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Original Research Article

Etiological study of blood stream infection in a maternal and child healthcare based tertiary hospital

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ABSTRACT

Purpose: Bloodstream infections by bacterial pathogens are major cause of morbidity and mortality in developing countries. The aim of this study was to identify the bacterial pathogens causing bloodstream infections in certain areas of Dhaka, Bangladesh and determine their antibiotic susceptibility pattern according to age group.

Materials and Methods: A total of 6095 blood samples were collected from patients attending at Ad-din Women's Medical College & Hospital from July 2019 to December 2020. All the blood samples were processed for culture using a BACT/Alert blood culture machine. Further identification & antimicrobial susceptibility tests were performed using standard microbiological procedures.

Results: Overall, 10.6% of the cultured blood samples were growth positive. Out of them, Gram-negative bacilli were predominant. Blood stream infection rate is highest among neonates, followed by the age group of 1-15 years, adult patients, with minimum rate observed among the age group of 1 month- 1 Year. *Salmonella Typhi* was the most frequently isolated among the Growth positive samples, followed by Coagulase negative *Staphylococci* (CONS), *Acinetobacter*, *S. paratyphae*, *Staphylococcus aureus* & *Klebsiella* species. The most frequently isolated pathogens among Neonates were Coagulase negative *Staphylococci* Spp, and *Acinetobacter* Spp; whereas *Salmonella Typhi* was most commonly isolated pathogen among pediatrics and adult age group (p=<.001). However, we observe a decreasing trend in the *S. Typhi* isolation & the percentage of multi drug resistance amongst *S. Typhi* spp. in the study period than studies conducted over previous years. A remarkable increase of susceptibility against chloramphenicol & cotrimoxazole has been observed in this study. However, Gram positive organisms show high sensitivity to imepenem, vancomycin and linezolid.

Conclusions: This study identified the bacterial pathogens involved with BSI in our hospital among different age groups and their antibiotic susceptibility patterns, so that, healthcare professionals can make proper decisions and provide better care for their patients.

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1. Introduction

Bloodstream infection (BSI) due to bacterial pathogens constitutes one of the most critical situations among infectious diseases. Continuous, intermittent, or transient presence of microorganisms in circulating blood is a threat

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to every organ in the body. It is often associated with increased length of hospital stay, a significant amount of healthcare related costs and most significantly, a high rate of morbidity and mortality.[1] The mortality rate for BSI varies between 4.0 and 41.5%[2–7] depending on the age, severity of infection and other risk factors. Current studies have reported a rapid increase in the number of bloodstream infections from both community and nosocomial sources.[8,9] Bloodstream infection is one of the major causes of neonatal mortality in developing countries. In some communities almost half of patients in neonatal intensive care units acquire infection.[10,11] The World Health Organization has estimated that 10 millions of neonates die during the first five days after birth. It is reported that one in five neonates in some developing countries is suffering from septicemia.[12] Neonatal blood stream infection can be acquired vertically from birth canal or environmentally due to lack of health facilities.

Prevalence and antimicrobial susceptibility of microorganism varies depending upon the geographic location, age, co-morbid illnesses and the use of antibiotics. The epidemiology and pathogen profile of BSIs changes in different regions.[13] So, constant analysis of local trends is required. The final outcome of disease might be much improved by available epidemiologic data for the most frequently isolated pathogenic organisms and their susceptibility to antimicrobial agents. Making of proper empirical treatment choices can also be achieved by understanding local epidemiological studies. This is especially true for Bangladesh and other developing countries where healthcare systems operate on poor hygiene system and there is gap in the facilities or policies of infection prevention and control (IPC) strategies to contain infections. The excessive and irrational use of antibiotics has led to an increase in the multidrug-resistant bugs and thus worsened the condition. Bloodstream infections have serious consequences like shock, disseminated intravascular coagulation, multiple organ failure, and even death. Increased hospital stay and associated costs are the most troublesome consequences.[14] The application of hospital-wide antibiograms to guide clinicians in the initial choice of antimicrobials is the usual approach adopted.

In this study, we aimed to identify the most prevalent bacterial pathogens involved in BSI in a maternal and child health care based hospital in Dhaka, Bangladesh according to age group. We also determined antibiotic susceptibility patterns of the pathogens to see the changing trend of antimicrobial susceptibility in this region.

2. Materials and Methods

In this retrospective study, blood samples were obtained from patients attending out-patient and in-patient departments at Ad-din Women's Medical College & Hospital, Dhaka, Bangladesh, which is famous for maternal and child health care services. A total of 6095 blood samples were processed from July 2019 to December 2020. All the blood samples were processed for culture using a BACT/Alert blood culture machine to identify the presence of bacterial pathogens. Manual method has been utilized as well. Antimicrobial susceptibility tests were performed on the isolated pathogens using Kirby-Bauer disk diffusion method.

2.1. Bacterial isolation

Collected blood samples were directly inoculated into adult (more than 12 years of age) and pediatric (up to 12 years of age) FAN blood culture bottle. Bottles were incubated in the BACT/Alert machine for up to 5 days. One drop of blood from growth positive culture bottles were directly inoculated onto MacConkey (MC) agar and blood agar (5% sheep blood) plates. MacConkey plates were then incubated at 37 °C in aerobic condition. Blood agar plates were incubated at 35 °C in aerobic condition. The bacterial isolates were identified and confirmed by using standard microbiological and biochemical tests like Gram staining, growth on selective media, colony morphology on culture media, lactose fermentation, indole, and citrate utilization, H_2S production, catalase, coagulase, oxidase, and urease test according to guidelines of World Health Organization.[15]

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (Merck, Germany) using disc diffusion (Kirby-Bauer's) technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines of 2015.[16] The antibiotic discs of ampicillin (Amp), cephradine (Ceph), cotrimoxazole (Cot), ciprofloxacin (Cip), levofloxacin (Lev), nalidixic acid (NA), ceftriaxone (CTR), chloramphenicol (Clo), amoxiclav (AMC), cefixime (CXM), cefotaxime (CTX), gentamicin (Gen), amikacin (AK), azithromycin (Az),ceftazidime (CAZ), meropenem (Mero), piperacillin-tazobactam (PIT), colistin (Col) were used for Gram negative bacteria and ampicillin (Amp), cephradine (Ceph), cotrimoxazole (Cot), ciprofloxacin (Cip), levofloxacin (Lev), cefotaxime (CTX), ceftriaxone (CTR), amoxiclav (AMC), gentamicin (Gen), amikacin (AK), imepenem (Ime), cefixime (CXM), oxacillin (Ox), cloxacillin (Clox), erythromycin (Ery), doxycycline (Do), vancomycin (Van), linezolid (Lz) were used for Gram positive bacteria. All antibiotic discs are obtained from Oxoid Ltd, Bashingstore, Hampire, UK.

2.3. Statistical analysis and ethical approval

Statistical analysis was performed by using the SPSS software (version 25, IBM). Differences between proportions were compared using Chi square test with

cut off for statistical significance at p = 0.05 and 95% confidence interval. Qualitative values were expressed as percentages.

2.4. Ethical statements

The research protocol was approved by Institutional Review Board (IRB) of Ad- din Women's Medical College & Hospital, code no AWMC IRB/21 July 2021/027.

3. Result

A total of 6095 blood culture samples were taken from patients, of them majority were female (3819 Female, 2276 Male) with male female ratio M: F=.1.5: 1. Distribution of the patient's sample according to age group was illustrated in Table 1. Almost 30% (1824/6095) of the sample were received from neonatal intensive care unit (NICU), 8% (492/6095) percent patients were aged between 1 months -1 year & 43.6% (2658/6095) from children (aged from 1 to 5 years), 18.4% (1121/6095) from adults (age >15 years) (Table 1). Pathogenic microbes were isolated from 10.6% (648/6095) of the blood cultures (Table 1).

Blood stream infection rate is highest among neonates (48.3%, 313/648), followed by, the age group of 1-15 Year (32.7%, 202/648), Adult patients (11.4%, 74/648) and the age group of 1 month- 1 Year (7.6%, 49/648) (Figure 1).

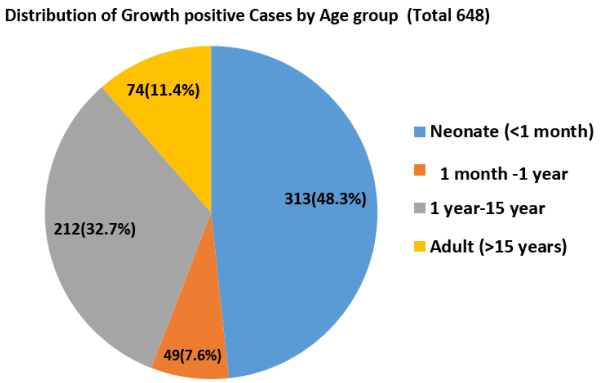

Fig. 1: Distribution of growth positive cases by age group

Among the growth positive cases, 64.2% (416/648) were infected by Gram negative bacilli while 35.8% (232/648) cases were infected by Gram positive cocci (Table 2). *S.Typhi* was the predominant isolates 207 (32%), followed by Coagulase negative *Staphylococci* (CONS) 194 (30%), *Acinetobacter* 98 (15.1%), *S. paratyphae* 36 (5.6%), *Staphylococcus aureus* 31 (4.8%) *& Klebsiella* spp. (3.9%). Few *Enterobacter*, *Proteus*, *Pseudomonas*, *Enterococci* species were also isolated.

The most frequently isolated pathogens in NICU were Coagulase Negative *Staphylococci* Spp (n =194), and *Acinetobacter* Spp (n=98); whereas, *Salmonella Typhi* was most commonly isolated pathogen among pediatrics and adult age group (n=207) [p=<.001]. These findings suggest that Coagulase negative *Staphylococci* Spp. and *Acinetobacter* Spp. are significantly associated with neonatal blood stream infection whereas, Salmonella Typhi is significantly associated with blood stream infection in childhood and adult patients (Table 2).

The rates of susceptibility to selected antimicrobial agents against Gram positive cocci and Gram negative bacilli are demonstrated in Table 4 respectively.

In this study, *S. Typhi isolates,* the predominant cause of BSI, showed higher sensitivity to meropenem (87%), cefixime (77.3%), amikacin (74.4%), doxycycline & azithromycine (71.5%), ceftriaxone (67.1%). Almost 30% (29.8%, 61/207) of the isolates are identified as MDR (Multidrug resistant = Resistant to ampicillin, cotrimoxazole and Chloramphenicol).

Staphylococci were responsible for majority of Neonatal blood stream infection cases; among these, *CONS* isolates were most frequently isolated. They showed high resistance to cephradin, nalidixic acid, erythromycin, and high sensitivity to, imepenem, vancomycin and linezolid (Table 4). *Staphylococcus aureus* is the second commonest Gram positive organism responsible for BSI.

Acinetobacter spp. is the second commonest pathogens in neonatal blood stream infection. They showed higher sensitivity to colistin (98%), meropenem (97%), piperacillin- tazobactum (80%), & amikacin (77.8%).

E. coli showed higher sensitivity to meropenem (95.2%), piperacillin- tazobactum (95.2%), amikacin (76%), gentamycin (76%) and high resistance to ampicillin, cephradin, chloramphenicol. Other Enterobacterieceae like *Klebsiella, Enterobacter, Proteus* has showed similar Sensitivity pattern.

4. Discussion

The complications related to blood stream infections and the rising resistances against commonly used antimicrobial agents are the compelling matters of the world now. In the present study most of the samples were collected from (Table 1) children and neonates (43.6%, 30%) and most patients were female.

The overall blood stream infection rate in this study was found to be 10.6% (Table 1) and anaerobic culture was not done. The predominance of BSI (Figure 1) is observed among neonates (48.3%) than other age groups which in accordance with another study[17] where they found 54% neonatal isolates. One of the reasons behind the fact that 30% of the samples were received from NICU; as our NICU is one of the referral centers for neonate from different hospitals of Bangladesh.

Patients admitted to ICUs have a higher risk of nosocomial BSIs than those admitted to other units. Neonates are more vulnerable to infection as they

Table 1: Distribution of patients with blood stream infection patients byage groups

Sample received	Neonate (<1 month) N (%)	1moth -1year N (%)	1 year-15 year N (%)	Adult (>15 years) N (%)	Total N (%)
Growth Positive	332 (18.2)	49 (10)	202 (8.2)	65 (5.8)	648(10.6)
Growth Negative	1492(81.8)	443(90)	2456(91.8)	1056(94.2)	5447(89.4)
Total	1824(100)	492(100)	2658 (100)	1121 (100)	6095(100)

Table 2: Frequency of bacterial pathogens causing bloodstream infection by age groups

Pathogen	Neonate (<1 month)	1moth -1 year	1 year-15 year	Adult (>15 years)	TotalN=648(100%) N (%)	P Value
Gram negative isolates						
S. Typhi	0	5	148	54	207 (32%)	
Acinetobacter	75	9	14		98(15.1%)	
E.coli	5	1	4	4	14(2.2%)	
S.paratyphae	0	4	19	13	36(5.6%)	
Klebsiella	17	1	5	2	25(3.9%)	
Enterobacter	18		3		21(3.2%)	
Proteus	5	2	2		9(1.4%)	**<.001***
Pseudomonas	4	1	1		6(0.1%)	
Total Gram negatives	124	23	196	73	416(64.2%)	
Gram positive isolates						
CONS	165	19	9	1	194(30%)	
Staphylococcus aureus	19	5	7		31(4.8%)	
S.pneumonie	3	1	0	0	4 0(.06%)	
Enterococci	2	1	0	0	3(0.05%)	
Total Gram positives	189	26	16	1	232(35.8%)	

[N.B: The p value was calculated between Age group 1 day - 1 year and >1 year. For statistical analysis we have merged neonate and infant in one group & children and adults in one group. *means statistically significant.]

Table 3: Susceptibility pattern ofgram positive organisms causing blood stream infection

Antibiotics	CONS N=(194) S (%)	S. aureus N=(31) S (%)	Enterococci (4) S (%)	S.pneumoniae (3) S (%)
Ampicillin	20(10.31)	3(9.68)	1(25)	0(0)
Cotrimoxazole	109(56.19)	17(54.84)	2(50)	1(33.33)
Ciprofloxacine	89(45.88)	19(61.29)	2(50)	1(33.33)
Doxycycline	116(59.79)	21(67.74)	3(75)	2(66.67)
Cefepime	108(55.67)	17(54.84)	2(50)	1(33.33)
Levofloxacine	96(49.48)	20(64.52)	2(50)	1(33.33)
Ceftriaxone	112(57.73)	16(51.61)	2(50)	1(33.33)
Cefotaxime	89(45.88)	15(48.39)	2(50)	1(33.33)
Amoxyclav	119(61.34)	18(58.06)	2(50)	2(66.67)
Oxacillin	51(26.29)	9(29.03)	1(25)	1(33.33)
Cloxacillin	48(24.74)	10(32.26)	2(50)	1(33.33)
Cefixime	62(31.96)	17(54.84)	3(75)	2(66.67)
Erythromycin	98(50.52)	14(45.16)	2(50)	2(66.67)
Amikacin	138(71.13)	24(77.42)	3(75)	2(66.67)
Imepenem	159(81.96)	26(83.87)	3(75)	3(100)
Gentamicin	138(71.13)	21(67.74)	3(75)	2(66.67)
Vancomycin	173(89.18)	28(90.32)	4(100)	3(100)
Linezolid	194(100)	31(100)	4(100)	3(100)

Table 4: Susceptibility pattern ofgram negative organisms isolated from Blood stream infection

Antibiotics	**S. Typhi (N=207)**	**Acinetobacter (N=98)**	**S.paratyphi (N=36)**	**Klebsiella (N=25)**	**Enterobacter (N=21)**	**Proteus (N=9)**	**Pseudomonas (N=6)**	**E.coli (N=14)**
	S(%)	S(%)	S(%)	S(%)	S(%)	S(%)	S(%)	S(%)
Ampicillin	46(22.2)	11(11.2)	3(8.3)	2(8)	2(9.5)	1(11.1)	(0)	3(21.4)
Cephradine	63(30.4)	19(19.4)	8(22.2)	3(12)	3(14.3)	1(11.1)	(0)	4(28.6)
Cotrimoxazole	103(49.8)	48(49)	16(44.4)	13(52)	12(57.1)	4(44.4)	4(66.7)	9(64.3)
Ciprofloxacin	107(51.7)	47(48)	18(50)	12(48)	11(52.4)	4(44.4)	3(50)	8(57.1)
Levofloxacin	110(53.1)	50(51)	18(50)	13(52)	12(57.1)	5(55.6)	3(50)	9(64.3)
Nalidixic acid	98(47.3)	44(44.9)	15(41.7)	12(48)	10(47.6)	4(44.4)	2(33.3)	8(57.1)
Ceftriaxone	139(67.1)	45(45.9)	20(55.6)	13(52)	12(57.1)	5(55.6)	4(66.7)	10(71.4)
Cefotaxime	108(52.2)	43(43.9)	14(38.9)	11(44)	11(52.4)	4(44.4)	2(33.3)	8(57.1)
Amoxyclav	124(59.9)	13(13.3)	17(47.2)	12(48)	12(57.1)	5(55.6)	3(50)	9(64.3)
Chloramphenicol	143(69.1)	36(36.7)	21(58.3)	6(24)	5(23.8)	4(44.4)	(0)	6(42.9)
Cefuroxime	113(54.6)	8(8.2)	16(44.4)	13(52)	13(61.9)	5(55.6)	(0)	8(57.1)
Amikacin	154(74.4)	62(63.3)	28(77.8)	19(76)	17(81)	7(77.8)	5(83.3)	12(85.7)
Meropenem	180(87)	73(74.5)	35(97.2)	22(88)	20(95.2)	8(88.9)	6(100)	13(92.9)
Gentamicin	118(57)	53(54.1)	21(58.3)	16(64)	16(76.2)	6(66.7)	4(66.7)	10(71.4)
Ceftazidime	120(58)	47(48)	16(44.4)	12(48)	12(57.1)	4(44.4)	2(33.3)	8(57.1)
Azithromycin	148(71.5)	NU	22(61.1)	(0)	(0)	(0)	3(50)	(0)
PiperacillinTazobactam	NU	79(80.6)	33(91.7)	24(96)	20(95.2)	8(88.9)	5(83.3)	14(100)
Colistin	NU	96(98)	(0)	25(100)	21(100)	9(100)	6(100)	14(100)
Cefixime	160(77.3)	43(43.9)	30(83.3)	13(52)	14(66.7)	5(55.6)	4(66.7)	8(57.1)
Doxycycline	148(71.5)	45(45.9)	19(52.8)	13(52)	9(42.9)	4(44.4)	(0)	7(50)

NU= Not Used

can acquire infection vertically from dealing with both prematurity and low birth weight is increasing.[18–20]

Salmonella Typhi was the predominant isolates, 207 (32%) in total blood samples and also isolated maximum (148) from the 1-15 years age group (Table 2). Similar finding was observed in couple of studies[21,22] who had found *S. Typhi* as predominant Gram negative organism and CONS as predominant Gram positive organism.

Several studies from Bangladesh have already identified *S. Typhi* as a common cause of blood stream infection in this region.[23–25]

S. Typhi, the causative agent of typhoid fever, is a major public health concern in Bangladesh and other developing Asian countries. *Salmonella* species was responsible for almost half of the disease burden associated with BSI in Dhaka, Bangladesh in past decades and about 80% of these infections were due to *S. Typhi*; but an overall decrease in Salmonella species isolation rate over the recent years[21] has been observed. This decrease may be attributed to the improved urban water management system and sanitation practices in Dhaka city over the past years and vaccination against *S. Typhi.*

The most frequently isolated pathogens among neonates were Coagulase negative Staphylococci Spp (52.7%, 165 /313) and *Acinetobacter* Spp (24%, 75 /313) in this study (Table 2).

Within the first week of life, neonates become rapidly colonized by environmental pathogen.[26,27] The risk of BSI is substantially increasing with CONS & *Acinetobacter* infection with the use of central venous catheters (CVC), mechanical ventilation, and parenteral nutrition, and with exposure to other invasive skin or mucosa-breaching procedures.[28,29] Consequently, infants admitted to a hospital obtain most of their microorganisms from the hospital environment, their parents, and staff.[30] Transmission via the hands of hospital staff can lead to endemic strains circulating for extended periods.[31] Antibiotic resistance in skin-residing strains has been found to be low at birth but it increases rapidly during the first week of hospitalization. The spectrum and antibiotic resistance pattern of microorganisms isolated from neonates depends on the selective pressure as a result of perinatal antibiotic exposure.[32]

CONS & *Acinetobacter* spp. blood infection can occur in the babies without being under intensive care or antibiotics, mechanical ventilation or having indwelling catheters.[3]

S. Typhi isolates, the predominant cause of BSI in older age group, showed higher sensitivity to meropenem (87%), cefixime (77.3%), amikacin (74.4%), doxycycline, azithromycin (71.5%) & ceftriaxone (67.1%). By mid-1990s, about half of the *S. Typhi* strains were MDR; these were resistant against three first line antibiotics - ampicillin, cotrimoxazole and chloramphenicol.[33] A decline in the percentage of MDR *S. Typhi* strains from 61.7 to 23.7% within 2005- 2014 has been observed in our country.[17] As indicated by recent studies from Bangladesh, *S. Typhi* still shows a high level of resistance against first line antibiotics.[23] In our study (Table 2), almost 30% (29.8%) of the isolates are identified as MDR (Multidrug resistant where Resistant to ampicillin, cotrimoxazole and chloramphenicol). These findings are concordant with other reports from our countries and neighboring countries like India & Nepal.[21,34,35]

However, we have observed a remarkable increase of susceptibility against chloramphenicol (69.1%) in this study. Cotrimoxazole sensitivity is also increased (50%) than the studies of previous decades[21]. Hopefully, if this trend continues, cheaper first line antibiotics to treat *S. Typhi* infections might be possible in near future.

Staphylococci spp., the major pathogen of neonatal BSI in this study, was ascertained with high resistance to cephradine, erythromycin, and high sensitivity to, imepenem, vancomycin and linezolid. *Staphylococcus aureus* remains as second most common Gram positive organism. Both vancomycin and linezolid are good treatment of choice against CONS and *S. aureus* which are usually resistant to commonly used antibiotics.

Acinetobacter spp. is the second commonest pathogens in neonatal blood stream infection. We have observed higher sensitivity (table) to colistin (98%), meropenem (97%), piperacillin- tazobactum (80%), & amikacin (77.8%).

In previous decades *Acinetobacter* remained as the most common isolate of Neonatal BSI in Bangladesh, but *Coagulase-negative staphylococci* (CONS) are found to be the most commonly isolated pathogens in the neonatal intensive care unit (NICU) in some other countries[36]. They are the major pathogen involved in Late Onset Neonatal Sepsis (LONS), particularly in infants born at a lower gestational age.

E.coli showed higher sensitivity to meropenem (92%), piperacillin- tazobactum (100%), amikacin (85.7%), and gentamycin (76%). So, carbapenems may be considered as a good choice of treatment for BSI caused by *E. coli. Klebsiella* species as it is showed the highest level of resistance against β-lactams, especially penicillins and third generation cephalosporins.

5. Limitation of the Study

Due to the lack of resources, we were not able to differentiate the samples received from indoor and outdoor patients. As a result, we could not show the difference between nosocomial and community acquired BSI. We were also not able to collect patient data on the clinical manifestations or any other patient characteristics, other than age and sex, which could be considered as risk factors for BSI. Also, we were not able to perform any molecular tests on received samples due to lack of required resources and adequate fund.

6. Conclusion

Major bacterial pathogens involved with bloodstream infections (BSI) occurring in Dhaka city among different age groups of patients and their antibiotic susceptibility patterns are demonstrated in our study. In a nutshell our study reveals that, Blood stream infection is higher amongst neonates. CONS are predominant pathogen for neonates and *S. Typhi* is predominant for 1-15 years age group children. Among 30% *S. Typhi* strains were found to be MDR. High resistance to cephradine, erythromycin, and high sensitivity to imepenem, vancomycin and linezolid were found among CONS strains. We hope that, our findings will help healthcare professionals to provide better care for their patients & also help the researchers and policy makers to make appropriate antibiotic policy to face future challenges of infectious diseases.

7. Source of Funding

This study was not funded.

8. Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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