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Short Communication **Open Access**

Prevalence and phenotypic characteristics of *Acinetobacter baumannii* isolated from critically ill patients in two healthcare facilities in Ebonyi State, Nigeria

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Abstract:

Background: The intrinsic property of *Acinetobacter baumannii* to survive in harsh conditions on environmental surfaces and its ability to resist commonly used antibiotics in hospitals make this pathogen to be one of the most prevalent causes of hospital infections. The present study was aimed at determining the prevalence of *A. baumannii* among critically ill patients in two tertiary hospitals; Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AE-FUTHA) and Mater Misericodiae Hospital Afikpo (MMHA) in Ebonyi State, southeast Nigeria.
Methodology: This was a hospital-based cross-sectional study of 300 consecutively selected critically ill hospitalized patients in the two hospitals over a period of 6 months, from whom a total of 300 different clinical samples were collected. The specimens were processed by standard microbiological culture methods at the Applied Microbiology Laboratory Unit of Ebonyi State University (EBSU), Abakaliki. All isolated bacteria from cultures were phenotypically screened for *A. baumannii* by conventional biochemical test scheme and antibiotic susceptibility of test (AST) of confirmed isolates was done using the Kirby–Bauer disc diffusion technique, with AST results interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline.
Results: Of the 300 critically ill patients, clinical samples of 21 (7.0%) were positive for *A. baumannii*, with 20 (10.0%) of 220 samples from AE-FUTHA and 1 (1.3%) of 80 samples from MMHA. Analysis of the different isolation sites showed that catheter urine (16.0%, 11/70) from AE-FUTHA and (2.0%, 1/50) from MMHA was the most frequent site of *A. baumannii* isolation. *A. baumannii* isolates showed high resistance rates to tetracycline (100.0%), trimethoprim-sulphamethoxazole (100.0%), ceftriaxone (81.0%) and amikacin (81.0%), while low resistance rate was demonstrated to meropenem (14.3%), imipenem (19.0%) and polymyxin B (33.3%). The multiple antibiotic resistance index (MARI) of the *A. baumannii* isolates was 12.1, with average MARI value of 0.57.
Conclusion: Early diagnosis of infection caused by *A. baumannii* and its treatment with meropenem, imipenem or polymyxin B can reduce the risks of mortality and morbidity in *A. baumannii* infection of critically ill patients.

Keywords: *Acinetobacter baumannii*, Critical illness, Prevalence, southeast Nigeria

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Prévalence et caractéristiques phénotypiques d'*Acinetobacter baumannii* isolées chez des patients gravement malades dans deux établissements de santé de l'État d'Ebonyi, Nigéria

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Résumé:

Contexte: La propriété intrinsèque d'*Acinetobacter baumannii* de survivre dans des conditions difficiles sur des surfaces environnementales et sa capacité à résister aux antibiotiques couramment utilisés dans les hôpitaux font

de cet agent pathogène l'une des causes les plus répandues d'infections hospitalières. La présente étude visait à déterminer la prévalence d'*A. baumannii* parmi les patients gravement malades dans deux hôpitaux tertiaires ; Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AE-FUTHA) et Mater Misericodiae Hospital Afikpo (MMHA) dans l'État d'Ebonyi, au sud-est du Nigeria.

Méthodologie: Il s'agissait d'une étude transversale en milieu hospitalier portant sur 300 patients hospitalisés dans un état critique sélectionnés consécutivement dans les deux hôpitaux sur une période de 6 mois, auprès desquels un total de 300 échantillons cliniques différents ont été collectés. Les échantillons ont été traités par des méthodes de culture microbiologique standard au laboratoire de microbiologie appliquée de l'Université d'État d'Ebonyi (EBSU), à Abakaliki. Toutes les bactéries isolées des cultures ont été analysées phénotypiquement pour A. baumannii par un schéma de tests biochimiques conventionnels et le test de sensibilité aux antibiotiques (AST) des isolats confirmés a été effectué à l'aide de la technique de diffusion sur disque de Kirby-Bauer, les résultats de l'AST étant interprétés conformément aux normes cliniques et de laboratoire. Lignes directrices de l'Institut (CLSI).

Résultats: Sur les 300 patients gravement malades, 21 échantillons cliniques (7,0%) étaient positifs pour *A. baumannii*, dont 20 (10,0%) sur 220 échantillons provenant d'AE-FUTHA et 1 (1,3%) sur 80 échantillons provenant de MMHA. L'analyse des différents sites d'isolement a montré que l'urine de cathéter (16,0 %, 11/70) de l'AE-FUTHA et (2,0%, 1/50) du MMHA était le site le plus fréquent d'isolement d'*A. baumannii*. Les isolats d'*A. baumannii* ont montré des taux de résistance élevés à la tétracycline (100,0%), au triméthoprime-sulfaméthoxazole (100,0%), à la ceftriaxone (81,0%) et à l'amikacine (81,0%), tandis qu'un faible taux de résistance a été démontré au méropénème (14,3%), à l'imipénème (19,0%) et polymyxine B (33,3%). L'indice de résistance multiple aux antibiotiques (MARI) des isolats d'*A. baumannii* était de 12,1, avec une valeur moyenne du MARI de 0,57.

Conclusion: Le diagnostic précoce de l'infection causée par *A. baumannii* et son traitement par méropénème, imipénème ou polymyxine B peuvent réduire les risques de mortalité et de morbidité liés à l'infection à *A. baumannii* chez les patients gravement malades.

Mots clés: *Acinetobacter baumannii*, Maladie grave, Prévalence, sud-est du Nigeria

Introduction:

The genus *Acinetobacter* is a group of Gram-negative bacteria belonging to the wider class of Gammaproteobacteria (1). This group of organisms can survive for prolonged periods of time in the environment and on the hands of healthcare workers (2). *Acinetobacter* is a clinically important pathogen with widespread resistance to various antibiotics. The bacterium is a key cause of infection among debilitated patients in the hospital (1).

Initially, the *Acinetobacter calcoaceticus-baumannii* (ACB) complex comprised four species; *Acinetobacter calcoaceticus* (genomic species 1)*, Acinetobacter baumannii* (genomic species 2), *Acinetobacter pittii* (previously named genomic species 3) and *Acinetobacter nosocomialis* (previously named genomic species 13 TU). Of these, *A. baumannii* is the most important clinically relevant species, responsible for 80% of *Acinetobacter* infections (3,4).

Acinetobacter baumannii has been reported as a notorious opportunistic pathogen, affecting debilitated patients especially at the intensive care units (ICU) and others with underlying illnesses, and the bacterium has consistently jeopardized many antibiotics (5). The clinical significance of *A. baumannii* as a hospital-acquired pathogen is undoubtedly related to its resistance to commonly used antibiotics and virulence potentials (4).

Although *A. baumannii* was initially considered as a low virulence pathogen, recent studies have shown *A. baumannii* as one of the most significant clinical pathogens associated with hospital-acquired infections (5-8). Pneumonia has been the major disease manifestation of nosocomial infections caused by this pathogen, resulting in significantly high mortality rate of patients. It is also responsible for wide range of other infections including septicemia, meningitis, urinary tract infection, endocarditis, and more recently, severe and deadly cases of necrotizing fasciitis (9).

Acinetobacter baumannii is recognized as one of the six ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) by the Infectious Diseases Society of America (IDSA). It has subsequently developed into a pan-drug resistant (PDR) pathogen and received rapid recognition as one of the most important bacterial pathogens causing healthcare-associated infections (10,11). This high MDR attributes and persistence make *A. baumannii* a serious threat to hospitalized patients.

The hallmark of extreme or extended drug resistance (XDR) phenotype is carbapenem resistance (CR), and carbapenem resistant *A. baumannii* (CRAB) constitute the major strains in many hospitals today (10,11), and are now reported as important cause of different types of infections including endocarditis, skin and soft tissue infections, meningitis, septicemia, respiratory and urinary tract infections. Carbapenem-resistant strains are often resistant to all other routinely available antibiotics except polymyxins (colistin), tigecycline, and sometimes aminoglycosides (10,11). Treatment of CRAB infections therefore involves the use of combinations of last resort antibiotics such as colistin (12).

High costs of treatment of CRAB infections, treatment failure, and high mortality have been reported in various health facilities

globally. In view of the paucity of information on infections caused by *A. baumannii* in Alex Ekwueme-Federal University Teaching Hospital Abakaliki (AE-FUTHA) and the Mater Misericodiae Hospital Afikpo (MMHA) Ebonyi State, Nigeria, this study was aimed at determining the prevalence and antibiotic susceptibility of *A. baumannii* isolates among critically ill patients hospitalized in the various wards and intensive care units of the two hospitals.

Materials and method:

Study area:

The study was conducted at the Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AE-FUTHA), and Mater Misericodiae Hospital Afikpo (MMHA), Ebonyi State, Nigeria. Ebonyi State is located in southeastern Nigeria within longitude 7.30' and 8.30'E and latitude 5.40' and 6.45'N. The State was created on October 1, 1996 from the former Abia and Enugu States, with Abakaliki as its capital. It is bounded to the north by Benue State, to the west by Enugu State, to the east by Cross River State and to the south by Abia State.

There are thirteen Local Government Areas (LGAs) in the State namely; Abakaliki, Ebonyi, Ishielu, Ohaukwu, Izzi, Ikwo, Ezza North, Ezza South, Afikpo North, Afikpo South, Ivo, Ohaozara and Onicha LGAs. There are many government-owned and some private health clinics are obtained from the study area.

Study design and period:

This study was hospital-based cross-sectional design involving 300 critically ill patients on admission in medical, surgical, and orthopedic wards, and the intensive care unit (ICU) of AE-FUTHA and MMHA. The study was conducted over a period of 6 months (September 1, 2022 – March 1, 2023)

Ethical clearance:

Ethical clearance was obtained from Research and Ethical Committee (REC) of AE-FUTHA (AE-FUTHA/REC/VOL3/2022/129) and written permission was obtained from the management of MMHA. Participation in the study was highly voluntary and participants who consented were at will to withdraw from participation at any point they felt uninterested. Informed consent was obtained from the participants or their spouse, parents or caregiver.

Sample size and participant selection:

The sample size was estimated using the Leslie Kish formula (13), which gave the calculated minimum number of participants as 300. The participants were critically ill patients who had been hospitalized for at least 14 days in male and female medical wards, male and female surgical wards, male and female orthopedic wards, burns, and intensive care units (ICU) of the hospital. The participants were recruited by consecutive sampling over the period of study.

Data and sample collection:

Relevant socio-demographic data and other information were obtained from the participants care giver or spouse. Demographic data collected included age, gender, marital status, duration of hospital stay and ward of admission. Different clinical samples as appropriate to each patient condition were aseptically collected using sterile urine container or by sterile swab sticks, and these included catheter urine, wound drain, and swabs of wound, skin, nose, and mouth. All samples were transferred to the Applied Microbiology Laboratory Unit of the Ebonyi State University (EBSU) for microbiological analysis.

Culture and isolation of *Acinetobacter:*

Wound swab was first inoculated onto Nutrient broth (Merck, Germany) and incubated aerobically for 24 hours followed by subculture on MacConkey agar (Merck, Germany) and further incubation for 24 hours at 37°C. Urine samples were directly inoculated onto MacConkey agar. *Acinetobacter* grew on MacConkey agar as non-lactose fermenter (colorless or slightly beige), and was presumptively identified as Gram-negative bacilli or coccobacilli (on Gram stain), oxidase negative, catalase positive and non-motile by hanging drop technique.

Acinetobacter isolate was phenotypically confirmed as *A. baumannii* by growth at 37°C and 42°C and by other biochemical tests (14). *Acinetobacter baumannii* ATCC1605 was used as a positive control for each test protocol.

Antimicrobial sensitivity testing:

Antibiotic susceptibility test on each isolate was done by the disk diffusion method as previously described (15). The colony suspension from each overnight culture was prepared using nutrient broth and compared with the turbidity of 0.5 McFarland standards. With the aid of a sterile swab stick, Mueller-Hinton agar plates were inoculated with suspension of the organism and allowed for 30 mins for prediffusion. Antibiotic impregnated discs (Oxoid, UK) including imipenem (10µg), tetracycline (10µg), meropenem (10µg), sulfamethoxazole-trimethoprim (25µg), amikacin (30µg), ciprofloxacin (5µg), polymyxin B (300 unit), ceftriaxone (30µg), doxycycline (10µg) and gentamicin (10µg), were placed on the surface of the media and incubated at 37° C for 24 hrs. The inhibition zone diameters were measured using a meter rule and the isolates were class-

ified as susceptible or resistant according to the Clinical and Laboratory Standards Institute guideline (16).

Multi-drug resistance (MDR) *A. baumannii* was taken as simultaneously resistance to three or more classes of antibiotics such as extended-spectrum cephalosporins (ceftriaxone, cefotaxime, ceftazidime, cefepime), fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin), aminoglycosides (gentamicin, amikacin), β-lactamase and β-lactamase inhibitors (ampicillin-sulbactam) and carbapenems (imipenem, meropenem, ertapenem).

Multiple antibiotic resistance index (MARI) was calculated and interpreted according to the method described by Ayandele et al., (17) using the formula; MARI=a/b, where 'a' is the number of antibiotics to which a particular isolate is resistant to and, 'b' is the total number of antibiotics tested against the isolate.

Results:

A total of 300 critically ill patients (220 from AE-FUTHA and 80 from MMHA) were recruited and clinical samples collected from each of them from the different wards of the two hospitals. Of these, 153 (51.0%) were males while 147 (49.0%) were females, with 113 males and 107 females from AE-FUTHA, 40 males and 40 females from MMHA. The age of the patients ranged from 18-69 years, with majority in age group 30-39 years (n=124) and 40-49 years (n=113), while the least is in the age group 60-69 years (n=1) (Table 1).

Of the 300 patients, 21 (7.0%) samples were positive for *A. baumannii* with 20 of the 220 participants (10.0%) from AE-FUTHA and 1 of the 80 participants (1.3%) from the MMHA being positive (Table 1). The most frequent *A. baumannii* infection occurred in the

Table 1: Frequency and demographic characteristics of critically ill patients with *A. baumannii* infections at Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA) and Mater Misericordiae Hospital Afikpo (MMHA), Ebonyi State, Nigeria

Characteristics	**AE-FETHA**		**MMHA**		**Total**	
	No of patients	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**
All patients	220	20 (10.0)	80	1 (1.3)	300	21 (7.0)
Gender						
Male	113	12 (10.6)	40	1 (2.5)	153	13 (8.5)
Female	107	8 (7.5)	40	0	147	8 (5.4)
Age group (years)						
18-29	31	3 (9.7)	22	0	53	3 (5.7)
30-39	88	4 (4.5)	36	1 (2.8)	124	5 (4.0)
40-49	94	11 (11.7)	19	0	113	11 (9.7)
50-59	6	2 (33.3)	3	0	9	2 (22.2)
60-69	1	0	0	0	1	0

Table 2: Frequency of critically ill patients with *Acinetobacter baumannii* infection by the hospital wards/units at Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA) and Mater Misericordiae Hospital Afikpo (MMHA), Ebonyi State, Nigeria

Ward of admission	**AE-FUTHA**		**MMHA**		**Total**	
	No of patients	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**
Medical ward	89	15 (16.9)	29	0	118	15 (12.7)
Orthopaedic ward	65	1 (1.5)	36	1 (2.8)	101	2 (1.9)
Intensive care unit	5	1 (20.0)	0	0	5	1 (20.0)
Surgical ward	61	3 (4.9)	15	0	76	3 (3.9)
Total	**220**	**20 (10.0)**	**80**	**1 (1.3)**	**300**	**21 (7.0)**

age group 50-59 years (22.2%, 2/9) followed by age group 40-49 years (9.7%, 11 of 113), while no infection was reported in the age group 60-69 years (Table 1).

Table 2 shows the different wards of admission and the frequency of isolation of *A. baumannii* from the clinical samples of patients. Most samples were collected from patients in medical (n=118) and orthopaedic (n= 101) wards followed by surgical ward (n=76) and very few from the intensive care unit (n=5). *A. baumannii* were isolated from all the wards and ICU of AE-FUTHA with most frequent being from the ICU (20.0%, 1/5) followed by medical (16.9%, 15/89), surgical (4.9%, 3/61) and orthopaedic (1.5%, 1/65) wards. However, the only *A. baumannii* isolated from MMHA was from orthopaedic ward.

As shown in Table 3, the most frequent source of clinical samples from the patients were catheter urine (n=120) and wound ulcer (n=82). *A. baumannii* were isolated most frequently from catheter urine (10.0%, 12 of 120), followed by wound drain (7.7%, 2/26), wound ulcer (7.3%, 6/82) and skin swab (6.7%, 1/15). *A. baumannii* were isolated from all the samples in AE-FTHA except from skin and nose swabs while the only *A. baumannii* isolated from MMHA was from catheter urine.

The antimicrobial susceptibility test of the 21 *A. baumannii* isolates to 10 commonly used antibiotics showed the highest levels of resistance to tetracycline (100.0%), trimethoprim-sulphamethoxazole (100.0%), followed by amikacin (80.9%), ceftriaxone (80.9%), gentamicin (57.0%), doxycycline (47.6%), and ciprofloxacin (42.8%), while resistance to meropenem, imipenem and polymyxin B were low 14.2%, 19.0% and 33.3%) respectively (Table 4).

The MARI value of the total *A. baumannii* isolates was 12.1 while the average MARI value was 0.57. Bacteria having MARI (>0.2) originate from a high-risk source of contamination where antibiotics are widely used. MARI value of ≤0.2 indicates strain originated from sources where antibiotics are seldom or never used.

Table 3: Frequency of critically ill patients with *A. baumannii* infections with respect to clinical samples at Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA) and Mater Misericordiae Hospital Afikpo (MMHA), Ebonyi State, Nigeria

Sample source	**AE-FUTHA**		**MMHA**		**Total**	
	No of patients	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**	**No of patients**	**No positive for *A. baumannii* (%)**
Catheter urine	70	11 (15.7)	50	1 (2.0)	120	12 (10.0)
Wound ulcer	67	6 (9.0)	15	0	82	6 (7.3)
Wound drain	24	2 (8.3)	5	0	26	2 (7.7)
Skin swab	13	1 (7.7)	2	0	15	1 (6.7)
Nose swab	20	0	5	0	25	0
Mouth swab	26	0	3	0	29	0
Total	**220**	**20 (10.0)**	**80**	**1 (1.0)**	**300**	**21 (7.0)**

Table 4: Antibiotics susceptibility of *A. baumannii* isolates from clinical samples of critically ill patients in Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA) and Mater Misericordia Hospital Afikpo (MMHA), Ebonyi State, Nigeria

Antibiotic	**Disc strength**	**No of isolates resistant (%)**	**No of isolates sensitive (%)**
Ciprofloxacin	5 µg	9 (42.9)	12 (57.1)
Tetracycline	5 µg	21 (100.0)	0
Trimethoprim-sulphamethoxazole	25 µg	21 (100.0)	0
Imipenem	10 µg	4 (19.0)	17 (81.0)
Gentamicin	10 µg	12 (57.1)	9 (42.9)
Amikacin	30 µg	17 (81.0)	4 (19.0)
Meropenem	10 µg	3 (14.3)	18 (85.7)
Doxycycline	10 µg	10 (47.6)	11 (52.4)
Polymyxin B	300 unit	7 (33.3)	14 (66.7)
Ceftriaxone	30 µg	17 (81.0)	4 (19.0)

Discussion:

Infection due to *Acinetobacter* species is a major public health challenge within the health care facilities and the community in general due to its multidrug resistance even to the most potent drugs such as carbapenems. Members of the genus *Acinetobacter* have not only shown increasing resistance to β-lactams but also to other classes of antibiotics such as aminoglycoside antibiotics and also thought to be a reservoir of antibiotic resistant genes in hospital environment (18,19). The prevalence of *A. baumannii* infection/colonization in the study is 7.0% (21/300), with the most frequent site of infection being the urinary tract and surface wound. This agrees with studies in Nigeria and elsewhere where urine and wound exudates/pus have been reported to be the most frequent clinical specimens sent for isolation of *A. baumannii* pathogen. However, Mohammad (20) and Neetu et al., (21) in Bangalore reported recovery of *A. baumannii* most frequently from blood samples

The number of critically ill patients in the intensive care unit (ICU) of the AE-FUTHA was relatively small, while there was no patient in ICU at MMHA during the entire period of the study. In spite of this, at AE-FUTHA, *A. baumannii* was more frequently isolated (20%, 1/5) from the ICU when compared to the medical (16.9%, 15/89) and surgical (4.9%, 3 of 61) wards. This is line with the findings of some studies conducted in different parts of Nigeria and in other parts of the world. In Nigeria, Nwadike et al., (18) reported ICU as the major point of isolation of *A. baumannii,* while a prevalence of 18.4 % was reported by Natalia et al., (8) at the surgical ICU of Maryland Medical Center, USA, and Neetu et al., (21) also reported high isolation rate from ICU of the hospital in Padmashree Bangalore. In contrast, Ikechukwu et al., (5) in Nigeria reported highest isolation rate from medical ward.

The antibiotic susceptibility test result showed that *A. baumannii* isolates in our study were mostly resistant to at least 3 classes of antibiotics, making them to be multidrug-resistant (MDR) isolates. The highest of resistance of the isolates was to tetracycline and trimethoprim-sulfamethoxazole while the least resistance was to meropenem, imipenem and polymyxin, which is in agreement with the finding of Ikechukwu et al., (5) who reported high *in vitro* efficacy of meropenem and imipenem against *A. baumannii*. Direkel et al., (22) and Eghbalimoghadam et al., (23) also reported similar low resistance rate to imipenem in Turkey and Iran respectively. Our findings and those of others suggest that meropenem and imipenem still remain the most potent antibiotics against *A. baumannii*.

The resistance to gentamicin was relatively high (57.1%), and quite high to ceftriaxone (80.0%) and tetracycline (100%) in the current study. A similar study by Muhammed (20) reported high resistance of *A. baumannii* to gentamicin (94.3%) and tetracycline (95%) but relatively low resistance rate to ceftriaxone (35.0%). Contrarily, the study by Al-Agamy et al., (24) reported that *A. baumannii* isolates were highly resistant to ciprofloxacin (80.0%) and imipenem (70.0%). The difference in resistance rates reported may reflect the degree of antibiotic exposure and use/ misuse in different settings.

The result of MARI of the isolates in our study agrees with that of Onuoha et al., (15) who reported average MARI of their isolates to be higher than 0.20. Bacteria having MARI > 0.2 tends to originate from a setting with high levels of antibiotics exposure, while those with MARI ≤ 0.2 tend to originate from setting with low antibiotic exposure.

Conclusion:

The prevalence of *A. baumannii* infection/colonization of critically ill patients in this study is 7.0%, which emphasizes the clinical importance of this pathogen in this group of patients. The most frequent sites of infection/ colonization are the urinary tracts and surface wounds, and the organism exhibited multidrug resistance pattern. However, meropenem and imipenem showed high *in vitro* activity and are recommended for treatment of infections caused by *A. baumannii* in critically ill patients.

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Contributions of authors:

OO conceived and designed the study, collected and analyze the samples; OSC wrote and reviewed the manuscript, DIE, OCN, EBO and OO carried out the literature search and critical review of the manuscript. All authors read and approved the final manuscript.

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Authors declare no conflict of interest

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