McFarland standard by comparing the clarity of the lines on a Wickerham card to obtain a turbidity that matches that of the standard (Enemali and Yilkahan, 2021)

Antibiotic Susceptibility test

Antimicrobial susceptibility testing was done using of Kirby Bauer disk diffusion method. The antibiotics used in this study include Rifampicin (RD) 20 µg, Ceftazidime (CTZ) 30 µg, Erythromycin (E) 30 µg, Gentamicin (CN) 10 µg, Cefuroxime (CEF) 30 µg, Ciprofloxacin (CPX) 10 µg, Levofloxacin (LEV) 20 µg, Azithromycin (AZM) 10 µg, Amoxil (AMX) 20 µg, Streptomycin (S) 30 µg. A loopful of the organism was emulsified in 5mls of sterile normal saline and mix well; the turbidity was compared to 0.5 Mac Farland standard. A sterile inoculating loop was used to inoculate the 18-24 hours old bacterial culture into 5 mL normal saline. Sterile swab sticks were then used to spread the suspension into already prepared Muller Hilton plates. The commercially prepared antibiotic disc was placed on the inoculated Agar plates using sterile forceps and incubated at 35-37 ºC for 18-24 hours after which the zone of inhibition for each antibiotic was measured using a meter rule in millimetre (mm) and interpreted with Clinical Laboratory Standards Institute (CLSI) 2023 guidelines. The reporting was done by indicating Resistant, Intermediate or Sensitive.

Multiple Antibiotics Resistance (MAR) Indexing.

Multiple antibiotics resistance index (MAR) was calculated for each *Staphylococcus* isolate recovered, after subjecting them to a panel of ten antibiotics. The formula used was x/y where 'x' represents the number of antibiotics to which the isolate is resistant, and y represents the total number of antibiotics to which the isolate was exposed (Okafor and Nweze, 2020). The *Staphylococcus* isolates resistant to three or more antibiotics were classified as multiple antibiotics resistant (MAR) isolates.

RESULTS

The identification of *Staphylococcus* species using biochemical characterization of the isolates are shown in Table 1 and indicated that 2 species of *Staphylococcus* were identified. The total prevalence rate of the two pathogens from this research was 80%. Of the 2 isolates, 87.5% $\left[\frac{14}{16}\right]$ and 12.5% $\left[\frac{2}{16}\right]$ were *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively as presented in Figure 1.

Antibiotic susceptibility test showed 16 (100%) of the *Staphylococcus* species isolates to be resistant to Ceftazidime. However, only 13 (81.3%) isolates, each, were resistant to Gentamicin, Ceftazidime, Erythromycin, Ciprofloxacin, Levofloxacin, Azithromycin,

Amoxil, Gentamicin and Streptomycin while 3 (18.5%) isolates, each, were resistant to Ceftazidime and Cefuroxime (Table 2).

About 15 (93.8%) *Staphylococcus* species isolates were seen to be multi-resistant as they were resistant to more than one of the 10 antibiotics used in this study. Based on the resistance profile; *Staphylococcus* species were shown to be multi-drug resistant.

Table 1: Phenotypic Characterization of *Staphylococcus* species

S/N	Gram reaction	Motility	Catalase	Oxidase	Urease	Coagulase	CUT	Haemolysis	Indole	DNase	MR	NR	VP	H_2S	Mannitol	Inference
1	+	-	+	-	+	+	+	+	-	+	+	+	+	-	+	*S. aureus*
2	+	-	+	-	+	-	-	-	-	-	-	+	+	+	-	*S. epidermidis*

KEY: CUT = Citrate utilisation test, MR = Methyl red, NR = Nitrate reduction, VP = Voges Proskauer, H_2S = Hydrogen sulphide

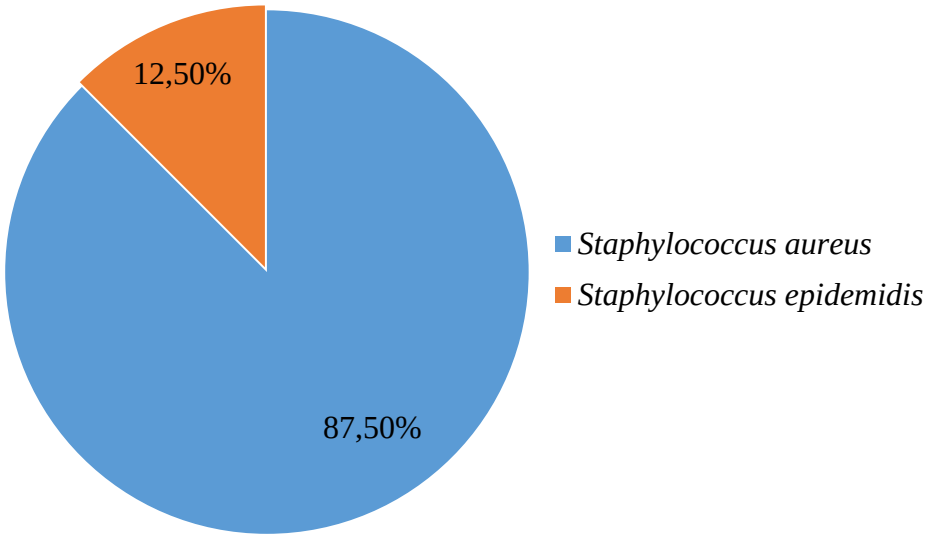


Fig. 1: Percentage Prevalence of Isolates Distributed on Toilet Seats

It is shown in this study that *S. aureus* is more prevalence than *S. epidermidis* and also the 10 isolates that showed most resistance was also *S. aureus*. The antibiotic resistance pattern of the isolates as presented in Table 3 showed that there was much discrepancy and wide range in resistance of *Staphylococcus* species to the antibiotic selected for the research.

The total multiple antibiotic resistant (MAR) index of the *Staphylococcus* species indicates that 87.5% $\left[\frac{14}{16}\right]$, 12.5% $\left[\frac{2}{16}\right]$ of *Staphylococcus aureus* and *Staphylococcus epidermidis* respectively had MAR index above 0.20 significant level of MAR of resistance pathogens as presented in Table 4.

Table 2: Antibiotic Susceptibility of the Isolates from Toilet Seats

		Staphylococcus aureus (n=14)			*Staphylococcus epidermidis* (n=2)		
S/No	Antibiotics (µg)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
1	Rifampicin (20)	0(0)	1(7.1)	13(92.9)	0(0)	0(0)	2(100)
2	Ceftazidime (30)	14(100)	0(0)	0(0)	2(100)	0(0)	0(0)
3	Streptomycin (30)	12(85.7)	0(0)	1(7.1)	1(50)	1(50)	0(0)
4	Azithromycin (10)	13(92.9)	0(0)	0(0)	2(100)	0(0)	0(0)
5	Amoxil (20)	13(92.9)	0(0)	1(7.1)	2(100)	0(0)	0(0)
6	Ciprofloxacin (10)	13(92.9)	0(0)	0(0)	2(100)	0(0)	0(0)
7	Erythromycin (30)	13(92.9)	0(0)	1(7.1)	2(100)	0(0)	0(0)
8	Levofloxacin (20)	13(92.9)	0(0)	0(0)	2(100)	0(0)	0(0)
9	Gentamicin (10)	13(92.9)	0(0)	0(0)	2(100)	0(0)	0(0)
10	Cefuroxime (30)	14(100)	0(0)	0(0)	1(50)	1(50)	0(0)

Table 3: Antibiotic Resistance Pattern of the Isolates from Toilet Seats

S/No	*Staphylococcus aureus*		S/No	*Staphylococcus epidermidis*	
	Resistance pattern	F		Resistance pattern	F
1	AMX, CPX, E, CN, CEF	1	1	CTZ, S, AMX, CPX, E, CN	1
2	CTZ, S, AZM, AMX, E, CN, CEF	1	2	CTZ, S, AZM, AMX, CPX, E, CN, CEF	1
3	CTZ, S, AZM, AMX, CPX, E, CEF	1			
4	CTZ, AZM, AMX, CPX, E, CN, CEF	1			
5	CTZ, S, AZM, AMX, CPX, E, CN, CEF	10			

KEY: F=Frequency, RD=Rifampicin, CTZ= Ceftazidime, E=Erythromycin, CN=Gentamicin, CEF=Cefuroxime, CPX=Ciprofloxacin, LEV=Levofloxacin, AZM=Azithromycin, AMX=Amoxil, S=Streptomycin.

Table 4: Multiple Antibiotic Resistant (MAR) Indices of the Isolates of *Staphylococcus* species

Organism	No. of multidrug resistant isolates	Classes of antibiotics to which isolates were resistant to	MAR index	Total MAR index of isolates greater than 0.20 significant value (%)
	1	5	0.5	7.1
	1	3	0.3	7.1
S. aureus	1	5	0.5	7.1
	1	5	0.5	7.1
	10	5	0.5	7.1
S. epidermidis	1	5	0.5	50.0
	1	5	0.5	50.0

DISCUSSION

The high prevalence of the *Staphylococcus* species from toilet seats swabbed in this research might be as a result of inadequate hygiene practices, cleaning and contact with contaminated hands and this could be one of the attributing factors of the distribution of the pathogen in toilet surfaces as reported earlier by Jaradat *et al.* (2020).

Staphylococcus bacteria are common skin inhabitants, and their presence on surfaces can transferred through direct contact. The prevalence rate in this research is lower compared to the one reported by Akinrotoye *et al.* (2019); they isolated 42 (50.60%) *S. aureus* out of the 300 door handles (including toilets) swabbed samples that were obtained from secondary schools in Abeokuta and its environs. This result is also similar another study by Sampson *et al.* (2020), on bacteriological assessment of toilet seats in a Nigerian University, where they isolated 32.5% *Staphylococcus* species out of the 37 isolates.

The high prevalence of *S. aureus* compared to *S. epidermidis* in this research may be due to the fact that *S. aureus* is a more common inhabitant of human skin compared to *S. epidermidis* (Byrd *et al.*, 2018). As people use toilet seats, skin shedding can lead to the deposition of bacteria, and if more individuals carry *S. aureus*, it may be more prevalent. Also, *S. aureus* tends to have more virulence factors that allow it to survive and persist in various environments, potentially making it more resilient on surfaces like toilet seats (Gehrke, *et al.*, 2023).

Staphylococcus aureus is the leading cause of bacterial infections, causing a wide range of diseases such as bacteraemia, wound infection, septicaemia, and pneumonia; but when it has not acquired the resistant gene (just the normal *S. aureus*), it is an important human-friendly microorganism which is often found as commensal on body surface but its transfer to other parts of the human body may lead to a wide variety of infections (Dayan *et al.*, 2016).

The antibiotic resistance in *Staphylococcus* species isolated from toilet seats in this research may be due to the overuse or misuse of antibiotics as individuals carrying antibiotic-resistant *Staphylococcus* strains use the toilet and shed these bacteria on to the seat, the resistant strains can persist and potentially spread (Jaradat *et al.*, 2020). Additionally, environmental factors, such as exposure to residual antibiotics or disinfectants, may contribute to the development of antibiotic resistance in bacteria on toilet seats (Wang *et al.*, 2023).

The multiple antibiotic resistance (MAR) indices give an indirect suggestion of the probable source(s) of the organism. The MAR indices in this research work were greater than 0.20, this confirms the report of Hammuel *et al.* (2014) that the MAR index greater than 0.20 indicates that the organisms must have been originated from an environment where
antibiotics are often used (Hammuel *et al.,* 2014). Thus, the result of the multiple antibiotic indexes in this research work can be reported that these pathogens might have been originated where these antibiotics are used.

As it is shown in this research, the multidrug resistance of *Staphylococcus aureus* from toilet seats was 93.75%, and was higher than 19.1% multidrug resistance of *S. aureus* as reported by Saka (2014). This finding corroborates the report of Guo *et al.* (2020) that among the Gram-positive microorganisms, staphylococci are the most frequently resistance pathogen to antibiotics.

CONCLUSION

The occurrence of *Staphylococcus aureus* on toilet seats was higher than *Staphylococcus epidermidis*. *Staphylococcus aureus* may pose serious health challenges; this is because it is one of the pathogenic strains which can infect both animal and human population. *Staphylococcus aureus* can pose a threat to the health of both students and staff within the

university community. The widespread use of antimicrobials, especially over- or inappropriate use of antibiotics, has contributed to an increased incidence of antimicrobial-resistant organisms or multidrug resistant microorganisms even on inanimate objects.

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