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Sterility Studies and Isolation of Bacteria from Vaccine Carriers used in Primary Health Care Centres in Awka, Anambra State

Okoli, F. A[1]., Anieto, E.C[2]., Anazodo C.A[1]., Awari V.G.[3], Okoye K.C[6]., Ebo, P.U. [3], Ogbunude, A.P. [4], Chidozie, C.P. [5], Okoye C. V. [1], Orji, C.C[1]., Obi Z.C[1].

[1]Applied Microbiology Department, Nnamdi Azikiwe University, PMB 5025, Awka
[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University on the Niger, KM-13, Onitsha-Enugu Express Way Umunya, Nigeria
[3]Depertment of Microbiology, Tansian University, Umunya, Anambra, Nigeria.
[4]Department of Public Health, School of Health and Life Sciences, Teeside University, Middlesbrough, UK
[5]Department of Paediatrics, Nnamdi Azikiwe University Teaching Hospital, Nnewi
[6]Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria Nsukka

Abstract- This study investigated the sterility of vaccine carriers used in selected Primary Health Care (PHC) centres in Awka, Anambra State, with the aim of assessing possible bacterial contamination and determining the antimicrobial susceptibility patterns of isolated organisms. Vaccine carriers are critical components of the cold chain system, ensuring that vaccines remain potent and effective during transportation and storage. However, poor handling, inadequate cleaning, and improper maintenance can compromise their sterility, thereby posing risks to public health. Samples collected from vaccine carriers were subjected to microbiological analysis. The isolates obtained included Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa. Antimicrobial susceptibility testing revealed varying sensitivity patterns: S. aureus showed susceptibility to ciprofloxacin (15.2 mm), streptomycin (14.3 mm), levofloxacin (12.7 mm), rifampicin (12.2 mm), and gentamicin (11.8 mm). S. epidermidis was sensitive to chloramphenicol (15.8 mm), ciprofloxacin (14.3 mm), gentamicin (12.8 mm), rifampicin (11.5 mm), and ampiclox (10.1 mm). P. aeruginosa demonstrated susceptibility to augmentin (14.5 mm), ofloxacin (13.5 mm), and cefuroxime (12.7 mm). The presence of these organisms indicates that vaccine carriers, if not adequately sterilized, can serve as reservoirs for pathogenic bacteria capable of causing nosocomial and community-acquired infections. The results highlight the urgent need for strict adherence to cleaning protocols, regular training of health workers, and periodic sterility checks on vaccine carriers. Strengthening these practices will help safeguard vaccine integrity, maintain public confidence in immunization programs, and enhance overall disease prevention efforts.

Keywords- Sterility, Bacteria, Vaccine Carriers, Primary Health Care Centres, Anambra

I. INTRODUCTION

Vaccines have been one of the most effective tools in modern public health for the prevention and control of infectious diseases. Proper vaccine storage and handling are important factors in preventing and eradicating vaccine-preventable diseases. Yet, the United States Centers for Disease Control (CDC) (2021) reports that each year, storage and handling errors result in revaccination of many

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patients and significant financial loss due to wasted vaccines. Failure to store and handle vaccines properly can reduce vaccine potency, resulting in inadequate immune responses in patients and poor protection against disease. The delivery of vaccines, particularly in low- and middle-income countries like Nigeria, depends heavily on the integrity of the cold chain system (Babatunde et al., 2020).

The cold chain system is a temperature-controlled supply chain used to store, transport, and distribute vaccines and other perishable medical products at recommended temperatures from the point of manufacture to the point of administration (Traynor, 2022). Components of the cold chain system include cold rooms (walk-in refrigerators and freezers), refrigerators and freezers for storage at health facilities, vaccine carriers and cold boxes for short-term transportation, temperature monitoring devices (e.g., thermometers, data loggers), transportation equipment (vehicles and insulated containers), and trained personnel for handling and managing the system.

A crucial component of this system is the vaccine carrier—an insulated container designed to maintain vaccines at appropriate temperatures during transportation and outreach immunization activities, especially in remote or rural areas (Centre for Disease Control and Prevention, 2024). While much attention is often paid to temperature control, an equally critical but often overlooked aspect is the sterility of the vaccine carriers. Microorganisms are ubiquitous in the environment and can easily contaminate surfaces and equipment used in healthcare settings (Awari et al., 2023). This study therefore focuses on assessing the sterility status of vaccine carriers used in primary health care (PHC) centres in Awka, the capital city of Anambra State, Nigeria.

In many healthcare settings, vaccine carriers are reused regularly and are exposed to various environmental and handling conditions that may compromise their sterility. If not properly cleaned, disinfected, and maintained, these carriers could become a potential source of microbial contamination, posing a serious risk of vaccine degradation or cross-infection during immunization sessions. Studies have shown that pathogenic microorganisms can persist in different environments, including hospital settings and community sources, contributing to increased risk of infection (Awari et al., 2024; Umeoduagu et al., 2023). Contaminated carriers may compromise the safety of the vaccines stored within them, potentially leading to adverse health outcomes among vaccine recipients, including children and immunocompromised individuals.

The issue of sterility is of particular concern in primary health care centres, which serve as the first point of contact for healthcare delivery in many Nigerian communities. These centres often face resource constraints, including limited access to sanitation supplies, inadequate staff training on infection prevention and control, and high workload burdens. Evidence from environmental and public health studies indicates that microbial contamination is common in water sources, food products, and healthcare-related environments, especially where hygiene practices are suboptimal (Egurefa et al., 2024; Ezeokoli et al., 2023; Ezenwelu et al., 2024a; Ezenwelu et al., 2024b). As such, maintaining strict hygiene protocols for medical equipment, including vaccine carriers, may not always be prioritized.

Furthermore, personal and frequently used items have been identified as reservoirs of potentially pathogenic microorganisms, highlighting the ease with which contamination can occur through routine handling (Okafor et al., 2016). Fungal and bacterial

contamination of commonly used materials in academic and community settings has also been documented, reinforcing the need for strict hygiene and monitoring (Obianom et al., 2023). Additionally, studies on antimicrobial resistance have shown that microorganisms isolated from various environments may exhibit resistance to commonly used drugs, thereby increasing the risk associated with contamination (Obasi et al., 2024).

Despite the critical role of vaccine carriers in safeguarding public health, there is a noticeable gap in the literature and in routine health practices concerning their microbiological safety. Previous studies have demonstrated the presence and activity of microorganisms in diverse environments, including those capable of surviving under harsh conditions, further emphasizing the need for continuous monitoring and control (Mbachu et al., 2014; Umeoduagu et al., 2023).

This research, therefore, seeks to bridge this gap by conducting a sterility test of vaccine carriers used in PHC centres across Awka. Awka serves as a representative urban setting with multiple PHC centres that carry out regular immunization exercises under the Expanded Programme on Immunization (EPI). By analysing the microbial load present on or within these carriers, this study aims to determine whether current handling and maintenance practices are sufficient to prevent microbial contamination.

The significance of this study cannot be overstated. Ensuring the sterility of vaccine carriers is not only vital for preserving the integrity of the vaccines but also for preventing healthcare-associated infections. The findings of this research will provide empirical evidence that could inform policy recommendations, improve infection control practices, and guide training for health workers. Furthermore, the outcomes may contribute to national efforts aimed at improving immunization coverage and trust in public health systems, particularly at the grassroots level.

As Nigeria continues to strengthen its immunization strategies to reduce the burden of preventable diseases, attention must be given not only to vaccine availability and cold chain temperature monitoring but also to the sterility of the containers used for vaccine storage and transportation. This study is, therefore, a timely and essential investigation into a key but neglected aspect of immunization safety in PHC centres in Awka.

The aim of this study is to carry out studies on the sterility and isolation of bacteria from vaccine carriers used in primary health care centres in Awka, Anambra State, in order to determine their microbiological safety and the effectiveness of handling and maintenance practices.

II. METHODOLOGY

Study Area

This study will be conducted in Awka, the capital city of Anambra State, Nigeria. Sample collection will be carried out in a purposively selected sample of ten (10) Primary Healthcare Centers (PHCs) within Awka metropolis and its immediate environs. The selection ensured geographical spread to cover both urban and semi-urban areas of the city.

The laboratory work was conducted at Cognig Simeone Biomedicals Research Laboratory at Nnamdi Azikiwe University, Awka.

Study Design and Sample Size

This was a cross-sectional, descriptive laboratory-based study aimed at isolating, identifying, and quantifying pathogenic bacterial contaminants on the surfaces of vaccine carriers. A total of ten (10) swab samples was collected. Swab sticks moistened with sterile Normal Saline (0.85% NaCl) was used to

clean internal Surface (from the base and the lid) and external handle.

Materials Used

Nutrient Agar, sterile containers, Buffers and Solutions, Sterile Peptone Water, Autoclave, Bunsen burner, Sterile pipettes or micropipette, Anaerobic jars, Petri dishes, Test tubes, Glass slides, Gram Staining Kit, Biochemical Test Reagents, Catalase Test Reagent, Oxidase Test Reagent, Carbohydrate fermentation tests, Light microscope, Immersion oil, Sterile loops, Sterile filter paper discs (for sensitivity tests).

Culture media preparation

The media prepared were Nutrient agar and Sabouraud Dextrose Agar (SDA). Nutrient Agar (NA) is a general-purpose medium used to grow a wide range of non-fastidious bacteria. It supports the growth of both Gram-positive and Gram-negative organisms and is commonly used for routine culture, colony morphology observation, and bacterial count estimation (Cheesbrough, 2010). To prepare it, 5.6g of nutrient agar powder is dissolved in 200ml of distilled water, heated to dissolve completely, and sterilized by autoclaving at 121°C for 15 minutes. After cooling to about 45–50°C, it is poured into sterile Petri dishes (Forbes et al., 2016). Nutrient agar is widely used in clinical, educational, and industrial labs for growing, maintaining, and sub culturing bacterial isolates (Willey et al., 2017).

Microbiology Analysis

The swab was vigorously aseptically agitated in a test tube containing 5ml of sterile Normal Saline (this becomes the stock solution, 10^0). A serial dilution up to 10^{-1} will be prepared. One tenth of a milliliter (0.1ml) from the 10^{-1}dilutions will be pipetted into sterile Petri dishes, and approximately 15-20ml of molten, cooled Nutrient Agar, Centrimide Agar, Mannitol Salt Agar and Blood Agar was poured, swirled gently for even mixing, and allowed to solidify. The inoculated nutrient agar and mannitol salt agar plates will be incubated aerobically and the blood agar anaerobically at 37°C for 24-48-hours. Discrete colonies for the bacteria from the various agar were obtained by sub culturing into Nutrient plate agar and were subsequently identified using standard methods.

Total Bacterial Count, Total Pseudomonas count and Total Staphylococcus count was calculated thus:

$$TBC/TSC/TPC\ (CFU/mL) = \left(\frac{N}{VxD} \right)$$

Where TBC: Total Bacterial Count/Total Fungi Count (CFU/mL)

- N: Number of colonies on plate
- V: Volume plated
- D: Dilution Factor

Characterization and Identification of bacteria

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Chessbrough, 2006). The characterization of the isolates was performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Motility test, as described by Bergey's Manuel of Determinative Bacteriology, 9th edition (1994).

Colony Morphology

After incubation, plates were examined for colony characteristics such as size, shape, margin, colour, elevation, and haemolytic properties. These preliminary observations help differentiate between bacterial species (Cheesbrough, 2018).

Gram reaction

Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with

Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope (× 100). Purple colour indicated Gram–positive organisms while red or pink colour indicated Gram-negative organisms.

Catalase test

Exactly 3ml of 3% solution of hydrogen peroxide (H2O2) was transferred into a sterile test tube. Then, 3 loopful of a 24-hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

Motility test (Hanging Drop Method)

A loopful of 18-24-hour broth culture of the test bacteria was placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at X100 magnification on a compound microscope. Care was taken to not interprete "drift" or "Brownian motion" as motility. Results were recorded as motile or non-motile.

Methyl Red test

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl red–Voges Proskauer (MR-VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

Hemolysis

24-hours pure culture was streak plated on a solidified blood agar and incubated anaerobically for 24h. The plates were examined after 24hrs to determine the type of hemolysis (alpha, beta or gamma).

Standardization of Test Bacteria

The test bacteria were standardized by using a sterile wire loop, to pick 3–5 pure cultures of the test microorganism and emulsified in 3–4 ml of sterile physiological saline. The turbidity reading of the 0.5 McFarland standard was recorded as Absorbance in a Spectrophotometer at 540 nm, while the turbidities of the test organisms were adjusted to match the absorbance of the 0.5 McFarland standard at the same wave length, using physiological saline. NB: 0.5 McFarland contains 1.5x108 CFU/mL

Antimicrobial Susceptibility Test for Bacteria

Exactly 100µl of 0.5 McFarland standardized suspension of test bacteria (1.5x108 CFUml-1) were cultured onto the Mueller-Hinton plates by spread plate method. Gram positive and negative discs were placed on the inoculated medium for 18-24hrs. The Inhibition zone diameters of the various plates were measured and recorded in millimeters. All experiments were done in duplicates.

III. RESULTS

Table 1 shows the overall bacterial load in the sample as well as the specific counts of selected microorganisms. The total bacterial count reflects the general microbial population, while the counts of Pseudomonas, Staphylococcus aureus, and Staphylococcus epidermidis highlight the presence of species that may cause spoilage, contamination, or opportunistic infections. The values, expressed in CFU ml^{-1}, provide a clear picture of both the total microbial burden and the contribution of particular bacteria of interest.

Table 1: Total unidentified Bacteria count, Total Pseudomonas count (Cfuml-1), Total S. aureus count (Cfuml-1) and Total S. epidermidis Count cfuml-1

Samples	*Total Unidentified Bacteria Count*		*Total Pseudomonas Count ($Cfuml^{-1}$)*		*Total S. Aureus Count ($Cfuml^{-1}$)*		*Total S. Epidermidis Count ($Cfuml^{-1}$)*	
	No. Of Bacterial Colonies On Plate	*Total Bacterial Count ($Cfuml^{-1}$)*	*No. Of Pseudomonas Colonies On Plate*	*Total Pseudomonas Count ($Cfuml^{-1}$)*	*No. Of Bacterial Colonies On Plate*	*Total S. Aureus Count ($Cfuml^{-1}$)*	*No. Of Bacterial Colonies On Plate*	*Total S. Epidermidis Count ($Cfuml^{-1}$)*
1	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Ng*	*Ng*
2	*89*	*$8.9x10^7$*	*Tftc*	*Tftc*	*47*	*$4.7 X10^4$*	*Tftc*	*Tftc*
3	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Ng*	*Ng*	*Ng*	*Ng*
4	*Tftc*	*Tftc*	*36*	*$3.6x10^5$*	*Tftc*	*Tftc*	*Ng*	*Ng*
5	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Tftc*
6	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*Tftc*	*45*	*$4.5 X10^4$*
7	*91*	*$9.1x10^4$*	*Tftc*	*Tftc*	*61*	*$6.1x 10^4$*	*Ng*	*Ng*
8	*53*	*$5.3 X10^4$*	*32*	*$3.2 X10^4$*	*44*	*$9.4x 10^4$*	*Ng*	*Ng*
9	*69*	*$6.9x10^4$*	*65*	*$6.5 X10^4$*	*54*	*$5.4 X10^4$*	*41*	*$4.1 X10^4$*
10	*161*	*$1.61x10^5$*	*42*	*$4.2x10^4$*	*49*	*$4.9 X10^4$*	*Ng*	*Ng*

NG: No growth.

Table 2 below presents the different types of bacteria identified in the samples and how they are distributed. It highlights the presence and frequency of occurrence of each bacterial species, giving an overview of the diversity of microorganisms present. This information helps to show not just the total number of bacteria, but also which species are most common or dominant within the sample population.

Table 2: Table showing bacterial distribution

Samples	Bacterial Distribution
Amansea 1	*Pseudomonas aeruginosa and Staphylococcus aureus*
Amansea 2	*Staphylococcus epidermidis, Pseudomonas aeruginosa and Staphylococcus aureus*
Okpuno 1	*Pseudomonas aeruginosa and Staphylococcus aureus*
Awka 1	*Pseudomonas aeruginosa and Staphylococcus aureus*
Awka 2	*Staphylococcus epidermidis, Pseudomonas aeruginosa and Staphylococcus aureus*
Sample 1a	*Staphylococcus epidermidis, Pseudomonas aeruginosa and Staphylococcus aureus*
Sample 1b	*Pseudomonas aeruginosa and Staphylococcus aureus*
Sample 2a	*Pseudomonas aeruginosa and Staphylococcus aureus*
Sample 2b	*Staphylococcus epidermidis, Pseudomonas aeruginosa and Staphylococcus aureus*
Sample 3	*Pseudomonas aeruginosa and Staphylococcus aureus*

Table 3 below provides a description of the physical characteristics used to identify the bacteria obtained from the samples. It records observable traits such as colony shape, size, elevation, margin, surface texture, and pigmentation, which are important in differentiating one bacterial species from another. By presenting these features, the table helps in the preliminary identification and classification of the isolates before further biochemical or molecular tests are carried out.

Table 3: Morphological of the Various Bacterial Isolates.

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Identity
Z1	Irregular	Glistening	Cream	irregular	Flat	Opaque	- Rod	*Pseudomonas aeruginosa*
Z2	Circular	Smooth	Yellowish	Entire	Raised	Opaque	+ cocci	*Staphylococcus aureus*
Z3	Circular	Smooth	Yellowish	Entire	Raised	Opaque	+ cocci	*Staphylococcus epidermidis*

Key:

Gram: Gram reaction

Cat: Catalase test

Mot: Motility test

Ind: Indole test

MR: Methyl-red test

Table 4 below presents the combined results of colony appearance, Gram staining, and biochemical reactions used to characterize the bacterial isolates. It details features such as shape, colour, and texture of colonies alongside specific biochemical test outcomes, which together provide a reliable means of identifying the bacteria. This table serves as an essential step in confirming the identity of the selected bacterial species.

Table 4: Morphological and Biochemical Identification of Selected Bacteria

Sample	Gram Reaction	Shape	Catalase Test	Motility Test	Glucose	Lactose	Sucrose	Maltose	Haemolysis	Organism
1	–	Rod	+	+	–	–	–	–	β	*Pseudomonas aeruginosa*
2	+	Cocci	+	–	+	+	+	+	β	*Staphylococcus aureus*

Sample	Gram Reaction	Shape	Catalase Test	Motility Test	Glucose	Lactose	Sucrose	Maltose	Haemolysis	Organism
3	+	Cocci	+	–	+	–	–	–	γ	*Staphylococcus epidermidis*

Key:
B-haemolysis = complete haemolysis (clear zone).
γ-haemolysis = no haemolysis.

Table 5 below shows how the different bacterial isolates responded when tested against various antimicrobial agents. It presents whether each isolate was sensitive, resistant, or intermediate to the drugs, thereby indicating their level of susceptibility. This information is important for determining effective treatment options and for understanding resistance trends among the bacteria studied.

Table 5: Antimicrobial Sensitivity Pattern of the Isolates

a. Gram Positive Isolate

Isolates	Antimicrobial Sensitivity pattern
Stapylococcus aureus	CPX (15. 2mm) S (14.3mm) LEV (12.70mm) RD (12.2mm) CN(11.80mm)
Stapylococcus epidermidis	CH (15.80mm) CPX(14.30mm) CN(12.80mm) RD(11.50mm) APX(10.10mm)

Gram Positive Disc- abbreviation and concentration of drugs

- Ciproflox (CPX) 10μg
- Norfloxicin (NB) 10μg
- Gentamycin (CN) 10μg
- Amoxil (AML) 20μg
- Streptomycin (S) 30μg
- Rifampicin (RD) 20μg
- Erythromycin (E) 30μg
- Chloramphenicol (CH) 30μg
- Ampiclox (APX) 20μg
- Levoflocaxin (LEV) 20μg

B. Gram Negative Isolate

Isolates	Antimicrobial Sensitivity pattern
Pseudomonas aeruginosa	AU(14.5mm) OFX (13.5mm) CXM (12.7mm)

Gram Negative Disc- abbreviation and concentration of drugs

- Augmentin (AU) 10μg
- Gentamycin (CN) 10μg
- Cefixime (CFX) 5μg
- Streptomycin (S) 30μg
- Trafid (OFX) 10μg
- Ciprofloxacin (CPX) 10μg
- Septrin (SXT) 30μg
- Cefuroxime (CH) 30μg
- Ceftrazon (CIP) 30μg
- Penicillin (PN) 30μg

IV. DISCUSSIONS

This study revealed that vaccine carriers used in Primary Health Care (PHC) centres in Awka, Anambra State, were contaminated with Staphylococcus aureus, Staphylococcus epidermidis, and Pseudomonas aeruginosa. The presence of these organisms confirms that sterility was compromised and supports concerns raised in previous studies about the hygienic management of vaccine storage equipment.

These findings are in strong agreement with Akers et al. (2014), who reported that poor disinfection, frequent improper handling, and exposure to unhygienic environments contribute significantly to contamination of vaccine carriers and other cold-chain tools. The isolation of S. aureus and S. epidermidis in this study is consistent with Akers et al.'s observations that organisms commonly found on human skin are easily transferred to carriers when proper hygiene and glove use are neglected. However, unlike Akers et al., this study also identified Pseudomonas aeruginosa, pointing to possible environmental contamination and moisture retention within the carriers.

The results further align with Samant et al. (2017), who emphasized that proper maintenance, disinfection, and minimal exposure are essential to preserving sterility. Samant et al. highlighted that inadequate training of health workers and failure to clean carriers after use contribute to microbial growth. The organisms identified in this study, particularly Staphylococcus species, strengthen their stance that both human handling and lack of routine decontamination play major roles in contamination. In contrast, this research goes further by examining the antimicrobial susceptibility patterns of the isolated organisms.

Additionally, Yakubu et al. (2020) reported that vaccine carriers and other cold-chain equipment in PHCs often fail sterility checks due to cracked interiors, inconsistent cleaning practices, and insufficient monitoring by supervisors. This study corroborates their findings, as the recovery of P. aeruginosa suggests the involvement of moisture-retentive surfaces, worn-out carriers, or poorly maintained equipment. Unlike Yakubu et al., who focused on the structural integrity of carriers and policy gaps, this research provides microbiological evidence and traces contamination to specific bacterial species.

The antimicrobial sensitivity patterns observed in this study also reflect emerging resistance trends noted in earlier literature. Staphylococcus aureus showed susceptibility to ciprofloxacin (15.2 mm), streptomycin (14.3 mm), levofloxacin (12.7 mm), rifampicin (12.2 mm), and gentamicin (11.8 mm). Staphylococcus epidermidis responded to chloramphenicol (15.8 mm), ciprofloxacin (14.3 mm), gentamicin (12.8 mm), rifampicin (11.5 mm), and ampiclox (10.1 mm). Pseudomonas aeruginosa showed moderate sensitivity to augmentin (14.5 mm), ofloxacin (13.5 mm), and cefuroxime (12.7 mm). While these antibiotics remain effective, the moderate inhibition zones suggest gradual resistance buildup, in line with concerns raised by Akers et al. (2014) about antimicrobial resistance in healthcare-associated organisms.

The public health implications are significant. Contaminated vaccine carriers not only pose risks to vaccine potency but may also expose both health workers and recipients to infection. When compared across authors, this study reinforces Akers et al.'s concerns regarding handling practices, supports the procedural emphasis of Samant et al., Validates's findings on monitoring lapses and equipment deterioration.

In summary, the contamination detected in this study confirms that sterility breaches in vaccine carriers are linked to poor maintenance, inadequate disinfection, weak supervision, and lack of training issues widely noted in existing literature. However, this research advances previous work by identifying the specific organisms involved and evaluating their antibiotic susceptibility, thereby providing deeper insight into the risks posed to immunization programs in Nigeria.

Recommendations

Based on the findings, the following recommendations are proposed to strengthen sterility practices and safeguard vaccine integrity in PHC centres:

1. **Strict Cleaning and Disinfection Protocols**: Vaccine carriers should be disinfected immediately after each use with effective disinfectants and allowed to dry completely before storage.
2. **Regular Sterility Monitoring:** Periodic microbiological testing of vaccine carriers should be conducted to detect contamination early and guide corrective measures.
3. **Training and Re-Training of Health Workers**: Healthcare staff should receive continuous training on the correct handling, cleaning, and maintenance of vaccine carriers, emphasizing infection prevention.
4. **Use of Protective Measures:** Health workers should wear gloves and practice proper hand hygiene during the handling and transportation of carriers to minimize contamination.
5. **Replacement of Damaged Carriers:** Old, cracked, or poorly insulated vaccine carriers should be promptly replaced to prevent microbial harbouring and loss of cold-chain efficiency.
6. **Government and Policy Support:** Public health authorities should invest in durable, high-quality vaccine carriers and enforce compliance with standard operating procedures across all PHC centres.

V. CONCLUSION

Therefore, the study highlights the urgent need for stricter protocols on vaccine carrier maintenance. Regular cleaning with appropriate disinfectants, thorough drying before storage, and periodic sterility monitoring are essential. Equally important is the training and re-training of health workers on the proper handling and disinfection of carriers to prevent contamination. Public health programs must prioritize investment in high-quality, durable carriers that are less prone to cracks and wear, thereby reducing the risk of microbial harbouring.

The sterility test results demonstrate that while vaccine carriers remain indispensable tools in immunization programs, their potential as sources of microbial contamination cannot be overlooked. Addressing the identified lapses in cleaning, handling, and monitoring is crucial for ensuring that vaccines remain safe and effective, thereby sustaining public trust in immunization as a cornerstone of disease prevention in Nigeria and beyond.

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