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RESEARCH ARTICLE

SUSCEPTIBILITY PROFILES OF BIOFILM-FORMING BACTERIAL ISOLATES FROM ARMPITS TO ANTIBIOTICS AND SELECTED ROLL-ON DEODORANTS MARKETED IN NIGERIA

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ABSTRACT

Background and Aim: Armpit malodor has been associated with bacteria capable of converting non-volatile apocrine secretions into volatile and odoriferous compounds and can be treated by the use of topical antibiotics, deodorants and antiperspirants. This work is aimed at evaluating the bacterial isolates from armpits of some students in a tertiary institution in southwestern Nigeria for their ability to form biofilm and their susceptibilities to antibiotics, and selected deodorants marketed in Nigeria.

Methods: Bacteria associated with armpit swabs of 50 students were isolated and characterised using conventional biochemical tests. The biofilm-forming capacity of the isolates was determined using the Congo red agar (CRA) method. Susceptibility profiles of the CRA-positive isolates to antibiotics and four selected roll-on deodorants were determined using the Kirby-Bauer disc diffusion and agar well diffusion methods, respectively.

Results: A total of 110 bacterial isolates obtained were basically *Staphylococcus* spp. (55.45%), *Bacillus* spp. (41.82%) and *Micrococcus* spp (2.73%). The CRA-positive isolates were 24 with significant resistance to penicillin G, fosfomycin, cefuroxime and meropenem. Of these, *Staphylococcus aureus* displayed highest level of antibiotic resistance and highest susceptibility to the selected roll-on deodorants.

Conclusion: The study concluded that roll-on deodorants could be effective in the management of body odour associated with biofilm-forming bacteria.

Key words: Bacterial isolates, antibiotic resistance, deodorant susceptibility, biofilm, armpit swab

INTRODUCTION

One common problem facing mankind and of public health concern is body odour which occurs when the body gives off an odour that people find offensive. Although body odours can originate from diverse sources such as axillae, perineum and toe webs, the most common and highly stigmatized with associated social and psychological impact is the axillary odour [1, 2]. Axillary odour has been attributed to bacterial degradation of too large to become volatile apocrine secretions containing long-chain fatty acids, fatty acids bound to amino acids, sulphur-containing amino acids and hormones, into odoriferous and volatile compounds [3]. However, the odour can be foul-smelling depending on the type of bacteria living in the axillary region [3]. Malodorous armpits have been attributed to *Staphylococcus* and *Corynebacterium* spp, the two abundant genera of bacteria in the axillary region [4]. For instance, *Staphylococcus hominis*, *Corynebacterium tuberculostearicum* and *Anaerococcus spp*. have been reported as important and abundant contributors to typical armpit malodour [5, 6]. Also, a significant positive correlations have been established between odour intensity and the relative cornucopia of *Staphylococcus epidermidis, Staphylococcus hominis*, and *Cutibacterium avidum* [1]. Other bacteria that have been reported in association with armpit region include *Staphylococcus aureus*, *Staphylococcus albus* and *Klebsiella* sp. [2]. However, these organisms vary in intensity with armpit harbouring about 500,000 bacteria per square inch and the forearm about 12,000 bacteria per square inch [7].

Nonetheless, one of the measures in the management of armpit malodour is the use of deodorants and antiperspirants. While the former reduce the bacterial counts in the axillae and mask the malodour formation by adding perfume, the later reduce the moisture production by blocking the eccrine sweat glands [3]. It has been reported that repeated exposure to these products as well as water, soaps, and detergents decreases the microbial load and diversity to a point where only the most adjusted bacterial genera survive [3]. One of the methods of survival is biofilm formation which when formed often displays high resistance to antimicrobial agents with the attendant consequences of increased cost of treatment, mortality and morbidity as well as length of hospital stay.

Despite the avalanche of literature reports on the isolates from armpits including their susceptibility profiles to antibiotics, information about their biofilm-forming capacity is lacking. This study, therefore, evaluated the bacterial isolates from the armpits of selected pharmacy students of Obafemi Awolowo University, Ile-Ife for their biofilm forming ability as well as their susceptibility to antibiotics and four selected Roll-on deodorants marketed in Nigeria.

MATERIALS AND METHODS

Study area and ethical clearance: The study was carried out at the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. Ethical Clearance (OSHREC/PRS/569T/629) was obtained from the Health Planning, Research and Statistics Department of Osun State Ministry of Health.

Collection of samples: This was done using the method of Uzeh *et al.* [8] with modifications. Samples were collected from both armpits of 50 randomly selected students at the Faculty of Pharmacy, Obafemi Awolowo University, over a three-week period (5^{th} - 23^{rd} July, 2023). Armpit swabs

were collected aseptically using sterile swab sticks, rotating each swab stick in each armpit of individual volunteers and transferring immediately into the sterile container, labelled and analysed in the Pharmaceutical Microbiology Laboratory of the Faculty.

Isolation of organisms from armpit samples: Swab samples were subjected to isolation of organisms according to the modified method of Uzeh *et al.* [8]. They were separately inoculated into 10 mL sterile peptone water solution and incubated at 37 ^{o}C for 24 hours. A loopful of the overnight-grown culture was streaked on sterile nutrient agar plate and incubated at 37 ^{o}C for 24 hours to give distinct colonies. Each morphologically distinct colony was identified using the method of Cowan and Steel [9] after which they were transferred onto agar slopes and stored in a refrigerator until needed.

Evaluation of isolates for biofilm-forming ability using the Congo Red Agar (CRA) method: Isolates were evaluated using the method of Podbielska *et al*. [10]. They were cultured on CRA plates prepared with brain heart infusion agar, Congo red and sucrose, and subsequently incubated at 37 ^{o}C for 24 hours. Production of black colonies with dry crystalline consistency was indicative of biofilm formation.

Antibiotic susceptibility test: All isolates capable of forming biofilm as detected by CRA method were evaluated for antibiotic susceptibility using the Kirby-Bauer disc diffusion technique as exemplified in the guidelines of the Clinical and Laboratory Standards Institute [11]. The antibiotic discs used included: gentamicin (30 µg), penicillin G (10 µg), nitrofurantoin (300 µg), cefuroxime (30 µg), fosfomycin (50 µg), ciprofloxacin (5 µg) and meropenem (10 µg). Discs were placed on the prepared Muller-Hinton agar surface pre-inoculated with standardised inoculum of each test organism. Isolates were categorised as susceptible or resistant according to the CLSI standard based on the diameter of the zone of inhibition.

Evaluation of the antibacterial activity of selected Roll-On deodorants: Four roll-on deodorants purchased in July 2023 from a Pharmacy shop located on campus were evaluated for their antibacterial activity against the CRA-positive isolates using the agar well diffusion method. An overnight-grown culture of the test isolate in Mueller-Hinton broth was appropriately diluted such that the final inoculum size in 20 mL Mueller-Hinton agar was 1.5 x 10^5 CFU/mL. Five wells with a diameter of 8 mm were punched aseptically with a sterile cork- borer and four drops each of the roll- on deodorant solutions were introduced. Ciprofloxacin solution (1 mg/mL) was the standard placed in the centre of the plate. Agar plates were incubated at 37 ^{o}C for 24 hours and then observed for the presence or otherwise of zones of inhibition measured in millimeters.

RESULTS

The 50 students used in the study comprised 58% male and 42% female aged between 15 and 34 years. However, majority (58%) of the students were within the range of 20 and 24, and those between 30 and 34 years were the least (6%). The distribution of the bacterial isolates from the study is as shown in Table 1. *Staphylococcus epidermidis* (25.45%) and *Staphylococcus haemolyticus* (23.64%) were the predominant species while *Micrococcus luteus* was the least (2.73%).

The percentage distribution of the ability of the isolates to form biofilm as detected by the CRA- method is as shown in Table 2. Out of

the 24 isolates that were CRA-positive, *S. epidermidis* has the highest percentage occurrence (37.5%) and *B. subtilis*, the least (4.2%). The percentage distribution of the resistance profiles of the CRA-positive bacterial isolates from the study is as shown in Table 3. Resistance to gentamycin was the least (12.5%) while all the isolates were resistant to penicillin G.

Table 1: Percentage distribution of bacterial isolates obtained from human subjects

S/N	Bacterial species	Number of isolates	Percentage (%) distribution
1	*Bacillus cereus*	3	2.73
2	*Bacillus flexus*	18	16.36
3	*Bacillus subtilis*	25	22.73
4	*Micrococcus luteus*	3	2.73
5	*Staphylococcus aureus*	7	6.36
6	*Staphylococcus epidermidis*	28	25.45
7	*Staphylococcus haemolyticus*	26	23.64
	Total	**110**	**100**

Table 3: Percentage distribution of resistance profiles of CRA-positive isolates to antibiotics (n = 24)

S/N	Antibiotics	Percentage (%) resistance
1	Penicillin G (10 µg)	100.00
2	Cefuroxime (30 µg)	79.17
3	Fosfomycin (50 µg)	75.00
4	Meropenem (10 µg)	75.00
5	Nitrofurantoin (300 µg)	29.17
6	Ciprofloxacin (5 µg)	16.67
7	Gentamicin (30 µg)	12.50

From Table 4, the resistance profiles of the different species differ. While all the isolates displayed absolute resistance to Penicillin G, only *B. cereus*, *B. subtilis*, *M. luteus*, *S. aureus* and *S. heamolyticus* gave absolute resistance to cefuroxime. Moreover, absolute resistance to meropenem was displayed by *B. subtilis*, *M. luteus*, and *S. aureus* while *B. cereus*, *M. luteus* and *S. aureus* were resistant to fosfomycin.

The container disclosures of the selected roll on deodorants are indicated in Table 5. All the deodorants used in the study had address of manufacturers, manufacturing and expiry dates, but Nivea® and Vanilla® in addition had NAFDAC numbers. However, the susceptibility profiles of the CRA-positive isolated species are presented in Table 6. Despite *S. aureus* having high resistance to some of the antibiotics used in the study, it also displayed susceptibility to three deodorants, Vanilla®, Rexona® and Sure®.

DISCUSSION

Despite that sweat contains long-chain fatty acids, lipoproteins, sulphur-containing amino acids (e. g. methionine, cysteine) and hormones, which are too large to become volatile, fresh sweat does not smell. However, sweat may produce odour with foul smells when bacteria break down these non-volatile components into volatile and malodourous compounds. Suffice it to say that malodour results from specific interactions between host-derived odour precursors and the microbial metabolism that they support [12]. Four groups of bacteria that dominate almost every part of the skin are diphtheroids; micrococci; streptococci and the enterococci. However, *Staphylococcus* and *Corynebacterium* spp. are two abundant genera in the axillary region [3]. In this study, 110 bacterial isolates obtained from 50 subjects whose ages ranged

between 15 and 34 years can be classified into 3 genera (*Bacillus, Micrococcus* and *Staphylococcus*) and 7 species (Table 1). The observation that *S. epidermidis* was the predominant bacterial isolate in this study is in agreement with the findings of Uzeh *et al.* [8].

Table 2: Percentage distribution of CRA-positive bacterial species isolated from armpit (n = 24)

Species	*Bacillus flexus*	*Bacillus cereus*	*Bacillus subtilis*	*Micrococcus luteus*	*Staphylococcus epidermidis*	*Staphylococcus aureus*	*Staphylococcus heamolyticus*
%	12.5 (3)	8.3 (2)	4.2 (1)	12.5 (3)	37.5 (9)	12.5 (3)	12.5 (3)

Table 4: Percentage distribution of resistance profiles of CRA-positive bacterial species to antibiotics

	Bacterial species						
Antibiotics	*Bacillus flexus* (n = 3)	*Bacillus cereus* (n = 2)	*Bacillus subtilis* (n = 1)	*Micrococcus luteus* (n = 3)	*Staphylococcus epidermidis* (n = 9)	*Staphylococcus aureus* (n = 3)	*Staphylococcus heamolyticus* (n = 3)
Cefuroxime	33	100	100	100	67	100	100
Ciprofloxacin	33	50	-	33	-	-	33
Fosfomycin	66	100	-	100	67	100	66
Gentamicin	-	50	-	33	-	-	33
Meropenem	66	50	100	100	67	100	66
Nitrofurantoin	33	-	-	-	11	66	66
Penicillin G	100	100	100	100	100	100	100

Table 5: Container disclosures of the selected Roll-on deodorants

S/N	Sample	Manufacturing date	Expiry date	NAFDAC no	Address of manufacturer
1	Nivea®	06/02/22	19/09/24	02-4139	Beiersdorf Nivea consumer products Nig ltd (BNCPNL); 21, Akinyemi Crescent, off fatai Atere way, Matori, Mushin, Lagos. Made in Nigeria
2	Vanilla®	18/06/21	17/05/24	02-3347	PAFRUMS XAVIER LAURENT; 29, Claredon Road Watford WD17 IHX England
3	Rexona®	21/01/21	22/12/23	-	Made in UK
4	Sure®	03/09/21	02/09/24	-	Made in UK

Table 6: Percentage distribution of resistance profiles of CRA-positive bacterial species to Roll-On deodorants

	Bacterial species						
Sample	*Bacillus flexus* (n = 3)	*Bacillus cereus* (n = 2)	*Bacillus subtilis* (n = 1)	*Micrococcus luteus* (n = 3)	*Staphylococcus epidermidis* (n = 9)	*Staphylococcus. aureus* (n = 3)	*Staphylococcus heamolyticus* (n = 3)
Nivea®	100	100	-	100	100	66	66
Vanilla®	33	-	-	-	44	-	66
Rexona®	-	100	100	33	11	-	33
Sure®	-	100	-	33	22	-	33

There have been reports of positive association between the presence of *S. epidermidis* and malodour of the arm pit due to the ability of the enzymes derived from *S. epidermidis* to be involved in a cascade of multiple metabolic pathways leading to the production of both sour odour-associated acetic acid and isovaleric acid [1]. Apart from being a normal flora of the skin, skin glands, anterior nares, and mucous membranes of humans and animals, *S. epidermidis* is also an opportunistic pathogen for humans and can cause urinary tract infections, wound infections, endocarditis, and septicemia [13]. *S. epidermidis* is also a major concern for people with catheters or other surgical implants because it is known to cause biofilms that grow on these devices [14]. Isolation of *Bacillus* species, especially *B. subtilis*, is an indication of poor personal hygiene as the organism is a ubiquitous bacterium commonly recovered from water, soil, air, and decomposing plant residue. It may however be found in the armpit through use of dirty under-wears. Although *B. subtilis* is known to have a low degree of virulence to humans, it has however been found associated with infections such, as endocarditis, pneumonia, bacteremia, septicaemia and some episodes of food poisoning [15].

The ability of the isolates to form biofilm was detected in this study by a simple, rapid and more reproducible CRA method with *S. epidermidis* having the highest capacity among the 24 bacteria that were CRA-positive (Table 2). Formation of biofilm results in reduced susceptibility to antimicrobial agents with attendant consequences of increased mortality and morbidity rate, increased cost of treatment and increased length of hospital stay [16, 17].

Antibiotic resistance profiles of the CRA-positive isolates revealed gentamicin (12.5 %) as the drug of choice in this study (Table 3). However, *S. aureus* isolates appeared to the most resistant species displaying resistance to four (cefuroxime, fosfomycin, meropenem and penicillin G) of the seven antibiotics tested (Table 4). Resistance to aminoglycosides can be mediated by enzymatic modification and inactivation; increased efflux; decreased permeability; and modifications of the 30S ribosomal subunit that interferes with binding of the aminoglycosides [18]. Enzymatic modification and inactivation can be mediated by aminoglycoside acetyltransferases, nucleotidyltransferases, or phosphotransferases as commonly observed across Gram-positive and Gram negative bacteria. In this study, however, resistance to gentamicin, an aminoglycoside, is possibly mediated by any or combination of the mechanisms.

Management of armpit malodour can be achieved through the use of deodorants and antiperspirants. This implies that fewer bacterial enzymes will be present when the bacterial growth is minimised with fewer apocrine sweat secretions being transformed into volatile compounds. It is important to note that deodorants and antiperspirants are non-selective and their application will result in elimination or reduction of all bacterial species.

The container disclosures of the selected deodorants are as shown in Table 5. These disclosures help in post-marketing surveillance of the products thereby making recall, in case of any untoward reaction to the product, easy. They also assist in determining

if the product is fake, adulterated or substandard. Vanilla® deodorant was the most active among the four deodorants tested, with *B. cereus*, *B. subtilis*, *M. luteus* and *S. aureus* being 100% susceptible (Table 6). However, all the CRA-positive *S. aureus* isolates were susceptible to Vanilla®, Rexona® and Sure® which indicates that biofilm formation by *S. aureus* can be prevented by most deodorants used in the study. Aside from focusing on bacterial load reduction in the control of malodour armpits, attention can be on sweat reduction which can be achieved through the usage of oral medication, antiperspirants, axillary liposuction or laser- and microwave-based ablation of the sweat glands, among others [3].

CONCLUSION

This study concluded that armpits can be colonised by different bacterial species having varying susceptibilities to antibiotics and deodorants. Some of these organisms also have capacity to form biofilm which can be prevented by some deodorants marketed in Nigeria. However, the influx of deodorants into the Nigerian market needs to be regulated as two of the four deodorants tested were not certified by National Agency for Food and Drugs Administration and Control. Also, public awareness for good personal hygiene is recommended.

Conflicts of interest: The authors declare there was no conflict of interest in the course of this work.

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