Original article

In vitro type 1 fimbriae expression and antibiotic resistance by uro-pathogenic bacterial isolates from human immunodeficiency virus infected patients in Uyo, Nigeria

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A R T I C L E I N F O

A B S T R A C T

Background: Some bacteria possess fimbriae that support their adherence to host cell surface, colonization of human epithelial cells and resistance to antibiotics. **Aim:** The expression of type 1 fimbriae and antibiotic resistance by isolates from human immunodeficiency virus (HIV) infected patients. **Methods:** Bacteriological analysis of mid-stream urine (MSU) samples of HIV infected patients (n=61) was determined using microbiological technique. *In vitro* type 1 fimbriae expression and antibiotic susceptibility of bacteria was determined using human erythrocytes with D-mannose and disc diffusion technique. **Results:** Of the 61 MSU samples from subjects, 31.1% had positive bacterial growth $\geq 10^5$ CFU/mL. Forty-nine (49) bacterial isolates, belonging to genera *Staphylococcus, Escherichia, Pseudomonas, Streptococcus, Klebsiella, Enterococcus* and *Proteus* were recovered from the MSU samples. *Escherichia coli* was the predominant isolate with 26.5%, followed by *S. aureus* (18.4%), while *E. faecalis* had the lowest occurrence (4.1%). Of the 29 isolates exhibiting hemagglutinating activity, type 1 fimbriae was expressed by 16 isolates, while 13 isolates showed P-fimbriae. The results showed 18.4 % isolates exhibiting resistance to gentamycin; between 30.6 % and 38.8% isolates were tetracycline and nitrofurantoin resistant, while $\leq$ 44.9% isolates were resistant to chloramphenicol and ampicillin. *Proteus* sp had a low level of resistance to ciprofloxacin, while ≥ 46.2 % *E. coli* were resistant to nitrofurantoin. There was significant relationship ($p < 0.05$) between isolates expressing type 1 fimbriae and resistance to gentamycin, nalidixic acid and ampicillin. **Conclusion**: This study has shown the phenotypic expression of type 1 fimbriae and antibiotic resistance by isolates from HIV infected patients.

Introduction

Many bacterial cell wall structures have short, external filamentous appendages known as Fimbriae [1]. The fimbriae, either located at the poles of a cell or evenly spread over its entire surface, aid bacteria in adhering to host cell surface, colonize human epithelial cells, tissue invasion, biofilm formation and cytokine induction [1]. Fimbriae are serologically categorized, by their hemagglutinating patterns and receptor specificities, as either mannose sensitive hemagglutination (type 1-fimbriae) or mannose resistant hemagglutination (P-fimbriae) [2]. Human Immunodeficiency Virus (HIV), an aetiological agent of Acquired Immunodeficiency Syndrome (AIDS), is one of the human T-cell lymphotrophic retroviruses, of the family Retroviridae. The HIV epidemic continues to be a

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burden worldwide and presents serious public health issue in developing countries [3]. The sexual networking practices, unprotected sex, poverty and blood transfusion have contributed to the spread of HIV [3, 4].

Bacterial infections occur more frequently in HIV positive individuals than in HIV negative individuals. The HIV positive individuals are at increased risk of both asymptomatic and symptomatic urinary tract infection (UTI) [5]. The UTI is caused when pathogenic organisms invade and multiply in the urinary tract, leading to an inflammation of the urothelium [6]. The incidence of UTI is gradually increasing amongst HIV positive patients and the low CD4 counts have put the HIV infected patients at higher risk of bacteriuria [7,8].

The UTI among HIV infected patients could lead to kidney diseases, infertility, cancer, sepsis, and neurologic complication [9, 10]. The species of *Escherichia, Proteus, Klebsiella, Pseudomonas, Enterococcus* and *Staphylococcus* have been reported as the causative agents of UTIs in people living with HIV [11]. The UTIs account for a large proportion of antibiotics consumption specifically among a significant number of HIV infected patients who visit the hospital daily [12]. The resistance of microorganisms due to widespread and injudicious use of frequently prescribed antibiotics is becoming a worldwide problem [13]. This study aimed at determining type 1 fimbriae expression and antibiotic resistance by uro-pathogenic bacterial isolates from HIV infected patients.

Materials and Methods

Collection of samples

This study was conducted between April and November, 2019 in Uyo, Akwa Ibom State. A total of sixty-one (61) 'clean-catch' mid-stream urine (MSU) samples were aseptically collected using sterile wide mouthed containers, covered with tight-fitting lids, from HIV patients between the ages of $\leq$ 20yrs and $\geq$ 51 yrs at different hospitals. Verbal informed consent was obtained from the subjects who had not received antibiotic treatment for the previous one week prior to sample's collection. The MSU samples were transported in cooler boxes to microbiology Laboratory for bacteriological analysis.

Bacteriological analysis of mid-stream urine samples

Each of the uniformly mixed, uncentrifuged, MSU samples was aseptically inoculated onto dried plate of cysteine lactose electrolyte deficient (CLED) agar using a sterile calibrated drop that delivered 0.002 ml

of urine sample. The plates were aerobically incubated at 37 °C for 24 hr. After incubation, the colonies on each plate were observed, enumerated and counts of >10^5 CFU/ml were considered as significant bacteriuria (SBU). The cultures with significant growth were further subcultured onto plates of nutrient agar, aerobically incubated at 37°C for 24 hr, maintained on nutrient agar slant at 4°C, characterized and identified using their colonial appearances, Gram staining reaction, biochemical and sugar fermentation tests [14].

Antibiotic sensitivity testing of bacterial isolates

The standardized Kirby-Bauer disk-diffusion technique was used to determine the antibiotic susceptibility of bacterial isolates [15]. Ten microliters (10 μL) of each isolate prepared directly from an overnight agar plate, adjusted to 0.5 McFarland turbidimetric standard of approximately 1.5 x 10^8 CFU/ml, was inoculated using sterile pipette onto each of the plates containing Mueller-Hinton Agar (MHA). The antibiotic discs: tetracycline (TET, 30μg), erythromycin (ERY, 15 μg), amoxycillin (AMO, 10μg), ciprofloxacin (CPX, 5μg), gentamicin (GEN, 10 μg), nalidixic Acid (NA, 30μg), nitrofurantoin (NIT, 300μg), ampicillin (AMP, 10μg), ceftriaxone (CEF, 30μg) and chloramphenicol (CHL, 10μg) were aseptically placed on surfaces of the culture plates. The plates were incubated at 37 °C for 18 hr, inhibition zones after incubation were observed and measured in millimeters (mm) using a ruler and interpretation of the measurement as sensitive and resistant was made according to the zone size interpretative manual [15].

Preparation of bacterial isolates for hemagglutination assay

The isolates were subcultured into Brain Heart Infusion (BHI) broth (5ml) and aerobically incubated for 72 hr at 37°C for maximum fimbriation [16]. The culture was centrifuged at 1500 rpm for 5 mins to sediment the bacteria and the supernatant was discarded. Each of the isolates was harvested, suspended into phosphate-buffered saline (PBS), pH 7.4 (10mM) and washed thrice in PBS at 1500 rpm for 5 mins. The bacterial suspension was adjusted to a McFarland Standard of approximately 1.5 x 10^8 CFU/ml.

Preparation of red blood cells (RBCs) and hemagglutination assay

The RBCs were obtained from apparently healthy individual with type O, Rhesus D positive
blood after centrifugation at 1500 rpm for 5 mins. The RBCs were washed thrice in sterile PBS, pH 7.4

(10 mM) and a 3 % vol/vol RBCs suspension was prepared with PBS [16]. The hemagglutination assay was carried out by mixing 20 μl of RBCs suspension with 20 μl of bacterial suspension on a chilled glass slide and was gently rocked for 5 mins at room temp. The isolates were considered positive for hemagglutination if a visible agglutination occurred and were considered negative if no visible agglutination was observed within 5 mins. The mixture of RBCs suspension (20 μl) and PBS (20 μl) on a chilled glass slide served as a control experiment [16].

Determination of mannose sensitive hemagglutination (type 1 fimbriae)
A drop of 2 % D-mannose was added to RBCs suspension (20 μl) and bacterial suspension (20 μl) on a chilled glass slide and was gently rocked for 5 mins at room temperature. The mixture of bacterial suspension (20 μl) and RBCs (20 μl) on a chilled glass slide served as positive control, while the mixture of RBCs suspension (20 μl) and PBS (20 μl) on a chilled glass slide served as a negative control. Presence of agglutination in the presence of D-mannose indicated mannose resistant hemagglutination (MRHA), while absence of agglutination indicated mannose sensitive-hemagglutination (MSHA) and expression of type 1 fimbriae by the isolates.

Results

The age and gender-wise distribution of SBU among HIV infected patients is presented in **Table 1**. Of the 61 MSU samples from the subjects, 31.1% had positive bacterial growth $\geq 10^5$ CFU/mL, indicating SBU; 41.0 % samples had bacterial growth $\leq 10^5$ CFU/mL, while 27.9 % samples had no bacterial growth. The prevalence of SBU was not significantly higher in females (12/35, 34.3%) than males (7/26, 26.9%) at $p > 0.05$. The highest SBU (40.9 %) was among age group 21-30 yrs and the lowest SBU (16.7 %) among the subjects aged ≤ 20 yrs. The observed difference in the age groups was not statistically significant ($p=0.845$).

A total of 49 bacterial isolates, belonging to 7 genera, were recovered from the MSU samples of HIV infected patients **(Figure 1)**. *Escherichia coli* was the predominant bacterial isolate from the MSU samples with prevalence of 26.5%, followed by *S. aureus* 18.4%, while *E. faecalis* had the lowest occurrence with 4.1%. The occurrence of other bacterial isolates in MSU samples in increasing order was as follows: CoN *Staphylococcus* sp (6.1%) < *K. pneumoniae* (8.2%) < *Streptococcus* sp (10.2%) <

Proteus sp (12.2%) < *P. aeruginosa* (14.3%) **(Figure 1)**.

The results of *in vitro* phenotypic expression of type 1 fimbriae and hemagglutinating activity by Gram positive (GPB) and Gram negative (GNB) bacteria from MSU samples of HIV infected patients are presented in **table (2)**. Of the 49 bacteria isolated, 55.1% were positive for haemagglutinating activity, while non-haemagglutinating activity were observed in 44.9 % isolates. The proportion of haemagglutinin producing GPB were as follows: *S. aureus* (n=4/9; 44.4%), CoN *Staphylococcus* sp (n=1/3; 33.3%), *Streptococcus* sp (n=2/5; 40.0%) and *E. faecalis* (n=1/2; 50.0%). Nineteen (19) GNB were positive for haemagglutinating activity, of which *K. pneumoniae* had the highest occurrence (75.0%), followed by *E. coli* (69.2%), *P. aeruginosa* (57.1%), while *Proteus* sp had the lowest occurrence (50.0%) **(Table 2)**.

Of the twenty-seven (27) bacterial isolates that displayed hemagglutination, 15 isolates comprising four (4) GPB isolates (*S. aureus*, n=1; *E. faecalis*, n=1 and *Streptococcus* sp, n=2) and eleven (11) GNB isolates consisting of *E. coli* (7), *Proteus* sp (2) and *P. aeruginosa* (2) phenotypically expressed type 1 fimbriae (mannose sensitive hemagglutination) **(Table 2)**.

The antibiotic resistance profiles of bacterial isolates from MSU samples to commonly used antibiotics are shown in **table (3)**. The results showed between 30.6 % and 38.8% isolates were resistant to tetracycline, erythromycin, amoxicillin and nitrofurantoin, while $\leq 44.9\%$ isolates were resistant to chloramphenicol and ampicillin. Of the 49 bacterial isolates, 24.5%, 18.4%, 28.6 % and 28.6 % were resistant to ciprofloxacin, gentamycin, ceftriaxone and nalidixic acid, respectively. *Enterococcus faecalis* showed a high level of resistance to tetracycline (n=2/2; 100%) and chloramphenicol (n=2/2; 100%); *Proteus* sp had a low level of resistance (n=1/6; 16.7%) to ciprofloxacin, gentamycin, chloramphenicol and nitrofurantoin; $\geq 46.2\%$ *E. coli* were resistant to ceftriaxone and nitrofurantoin; while between 42.9% and 60.0% *Proteus* sp, *P. aeruginosa* and *Streptococcus* sp were resistant to erythromycin **(Table 3)**.

The relationship between antibiotic resistance and virulence markers of bacterial isolates is shown in **table (4)**. Among the haemagglutinating isolates (n=27), the highest resistance was observed to chloramphenicol (55.6%), followed by ampicillin (48.1%), tetracycline and nitrofurantoin with 44.4% each, while $\leq 29.6\%$ were resistant to ceftriaxone and

gentamycin. There was a significant relationship between the haemagglutinating bacteria and resistance to tetracycline, nitrofurantoin and chloramphenicol (p < 0.05). the isolates expressing type 1 fimbriae were highly resistant to chloramphenicol (66.7%) and ampicillin (60.0%), but showed low resistance (13.3%) to gentamycin. There was significant relationship (*p<0.05*) between isolates expressing type 1 fimbriae and resistance to gentamycin, nalidixic acid and ampicillin **(Table 4)**.

Table 1. Age and gender-wise distribution of significant bacteriuria among HIV infected patients.
SBU: Significant Bacteriuria; Values in parenthesis indicated percentages.

Variables	Categories	No of sample collected	Samples with SBU	Samples without SBU	Samples Without bacterial growth	χ2	*p-value*
			No (%)	No (%)	No (%)		
Gender	Male	26	7 (26.9)	9 (34.6)	10 (26.9)	2.6	*0.107*
	Female	35	12 (34.3)	16 (45.7)	7 (20.0)		
	Total	61	19 (31.1)	25 (41.0)	17 (27.9)		
Age (yrs)	≤ 20	6	1 (16.7)	3 (50.0)	2 (33.3)	1.4	*0.845*
	21-30	22	9 (40.9)	8 (36.4)	5 (22.7)		
	31-40	15	4 (26.7)	6 (40.0)	5 (33.3)		
	41-50	10	3 (30.0)	5 (50.0)	2 (20.0)		
	≥ 51	8	2 (25.0)	2 (25.0)	4 (50.0)		
	Total	61	19 (31.1)	24 (39.3)	18 (29.5)		

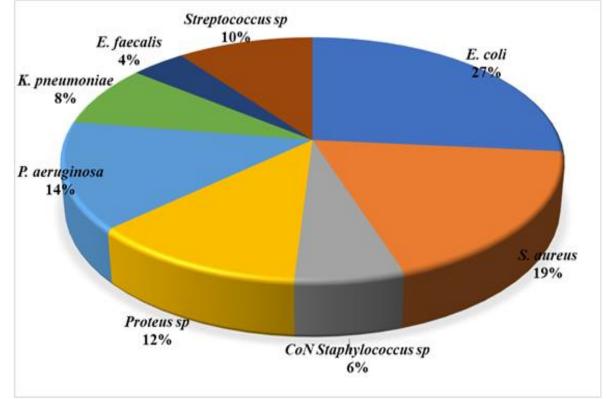**Fig 1.** Percentage occurrence of bacterial isolates in urine specimens of HIV infected patients.

Table 2. Occurrence of bacterial isolates expressing type 1-fimbriae in urine samples of HIV infected patients

Bacterial Isolates	No of Occurrence	HA	NHA	MSHA Type 1 Fimbriae	MRHA Type P-Fimbriae
		No (%)	No (%)	No (%)	No (%)
S. aureus	9	4 (44.4)	5 (55.6)	1 (11.1)	3 (33.3)
CoN *Staphylococcus* sp	3	1 (33.3)	2 (66.7)	0 (0.0)	1 (33.3)
E. coli	13	9 (69.2)	4 (30.8)	7 (53.8)	2 (15.4)
Proteus sp	6	3 (50.0)	3 (50.0)	2 (33.3)	1 (16.7)
P. aeruginosa	7	4 (57.1)	3 (42.9)	2 (28.6)	2 (28.6)
K. pneumoniae	4	3 (75.0)	1 (25.0)	0 (0.0)	3 (75.0)
E. faecalis	2	1 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)
Streptococcus sp	5	2 (40.0)	3 (60.0)	2 (40.0)	0 (0.0)
Total	49	27 (55.1)	22 (44.9)	15 (30.6)	12 (24.5)

HA: Haemagglutination, NHA: Non-Haemagglutination, MSHA: Mannose Sensitive Haemagglutination, MRHA: Mannose Resistant Haemagglutination.

Table 3. Antibiotic resistance profile of bacterial isolates from urine samples of HIV infected patients.

CIP: Ciprofloxacin, TET: Tetracycline, GEN: Gentamycin, ERY: Erythromycin, AMO: Amoxycillin, CHL: Chloramphenicol,   CEF:

Bacterial Isolates	Antibiotics / No / % Antibiotic resistant isolates									
	CIP	**TET**	**GEN**	**ERY**	**AMO**	**CHL**	**CEF**	**NA**	**AMP**	**NIT**
S. aureus	2 (22.2)	3 (33.3)	2 (22.2)	0 (0.0)	3 (33.3)	5 (55.6)	3 (33.3)	1 (11.1)	2 (22.2)	4 (44.4)
CoN *Staph.* sp	1 (33.3)	2 (66.7)	0 (0.0)	1 (33.3)	2 (66.7)	1 (33.3)	0 (0.0)	2 (66.7)	3 (100)	1 (33.3)
E. coli	3(23.1)	4 (30.8)	3(23.1)	5 (38.5)	5 (38.5)	6 (46.2)	9 (69.2)	3(23.1)	6 (46.2)	6 (46.2)
Proteus sp	1 (16.7)	2 (33.3)	1 (16.7)	3 (50.0)	2 (33.3)	1 (16.7)	3 (50.0)	2 (33.3)	3 (50.0)	1 (16.7)
P. aeruginosa	2 (28.6)	1 (14.3)	2 (28.6)	3 (42.9)	3 (42.9)	4 (57.1)	2 (28.6)	1 (14.3)	3 (42.9)	3 (42.9)
K. pneumoniae	1 (25.0)	2 (50.0)	0 (0.0)	0 (0.0)	1 (25.0)	2 (50.0)	1 (25.0)	2 (50.0)	2 (50.0)	1 (25.0)
E. faecalis	1 (50.0)	2 (100)	0 (0.0)	0 (0.0)	1 (50.0)	2 (100)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)
Streptococcus sp	1 (20.0)	2 (40.0)	1 (20.0)	3 (60.0)	2 (40.0)	2 (40.0)	1 (20.0)	2 (40.0)	2 (40.0)	1 (20.0)
Total	12(24.5)	18(36.7)	9(18.4)	15(30.6)	19(38.8)	23(46.9)	14(28.6)	14(28.6)	22(44.9)	17(34.7)

Ceftriaxone, NA: Nalidixic Acid, AMP: Ampicillin, NIT: Nitrofurantoin.

Table 4. Relationship between antibiotic resistance and virulence markers of bacterial isolates in HIV infected patients.

Virulence Markers		No of isolates	Antibiotics (No / %)									
			CIP	**TET**	**GEN**	**ERY**	**AMO**	**CHL**	**CEF**	**NA**	**AMP**	**NIT**
HT	positive	27	8(29.6)	12(44.4)	6(22.2)	10(37.0)	9(33.3)	15(55.6)	8(29.6)	8(29.6)	13(48.1)	12(44.4)
	negative	22	4(18.2)	6(27.3)	3(13.6)	5(22.7)	10(45.5)	8(36.4)	6(27.3)	6(27.2)	9(40.9)	5(22.7)
	p-value		NS	*0.03**	NS	NS	NS	*0.024**	NS	NS	NS	*0.01**
Fimbriae	type 1	15	5(33.3)	8(53.3)	2(13.3)	4(26.7)	7(46.7)	10(66.7)	3(20.0)	2(13.3)	9(60.0)	6(40.0)
	type P	12	3(25.0)	4(33.3)	4(33.3)	6(50.0)	2(16.7)	5(41.7)	5(41.7)	6(50.0)	4(33.3)	6(50.0)
	p-value		NS	NS	*0.03**	NS	NS	NS	NS	*0.03**	*0.01**	NS

HT: Hemagglutination, CIP: Ciprofloxacin,TET: Tetracycline, GEN: Gentamycin, ERY: Erythromycin,   AMO: Amoxycillin, CHL: Chloramphenicol, CEF: Ceftriaxone, NA: Nalidixic Acid;, AMP: Ampicillin;, NIT: Nitrofurantoin. *p*-values (by Fisher's exact test) are shown where *p < 0.05,* NS: Not Significant.

Discussion

Mid stream urine samples are frequently sent to the laboratories for microbiological analysis so as to determine the case of bacteriuria and UTI among population [6]. In this study, a high prevalence of SBU, indicating UTI, was observed as 31.1% of HIV patients had positive bacterial growth $\geq 10^5$ CFU/mL. This value was relatively higher than 11.9 % reported by **Alemu et al**. [17] in Gondar, Ethiopia; < 9.5 % obtained by **Banu and Jyothi** [7], but was lower than 77.5 % reported by **Xavier et al**. [18] in Tamil-Nadu, India. There was no significant difference in the occurrence of SBU with respect to gender (*p=0.107*) and age group (*P=0.845*) and this result agrees with **Essien et al.** and **Zakka et al**. [19, 20] but disagrees with the reports of **Kanu et al**. [21] in Aba, Nigeria. The highest occurrence of SBU among HIV patients within ages 21-30 yrs in our study corroborated the findings of **Chedi et al**. [22] in Kano, Nigeria. The high occurrence of SBU in age group 21-30 yrs might be related to their sexual activity which predispose them to bacteriuria and UTIs [5].

Escherichia coli was predominant bacterial isolate from MSU samples of HIV patients in our study and a similar finding was reported by **Alemu et al**. [17] in Gondar, Ethiopia and **Turpin et al**. [23] in Kumasi, Ghana. This result is inconsistent with the finding of **Olowe et al**. [5] in Osogbo, South-western Nigeria and **Xavier et al.** [18] in Tamil-Nadu, India who found *K. pneumoniae* and *S. aureus* as the commonest urinary tract pathogens in HIV patients, respectively. The preponderance of *E. coli* could be attributed to poor genital hygienic practices by the subjects and presence of a unique structure that aids its attachment to uroepithelial cells, and multiplication and tissue invasion [24].

The high sensitivity of *S. aureus* and *E. coli* to ciprofloxacin in this study is similar to the results of **Ehinmidu** [25], but this finding contradicts the reports of **Shill et al**. [26] in Dhaka, Bangladesh, where high ciprofloxacin resistant *E. coli* was obtained. The low gentamycin resistant isolates obtained in our study substantiates the reports of **Mbata** [27] in Nsukka, Nigeria, while the high ampicillin and tetracycline resistant isolates is in conformity with **Randrianirina et al.** [28] and **Edoh and Alomatu** [29] in Madagascar and Ghana, respectively. The high erythromycin resistant *Proteus* sp and *P. aeruginosa* in our study corroborates the finding of **Kyabaggu et al**. [30] in Uganda. The observed antibiotic resistance is an indication of earlier exposure of these isolates to these antibiotics and / or indiscriminate use of antibiotics among the subjects, which has favoured the emergence of resistant strains.

The pathogenicity of bacteria has been related to a number of virulence factors such as fimbriae and hemagglutinin which may be encoded on chromosomal DNA, plasmids or transposons. Pathogenic bacteria develop surface structures whose primary function is to interact with receptors in the membranes of target cells [31]. In this study, 55.1 % isolates expressed hemagglutinating activity and this is in agreement with **Hager et al.** [32] who reported hemagglutination properties of some bacterial pathogens isolated from clinical samples. This study showed 30.2 % type 1- and 24.5 % P-fimbriae producing bacterial isolates and these values were higher than $\leq$ 15 % type 1- and $\leq$ 12 % P- fimbriae expressing isolates reported by **Nachammai et al.** [33]. The occurrence of type 1- and P- fimbriae in *E. coli* in our study agrees with the reports of **Charles et al**. [34] and **Tiba et al.** [35] in Brazil.

Conclusion

The bacterial isolates from urine specimens of HIV infected patients exhibited varied levels of antibiotic resistance and also phenotypically expressed hemagglutinating activity and type-1 fimbriae for invasion of the host cells.

Recommendation

Further studies on putative virulence factors besides the production of hemagglutinin and expression of type 1 fimbriae by uro-pathogenic isolates should be investigated. Indiscriminate use of antibiotics should also be avoided so as to reduce the spread of antibiotic resistant isolates.

Competing Interest

Authors have declared that no competing interests exist

Authorship

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

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