Original Research Article

Identification and Antibiotic Resistance Profile of Biofilm-forming Methicillin Resistant *Staphylococcus aureus* (MRSA) Causing Infection among Orthopedic Wound Patients.

ABSTRACT

Background and Objectives: The biofilm-forming ability of Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains have demonstrated the involvement of MRSA biofilm in antibiotic resistance, recalcitrant and persistent infections in humans. Despite a deeper understanding of the biofilm-forming ability of MRSA strain, it is still essential to extend the research on the identification and antibiotic resistance profile of biofilm-forming MRSA causing infection among orthopedic wound patients.

Methodology: A total of three hundred and thirty (303) patient-isolate of non-repeatable *Staphylococcus aureus* strains were obtained during the period of 2021 until 2022 from fracture and post-surgical orthopedic wound patients with wound duration >2months at the National Orthopedic Hospital, Enugu (NOHE). *S. aureus* were identified using conventional microbiological cultures Technique followed by confirmation of MRSA strain through Brilliance MRSA 2 Agar. Antibiotic Susceptibility testing (AST) of biofilm-forming MRSA was performed using the Kirby–Bauer disk diffusion method and the results were interpreted using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints. Multidrug Resistance (MDR) was determined for biofilm-forming MRSA.

Result: Of the 303 isolate of *S. aureus*, MRSA strain accounted 86(28.4 %) and 78(25.7 %) from post-surgical wound and fracture wound respectively while biofilm forming MRSA was identified in 101(33.4%) MRSA strain consisting of high proportion 66(21.8 %) from Post-surgical wound followed by fracture wound samples recording 35(11.6 %). Association between MRSA production and biofilm formation was considered statistically significant at $P < .05$. The proportion of biofilm-forming MRSA resistance to β-lactam accounted 71.4-100%, macrolide resistance recorded 65.7-92.4 %, lincosamide resistance 74.3-100 %, glycopeptide resistance proportion ranged from 62.8-100 % while low level of resistance to fluoroquinolones 19.7-42.9 % and Aminoglycoside 8.6-10.6 % was observed. Biofilm-forming MRSA isolate were MDR to one or more antibiotic antimicrobial agents in at least three categories with MDR Index range ≥ 0.3 but majority of the isolate were 91.4% and 100% susceptible to Gentamicin and Imipenem.

Conclusion: The *in vitro* expression of biofilm formation among MRSA strain and their antibiotic resistance profile in this study makes them a potential threat and challenging pathogens with the ability to cause persistent infections in humans, especially among orthopedic wound patients. Thus the development of an antimicrobial stewardship program and regular detection of biofilm production is needed for timely intervention while judicious use of Imipenem and Gentamicin as a drug of choice for effective treatment of infection caused by biofilm-forming MRSA among orthopedic patients will avert the severity of infection. Further research of these sort should investigate the genotyping expression of a biofilm gene variant in other human diseases, different bacteria species, and orthopedic medical implant devices.

Keyword: *Biofilm-forming, Methicillin Resistant Staphylococcus aureus, antibiotic resistance*

1. INTRODUCTION

This strain referred to as Methicillin Resistant *Staphylococcus aureus* (MRSA) are strain encoding resistant to methicillin and other β- lactam drugs [1, 2, 3]. "This resistance is mediated by an altered penicillin binding protein (PBP2a) which is encoded by the *mec A* gene" [3]. "The *mec* A gene is found as part of a mobile genetic element found in MRSA strain called *Staphylococcal* cassette chromosome *mec* (SCC mec)" [2, 3]. "MRSA is recognized worldwide as an important bacterial pathogen causing mild infections often associated with skin or soft tissue" [4]; however, "it can cause more severe infections such as pneumonia, osteomyelitis, cerebral abscess and sepsis, resulting in high rates of morbidity, high economic burden and possible mortality" [5, 6]. "MRSA generally has been implicated in bone and wound infections encountered in orthopedic practice" [1, 7, 8], for example, "osteomyelitis, as well as in postoperative wound infections [9] where they are known to lead to delayed healing of wounds, delayed union, or even nonunion of bones which may lead to the amputation of such bones". "Precisely patients with surgical wounds have been reported to be at high risk of MRSA infection" [10, 11] "Compounding the problem even further is the fact that MRSA can form biofilms on biotic and abiotic surfaces" [12]. "A biofilm can be described as a complex and well-structured aggregation of microorganisms of one or more species" [11].

"Biofilms are found adherent to biotic (host tissue) and abiotic (implant/biomaterial) surfaces or as floating aggregates, all of which are encased in a self-produced matrix of polymeric substances" [13]." It's well documented, that microorganisms such as MRSA, under stressful conditions, cooperate and communicate with each other, sharing the same biological niche or body district, guaranteeing their mutual survival" [14, 15]. "The biofilm represents one of the most complicated factors implicated in wounds healing, with a predominance rate of 60% and 100% in chronic wounds" [8]. "The infections associated with biofilms are debilitating for patients since they can persist for months causing patients to lose hope of recovery" [8]. "The biofilm matrix protects MRSA from host immune system and as well increased bacterial antibiotic resistance and/or tolerance. MRSA biofilm formation is also related to increased bacterial antibiotic resistance and tolerance. This biofilm forming MRSA strain are difficult to eradicate since these strains are often multi-drug resistant (MDR) compromising the effectiveness of most antibiotics antimicrobial agent leading to poorer patient outcomes" [11]. "Biofilm-specific antibiotic resistance and tolerance mechanisms are multifactorial, varying depending on the particular antimicrobial agent; the bacterial strain and species; the age and developmental stage of the biofilm; and the biofilm growth conditions" [16]. "Individually, no single mechanism can account for the heightened antibiotic recalcitrance that is characteristic of biofilms. In combination, however, these resistance and tolerance mechanisms severely limit the ability to effectively treat biofilm-forming MRSA infections with the antimicrobial arsenal that is currently available" [17]. Hence continue screening of available antibiotic agent in this era of heightening antibiotic resistant prevalence, will aid in understanding the trend of resistant by biofilm-forming MRSA causing infection in orthopedic wound patients.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of the Strains

Aseptically, three hundred and thirty (303) patient-isolate of non-repeatable *Staphylococcus aureus* strains were obtained during the period of 2021 until 2022 from fracture and post-surgical orthopedic wound patients with wound duration >2months at the NOHE located at latitude 6°27'59.4"N and longitude 7°31'30.7"E. *S. aureus* were identified using conventional microbiological cultures Technique described in Microbiology Practical Handbook [18].

2.2 Phenotypic Detection of MRSA

2.2.1 Brilliance MRSA 2 Agar

Confirmation of MRSA strain through Brilliance MRSA II agar (bioMérieux, France) was performed according to manufacturer's guideline. A colonies of an overnight culture of *S. aureus* isolates were aseptically streaked onto plates of sterilized Brilliance MRSA 2 agar. The inoculated plates were kept in 24 hours incubator (Edmund Bühler GmbH, Hechingen, Germany). Growth of blue colony after overnight incubation at 35^{O}C infer MRSA positive strains. Absence of blue colony is indicative of MRSA negative strain [3].

2.3 *In Vitro* Biofilm Production Assay

2.3.1 Qualitative Assay (Congo Red Method)

Qualitative assay of biofilm-forming MRSA was performed by the growing the MRSA on Congo red agar (CRA). Briefly, the Brain Heart Infusion (Thermo Fisher Scientific, Inc., USA). The broth (37g/l) was supplemented with sucrose (50 g/l) (Sigma-Aldrich, Germany), agar (10 g/l) and Congo red dye (0.8 g/l) was used for CR agar method [19]. Aqueous concentrated solution of Congo red was prepared and autoclaved separately from other constituents. After cooling to 55^{O}C it was added to the other mixture. The isolate were plated on the sterilized solidified CR agar. The strains were kept in 24 hours incubator (Edmund Bühler GmbH, Hechingen, Germany). After overnight incubation, "the results were interpreted as follows: red and Bordeaux red with smooth colonies was considered to be non-biofilm producers while strains producing intensive black, black, and reddish black colonies with a rough, dry, and crystalline consistency was classified as biofilm producers" [19].

2.4 Antibiotic Susceptibility Testing

This was aseptically carried out using Kirby-Bauer disk diffusion method, and in conformity to the recommended standard of Clinical and Laboratory Standard Institute (CLSI, 2019). A suspension was made from a 24 hour growth of the test organisms in sterile water to match the 0.5 McFarland turbidity standard. This was seeded on the entire surface of solidified Mueller-Hinton agar (Thermo Fisher Scientific, U.S.A) plates. The following antibiotic discs with potencies was used: Ampicillin (30 µg), Amoxicillin (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Imipenem (10 µg), Erythromycin (15 µg), Lincomycin (15 µg), Clindamycin (15 µg), Ciprofloxacin (5 µg), Gentamicin (15 µg), Vancomycin (30 µg), (Oxoid UK). The Mueller-Hinton agar plates were incubated at (35°C) in an aerobic atmosphere for 24 hour, after percentage susceptibility and resistance was interpreted from the inhibition zone diameters (IZDs) produced by the antibiotic disks against the test isolates [3, 20].

2.5 Determination of Multidrug Resistance (MDR)

MDR isolates earlier described by the CDC as acquired non-sensitivity to one or more agents in at least three categories of antimicrobials was determined i.e.,

(x) number of antibiotics to which test isolate displayed resistance

(y) the total number of antibiotics to which the test organism has been evaluated for sensitivity [3, 21].

2.6 Data Analysis

The data collected were analyzed by SPSS software statistical application version 20 (SPSS INC, Chicago, IL, USA). Spearman's Rho Correlation was used to evaluate Association between MRSA production and biofilm formation. A *P*-value <0.05 was considered statistically significant.

3. RESULTS

3.1 Distribution of biofilm-forming MRSA among patients in NOHE according to wound source

Of the 303 isolate of *S. aureus*, MRSA strain accounted 86(28.4 %) and 78(25.7 %) from post-surgical wound and fracture wound respectively (Table 1). Biofilm forming MRSA was identified in 101(33.4%) MRSA strain consisting of high proportion 66(21.8 %) from Post-surgical Wound followed by fracture wound samples recording 35(11.6 %). Association between MRSA production and biofilm formation was considered statistically significant at $P < .05$

3.2 Antibiotic Resistance profile of biofilm-forming MRSA isolates from Post-surgical and Fracture wound patients in NOHE

In Post-surgical wound isolate, β-lactam resistance were as follows: Ampicillin 100 %, Amoxicillin 100 %, Cefotaxime 89.4% and Ceftazidime 86.4 %. Macrolide resistance include: Erythromycin 92.4 %. Lincosamide resistance was noted in both Lincomycin and clindamycin accounted 100 % while Glycopeptide resistance was found in Vancomycin 100%. The isolate resistance to fluoroquinolones (Ciprofloxacin 19.7 %) and aminoglycoside (Gentamicin 10.6 %) was relatively low (Table 2). Biofilm forming MRSA isolate from Fracture wound patients were highly resistance to β-lactam antiobiotic: Ampicillin and Amoxicillin both recorded 100 %, Cefotaxime 82.9 % and Ceftazidime 71.4%. Resistance to Macrolide resistance was demonstrated against Erythromycin 65.7 %. Lincosamide resistance proportion of 74.3 % and 97.1 % was observed in Lincomycin and clindamycin respectively. Glycopeptide resistance proportion of 62.8% was observed in vancomycin while the isolate resistance to fluoroquinolones accounted 42.9 % and aminoglycoside 8.6 %.

3.3 Multidrug Resistant (MDR) Index of biofilm forming MRSA isolates from patients in NOHE

Biofilm-forming MRSA isolate were MDR i.e., non-sensitivity to one or more agents in at least three categories of antimicrobials with Index range ≥ 0.3 (Table 3). This indicate that these isolate emanate from source were antibiotic are frequently used.

Table 1: Distribution of biofilm-forming MRSA among patients in NOHE according to wound source

Clinical Sample	**Musculoskeletal Region**	**No. of *S. aureus* (%)**	**MRSA (%)**	**Biofilm (%)**	**Non-biofilm (%)**	***P-value****
Post-surgical Wound	Legs	97(32.0)	50(16.5)	42(13.9)	8(2.6)	.04397
	Hands	52(17.2)	36(11.9)	24(7.9)	12(4.0)	
		149(49.2)	**86(28.4)**	**66(21.8)**	**20(**6.6**)**	
Fracture Wound	Legs	84(27.7)	58(19.1)	20(6.6)	38(12.5)	
	Hands	70(23.1)	20(6.6)	15(5.0)	5(1.7)	
		154(83.7)	**78(25.7)**	**35(11.6)**	**43(14.2)**	
Total		**303(100)**	**164(54.1)**	**101(33.4)**	**63(20.8)**	

Spearman's Rho Correlation r_s =0.82353, *p* (2-tailed)= 0.04397.

Key: MRSA-Methicillin Resistant *Staphylococcus aureus*

Table 2: Antibiotic Resistance profile of biofilm-forming MRSA isolates from Post-surgical and Fracture wound patients in NOHE

Wound Source		Post-surgical (n=66)		Fracture (n=35)	
Categories	**Antibiotics (µg)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
β-lactam	Ampicillin (30)	66(100)	0(0.0)	35(100)	0(0.0)
	Amoxicillin (30)	66(100)	0(0.0)	35(100)	0(0.0)
	Ceftazidime (30)	57(86.4)	9(13.6)	25(71.4)	10(28.6)
	Cefotaxime (30)	59(89.4)	7(10.6)	29(82.9)	6(17.1)
	Imipenem (10)	0(0.0)	66(100)	0(0.0)	35(100)
Macrolide	Erythromycin (15)	61(92.4)	5(7.6)	23(65.7)	12(34.3)
Lincosamide	Lincomycin (15)	66(100)	0(0.0)	26(74.3)	9(25.7)
	Clindamycin (15)	66(100)	0(0.0)	34(97.1)	1(2.9)
Fluoroquinolones	Ciprofloxacin (5)	13(19.7)	53(80.3)	15(42.9)	20(57.1)
Aminoglycoside	Gentamicin (15)	7(10.6)	59(89.4)	3(8.6)	32(91.4)
Glycopeptide	Vancomycin (30)	66(100)	0(0.0)	22(62.8)	13(37.1)

Key: R-Resistance, **S**-Susceptible, **%**-Percentage, **n**-number of isolate

Table 3: Multidrug Resistant (MDR) Index of biofilm-forming MRSA isolates from patients in NOHE

Categories	Mean Multidrug Resistant Index (MDRI)	
	Post-surgical Wound	Fracture Wound
β-lactam	0.7	0.6
Macrolide	0.4	0.4
Lincosamide	0.6	0.5
Fluoroquinolones	0.3	0.5
Aminoglycoside	0.3	0.3
Glycopeptide	0.7	0.5
MEAN	**0.5**	**0.47**

4 DISCUSSION

As shown in the results section, this study reported a high phenotypic prevalence of MRSA (54.1%) in orthopedic patients, such high prevalence was reported in Kano, Nigeria (67.9 %) in orthopedic patients, (61.0 %) in Iran [22], 75% from surgical wounds in Algeria [23] 80.0 % in Peru [24] and in a setting in Colombia 90.0 % [25] but in contrast to these findings, a study from Ethopia 9.8 % [26], Eritrea [surgical wound 35.6%] [9], 18.8 % in Mwanza-Tanzania [27], 25.0 % in Jinja-Uganda [28], 37.4% in Madinah kingdom of Saudi Arabia [29]

The variation in the prevalence of MRSA across these countries indicates a disparity in the control measures applied, source of bacteria, the nature of the study participants, the laboratory methods used, and the study methods applied. MRSA was more predominant in post-surgical wound 28.4 % than other wound sample in the study. The observed increased prevalence of MRSA in this study may be linked to the fact that post-surgical wound patients may be predisposed to toxigenic equipment carriage and antibiotic-resistance clonal strain of *S. aureus* [11, 30, 31]. Also, due to the high rate of certain antibiotics use as prophylaxis and treatment either due to availability or cost-effectiveness issues may increase risk of MRSA colonization among these patients. "Further studies may provide useful insights into the virulence potential and nature of MRSA populations from post-surgical wound patients. Given the detection of a significant amount of toxin genes including *tst* gene in post-operative patients hospitalized in the surgical wards" [32]. Post-surgical wound patient in this study may likely be at risk of toxic shock syndrome [11, 33, 34] which result in delay wound healing and prolong hospitalization.

The qualitative method (CRA) showed 101(33.4 %) biofilm formation in MRSA isolates. The biofilm *in vitro* CRA model used in this study is well established and has been used by several other authors for studying biofilm formation. This finding substantiate CRA method reported prevalence of biofilm forming MRSA 50%, 52.7% and 76.02% [7, 11, 35] with strong ability of biofilm production seen among the identified strain. Although this study reported low prevalence of biofilm formation, it's worth noting that, phenotypic switch from a free-swimming, planktonic lifestyle to a sessile existence in a biofilm depends on many factors such as environment, availability of nutrients, geographical origin, types of specimen, surface adhesion characteristics and genetic makeup of the organism [36, 37].

"Occurrence of biofilm forming strain reported in this study could be linked to the slow progression in wound healing process among orthopaedic patients. The biofilm matrix is known to be a vital factor in preventing antibiotics from reaching the infecting organisms within the matrix" [38], thereby conferring resistance on the bacteria within the biofilm matrix. Biofilm forming MRSA was identified consisting of high proportion 21.8 % from post-surgical wound followed by fracture wound sample recording 11.6 %. It is important to explain that the presence of orthopedic implant device in most Post-surgical wound patient may have increase the observed proportion. In that biofilms strains are known to adherent to biotic (host tissue) and abiotic (implant/biomaterial) surfaces. After maturation on implant/biomaterial, they may disperse to recolonize other host tissue.

In this study, the Spearman's Rho Correlation statistical tool showed association between MRSA production and biofilm formation to be statistically significant at $p < .05$. This may be a sign that "due to the proximity or adherence of bacteria (MRSA) cells to each other within biofilms, resistant genes that confer resistance such as the *mecA* gene are easily transferred from one cell to other cells through HGT, thereby making the whole biofilm community resistant to methicillin and other antibiotics" [38].

In this study, high percentage of biofilm forming MRSA were resistant to Erythromycin, Lincomycin and clindamycin ranging from 65.7%-100 % similar to previous studies in Nepal reported by Gaire *et al.* [37] were Erythromycin resistant accounted for

86.6%, also in Northern India 76.5 % and 66.7 % resistance was seen against Erythromycin and Clindamycin, respectively [39], In Ethopia Erythromycin 61.5% was reported [26], while from 2015-2017 in Poland Hospital, a large number of MRSA isolates showed resistance to erythromycin (77.7 %) and clindamycin (72.3%) [40], while In Mexico, Uribe-García *et al.* [41] reported biofilm forming *S. aureus* strain resistant to Erythromycin 86.0 %, Alli *et al.* [42] in a study conducted in Nigeria, reported resistance rates of 49.4 % and 25 % for erythromycin and clindamycin, respectively while the results from Mohammadi *et al.* [43] indicated high prevalence rates of resistance to erythromycin (72.3 %), clindamycin (75.9 %) while resistant to lincomycin substantiate or echoes with that of earlier studies [9, 29, 44, 45, 46]. This study shows that biofilm forming MRSA exhibit phenomenal inducible clindamycin resistance which mediate resistant to macrolides (that induce *erm* expression) and lincosamide. However, Erythromycin resistance may be due to its random use to cure generalized and pyogenic infections [37]. Result from this study implies that both Clindamycin, lincomycin and erythromycin cannot be used in these patients.

Biofilm-forming MRSA was found to be 62.8-100 % resistant towards Vancomycin. Like other studies conducted in the Nigeria [47, 48, 49, 50] this study confirms the presence of vancomycin-resistant among wound patient in Enugu. Low vancomycin-resistant MRSA from wound 11.0 %, 22.0 % and 21.88 % in Asmara Eritrea, Ethopia and Dhaka, Bangladesh respectively has been documented [9, 51, 52]. In contrast, some study has reported 50-100 % susceptibility of biofilm-forming MRSA to vancomycin [29, 35, 39, 53, 54, 55, 56, 57, 58]. Falagas *et al*. [59] earlier estimated that "the susceptibility of these in Africa to VRSA is between 82.0 % and 100 %" [59]. "These estimates and the findings of this study also contradict an earlier conclusion by Kong *et al*. (2016) that VRSA strains are rare and that there is limited evidence of increasing frequency" [60]. "The main variation in vancomycin antibiogram patterns among different studies might be due to the indiscriminate use and availability of these antibiotics in a certain area. The variation of resistance rate among different areas indicates that resistance pattern of antibiotics varies according to regional and geographical location and also changes through time. Additionally, the cause of vancomycin resistance may be due to the activation of van A and van B gene [61] which seem to function independently of *mecA*".

Data on the antimicrobial resistance to other antibiotics were as follows: Amipicillin and amoxicillin 100 %, Cefotaxime and ceftazidime 71.4-89.4 %. Our finding echoes with the previous studies reported in Mexico, Tehran, Ethopia, Abakaliki and Zaria, Nigeria [1, 41, 43, 51, 62]. "It worth noting that unregulated use of the aforementioned antibiotics and over the counter sales of this antibiotics without prescription is rife in Nigeria. The cumulative effect of these over time may have been responsible for this high prevalence of resistant to most antibiotic documented in this study. Additionally, it is clear that the evolution of MRSA strain has been traced to the acquisition of the exogenous gene (*mec*A) which is part of the staphylococcal cassette chromosome *mec* (SCCmec) (types I–VII) and is under the control of *Mec*I (a repressor) and *Mec*R1 (a transducer) and represent the regulatory/signalling proteins of the *blaZ* system" [3, 19]. "The *mecA* gene codes for additional penicillin-binding protein (PBP2a), a peptidoglycan transpeptidase, which can confer resistance to all *β*-lactam and other antibiotics class" [3, 19] as evidence in this study. "Other isolates containing a particular variant of *SCCmec* types II and III have expanded range of resistance due to the presence of additional resistance genes" [9]. However, the ability of MRSA to form biofilms may have contributed to the highest prevalence of antibiotic resistant observed in this study.

Here, "MDR with MAR index of 0.3-0.7 were found in biofilm-forming MRSA. The increase in MDR in MRSA may be due to a distinctive feature of MRSA, i.e. their resistance to β-lactam antibiotics. Therefore, once the *S. aureus* is resistant to Methicillin, it may also show resistance towards other antibiotic classes like: aminoglycosides, macrolides, Glycopeptide, flouroquinolones and lincosamide. The data obtained from this research was found to be similar to the study conducted by other researchers" [26, 37, 61]. Also, the higher prevalence of MDR may be due to haphazard use of antibiotics for treatment which is common practice in Nigeria. The greatest problem with the control of resistant organisms in Nigeria has remained that of education. Very high indiscriminate use of antibiotics (without prescription) is common knowledge. This explains why the MAR index is high pointing to an internal (hospital) and external (community) source of contamination. Education and more education especially of the local populace remain the most important step to halting a rise in this infection.
Following MDR, majority of the biofilm forming MRSA were exceptionally sensitive to Gentamicin 91.4% and Imipenem 100% which echoes with earlier literature indicating MRSA susceptibility to imipenem 73.2% and Gentamicin 79.32% in Nigeria and Nepal [56, 63]. As such, imipenem and Gentamicin could be considered for judicious use in the treatment of wound infection harboring biofilm forming MRSA.

4 Conclusion

This study indicate that biofilm-forming MRSA accounted for 33.4% among different orthopedic wound sample. The *in vitro* expression of biofilm formation among MRSA strain in this study makes them a potential threat and challenging pathogens with ability to causing persistent infections in humans, especially among orthopedic wound patients. This may result in treatment failure and persistency of infections among community and hospital inhabitants. Thus development of antimicrobial stewardship program and regular detection of biofilm production is the need for the timely intervention while judicious use of imipenem and gentamicin will aid in effective treatment of infection cause by biofilm-forming MRSA among orthopedic patients. The study is, therefore, an opening to facilitate epidemiological studies base the current findings establishing correlation between MRSA and

biofilm formation. Further research of this sort should investigate the genotyping expression of biofilm gene variant in other human disease, different bacteria species and orthopedic medical implant device.

Ethical Approval
The protocol used in this study was approved by the Orthopedic Ethics Research Committee of the NOHE conveyed with ethical clearance number NOHE/HREC/21/2021/95.

Consent
As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

Disclaimer
The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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