

ORIGINAL ARTICLE

Colistin resistance in CRE infections: Analysis of MIC patterns and associated risk factors in a tertiary care hospital in Saudi Arabia

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ABSTRACT

Background: Gram-negative bacteria represent a significant challenge to the public health as a result of their elevated resistance rates and the associated risk of mortality. The inappropriate use of antibiotics has driven the rise of multidrug-resistant (MDR) bacterial strains. The objectives of this work are to investigate the colistin susceptibility among carbapenem-resistant Enterobacterales (CRE) clinical isolates and to analyze associated risk factors.

Methods: A retrospective evaluation was carried out on 37 patients with their first episode of CRE infection. Clinical isolates obtained from different body sites were identified using the VITEK MS system. Colistin minimum inhibitory concentrations (MICs) were measured using the reference broth microdilution (BMD) method. Demographic, clinical, and microbiological data were interpreted using descriptive statistics.

Results: The mean age of patients was 61.3 years, with 62.2% males and *Klebsiella pneumoniae* represented the large majority of strains (89.2%), followed by *Escherichia coli* (10.8%). Colistin resistance was identified in 17% of isolates, and the highest resistance rates were observed for Amoxicillin-Clavulanate (100%), Meropenem (100%), and Ciprofloxacin (89.2%). The colistin MIC₅₀ and MIC₉₀ were 0.25 µg/mL and 4.0 µg/mL,



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respectively. Mortality was significantly associated with ICU admission and within patients with history of antibiotics consumption.

Conclusion: This study underscores the emerging issue of colistin resistance among CRE strains in Saudi Arabia. The findings suggest that patient acuity and underlying comorbidities are critical determinants of mortality. The study emphasizes the need for coordinated efforts to address this public health issue and improve patient outcomes through effective therapies and infection control strategies. (www.actabiomedica.it)

Key words: colistin resistance, carbapenem-resistant, *Enterobacterales*, risk factors, broth microdilution

Introduction

Antimicrobial resistance (AMR) among Gram negative strains represents a major global dilemma. Enterobacterales, particularly *Escherichia coli* and *Klebsiella pneumoniae*, are well-known for their ability to develop resistance to multiple antibiotics, especially carbapenems, therefore classified among WHO critical pathogen (1). The resistance is mainly due to the production of hydrolytic enzymes that destruct the antimicrobial molecules and their unique cell wall structure, specifically an outer layer rich in lipopolysaccharides (LPS) which acts as a strong barrier, preventing many antibiotic molecules from penetrating the cell and reducing their efficacy (2). Carbapenems, an important class of beta-lactam antibiotics that are extensively used to manage infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB), however, the overuse of this class in the clinical settings has resulted in the development of carbapenem-resistant bacteria, especially among members of Enterobacterales (CRE) (3). Regrettably, the worldwide dissemination of carbapenem-resistant strains has been linked to resistant genes, frequently located on plasmids, which can be exchanged among many bacterial species via horizontal gene transfer (2). With the harsh waves of AMR reports, colistin appeared to be a choice to manage complicated infections caused by MDR-GNB despite its nephron- and neurotoxicity, however reports documenting colistin resistance continue to be detected (4). Resistance to colistin may be mediated by alteration of bacterial outer membrane (Lipopolysaccharide component), upregulation of

efflux pumps, CrrAB and PhoQ/PhoP signaling system however, horizontal gene transfer of colistin resistant gene (*mcr-1-10*) represents a significant clinical concern (5). This resistance is likely mediated by chromosomal mutations (e.g., *mgrB*, *pmrA/B*, *phoP/Q*) or plasmid-borne *mcr* genes, mechanisms well-documented in regional studies (6,7). Additionally, assessment of colistin drug susceptibility is challenging therefore, the Clinical and Laboratory Standards Institute guidelines states that broth microdilution method (BMD) is the reference method to evaluate colistin minimum inhibitory concentration (MIC) since disk and gradient diffusion methods continue to show inconsistency (8). The worldwide incidence of colistin resistance has been progressively rising, with reports from Asia, Europe, the Americas, and Africa (9). The widespread use of colistin in non-clinical settings such as animal health and food industry complicate the problem and facilitate the spread of resistant strains to the humans through the direct contact or food chain interactions (10). Regional studies, including those from Saudi Arabia, Egypt, and Jordan, have reported varying rates of colistin resistance, often linked to the mobile *mcr* genes and other chromosomal resistance mechanisms (11-13). These findings underscore the necessity of region-specific methods to mitigate the proliferation of colistin-resistant bacteria. The difficulties in identifying and controlling colistin-resistant isolates are exacerbated by the scarcity of effective antibiotics, resulting in increased morbidity, mortality, and healthcare expenses (14). Molecular characterization by methods such PCR (polymerase chain reaction), WGS (whole-genome sequencing), and plasmid

profiling are essential for identifying resistance genes and understanding their genetic context. These methods provide insights into resistance mechanisms, facilitate the development of diagnostic assays, and support the creation of targeted treatment protocols. Understanding the genetic environment of resistance genes, including their association with mobile elements like plasmids, is vital for preventing their further spread (15). This study aims to evaluate colistin susceptibility among carbapenem-resistant Gram-negative clinical isolates. It further seeks to identify the distribution of resistant strains across different specimen types, assess associated clinical risk factors and comorbidities, and analyze the outcomes in relation to colistin resistance using standardized laboratory and statistical methods.

Materials and Methods

Selection of bacterial isolates

A total of 37 non-replicate, carbapenem-resistant Enterobacterales (*E. coli*; n=4 and *Klebsiella pneumoniae*; n=33) were collected prospectively from the first clinical episode of patients admitted to King Fahad hospital of the University (KFHU) between December 2020 and September 2021.

Bacterial identification and antibiotic susceptibility testing

Strains were identified using the automated VITEK MS platform (BioMérieux, Craaponne, France) and antibiotic susceptibility testing (AST) was performed using the VITEK 2 system, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI 2020) guidelines using control strains in each AST run: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), and *Pseudomonas aeruginosa* (ATCC 27853). Antibiotics tested included imipenem and meropenem (carbapenem); cefepime and ceftazidime (cephalosporin class); levofloxacin and ciprofloxacin (fluoroquinolone class); amikacin and gentamicin (aminoglycoside class). Given that CRE are resistant to all beta-lactams, lower-generation beta-lactams like

amoxicillin-clavulanate were excluded from the final analysis to focus on clinically relevant resistance patterns. Colistin was evaluated separately using the broth microdilution method. Colonies were stored at -80C for further investigation.

Determination of colistin minimum inhibitory concentration (MIC)

Colistin susceptibility was assessed using the broth microdilution (BMD) method following CLSI guidelines. Minimum inhibitory concentrations (MICs) were determined using the ComASP™ colistin panel (Liofilchem, Italy). Colistin susceptibility was defined according to CLSI breakpoints: MIC ≤ 2 µg/L (intermediately susceptible) and MIC ≥ 4 µg/L (Resistant). (8) For quality control, *E. coli* ATCC 25922 was used as the susceptible control and *E. coli* NCTC 13846 as the resistant control (mcr-1 positive), following manufacturer's.

Data collection

Clinical and demographic data were retrieved from the hospital's electronic medical files system. Information collected included patient age, gender, admission details, and primary diagnosis. The type and date of specimen collection were recorded, along with the presence of comorbidities such as diabetes mellitus, hypertension, and renal disease. Risk factors assessed included ICU admission, mechanical ventilation, central venous catheter placement, recent surgery, and prior antibiotic use.

Risk factor and outcome assessment

Risk factors associated with colistin resistance, such as ICU admission, mechanical ventilation, and prior antibiotic exposure, were evaluated. Patient outcomes were also recorded, including 30-day mortality and length of hospital stay. Colonization was specified as the presence of CRE in a clinical specimen without signs or symptoms of active infection, whereas infection was described by the presence of compatible clinical signs and symptoms supported by microbiological findings and resulted in initiating targeted therapy as per standard institutional criteria.

Statistical analysis

Descriptive statistics were determined to summarize the clinical and demographic features. Association between colistin resistance, risk factors, and patient outcomes were analyzed using Pearson's chi-square and Fisher's exact test. Given the exploratory nature of this study and the small sample size, no adjustment for multiple comparisons was applied to the p-values; results should therefore be interpreted as generating hypotheses rather than confirming associations. Statistical significance was set at $p < 0.05$. Data were analyzed using SPSS version 22 (IBM, USA).

Results

The study included 37 participants, ranging in age from 7 months to 92 years, with a mean age of 61.3 years (SD = 22.1). The majority of participants were male 62.2% (n = 23). Clinical specimens were collected from various sources, with respiratory samples accounted for the most frequent source (n=12; 32.4%), followed by urine (n=7; 18.9%), blood (n=6; 16.2%) and other samples (n=8; 21.6%)

The antimicrobial susceptibility result showed that all strains were resistant to amoxicillin-clavulanate (100%) and meropenem (100%). Elevated levels of resistance were also observed for ciprofloxacin (89.2%) and Cefepime (83.8%). Resistance to colistin was detected in 17% of isolates all of which were *K. pneumoniae*. The colistin MIC₅₀ and MIC₉₀ for all isolates were 0.25 µg/mL and 4.0 µg/mL, respectively. The AST results are presented in Table 1 Comorbid conditions were noted among participants, with hypertension reported in 43.2% and diabetes mellitus in 16.2%. All patients were hospitalized and the 30-day mortality rate was 29.7% (n = 11). 76% (n = 28) of the patient required ICU admission, mechanical ventilation, and central line placement. Additionally, 29% (n = 10) had a history of hospitalization in the six months prior to infection onset. Antibiotics consumption in the previous 30 days was reported for 13 cases, 3 patients had a history of carbapenem and 2 had combinational therapy of which colistin was one of the drugs.

Table 1. Descriptive Statistics of participants

Antibiotic Susceptibility Pattern			
Amoxicillin-Clavulanate (AMC)	R	37	100.0
	S		
Ceftazidime (TAZ)	R	35	94.6
	S	1	2.7
Cefepime (FEP)	R	31	83.8
	S	1	2.7
	I	2	5.4
Imipenem (IMP)	R	32	86.5
	S	2	5.4
Ciprofloxacin (CIP)	R	33	89.2
	S	1	2.7
Meropenem (MEM)	R	37	100.0
Colistin (COL)	R	6	17
	S	30	83
Comorbidities			
Diabetes mellitus (DM)	Yes	6	16.2
	No	31	83.8
Hypertension (HT)	Yes	16	43.2
	No	21	56.8
30 days mortality rate	Dead	11	29.7
	Survived	26	70.3
RISK FACTORS			
Admission	Yes	37	100
Intensive care unit (ICU)	Yes	28	76
	No	9	24
Mechanical Ventilator (MV)	Yes	28	76
	No	9	24
Central Line	Yes	28	76
	No	9	24
Hospitalization in 6 months	Yes	10	29
	No	25	71

Comparison between colonization and infection cases

A comparison of antibiotic susceptibility patterns between CRE colonization and true infection cases showed higher resistance rates in infection cases for most antibiotics, although the differences were not statistically significant. Resistance to Trimethoprim-Sulfamethoxazole (SXT) was 73% in colonization cases

versus 96% in infection cases ($p = 0.076$). Levofloxacin (LEVO) resistance was universal in both groups (100%). Tigecycline (TGC) resistance was observed in 25% of colonization cases compared to 38% of infection cases ($p = 0.5$). Colistin resistance was comparable between the two groups, at 18% in colonization cases and 16% in infection cases ($p = 0.609$). Risk factors analysis regarding ICU admission, mechanical ventilation, and hospitalization within six months showed no significant differences between colonization and infection cases ($p > 0.05$). Similarly, comorbidities such as diabetes mellitus, hypertension, renal disease, neurological disorders, and malignancy did not show statistically significant differences between the cohorts (Table 2).

30-Day mortality analysis

Analysis of 30-day mortality (Table 3) indicated a higher mortality rate among patients with recent hospitalization (40%) compared to those without (24%), although this was not statistically significant ($p = 0.292$). However, the presence of comorbidities notably influenced mortality outcomes. A significantly higher mortality rate was noted among patients with diabetes mellitus (67% vs. 23%, $p = 0.031$) and those with hypertension had an even more pronounced increase in mortality (63% vs. 5%, $p = 0.001$). Also, Patients who previously received antibiotics had a higher mortality rate (54%) compared to those who did not (17%) ($p = 0.025$). A higher mortality rate, yet not significant, was noted among colistin resistant group (50% vs. 27% in susceptible cases; $p = 0.252$). Mortality was also higher among those who received antibiotics (54%) compared to those who did not (17%), and this difference was statistically significant ($p = 0.025$). However, there was no significant difference when colonization and true infection cases were correlated with the mortality risk (35% infection cases vs. 18%, colonization cases $p = 0.278$).

Logistic regression analysis of mortality predictors

Multiple logistics regression analysis was carried out to detect predictors of 30-day mortality (Table 4).

ICU admission was significantly associated with increased mortality (OR = 4.890, 95% CI: 1.469-16.282, $p = 0.010$). Similarly, a significant association was detected between hypertension, prior exposure to antibiotics and the 30-day mortality rate (Table 4).

Table 5 summarizes the microbiological and clinical characteristics features of colistin resistant strains. 3 cases showed elevated MIC levels $\geq 16 \mu\text{g/ml}$ (all did not survive) and the other 3 had lower level of resistance (MIC=4 $\mu\text{g/ml}$). All cases were admitted to the hospital, and 4 patients had a history of ceftazidime-avibactam (n=3), and tigecycline, trimethoprim-sulpha (n=1) consumption.

Discussion

This study points out the growing global health problem of colistin resistance in carbapenem-resistant Gram-negative bacteria (CRE-GNB), focusing on clinical isolates from a single center in Saudi Arabia. A key strength of our work is the detailed reporting of local MIC distributions and their correlation with patient outcomes, providing a granular view of the challenge. Strains belonging to the CRE group represent a challenge in modern healthcare. Their resistance characterizes them to almost all β -lactam antibiotics, including carbapenems, which are typically used for severe multidrug-resistant infections. Our findings align with national trends, as 89.2% of CRE isolates in this study were *K. pneumoniae*, all exhibiting substantial resistance to meropenem (100%) and high imipenem resistance (86.5%), underscoring the critical need for alternative therapies, positioning colistin as a last-resort agent in such scenarios. (16) Our study observed colistin resistance in 17% of CRE isolate with an MIC₉₀ of 4.0 $\mu\text{g/mL}$ for the entire cohort, indicating a subset of isolates with elevated MICs even within the susceptible range. However, pooled data on colistin resistance trend among members of Enterobacterales are variable, and increasing over decades (17–19). This trend is further supported by a very recent 2024 review (20). In a recent meta-analysis, the aggregated prevalence of colistin resistance was estimated to be 8.34% for *Klebsiella* spp. and 3.44% for *E. coli* and among blood stream infections (BSI), *K.*

Table 2. Comparison of antibiotic susceptibility/co-morbidities/risk factors to colonization and true infection cases

Antibiotics	Susceptibility Pattern	CRE		P-value
		Colonization	Infection	
Sulfamethoxazole (SXT)	R	8(73)	24(96)	0.076
	S	3(27)	1(4)	
Levofloxacin (LEVO)	R	7(100)	15(100)	-
Tigecycline (TGC)	R	2(25)	3(38)	0.5
	S	6(75)	5(62)	
Amoxicillin-Clavulanate (AMC)	R	11(100)	26(100)	-
Ceftazidime (TAZ)	R	0(0)	1(4)	0.639
	S	11(100)	24(96)	
Cefepime (FEP)	R	9(90)	22(92)	0.198
	S	1(10)	0	
	I	0	2(8)	
Imipenem (IMP)	R	10(100)	22(92)	0.492
	S	0	2(8)	
Ciprofloxacin (CIP)	R	10(91)	23(100)	0.324
	S	1(9)	0	
Gentamicin (GENT)	R	5(46)	13(50)	0.543
	S	6(55)	13(50)	
Amoxicillin-clavulanic acid (AMK)	R	7(64)	9(41)	0.195
	S	4(36)	13(59)	
Meropenem (MEM)	R	11(100)	26(100)	-
Colistin (COL)	R	2(18)	4(16)	0.609
	S	9(82)	21(84)	
RISK FACTORS				
Intensive care unit/Mechanical ventilation (ICU/MV)	Yes	10(91)	18(69)	0.163
	No	1(9)	8(31)	
Hospitalization in 6 months	Yes	1(9)	9(38)	0.089
	No	10(91)	15(62)	
CO-MORBIDITIES				
Diabetes mellitus (DM)	Yes	0(0)	6(23)	0.099
	No	11(100)	20(77)	
Hypertension (HT)	Yes	3(27)	13(50)	0.182
	No	8(73)	13(50)	
Renal	Yes	2(18)	0(0)	0.083
	No	9(82)	26(100)	
Neurological	Yes	0(0)	2(8)	0.498
	No	11(100)	24(92)	
Malignancy	Yes	0(0)	2(8)	0.488
	No	11(100)	24(92)	

Table 3. Comparison of 30-day mortality with the antibiotic received and CRE true colonization

Factors		30-day mortality		P-value
		Yes (survived)	No	
Hospitalization in 6 months	Yes	4(40)	6(60)	0.292
	No	6(24)	19(76)	
Intensive care unit (ICU)	Yes	9(32)	19(68)	0.454
	No	2(22)	7(78)	
Colistin resistance	S	8(27)	22(73)	0.252
	R	3(50)	3(50)	
Antibiotic consumed in the previous 30 days	Yes	7(54)	6(46)	0.025*
	No	4(17)	20(83)	
Diabetes mellitus (DM)	Yes	4(67)	2(33)	0.031*
	No	7(23)	24(77)	
Hypertension (HT)	Yes	10(63)	6(37)	0.001*
	No	1(5)	20(95)	

*Statistically Significant

Table 4. Multiple linear logistic regression for Risk factors associated with mortality in 30 days

Variables	p-value	OR	95% C.I. for OR	
			Lower	Upper
ICU (Yes vs No)	0.010*	4.890	1.469	16.282
Hospitalization in 6 months (Yes vs No)	0.530	1.730	0.313	9.559
DM (Yes vs No)	0.280	0.289	0.031	2.740
Hypertension (Yes Vs No)	0.026*	0.178	0.039	0.817
Colistin AST result (S or R)	0.933	1.01	0.140	7.259
Antibiotic consumed in the previous 30 days (yes/no)	0.041*	5.66	1.070	29.92
Central line (yes/no)	0.504	1.946	0.135	13.73
Mechanical ventilation (yes/no)	0.574	1.658	0.285	9.639
Catheter (yes/no)	0.596	9.44	0.022	8.997

*Statistically significant

pneumoniae represented 3.1%, 95% CI (1.5–4.7%) of colistin resistant strains (18,19). In addition, the prevalence of colistin resistant *K. pneumoniae* strains has been documented to rise from 6% in 2010 to 18% in 2023 based on data pooled from 682 studies (17). In Saudi Arabia, a multi-center, extensive work analyzed the phenotypic and genomic features of *K. pneumoniae* isolates collected from 34 hospitals across the country identified a high resistance level indicated by the detection of ESBL (72%), carbapenemases (77%) and

colistin resistance (27%) (21). Drug resistance and its association with risk factors have also been investigated in this study. A significant association was observed between antibiotic consumption within the preceding 30 days and increased 30-day mortality (54% vs. 17%, $p = 0.025$). This aligns with global evidence that prior antibiotic exposure, particularly to broad-spectrum agents, selects for infections with more resistant and virulent strains (22). The temporal relationship suggests that recent antibiotic use may deplete commensal

Table 5. Microbiological and clinical features of Colistin resistant- *K. pneumoniae* cases

Patient	Specimen	Colistin (MIC)	Infection/ Colonization	Diagnosis	Risk factors	Hospitalization in 6 months	Antibiotics taken in 30 days	Co-morbidities	30-day mortality
57 Years-Male	Blood	16	Infection	Epilepsy	ICU/MV *	Yes	Yes	Diabetes	NO
					Central line Catheter			Hypertension	
7 Years-Male	Tissue	4	Infection	Diabetic ketoacidosis	ICU/MV	Yes	No	Diabetes	Yes
					Central line Catheter				
63 Years-Female	Catheter tip	4	Infection	Brain abscess	ICU/MV	No	Yes	Hypertension	Yes
92 Years -Male	Tracheostomy	> 16	Infection	Pneumonia	No	Yes	Yes	Hypertension	NO
					ICU/MV	No	No	Hypertension	Yes
69 Years-Male	Urine	4	Colonization	Trauma	Central line Catheter		No	Hypertension	Yes
					ICU/MV		No	Hypertension	Yes
83 Years-Female	Trans tracheal	> 16	Colonization	Trauma	ICU/MV	No	Yes	Hypertension	NO
					Central line Catheter		No	Hypertension	NO

*Intensive care unit/ Mechanical ventilation (ICU/MV)

microbiota, facilitating CRE colonization and subsequent invasive disease. Furthermore, as observed in previous cohorts, inappropriate empiric therapy is common in settings with high resistance rates, which likely contributed to poorer outcomes (23). Although cases carrying colistin resistant strains seems to have elevated risk of mortality rate relative to patients infected with sensitive isolates, the small sample size made this conclusion hard to achieve (24). In our cohort, colonization and infection cases showed similar antimicrobial resistance pattern including that to colistin (18% in colonization relative to 16% in infected patients). Studies in this regard described variable findings with some identified clinical infection cases to exhibit higher resistant profile to advanced antimicrobial agents (25), while others detected similar resistance pattern suggesting the role of virulence features (e.g. toxins and biofilm production capabilities) as a major drive for true infection cases (26). Risk factors, such as ICU admission, mechanical ventilation, or device use did not reveal significant association with colonization and true infection. This suggests that colonized patients may be at the same risk of contracting true infections, highlighting the significance of early detection and stringent infection prevention and control policies, although this may be hard to conclude due to the small sample size. This is consistent with other studies suggesting that the relationship between specific comorbidities and CRE infections may be complex and affected by several factors, including illness severity and healthcare exposure (22). Also, in our cohort, all cases were derived from admitted patients with various clinical conditions. Admission to the ICU setting was significantly correlated with increased 30-day mortality rate in agreement with studies linking (27). Our analysis suggests that mortality was heavily influenced by host factors, including acuity of illness (as indicated by the strong association with ICU admission) and underlying comorbidities like diabetes and hypertension, rather than by the colistin resistance profile or CRE infection status alone. This is a critical consideration for clinicians managing these complex cases. The study delivers significant understanding of the prevalence and clinical implications of colistin resistance in Saudi Arabia. Nevertheless, specific

limitations must be acknowledged when interpreting the results. The single-center methodology and modest sample size (n=37) limit the statistical power, particularly for the multivariable logistic regression, which should be considered exploratory. While molecular profiling of resistance mechanisms (e.g., for *mcr* genes or chromosomal mutations) was not performed in this phase of the study, the phenotypic results provided a robust basis for comprehending resistance trends. Future work should aim to correlate these phenotypic findings with genotypic analysis to identify the prevalence of *mcr* genes versus chromosomal mutations (e.g., in *mgrB* or *phoP/Q*). Moreover, data collection predominantly utilizes electronic medical records, which are extensively employed and realistic, although they may occasionally restrict clinical depth. The 30-day follow-up period was selected to maintain consistency in result evaluation; however, future research may benefit from prolonged follow-up intervals to investigate long-term prognosis more thoroughly. Finally, the unadjusted p-values reported for multiple comparisons should be interpreted with caution as hypothesis-generating for further research. With the wide spread of CRE and MDR phenotypes, and the steadily increasing resistance to colistin, more comprehensive studies are required to evaluate the combinational therapy to manage hard to treat infections.

Conclusion

The current work highlights the pressing global challenge of colistin non-susceptibility among carbapenem-resistant Gram-negative clinical isolates in Saudi Arabia. The observed high resistance rates and the quantitative MIC data mirror both national and international data, reinforcing the urgent need for targeted antimicrobial strategies. Our findings underscore that patient survival is multifactorial, heavily dependent on host acuity and comorbidities. This research advances the understanding of CRE epidemiology and underscores the importance of robust antimicrobial stewardship, consideration of combination therapies as per latest guidelines, and stringent infection control practices in healthcare settings.

Ethical Approval: The study was approved by the Institutional Review Board (IRB) of Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia (IRB-2020-03-163).

Conflict of Interest: All authors declare that they have no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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