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ORIGINAL ARTICLE

Detection of carbapenemase production by blood culture carbapenem inactivation method (bcCIM) compared to modified carbapenem inactivation method (mCIM) in bloodstream infections

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Carbapenemase, bloodstream infection, phenotypic test, Gram-negative bacilli

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ABSTRACT

Background: *Early detection of bloodstream infections (BSIs) caused by carbapenemase-producing organisms can guide rapid lifesaving appropriate therapy. A new variant of the modified Carbapenem Inactivation Method (mCIM) test, named blood culture CIM (bcCIM), can directly detect carbapenemase enzymes from positive blood culture.* ***Objective:*** *This study aimed at comparing detection of carbapenemase activity in Gram-negative bacilli (GNB) by Carbapenem Inactivation Method on positive blood culture and isolated colonies.* ***Methodology:*** *bcCIM test was used to detect carbapenemase production in 38 positive blood cultures. Subculture of blood samples followed by isolation and identification of causative pathogens was done, antimicrobial susceptibility by disk diffusion test as well as performing mCIM test were applied to all isolated GNB. Results: Forty-one GNB were isolated. Klebsiella spp. was the most common isolated GNB 16 (39%). Most isolated GNB were carbapenem-resistant 31(75.6%). For monomicrobial bloodstream infection, there was fair (kappa =0.327), moderate (kappa=0.429), and slight agreement (kappa=0.158) between results of mCIM and results of bcCIM as regards all GNB, Enterobacteriaceae, and Pseudomonas, respectively.* ***Conclusion:*** *bcCIM is a promising test for direct detection of carbapenemase activity, particularly in carbapenem-resistant Enterobacteriaceae. Further studies are needed for the standardization of this method.*

INTRODUCTION

Gram-negative bacilli (GNB), particularly *Enterobacteriaceae*, are the causative agents of a wide range of nosocomial as well as community-acquired infections that raise a significant public health concern [1].

The irrational wide use of antimicrobials, especially in developing countries, leads to the emergence of variety resistant GNB. The identification of these resistant phenotypes is mandatory for the proper implementation of infection control measures and better strategies for early patient management [2].

Carbapenems are considered a cornerstone treatment for GNB infections, and they are used as empirical therapy for bloodstream infections, especially when suspected to be caused by extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriales*. [3,4].

Several mechanisms are proposed for resistance to Carbapenems e.g., alteration of cell membrane porin channels, efflux pumps, and target-site mutation. Still, carbapenemase production is of utmost importance as they are usually carried on mobile elements having the risk of rapid transmission and emergence of outbreaks [4].

Different assays were developed to shorten the time needed to identify causative pathogens and detect their resistance pattern directly from blood culture, e.g., SeptiFast, SepsiTest, and VYOO [5].

Carbapenem-resistant organisms increase morbidity and mortality rates in infected patients compared to carbapenem susceptible pathogens [6,7] and carbapenemase-producing GNB have the worst outcomes compared to non-carbapenemase producing GNB [8]. The delay in starting antimicrobial therapy in BSI patients increases the probability of devastating outcomes [9].

In regions with higher levels of carbapenem resistance, early detection of the carbapenem resistance mechanism could be considered cost-effective in decreasing hospital stay and risk of mortality [7].

In national surveillance, including 28 hospitals in Egypt, bloodstream infection was the most common healthcare-associated infection (HAIs) in 91 intensive care units (30%). *Klebsiella* spp. was the most common isolated pathogen in all HAIs (28.7%), with carbapenem resistance detected in (48.1%) of them. In more comprehensive, recent surveillance, the percentage of carbapenem-resistant *Klebsiella* spp. was (53.7%) with

blood representing the most common specimen containing carbapenem-resistant *Enterobacteriaceae* (CRE) [10–12].

Several phenotypic and genotypic tests are available for direct detection of carbapenemase from positive blood culture, e.g., colorimetric methods such as β CARBA® and NeoRapid CARB, which are limited by decreased specificity of the tests. Immunochromatographic test as NG-Test® CARBA 5. and molecular assays, e.g., Xpert® Carba-R, are helpful, but they detect only the five common carbapenemases[13–15].

Meier & Hamprecht [13] proposed a modification on the phenotypic test recommended by CLSI for carbapenemase detection; modified carbapenem inactivation method (mCIM) to be applied directly on positive blood cultures. The test is simple, cost-effective and does not require any equipment, and showed high sensitivity and specificity in detecting carbapenemase production in *Enterobacteriaceae* compared to three other phenotypic tests but compared them; the test carries the disadvantage of longer detection time.

In their publication, Meier & Hamprecht [13] used blood samples spiked with *Enterobacteriaceae* only. Yet, no other publications evaluated this phenotypic test directly on clinical samples and/or on GNB other than *Enterobacteriaceae*. Moreover, clinical samples carry the unlimited possibilities of mixed infection that is difficult to assess in spiked samples.

This study aimed at comparing the detection of carbapenemase activity in GNB by the Carbapenem Inactivation Method on positive blood culture and isolated colonies.

METHODOLOGY

This is a cross-sectional study conducted at Ain Shams University Hospitals during November and December 2020 on 300 positively cultured blood samples collected from patients suspected to have bloodstream infections. The Ethical Committee of Ain Shams University approved the study.

Blood culture:

Blood samples were cultured using DL-Bt Auto Blood Culture Detection System (Zhuhai DL Biotech, China). Gram-stained films of all positive cultures were examined, and blood culture bottles showing GNB detected by Gram stain were further processed.

Phenotypic detection of carbapenemase production directly from positive blood culture (bcCIM)

A 10-µg meropenem disk (Oxoid, England) was immersed in 1 mL of the blood culture fluid containing ten µL of 10 mM $ZnSO_4$ and incubated at 37°C for two h.

A suspension equivalent to 0.5 McFarland of carbapenem susceptible *E. coli* reference strain ATCC® 25922, was prepared and inoculated on a Muller Hinton agar plate (MHA) (Himedia, India), then left to dry for 3 minutes.

After incubation, the meropenem disk was pulled and placed on the inoculated MHA plate, then incubated at 37°C for 24 h.

The sample was considered as carbapenemase positive if clear zone diameter measured 6-15 mm. Carbapenemase negative sample was considered if clear zone diameter was ≥19 mm. The result was recorded as indeterminate if zone diameter ≥ 19 mm with pinpoint colonies or 16-18 mm clear zone (considered positive if pinpoint colonies present)[13].

Isolation and identification of GNB

Subculture from positive blood cultures on blood and MacConkey's agar plates (Oxoid, England) was done. Isolated colonies were identified through conventional microbiological methods, i.e., Gram stain and suitable biochemical reactions[16].

Detection of antimicrobial susceptibility

Detection of antimicrobials susceptibility of isolates by disk diffusion method on MHA was done and interpreted according to CLSI guidelines [17]. Table (1) shows the used antimicrobial disks for different GNB (Oxoid, England).

Table 1: Antibiotic disks used for different GNB

Antibiotic	Disk content
Amoxicillin [a]	10 µg
Amoxicillin/Clavulanic Acid [a]	20/10µg
Piperacillin/Tazobactam [a,b,c]	100/10µg
Ceftriaxone [a,c]	30 µg
Ceftazidime [a,b,c]	30µg
Ampicillin/Sulbactam [c]	10/10 µg
Cefepime [a,b,c]	30µg
Meropenem [a,b,c]	10 µg
Amikacin [a,b,c]	30µg
Gentamicin [a,b,c]	10µg
Sulfamethoxazole/Trimethoprim[a,c]	25µg
Ciprofloxacin [a,b,c]	5µg
Doxycycline [c]	30µg

[a]: used for *Enterobacteriaceae*
[b]: used for *Pseudomonas* spp.
[c]: used for *Acinetobacter* spp.

Phenotypic detection of carbapenemase production in GNB isolates using (mCIM) method

The test was performed for all isolated strains and interpreted according to CLSI guidelines[17]

Briefly, for each isolate, a 1- µL loopful of bacteria for *Enterobacteriaceae* and 10 µL for *P. aeruginosa* and *A. baumannii*, was emulsified in 2 mL Tryptone Soya broth (TSB) (Himedia, India), then vortexed for 10 – 15 seconds.

Meropenem disk (10µg) was added to each tube and processed and interpreted as for bcCIM. Additionally, the mCIM test was repeated for strains reported to be indeterminate for carbapenemase production [18]

Carbapenemase positive and negative strains were used as control while performing mCIM and bcCIM tests.

Statistical analysis

Data were analyzed using SPSS (statistical package for social science) version 25 (Armonk, NY: IBM Corp) software for Windows. Means and standard deviations were calculated for continuous variables. Percentages were used for categorical variables.

The Chi-square test was used to study the comparison and association between two qualitative variables. A P-value of <0.05 was considered statistically significant.

Agreement between mCIM and bcCIM were tested with kappa statistics; cut-off values for the kappa was interpreted as values ≤ 0 indicating no agreement and 0.01–0.20 as slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement.[19]

RESULTS

Out of 300 positively signaled blood culture bottles, 38 samples were positive for GNB (Fig1). The age of the 38 patients ranged from 24 to 73 years with a mean ± SD of 49.92 ± 15.13; they were 17 females (44.7%) and 21 males (55.3%).

Forty-one GNB were isolated from positive blood culture bottles. Isolates were distributed as follows: *Klebsiella* spp.16 (39%), *Pseudomonas* spp. 9(22%), *E.coli* 8(19.5%) , *Acinetobacter* 7 (17.1%) and *Proteus* spp. 1(2.4%).

Mixed infection was detected in 3 samples (two samples contained *E. coli* and *Klebsiella* spp. while one sample contained *Pseudomonas and Proteus* spp.

For all GNB highest resistance was recorded for Ceftazidime, while the least resistance was against amikacin (Fig2).

Most of the isolated GNB were carbapenem-resistant 31(75.6%), all *Acinetobacter* isolates were carbapenem-resistant 7(100%), while 7(77.8%) and 17(68.0%) of *Pseudomonas* spp. and *Enterobacteriaceae* isolates were carbapenem-resistant, respectively.

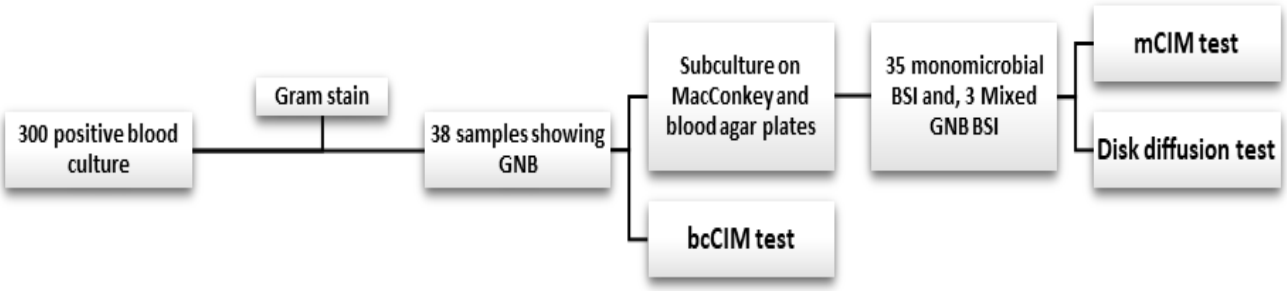

Fig. 1: Flow chart for samples processing

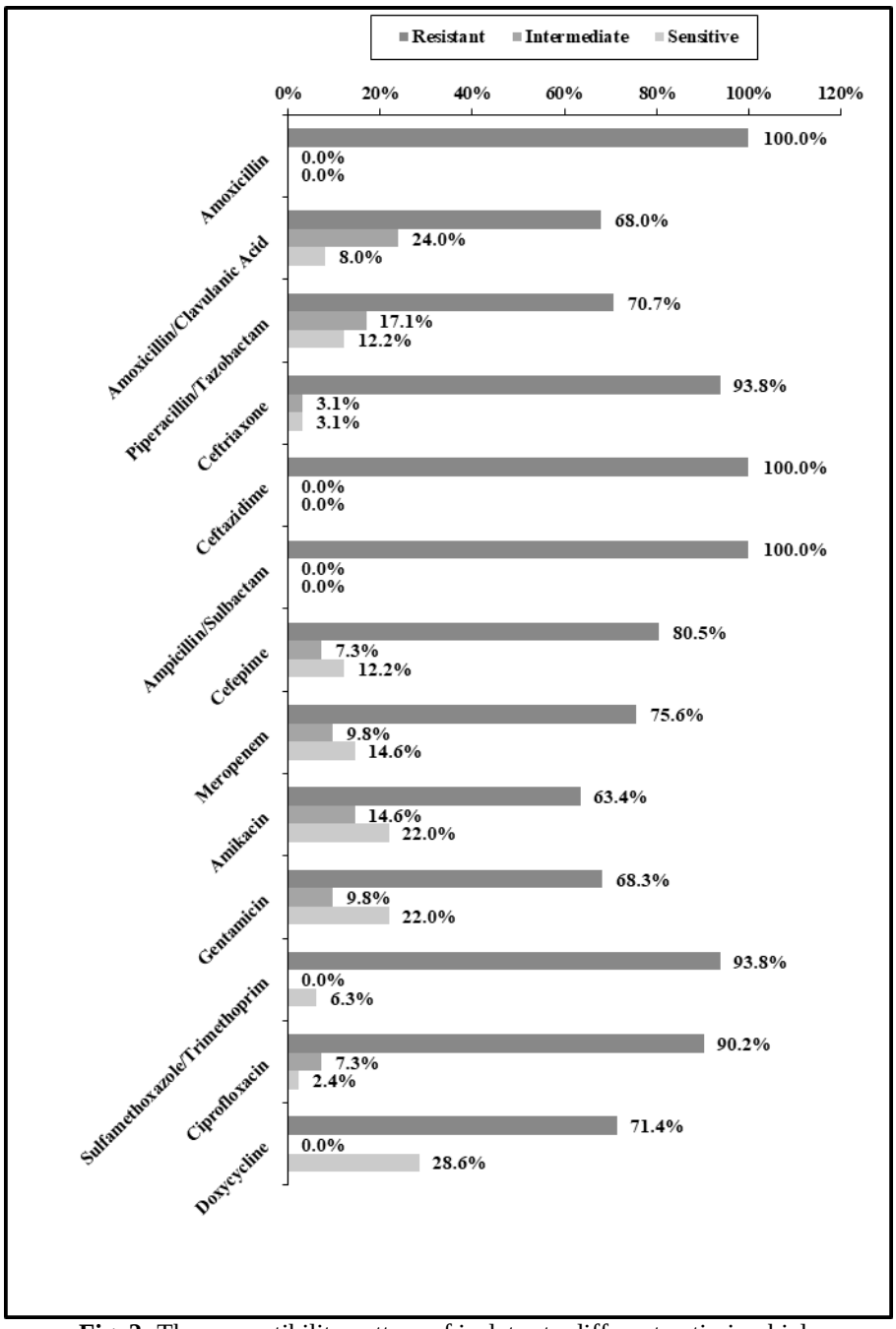

Fig. 2: The susceptibility pattern of isolates to different antimicrobials

For the 41 isolates tested by mCIM; 31 (75.6%) of GNB were positive for carbapenemase production while 6 (14.6%), 4(9.8%), were negative, indeterminate for carbapenemase production, respectively. For the 38 blood samples tested using bcCIM, half of the samples, 19(50%), were negative, and nine samples (23.7%) tested positive for carbapenemase production, while ten samples (26.3%) gave indeterminate results

Table (2) demonstrates results of carbapenem susceptibility, mCIM, and bcCIM in different isolated GNB.

When comparing bcCIM and mCIM, samples with mixed GNB infection (3 samples) were excluded. Indeterminate results were considered as positive results.

There was a fair agreement between mCIM and bcCIM for GNB; a moderate agreement was noticed for *Enterobacteriaceae* while for *Pseudomonas* spp. there was a slight agreement between both tests (table 3).

Table 2: Results of carbapenem susceptibility, mCIM, and bcCIM in different GNB

	Meropenem			Modified CIM (mCIM) results			Related results of Blood culture CIM (bcCIM)		
	Resistant	Sensitive	Intermediate	Negative	Indeterminate	Positive	Negative	Indeterminate	Positive
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Pseudomonas	7 (77.8%)	1 (11.1%)	1 (11.1%)	1 (11.1%)	1 (11.1%)	7(77.8%)	5 (55.6%)	4 (44.4%)	0 (0.0%)
Acinetobacter	7 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	7(100%)	2 (28.6%)	2 (28.6%)	3 (42.8%)
Enterobacteriaceae	17 (68%)	5 (20%)	3 (12%)	5 (20%)	3 (12%)	17(68%)	13 (52%)	5 (20%)	7 (28%)

Table 3: Correlation between results of mCIM and bcCIM

mCIM		bcCIM		Total	P-value	The measure of Agreement Kappa
		Negative	Positive*			
GNB	Negative	6	0	6	0.009	0.327
	Positive*	12	17	29		
Total		18	17	35		
Enterobacteriaceae	Negative	5	0	5	0.020	0.429
	Positive*	6	9	15		
Total		11	9	20		
Pseudomonas	Negative	1	0	1	0.408	0.158
	Positive*	4	3	7		
Total		5	3	8		
Acinetobacter	Negative	0	0	0	NA	NA
	Positive*	2	5	7		
Total		2	5	7		

*Indeterminate results were considered as positive [20], NA: Not applicable

DISCUSSION

Bloodstream infection is responsible for significant short and long-term morbidity and mortality worldwide [21]. Higher rates of resistance to antimicrobials are documented in GNB causing BSIs, especially towards carbapenem antimicrobials [11,22].

In this study, GNB constituted (13.9%) of all documented BSIs. In 38 blood samples, *Klebsiella* spp. (39%) was the predominant pathogen followed by *Pseudomonas* spp. (21.9%). Similar results were reported by other studies in Egypt and nearby countries as *Klebsiella* spp. represented the most common GNB in BSIs[23,24].

Isolated GNB showed high resistance to most antibiotics. The least resistance detected was for amikacin antibiotic. Similar results were reported by other studies on GNB BSIs in Egypt [23,25]. Amikacin antibiotic demonstrate good activity against GNB because it is less susceptible to inactivating enzyme due to its chemical structure [26].

In the present study, most of the isolated GNB (75.6%) were carbapenem-resistant, and all *Acinetobacter* spp. were carbapenem-resistant. Similar results were obtained by Tohamy and his colleagues who studied BSIs in cancer patients[27].Carbapenem resistance in *Enterobacteriaceae* (68.0%) and *Pseudomonas* spp. (77.8%) where higher than detected elsewhere. [11,25] but in a more recent study conducted at ICUs of a tertiary care hospital in Egypt, similar high rates for carbapenem resistance were detected [28].

Bloodstream infections by carbapenemase-producing GNB show variable rates in different world regions [13,29–31]. According to the results of mCIM, 31 (75.6%) of GNB were positive for carbapenemase production. This finding is higher than reported in a similar study in Egypt by Abdulall and his colleagues, but they used different phenotypic tests for carbapenemase detection [25].

All strains that were resistant to carbapenem were carbapenemase producers. This finding is higher than reported in studies by Kamel and later on by Raheel and their colleagues. They found that (67.9%) (46.6%) respectively of the CRE isolates from different HAIs were positive for carbapenemase production by the mCIM test [32,33].

In nationwide surveillance in Italy (65.1%) of CRE-causing BSIs were confirmed carbapenemase producer phenotypically[34]. At the same time, in the PANORAMA study, carbapenemase-encoding genes were detected in (88%) of CRE-causing BSIs in 10 low-income and middle-income countries, including Egypt [35]. Carbapenem-resistant organisms are difficult to treat than carbapenem-sensitive ones, and carbapenemase-

producing GNB has the worst outcomes compared to non-carbapenemase-producing GNB [8].

Several variations exist of the phenotypic assay Carbapenem Inactivation Method (CIM), i.e., modified CIM (mCIM), rapid CIM (rCIM), Simplified CIM (sCIM), (CIMTris), and (CIMTrisII) for detection of carbapenemase-producing GNB[17,20,36,37]. Moreover, sodium mercapto acetate (SMA-mCIM), EDTA (eCIM) and CIMplus tests are modified versions of the test designed to discriminate different types of carbapenemases [17,38]. A recent variation of this test (bcCIM) was applied directly on positive blood culture aiming at early detection of carbapenemase activity before culture results appear [13].

Different CIM tests share the main principle with slightly different procedures. These variations in steps and materials used lead to variable sensitivity and specificity towards detection of carbapenemases in GNB culture. for instance, the difference between mCIM and CIM was using tryptone soy broth instead of water and different inoculum and incubation time[20].

In the present study, there was a fair agreement (kappa =0.327) between mCIM as a reference method recommended by CLSI and bcCIM in detecting carbapenemase-producing GNB. The decreased performance of bcCIM compared to mCIM, and to the results of Meier & Hamprecht could be due to several factors, e.g., using different blood culture system, besides clinical samples differ from spiked samples in terms of inoculum used and other host factors [13].

Similar results were reported by De Lima-Morales et al.[39] comparing another carbapenemase detecting phenotypic test directly on the blood and on pure culture, where applying the test directly on blood detected only 80% of carbapenemase positive isolates identified by the same test on pure culture.

In the present study, results of mCIM correlated better with bcCIM as regard *Enterobacteriaceae* (moderate agreement, kappa=0.429) compared to *Pseudomonas* spp. (slight agreement, kappa=0.158).

Previous studies recommended when performing the mCIM test to use a higher inoculum (10ul) for reliable detection of carbapenemase production by *P. aeruginosa* and *A. baumannii* than the inoculum used for the mCIM test in *Enterobacteriaceae* (1ul) [17,18].

In blood samples, the causative agent couldn't be identified before performing bcCIM, and worth noting that blood culture bottles that signaled positive might not be processed immediately due to the limitation of working hours, i.e., not processed until next working hours. This may affect the initial test inoculum and, subsequently, the results. This study is the first to test carbapenemase production using bcCIM on blood samples containing *Pseudomonas* and *Acinetobacter* spp. Future studies are needed to determine the optimum processing time for best results for different GNB.

Acinetobacter baumannii is an important cause of BSI. The mCIM test is not standardized for testing carbapenemase production among *Acinetobacter* spp. as the most common types of carbapenemases produced by *Acinetobacter* have weak carbapenemase activity [18].

In the present study, all *Acinetobacter* isolates tested positive for carbapenemase production using mCIM. The results of the bcCIM test matched the results of mCIM test in 5 (71.4%) samples. Unfortunately, the statistical correlation between the two tests couldn't be performed as none of the *Acinetobacter* isolates was negative for carbapenemase production using the mCIM test.

The present study included three samples with mixed GNB infection. Results of bcCIM in two samples correlated with results of mCIM. The third sample tested negative for bcCIM while both strains were positive by mCIM. Different combinations of various types of carbapenemases may affect reporting of results, and future large-scale studies on mixed GNB bacteremia are needed to revise the cut-off points of reporting bcCIM in these situations.

Although simplicity and feasibility of performing mCIM and bcCIM tests, both detect only carbapenemases production, which is one of the mechanisms responsible for carbapenem resistance in GNB, besides no differentiation between different types of carbapenemases can be achieved using mCIM, bcCIM tests alone [8,13].

Limitation of the current study includes the small number of isolates tested and the lack of molecular characterization of the types of carbapenemases present in isolated strains.

CONCLUSION

The relatively high incidence of carbapenem-resistant GNB causing BSI warrants the need for effective antimicrobial stewardship as well as effective implementation of an infection control program.

Delay in starting treatment may lead to catastrophic consequences. Phenotypic tests for carbapenemase production give a good alternative to genotypic methods that are sometimes nonapplicable, especially in low-resource countries. The bcCIM test could be a promising test for the early detection of carbapenemase production, particularly in CRE. Further studies are needed for the optimization of culture conditions and cut-off points for reporting results.

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- Each author listed in the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for it.

- This article has not been published anywhere and is not currently under consideration by another journal or a publisher.

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