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

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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

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. 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). 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. 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).

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 was conducted in Awka, the capital city of Anambra State, Nigeria. Sample collection was 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 Conig-simonne 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 were collected. Swab sticks moistened with sterile Normal Saline (0.85% NaCl) were used to

swab the internal surfaces (base and lid) and the external handle.

Materials Used

Nutrient Agar, mannitol salt agar, blood 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, carbohydrate fermentation tests, light microscope, immersion oil, sterile loops, sterile filter paper discs (for sensitivity tests).

Culture media preparation

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., 2007). Nutrient agar is widely used in clinical, educational, and industrial labs for growing, maintaining, and sub culturing bacterial isolates (Willey et al., 2017). In addition, other agar that were used include; cetrimide agar, mannitol salt agar and blood agar.

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, cetrimide 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 were incubated aerobically and the blood agar anaerobically at 37°C for 24-48-h. Discrete colonies for the bacteria from the various agar were obtained by subculturing into nutrient plate agar and were subsequently identified using standard methods.

Total bacterial count, total Pseudomonas count and total Staphylococcus count were calculated thus:

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

Where TBC: Total Bacterial 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 (Cheesbrough, 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 & Holt (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, 2006).

Gram reaction

A thin smear of the isolate was made on a clean, non-greasy, dust-free slide. The slide were 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 Gram's iodine, allowed to remain for 60 seconds and washed off. The smear was decolourized with acetone-alcohol mixture. It was

counter-stained with safranin for 60 seconds and rinsed with tap water and allowed to air-dry. The slide was then viewed under oil immersion objective lens (×100). Purple colour indicated Gram–positive organisms while red or pink colour indicated Gram-negative organisms. (Chessbrough, 2006)

Catalase test

Exactly 3mL of 3% solution of hydrogen peroxide (H_2O_2) was transferred into a sterile test tube. Then, 3 loopfuls 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 reaction, while no bubbling indicated a negative reaction. (Chessbrough, 2006)

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 not to interpret "drift" or "Brownian motion" as motility. Results were recorded as motile or non-motile. (Cappuccino and Sherman, 2014).

Methyl Red test

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48-hour culture of the isolate in Methyl red–Voges Proskauer (MR-VP) broth. The production of a bright red colour indicated a positive test while yellow colour indicated a negative test after vigorous shaking. (Cheesbrough, 2006)

Sugar Fermentation Test

Each of the isolates was tested for its ability to ferment a specific sugar. One gram (1g) of the sugar and 1g of peptone water were dissolved in 100mL of water. Five milliliters (5ml) of the solution was transferred into clean test-tubes using sterile pipettes. Within the test-tubes containing peptone water and sugar, Durham tubes were inserted in an inverted position and bromothymol blue served as an indicator. These were sterilized for 10 minutes and allowed to cool before inoculating with the test organism. The test tubes were incubated for 3 days. 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 the presence of gas in the Durham's tubes (Cheesbrough, 2006)

Hemolysis

Twenty-four (24-hour) pure culture was streak plated on a solidified blood agar and incubated anaerobically for 24 hours. The plates were examined after 24 hours to determine the type of hemolysis (alpha, beta or gamma). (Cheesbrough, 2006)

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 wavelength, using physiological saline. NB: 0.5 McFarland contains $1.5x10^8$ CFU/mL (Matuschek *et al.*, 2014)

Antimicrobial Susceptibility Test for Bacteria

Exactly 100µL of 0.5 McFarland standardized suspension of test bacteria ($1.5x10^8$ CFU/ml) were cultured onto the Mueller-Hinton plates by spread plate method. Gram positive and negative discs were placed on the inoculated medium for 18-24h. The Inhibition-zone diameters of the various plates were measured and recorded in millimeters. All experiments were performed in duplicates. (Matuschek *et al.*, 2014)

III. RESULTS

Table 1 shows the overall bacterial load in the sample as well as the specific counts of selected bacteria. The total bacterial count reflects the general microbial population, while the counts of *Pseudomonas* spp, *Staphylococcus aureus*, and *Staphylococcus epidermidis* highlight the presence of species that may cause spoilage, contamination, or opportunistic infections. The values, expressed in CFU/mL, 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*	*$8.9x10^{4}$*	*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*	*$4.4x 10^{4}$*	*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*

Key:
Tftc: Too few to count
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 identification tests are carried out.

Table 3: Morphological Characteristics 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*
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 Susceptibility Pattern of the Isolates

a. Gram Positive Isolates

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 (CFM) 5µg
- Streptomycin (S) 30µg
- Trafid (OFX) 10µg
- Ciprofloxacin (CPX) 10µg
- Septrin (SXT) 30µg
- Cefuroxime (CXM) 30µg
- Ceftriaxone (CRO) 30µg
- Penicillin (PN) 30µg

IV. DISCUSSIONS

This study revealed that vaccine carriers used in Primary Health Care (PHC) centres in Awka, Anambra State, were in some cases 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.

Published research regarding the direct microbial contamination of vaccine-carrier surfaces is scarce. Tabatabaei *et al.* (2017) revealed bacterial contamination in multidose vaccine vials, including *E. coli* found in a vial within a vaccine carrier; however, the surfaces of the carrier were not subjected to microbiological analysis. The identification of *S. aureus*, *S. epidermidis*, and *P. aeruginosa* from vaccine-carrier surfaces in this work thus offers insights into a hitherto overlooked aspect of the vaccine cold chain.

The bacterial contamination reported in the present study is in agreement with the concerns surrounding the microbial contamination due to vaccine handling and maintenance of proper aseptic measures. In Tabatabaei *et al.* (2017) investigation, bacteria such as *Staphylococcus epidermidis* and *Escherichia coli* were identified from multidose vaccination vials used in immunization centers and findings were linked to the breaches in safe injection practices and non-compliance with aseptic techniques. The isolation of *S. epidermidis* in the present work could point to handling-related contamination, as this bacterium is a well-known member of the natural microbiota of human skin (Severn & Horswill, 2023). Similarly, S. aureus is a prevalent human coloniser and can be transmitted from humans to frequently touched surfaces through human contact (Howden *et al.*, 2023). The extra isolation of *Pseudomonas aeruginosa* might suggest an environmental source, particularly if moisture remains in improperly dried vaccine carriers, since *P. aeruginosa* is known to be able to survive in damp hospital settings (Schärer *et al.*, 2023). The findings emphasize the necessity of routine cleaning, proper drying, suitable handling and sanitary upkeep of vaccine carriers.

The findings of the present study may be considered alongside those of Samant *et al.* (2007), who identified deficiencies in the condition and maintenance of vaccine carriers used within the cold chain. Their study showed that some vaccine carriers failed to meet recommended standards for physical condition, temperature maintenance, and ice-pack adequacy, underscoring the importance of proper maintenance of vaccine carriers in preserving vaccine quality. Although Samant *et al.* (2007) did not investigate microbial contamination, the recovery of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* in the present study demonstrates an additional aspect of vaccine-carrier management that requires attention. The present study therefore extends previous work on vaccine-carrier maintenance by providing microbiological evidence of contamination and by evaluating the antimicrobial susceptibility patterns of the bacterial isolates.

The findings of the present study are consistent with established concerns regarding the maintenance and handling of vaccine carriers. The World Health Organization recommends that vaccine carriers be thoroughly dried after use because residual moisture and damp conditions may promote mould formation and contribute to contamination (WHO, 2015). WHO guidance also notes that cracks in the walls or lids of vaccine carriers may compromise their structural integrity and expose the insulating material. Similarly, Adebimpe and Adeoye (2021) identified gaps in the knowledge and practice of vaccine cold-chain logistics among primary health-

care workers in Nigeria, highlighting the need for improved training and appropriate management of cold-chain equipment. The isolation of *Pseudomonas aeruginosa* in the present study may indicate environmental contamination, particularly under moist conditions, although the source of contamination cannot be confirmed from the present study alone. Unlike previous studies that largely evaluated cold-chain management practices and equipment performance, the present study provides direct microbiological evidence of bacterial contamination of vaccine carriers and further characterizes the antimicrobial susceptibility patterns of the recovered isolates.

Antimicrobial susceptibility testing revealed varying inhibition-zone diameters among the bacterial isolates. *Staphylococcus aureus* produced inhibition zones of 15.2 mm for ciprofloxacin, 14.3 mm for streptomycin, 12.7 mm for levofloxacin, 12.2 mm for rifampicin, and 11.8 mm for gentamicin. *Staphylococcus epidermidis* showed inhibition zones of 15.8 mm for chloramphenicol, 14.3 mm for ciprofloxacin, 12.8 mm for gentamicin, 11.5 mm for rifampicin, and 10.1 mm for ampiclox, while *Pseudomonas aeruginosa* produced inhibition zones of 14.5 mm, 13.5 mm, and 12.7 mm against augmentin, ofloxacin, and cefuroxime, respectively. These findings demonstrate variations in the antimicrobial responses of the recovered organisms; however, interpretation as susceptible, intermediate, or resistant should be based on appropriate organism-specific standard breakpoints. The findings are relevant to wider concerns regarding antimicrobial resistance, as Ezeh *et al*. (2023) documented substantial resistance of *S. aureus* to several commonly used antimicrobial agents in Nigeria, while multidrug resistance has also been increasingly recognized in *S. epidermidis* (Siciliano et *al*., 2023) and *P. aeruginosa* (Karruli *et al*., 2023). The susceptibility findings in the present study therefore highlight the importance of standardized antimicrobial susceptibility testing and continued surveillance of bacterial contaminants recovered from vaccine carriers.

These discoveries have public health consequences that deserve consideration. The isolation of *S. aureus, S. epidermidis* and *P. aeruginosa* from the vaccine carrier surfaces indicates that these commonly handled objects could be contaminated and possible sites of indirect transfer of microorganisms. Inanimate surfaces contaminated with bacteria have been considered as potential reservoirs and may have a role in transmission by contact with health workers (Kramer *et al*., 2024). The presence of bacteria on the surfaces of vaccine carriers, however, does not itself establish transmission of infection to health professionals or vaccine recipients.

However, the maintenance of vaccine carriers is still important to vaccine quality. The WHO recommends that vaccine carriers be dried properly after use, as moisture and wet environments can encourage the growth of microorganisms, which can contaminate vaccinations. Physical damage, such as cracks in the carrier walls and lids, may expose insulation and raise the danger of inappropriate temperature exposure. These issues are comparable with findings of Samant *et al*. (2007) who reported impairments in the physical condition and capacity of some vaccination carriers to keep temperature. Similarly, Ogboghodo *et al*. (2017) reported that cold-chain practices were substantially associated with health-worker training, supportive supervision and availability of working cold-chain equipment in Nigeria. Adebimpe and Adeoye (2021) also discovered persisting gaps in knowledge and implementation of vaccination cold-chain logistics among primary health-care personnel.

In summary, the bacterial contamination seen in the present investigation indicates possible lapses in the handling of vaccine carriers. Although the study did not specifically identify sources of contamination,

nor did it prove that poor cleaning, supervision, or training were direct causes of the contamination seen, these factors are still important to consider for effective management of vaccine cold-chain equipment. The present study expands prior research by giving direct microbiological proof of the contamination of surfaces of vaccine-carriers, identifying the bacterial species involved and analyzing their antimicrobial susceptibility patterns. These findings further underscore the importance of frequent cleaning and drying of vaccine carriers, proper equipment maintenance, staff training and continuous supervision in 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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