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Original article

MIC and MBC in the evaluation of the clinical relevance of beta-lactams produced in Lubumbashi in the DR of Congo

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ABSTRACT

Faced with the recurring problems of bacterial resistance to antibiotics and the ineffectiveness of medical therapies used to cure patients with bacterial infections, it is urgent not only to think about the development of new therapeutic strategies to guarantee the future, but above all, for the present to set up mechanisms allowing a judicious selection of antibiotics according to various parameters, in particular microbiological: The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Methodology: The determination of the MIC and MBC was carried out according to the recommendations of the Clinical & Laboratory Standards Institute (CLSI). The bacterial population consisted of strains typed Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) conditioned under an adjusted turbidity of 0.5 McFarland. We used Mueller Hinton broth and Mueller Hinton agar for MIC and determination of inhibition diameters and trypcase soy agar for subculture of the MBC determination. And a MBC/MIC ratio was calculated for determination of the potency of antibiotics. Results: After determination of the MIC and MBC values, calculation of the MBC/MIC ratio, it appears that only Meropenem has a bactericidal action on the two ATCC bacteria and for the antibiotics produced by the two firms; Ceftriaxone demonstrated a bactericidal action on Staphylococcus aureus for both firms. For the rest, the antibiotics demonstrated essentially bacteriostatic activity for both bacteria and for both firms. Conclusion:In this study, their measurement allowed to evaluate the quality of some antibiotics manufactured by two local firms by specifying the power of their activity on the reference strains. These results and practices are recommended in the daily practice of microbiology laboratories as an element of quality control of the routine antibiogram technique. This practice can be extended to the search for MIC and CMB on the serum of patients in order to ensure the good crossing by the antibiotic of the different biological barriers and thus guarantee the effectiveness of the treatment, particularly in intensive care units.

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Introduction

The World Health Organization (WHO) has issued a warning that the world is "running out of antibiotics," raising fears that global antibiotic resistance is reaching new heights.[1] Antibiotic resistance is on the rise in all regions of the world, with bacterial infections a leading cause of disease and death.[2]

The increasing resistance of bacteria to antibiotics and the increasingly frequent failures of treatments against infections require identifying the underlying causes of this problem and, in addition, seeking ways to reduce it and improve the effectiveness of therapies against infections.[3] One of the recognized reasons for the failure of therapies is the pressure on the choice of drugs, especially when they are poorly chosen and administered at too low doses, which leads to the survival of a resistant bacterial population or induces antibiotic resistance mechanisms.[4,5]

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The ineffectiveness of antibacterial therapies requires not only the active search for new therapeutic strategies, but above all the judicious choice of antibiotics based on various parameters, particularly microbiological ones.[6]The minimum inhibitory concentration (MIC) defines the in vitro levels of susceptibility or resistance of specific bacterial strains to the applied antibiotic.[7]

A reliable assessment of the MIC significantly impacts the choice of a therapeutic strategy, which affects the efficacy of an anti-infective treatment.[8] In order to obtain a credible MIC, many elements must be considered, such as the appropriate choice of the method, compliance with labeling rules and a competent interpretation of the results.[9]

In this article, we proposed to evaluate the MIC of beta-lactams marketed in pharmacies of the city of Lubumbashi by dilution method and the possibilities of using the MIC in clinical practice, considering the pharmacokinetic/pharmacodynamic parameters, were studied.

Due to the problems associated with determining PK in individual patients, a statistical estimation of the possibility of achieving the PK/PD index, based on the Monte Carlo method, was discussed. In order to provide comprehensive information, the possible limitations of the MIC, which scientists are aware of, were described.

Methodology

1. Preparation of antibiotics

The first concentration of 50mg/ml was prepared from the initial concentration, the concentration ranges were prepared in a series of 15 wells of the microplate by the method of double dilution in liquid medium. These concentration ranges vary from 25mg/ml to 0.000763 mg/ml, distributed as follows in a series of dilution from half to half :

Table 1: Dilution of antibiotics

Well	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Concentration	25	12.5	6.25	3.13	1,563	0.78125	0.390625	0.195313	0.097656
Well	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
Concentration	0.097656	0.048828	0.024414	0.012207	0.006104	0.003052	0.001526	0.000763	

Dilution methods

To determine MIC values, we used Mueller-Hinton broth (MHB). Antibiotics were diluted in sterile distilled water to obtain an appropriate starting concentration, and the dissolved and diluted antibiotics are used to prepare working solutions in Mueller-Hinton broth. [10,11]

Working solutions should contain double dilutions of antibiotics, the range of concentrations used for testing depending on the drug concerned and should consider the critical MIC values for reference strains. [10] Subsequent double dilutions of the antibiotic should be performed using the schemes available in the documents [10] and proposed by EUCAST. [12]

In the broth microdilution method, working solutions prepared with twofold dilutions of antibiotics are distributed into appropriate wells of microtiter plates and in this form can be used directly for MIC determinations or stored in plastic bags for up to three months at a temperature ≤ −60 °C.[10]

Bacterial inoculum

Strains selected according to the recommendation of the French Society of Microbiology and Staphylococcus aureus CIP 76.25 (ATCC 29213), Escherichia coli CIP 76.24 (ATCC 25922).[13]

The inoculum to be treated with subsequent dilutions of antibiotics to final values of 5×10^5 CFU (colony forming units)/ml [10] from one of the 0.5 MFU (McFarland Units) bacterial suspensions corresponds approximately to a culture density of 1.5×10^8cells/ml. and proceed to dilutions of the order of 100× to a density of 10 6 CFU/ml (9.9 ml of broth + 0.1 ml of 0.5 McFarland suspension) and then poured into wells containing the appropriate antibiotic concentrations in the broth (50 µL of bacterial inoculum + 50 µL of liquid medium with antibiotic or 10 µL of inoculum per 100 µL of diluted antibiotic).

If commercial tests with lyophilized antibiotic are used in the wells, a 5 × 10 5 suspensions should be obtained immediately by adding 50 µL of 0.5 McFarland suspension to 10 mL of broth).[14].

Obtaining a 0.5 McFarland suspension is controlled by measurements in a densitometer or spectrophotometer, where the absorbance at a wavelength of 625 nm should be between 0.08 and 0.13 .[15,16] The inoculum obtained in the wells of the microtiter plate should also be controlled. For this purpose, when using broth microdilution, 10 µL should be sampled from the growth control well (MHB medium with bacterial suspension and without antibiotic). Obtaining a growth of 20 to 80 colonies of a given bacterial strain proves the density of 5 × 10 5 CFU/ml . [10]

A quality control should be performed by checking the sterility of the medium, the growth of the strain and the quality of the results obtained by evaluating the MIC of the tested antibiotic for the reference strains according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical & Laboratory Standards Institute (CLSI) .[17, 18] The MIC values obtained for the reference strains should be within the concentration range recommended by EUCAST and CLSI. [18, 19]

The choice of Escherichia coli and Staphylococcus aureus strains was motivated by the results of several studies worldwide, in Africa and locally reporting their massive isolation and the first-line use of beta-lactams for their treatment .[20-25]

Reading the results

The MIC value is the lowest concentration of an antibiotic at which bacterial growth is completely inhibited. In the broth microdilution method, for some antibiotics, separate rules for reading the MIC value are used [26], among which is the use of resazurin (a weakly fluorescent blue dye), which is reduced by active bacteria to fluorescent (pink) resorufin .[27]

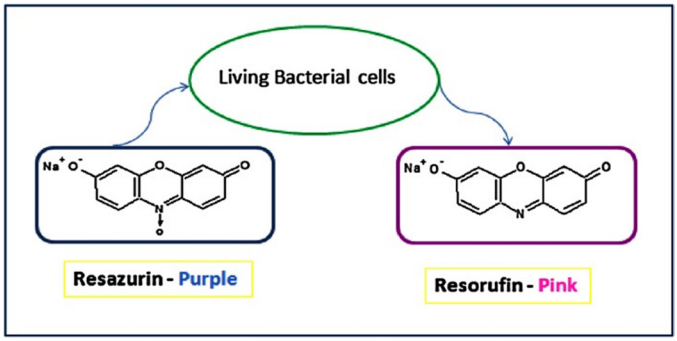


Fig 1: Active living cells cause the reduction of resazurin (purple blue) to resorufin (pink-colorless).[28]

Diffusion Method: Determination of Inhibition Zones

To complete and counter-expertly assess the dilution method and with the same aim of assessing the effectiveness of certain antibiotics dispensed in community pharmacies in the South of the DR Congo, we associated a disk diffusion test with the effect against strains typed Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) [13] for the products (antibiotics) of each of the two manufacturers under study, adapting the concentration of the disks according to EUCAST for each antibiotic as follows.

Preparation of antibiotic disks for disk diffusion test

Discs with a diameter of 6.0 mm were punched out and heat sterilized in Bijou screw cap bottles for each drug brand at 140 °C in an oven for 60 minutes and then cooled to room temperature . The antibiotic solution was prepared using dimethyl sulfoxide (DMSO) as the diluent.

The discs were impregnated with the antibiotic solutions so that 1.0 ml contained 100 times the amount of active ingredient required for each disc of the different drugs. The final amount of drug was 5 µg (Cefixime), 10 µg (Ampicillin and Meropenem), or 30 µg (Ceftriaxone)and Cefotaxime). The discs were stored at 25 °C under humid conditions before using.[29]

Standardization of bacterial inoculum

Subcultures of ATCC strains of E. coli and S. aureus were performed by carefully picking a colony using a sterile inoculation loop and then inoculating it onto the surface of nutrient agar for 24 h. Using a sterile swab, the overnight nutrient broth culture was diluted with saline so that the turbidity was adjusted to the McFarland standard of 0.5 .

This was found to yield a bacterial suspension with an average microbial population density of 3.3 × 106 colony forming units (CFU)/ml. The inoculum was determined by comparison with the turbidity of a barium sulfate solution (1%, v/v), as described and performed previously. [29]

Disk Diffusion Test Procedure

A standard sterile swab was dipped into a suspension of the prepared inoculum solution. Then, the swab was spread evenly and aseptically on the surface of the Mueller Hinton agar plate, rotating the plate to ensure uniform distribution. The prepared antimicrobial disks were then aseptically pressed with forceps.

After 10 min of prediffusion, the plates were incubated in a microbiological incubator at 37 °C for 18 h. The diameters of the inhibition zones (ratios in mm) were measured in replicates using a Vernier caliper. Data is given as the mean ± standard deviation of n = 6 independent experiments .[29]

Determination of the minimum bactericidal concentration (MBC)

The broth dilution method was used to calculate the minimum biological concentration (MBC) of antimicrobials .[29] A volume of 0.1 ml was taken from the wells of microtiter plates (Oy Growth Curves Ab) where no growth was observed after 48 h of incubation at 37 °C and then inoculated onto the surface of Tryptone Soy Agar (TSA) plates (Oxoid).

They were incubated for 48 h at 37 °C, the MBC being considered as the lowest concentration of the substance at which no colonies formed under these conditions. Since the detection limit of this technique is 10 cfu/ml, the absence of growth on a TSA plate indicated that the concentration was below this value.

The initial concentration of 10 5 cfu/ml had thus been reduced to less than 10 cfu/ml. Therefore, the MBC was effectively considered as the minimum concentration of antimicrobial capable of inactivating more than 99.99% of the bacteria present. Three replicates were performed for each strain and antimicrobial compound.

NB: The intrinsic activity of an antibiotic is defined by the ratio: MBC / MIC

MBC / MIC ≤ 4	Bactericidal antibiotics
MBC / MIC = 4–32	Bacteriostatic antibiotic
MBC / MIC => 32	Antibiotic tolerant bacteria

Ethical statement

The study was approved by the medical ethics committee of the University of Lubumbashi under number UNILU/CEM/033/2024

Limit of the study

The limit of our study is that of having worked only on a single production batch of each of the antibiotics available on the market during the period of our study for each of the pharmaceutical companies and of only considering the concentration in millicule base that the declaration of the label of the manufacturer.

Results and discussion

The results thus presented constitute the average of the values titrated on 7 different batches of each antibiotic.

Table 2 Distribution of CMI results by firm dilution method

Antibiotic	Escherichia coli ATCC 25922 MIC in mg/l (µg/ml)			Staphylococcus aureus ATCC 29213 MIC in mg/l (µg/ml)		
	Firm 1	Firm 2	Standard CLSI	Firm1	Firm2	CLSI Standard
Ampicillin	12.5	12.5	2-8	12.5	25	0.5-2
Cefotaxime	3.125	3,125	0.03-0.125	1.5625	1.5625	1-4
cefixime	6.25	1.5625	0.25- 1	0.78	3,125	8-32
Ceftriaxone	6.25	12.5	0.03-0.12	3,125	6.25	1-8
Meropenem	0.0244	0.0488	0.008-0.06	0.006	0.0488	0.03-0.12

The MIC values ranged from 12.5mg/l to 0.0244 mg/l for Escherichia coli for firm 1 and from 12.5mg/l to 0.0488 mg/l for firm 2. For Staphylococcus aureus the values for firm 1 ranged from 12.5mg/l to 0.006 mg/l and 25mg/l to 0.0488 mg/l for firm 2.

MICs define the clinical breakpoint, the concentration of antibiotic used to indicate whether an infection with a particular bacterial isolate is treatable in a patient. Clinical breakpoints are used by clinical microbiology laboratories to define patient isolates as susceptible (S), intermediate (I), or resistant (R) to a panel of antibiotics. Thus, the MIC test is the gold standard to guide physicians' treatment practices.

Referring to the tables of Clinical and Laboratory Standard Institutes which define the standard performances of antimicrobials by the determination in particular of the MIC evaluated in mg/l or in µg/ml, it should be noted that the activities of the following antibiotics: ampicillin, cefotaxime, cefixime and ceftriaxone have their MIC higher than the expected range against the standard germs Escherichia coli ATCC25922 and Staphylococcus aureus ATCC29213, except for the latter, Ceftriaxone and cefixime have a MIC still within the expected range. Meropenem shows good activity against both bacteria by its MIC still located within the expected ranges [29] this same observation is that of the standards validated by EUCAST .[30]

This situation demonstrates the urgent need to evaluate ways to reduce the global spread of bacterial resistance and to select the most effective antibiotic during the initial phase of infection.[31]

Most of the studies carried out to date and demonstrating or suggesting the interest in determining the MIC have been based essentially on a "theoretical" MIC; generally the highest MIC for the chosen antibiotic and found in the species responsible for the infection, however, carries a risk of individual toxicity and collective overconsumption of antibiotics.[32]

Indeed, MIC determination helps researchers and clinicians detect new resistance patterns and can therefore be anticipated if treatment needs to be changed or new antibiotics need to be developed.[33] MIC can therefore be an important surveillance tool to monitor the spread of resistant pathogens and to inform infection control strategies.[34]

Although it is difficult to predict the clinical outcome of an infection based solely on the MIC value, it can help to choose the most appropriate treatment. It defines in vitro the levels of susceptibility or resistance of specific bacterial strains to a targeted antibiotic .[35] However, the determination of the MIC of β-lactams to define the most appropriate treatment is more justified when a practitioner is faced with severe or invasive infections (such as bacteremia or meningitis), clinical failure and/or a suspected isolate with reduced susceptibility to penicillin (S. pneumoniae) or MPA (H. influenzae). EUCAST guidelines recommend testing the β-lactams of interest, especially in these cases .[36] And

instead, the use of β-lactam/β-lactamase inhibitor combinations or cephalosporins has been proposed as an alternative to carbapenems .[37] Despite controversies [38, 39], the success of using these antibiotics depends on several factors, including microbial species, site of infection [40], and MIC .[41]

Also, a posteriori adaptation by concentration measurement is thus recommended for many antibiotics .[42] Particularly in cases where there is an acute risk of toxicity with antibiotics with a narrow therapeutic window or, conversely, a risk of underexposure due to PK characteristics (such as increased renal clearance) or PD in the case of a difficult-to-reach infectious site.[35]

A low MIC can help in the case of a narrow therapeutic window, flexibility is reduced.[43]and that by measuring the MIC at the epidemiological threshold, i.e. the highest MIC value observed in organisms without phenotypically detectable resistance, could be assumed instead of a measured wild-type MIC.[44]

This allows to bypass the problems related to the resulting limited precision and reproducibility as well as to variations in assays, because it takes into account the potential variations in MIC for assay decisions and to consider MIC values in the context of clinical MIC breakpoints and MIC distributions.[45]

Table 3: Distribution of inhibition diameter results by diffusion method

Antibiotic	Disk content µg	*Escherichia coli* ATCC 25922 *Inhibition diameter in mm*			*Staphylococcus aureus* ATCC 25923 *Inhibition diameter in mm*		
		Firm 1	**Firm 2**	**CLSI 2024). [19]**	**Firm1**	**Firm2**	**CLSI 2024). [19]**
Ampicillin	10	13	14	15-22	20	21	27-35
Cefotaxime	30	16	17	29-35	19	20	25-31
cefixime	5	18	18	20-26	20	20	
Ceftriaxone	30	30	35	29-35	23	23	22-28
Meropenem	10	35	35	28-35	30	30	29-37

The values of the inhibition diameters varied between 13 mm to 35 mm for Escherichia coli for firm 1 and from 14 mm to 35 mm for firm 2. For Staphylococcus aureus the values for firm 1 were between 20 mm to 30 mm and 21 mm to 30 mm for firm 2 .

A good overall correlation between the MIC value and the disk inhibition zone was found for Meropenem and Ceftriaxone from both companies. Although for Ampicillin, Cefotaxime and Cefixime, the values are out of the expected range m, this reflects a serious problem in the dosing process of these antibiotics during the manufacturing process .

We have finally determined the Minimum Bactericidal Concentration (MBC)

Table 4: Distribution of CMB results

Antibiotic	**Escherichia coli ATCC 25922 MBC in mg/l (µg/ml)**		**Staphylococcus aureus ATCC 29213 MBC in mg/l (µg/ml)**	
	Firm 1	**Firm 2**	**Firm1**	**Firm2**
Ampicillin	-	-	-	-
Cefotaxime	-	-	-	-
cefixime	-	-	-	-
Ceftriaxone	-	-	6.25	6.25
Meropenem	0.0244	0.0488	0.006	0.0488

he CMB values varied between 3.125mg/l and 25 mg/l for Escherichia coli for firm 1 and from 0.195mg/l to 25 mg/l for firm 2. For Staphylococcus aureus the values for firm 1 were between 25mg/l to 0.0488 mg/l and 1.5625mg/l to 0.0788 mg/l for firm 2. Bacterial growth was observed on all dilutions of ampicillin for firm 1 on Escherichia coli and on Staphylococcus aureus for firm 2. Same observation for Cefixime and cefuroxime respectively for firm 1 and firm 2 on Escherichia coli.

Beta-lactam antibiotics inhibit the final step of peptidoglycan synthesis by acylating the transpeptidase involved in cross-linking peptides to form peptidoglycan. The targets of beta-lactam antibiotic actions are known as penicillin-binding proteins (PBPs). This binding, in turn, interrupts the terminal transpeptidation process and induces loss of viability and lysis, also through autolytic processes within the bacterial cell. [46]

Table 5: Results of the intrinsic activity of an antibiotic is defined by the ratio: CMB / MIC

Antibiotic	**Escherichia coli ATCC 25922 MBC/MIC**		**Staphylococcus aureus ATCC 25923 MBC/MIC**	
	Firm 1	**Firm 2**	**Firm1**	**Firm2**
Ampicillin	-	-	-	-
Cefotaxime	-	-	-	-
cefixime	-	-	-	-
Ceftriaxone	-	-	2	1
Meropenem	1	1	1	1

The bactericidal effect has been shown to be maximal at 4–5 × MIC and to reach a plateau at concentrations above this threshold.[47] In order to define whether an antibacterial agent is bactericidal or bacteriostatic in vitro , the MBC/MIC ratio can be used. If the MBC/MIC ratio is ≤ 4, the effect is considered bactericidal, and if the MBC/MIC ratio is > 4, the effect is defined as bacteriostatic .[48]

Thus, from Table 4 , it is clear that only Meropenem has bactericidal action on both ATCC bacteria and for antibiotics produced by both companies; Ceftriaxone has demonstrated a bactericidal action on Staphylococcus aureus for both companies. For the rest of the antibiotics have demonstrated an essentially bacteriostatic activity for both bacteria and for both companies .

From the above, therapeutic monitoring of beta-lactams as a tool for dose optimization and individualization has been recommended to overcome this variability in exposure. This involves measurement of drug concentration to help inform optimized dosing, with the aim of improving patient outcomes and minimizing toxicity.[49].

Hence the need to rapidly determine MBC values is increasing due to several factors including the global increase in antimicrobial resistance requiring accurate and effective use of new and existing antibiotics. [50]

MBC values help to select the most effective antibiotic options in specific scenarios. They also help to decide the optimal doses of antimicrobial agent to use in relation to resistant strains. [51] Collectively, this can help to slow the spread of antimicrobial resistance. [52]

Thus, it should be noted that the choice of antimicrobial agents, the timing and dosage of effective antimicrobial agents are also very important.[53] Because neither too high nor too low a dose of antibiotics is the optimal treatment regimen: too high a dose may lead to increased resistance, and too low a dose will not achieve the desired effect of antibiotic treatment. [54] The minimum inhibitory concentration (MIC) indicates the appropriate dosage of antibiotics.[45]

The MIC is an important index to measure both the effectiveness of antimicrobial agents and bacterial drug resistance. [55] Treatment with the optimal dose of effective antibiotics as early as possible after infection is the key to curing an infection.[56] Therefore, the time required to determine the MIC is an important factor in determining whether antibiotics can be used in the early stage of infection.[57] Because treatment is more effective when effective antibiotics are administered early.[58]Clinically, the MIC is the most commonly used PD parameter to describe the relationship between antimicrobial drug and physiological activity.[59]

It is considered the most useful guide to the efficacy of antimicrobial therapy.[60] Its measurement in routine clinical laboratories facilitates the interpretation of disc susceptibility tests, by confirming susceptibility in cases of unexpected treatment failures.[33] MICs are used to study new drugs to determine their likely clinical value, and they currently represent a reasonable measure to define antimicrobial activity.[61]

Indeed, among the variety of clinical microbiology methods used for antibiotic susceptibility testing, minimum inhibitory concentration (MIC) tests have become the gold standard in clinical practice.[60] However, MIC determination is based on two fundamental assumptions that do not reflect the clinical situation and that constitute its limitations. These assumptions include a static concentration-time profile (fixed concentrations) and drug-free evaluation, i.e., the culture medium does not contain proteins to mimic the biological environment.[62] Therefore, CBM, which applies to bactericidal antibiotics and is a useful measure for AMR manifested by heteroresistance or high survival (tolerance and/or persistence) [63], is complementary to MIC, because bacterial susceptibility to antimicrobials may need to be described by a combination of parameters rather than a single MIC parameter. [62]

Conclusion

Faced with limited availability of antibiotics to treat multidrug-resistant Gram-negative and Gram-positive bacterial infections remain a serious problem. Therefore, it is imperative to develop new agents or new therapeutic strategies capable of overcoming drug resistance in these organisms. And the measurement of MIC and CMB gives a quantitative assessment of the potency of an antibiotic. In this study, their measurement allowed to assess the quality of some antibiotics manufactured by two local firms by specifying the potency of their activity on reference strains.

These results and practices are recommended in the daily practice of microbiology laboratories as an element of quality control of the routine antibiogram technique. This practice can be extended to the search for MIC and CMB on patient serum to ensure the proper crossing of the antibiotic of the various biological barriers and thus guarantee the effectiveness of the treatment especially in intensive care units .

Their performance and interpretation must be carried out in strict compliance with the principles published by the Clinical and Laboratory Standards Institute (CLSI) which provides specific advice on the measurement and interpretation of MIC results and established protocols and standards for establishing MIC and MBC in products including quality control approaches to ensure interlaboratory reliability.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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