

ORIGINAL ARTICLE

Bacteriological Profile and Antimicrobial Resistance of Semen Infections among Infertility Cases before and during COVID-19 Pandemic: A Comparative Study

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ABSTRACT

Key words:
COVID-19, AMR, Semen culture, Bacteriospermia, Male infertility

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Background; Antimicrobial resistance (AMR) among bacteria causing male genitourinary infections has the potential to affect male fertility. The objective of this work is to determine differences in AMR patterns of bacteria causing bacteriospermia in infertile men before and during the COVID-19 pandemic. **Methods:** This is an observational comparative study in which AMR of non-duplicate semen cultures from infertility cases before and during COVID-19 is presented. The COVID-19 cases are defined as per the protocol of the Ministry of Health and Population (MOHP) in Egypt. Antimicrobial susceptibility reporting was performed in agreement with the Clinical and Laboratory Standard Institute (CLSI, 2020). **Results;** A total of 176 samples were processed during the study period. 108 (61.4%) and 68 (38.6%) were of pre- and during the COVID-19 era. The commonest pathogens isolated were *S. Aureus* [60 (55.6%) and 40 (58.8%)], *S. epidermidis* [12 (11.1%) and 8 (11.8%)] and for *N.gonorrhoea* [11 (10.2%) and 6 (8.8%)] in pre-and during the COVID -19 period respectively. Significant antimicrobial resistance were observed for Nalidixic acid (P <0.001), Erythromycin (P <0.001) and Clarithromycin (P <0.001). Similar significant resistance were observed for Cefepime (P <0.001) and Meropenem (P <0.001) antibiotics. Further, 22 (20.4%) isolates were multi-drug-resistant during the COVID-19 period. **Conclusion;** Pathogens presented significant levels of AMR in pre- and during the COVID-19 era. Further research with control groups is needed to better understand the effects of COVID-19 on male fertility.

INTRODUCTION

SARS-CoV-2 (severe acute respiratory syndrome, coronavirus) is an enveloped RNA-coronavirus first recovered from Wuhan City in China¹. The virus produces severe pneumonia with clinical signs distinct from those seen with other members of coronavirus families, such as SARS-CoV and MERS-CoV². Co-infection with other viruses, bacteria, and fungi complicates diagnosis, treatment, and increase the death rate among the COVID-19 cases³. The extensive and incorrect usage of antibiotics during COVID-19 era or otherwise lead to antibiotic resistance (AMR)⁴.

A study of mild/asymptomatic COVID-19 cases have documented adverse effects on sperm quality, count, motility and morphology⁵. Red blood cell exudation, congestion, and interstitial edema were seen

in the testes of COVID-19 autopsied testicular specimens. Testicular tissue's interstitial cells contained higher levels of CD3+ and CD68+, oligozoospermia was seen in 39.1% of the specimens, and a notable rise in leucocytes in semen in 60.9% of the specimens⁶.

One out of every five males seeking medical attention for a couple experiencing primary infertility have an asymptomatic bacterial semen infection⁷. Semen culture is a main diagnostic tool used to detect genitourinary tract infections which causes male infertility⁸. Microorganisms seen in semen originate from the urinary tract or may be sexually transmitted, as in the cases of Chlamydia trachomatis and N. gonorrhoeae infections. Concentrations greater than 10³ bacteria/ml ejaculate is considered an active infection⁹. Enterobacteriaceae such as *E. coli*, *Klebsiella* species are associated with epididymitis, orchitis, and

prostatitis¹⁰. Gram positive cocci such as *Enterococci*, *Streptococci*, and *Staphylococci* are associated with prostatitis and epididymitis and infertility¹¹. Yasser and colleagues reported that *S.aureus* was the commonest isolated pathogen among positive semen specimens then urogenic gram-negative pathogens¹⁰. Silago et al¹², reported a very high resistance (around 100%) to non-beta lactam antibiotics between Gram-negative bacteria associated with male infertility¹². The abuse of antibiotics during COVID-19 period might have an effect on AMR among bacteria causing semen infection. The current study looked for variations in drug resistance patterns of bacteriospermia-causing bacteria among infertile patients pre- and during COVID-19 outbreak.

METHODOLOGY

Setting and design

This observational comparative study aimed to determine MAR among bacteria recovered from semen cultures of primary and secondary infertility cases before and during COVID-19 pandemic. This study was done at the Suez-Canal University hospital (SCUH) from June 1, 2017 to December 31, 2018 (pre-COVID19) and June 1, 2020 to December 31, 2021 (during COVID-19 pandemic).

Data collection

The study included all non-duplicate antimicrobial sensitivity reports of bacterial cultures of semen during the specified period. The COVID-19 cases were defined in agreement with COVID-19 MOHP protocol in Egypt¹³. Excluding the sensitivity reports of polymicrobial cultures, and reports with incomplete data and positive fungal cultures. In addition, data on age, smoking status and type of infertility were recorded. The research protocol was approved by the Ethical Committee, Faculty of Medicine, Suez Canal University dated 20th December 2022. At the time of recruiting, each participant gave their informed consent.

Semen analysis

Semen was collected and analysed according to Basic semen examination (Preparations – Pre-

examination procedures, Examination procedures and Post-examination procedures) methods and standards outlined by WHO guidelines 2021¹⁴. Leukocytospermia was defined wherein peroxidase-positive cells were equal or greater than 1.0×10^6 per ml (WHO, 2021)¹⁴.

Semen culture

Chocolate agar cultures were incubated at 5% CO₂ candle jar for 24-48 hours, whereas semen samples were cultivated on blood and MacConkey agar within one hour of specimen collection and aerobically incubated at 37 C for 24-48 hours^{11,15}. The bacterial concentration of more than 10^3 CFU/ml for gram negative rods and greater than 10^4 for gram positive cocci¹⁶ were considered significant. The Kirby-Bauer disk diffusion method was used to detect antibiotic susceptibility, and the results were reported in accordance with the Clinical and Laboratory Standard Institute (CLSI)¹⁷. The antimicrobial discs were obtained from Oxoid (Basingstoke, UK) and included Ampicillin, Cefotaxime, Cefuroxime, Cefaclor, Penicillin, Gentamycin, Trimethoprim/Sulfamethoxazole, Chloramphenicol, Teicoplanin, Meropenem, Erythromycin, Clarithromycin, Cefepime, Nalidixic acid, Pefloxacin, Ciprofloxacin, Doxycycline and Tetracycline.

Definition of Multidrug- resistant organisms [MDROs]

The terms "acquired non-susceptibility to at least one agent in three or more antimicrobial categories," "extensive drug resistance" (XDR), "pan-drug resistance" (PDR), and "non-susceptibility to all agents in all antimicrobial categories" (XDR) were used to characterize their respective levels of resistance.¹⁸

Statistical analysis

IBM Statistical Package for Social Sciences (SPSS) software version 20.0 for Windows® (SPSS, Inc., Chicago, IL, USA) was used for data analysis. The data is provided as a mean, Median, standard deviation and frequencies and percentage. The study used Chi-squared test to compare various semen parameters pre-and during COVID-19 period. A *P*-value of 0.05 was considered significant.

RESULTS

Table 1: Bacteriological profile of semen infections before and during COVID 19 period (n=176)

Category	Pre-Covid-19 n=108 (61.4%)	During Covid-19 n=68(38.6%)	*P-value
Bacteriological profile			
<i>S. aureus</i>	60 (55.6%)	40 (58.8%)	0.670
<i>S. epidermidis</i>	12 (11.1%)	8 (11.8%)	0.894
<i>N. gonorrhoea</i>	11 (10.2%)	6 (8.8%)	0.766
<i>E. coli</i>	7 (6.5%)	2 (2.9%)	0.485
NHS <i>S. bovis</i>	5 (4.6%)	4 (5.9%)	0.736
<i>Klebsiella</i>	4 (3.7%)	3 (4.4%)	1.000
<i>Enterobacter</i>	4 (3.7%)	0 (0.0%)	0.160
BHS <i>S. agalactia</i>	4 (3.7%)	3 (4.4%)	1.000
<i>Alpha hemolytic streptococcus</i>	1 (0.9%)	0 (0.0%)	1.000
<i>Pseudomonas</i>	0 (0.0%)	1 (1.5%)	0.386
<i>Corynebacteria</i>	0 (0.0%)	1 (1.5%)	0.386
Resistance category			
MDR	12 (17.7%)	22 (20.4%)	0.543
XDR	2 (1.8%)	0 (0.0%)	

NHS: Non-hemolytic; BHS: Beta-hemolytic; MDR: Multidrug-resistance; XDR: Extreme drug resistance

*Statistical tests: (χ^2) Chi-square test and Fishers exact test.

Of the 176 samples processed, 108 (61.4%) and 68(38.6%) were from the pre-and during COVID-19 period. *S. aureus* was the predominant pathogen isolated in pre - and during COVID-19 period [60 (55.6%) and 40 (58.8%)] respectively. The other common pathogens were *S. epidermidis* [12 (11.1%) and 8 (11.8%)] and *N. gonorrhoea* [11 (10.2%) and 6 (8.8%)] in pre-and During

COVID -19 period. 22 (20.4%) MDR isolates were recovered in the pre-COVID-19 period.

S. aureus was the predominant pathogen isolated in pre - and during COVID-19 period [60 (55.6%) and 40 (58.8%)] respectively. The other common pathogens were *S. epidermidis* [12 (11.1%) and 8 (11.8%)] and *N. gonorrhoea* [11 (10.2%) and 6 (8.8%)] in pre-and during COVID -19 period.

Table 2: Antimicrobial resistance pre-and during COVID-19

Antibi otic	Pre-COVID (n = 108)			During-COVID (n = 68)			χ^2	P value
	Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive		
NA	31 (28.7%)	21 (19.4%)	56 (51.9%)	26 (38.2%)	38 (55.9%)	4 (5.9%)	43.563	<0.001
PEF	37 (34.3%)	38 (35.2%)	33 (30.6%)	14 (20.6%)	25 (36.8%)	29 (42.6%)	4.452	0.108
CIP	20 (18.5%)	40 (37.0%)	48 (44.4%)	5 (7.4%)	15 (22.1%)	48 (70.6%)	11.887	0.003
DO	26 (24.1%)	34 (31.5%)	48 (44.4%)	12 (17.6%)	27 (39.7%)	29 (42.6%)	1.643	0.440
TE	9 (8.3%)	16 (14.8%)	83 (76.9%)	18 (26.5%)	19 (27.9%)	31 (45.6%)	18.860	<0.001
TEC	42 (38.9%)	57 (52.8%)	9 (8.3%)	21 (30.9%)	35 (51.5%)	12 (17.6%)	3.795	0.150
MEM	31 (28.7%)	19 (17.6%)	58 (53.7%)	9 (13.2%)	45 (66.2%)	14 (20.6%)	42.664	<0.001
E	51 (47.2%)	44 (40.7%)	13 (12.0%)	18 (26.5%)	21 (30.9%)	29 (42.6%)	22.065	<0.001
CLR	46 (42.6%)	45 (41.7%)	17 (15.7%)	22 (32.4%)	12 (17.6%)	34 (50.0%)	25.467	<0.001
FEP	24 (22.2%)	80 (74.1%)	4 (3.7%)	15 (22.1%)	23 (33.8%)	30 (44.1%)	46.831	<0.001
AMP	53 (49.1%)	23 (21.3%)	32 (29.6%)	24 (35.3%)	30 (44.1%)	14 (20.6%)	10.333	0.006
CTX	28 (25.9%)	57 (52.8%)	23 (21.3%)	16 (23.5%)	25 (36.8%)	27 (39.7%)	7.370	0.025
CEF	35 (32.4%)	21 (19.4%)	52 (48.1%)	21 (30.9%)	17 (25.0%)	30 (44.1%)	0.772	0.680
CEC	37 (34.3%)	35 (32.4%)	36 (33.3%)	23 (33.8%)	9 (13.2%)	36 (52.9%)	10.059	0.007
P	50 (46.3%)	33 (30.6%)	25 (23.1%)	25 (36.8%)	33 (48.5%)	10 (14.7%)	5.980	0.050
CN	54 (50.0%)	30 (27.8%)	24 (22.2%)	26 (38.2%)	29 (42.6%)	13 (19.1%)	4.214	0.122
SXT	30 (27.8%)	58 (53.7%)	20 (18.5%)	25 (36.8%)	38 (55.9%)	5 (7.4%)	4.777	0.092
C	24 (22.2%)	16 (14.8%)	68 (63.0%)	21 (30.9%)	43 (63.2%)	4 (5.9%)	63.641	0.001

NA: Nalidixic acid, PEF: Pefloxacin, CIP: Ciprofloxacin, DO: Doxycycline, TE: Tetracycline, TEC: Teicoplanin, MEM: Meropenem, E: Erythromycin, CLR: Clarithromycin, FEP: Cefepime, AMP: Ampicillin, CTX: Cefotaxime, CEF: Cefuroxime, CEC: Cefaclor, P: Penicillin, CN: Gentamycin, SXT: Trimethoprim/ Sulfamethoxazole, C: Chloramphenicol

χ^2 = Chi-square test

Significant differences in antimicrobial resistance were observed for Nalidixic acid ($P < 0.001$), Tetracycline ($P < 0.001$), macrolides like Erythromycin ($P < 0.001$) and Clarithromycin ($P < 0.001$) respectively in pre-and during COVID-19 periods. Furthermore, significant differences were observed for cephalosporin, 'Cefepime' ($P < 0.001$) and Carbapenems, 'Meropenem' ($P < 0.001$). (Table 2)

In our study, pathogens showed resistance to nalidixic acid (38.2% vs 28.7%), tetracycline (26.5% vs 8.3%), trimethoprim/sulfamethoxazole (36.8% vs 27.8%), and chloramphenicol (30.9% vs 22.2%) during COVID-19 and pre COVID period respectively. Multi drug-resistant (22 (20.4%) bacteria were frequent in the COVID-19 period.

Table 3: Comparison of semen parameters between pre- and during COVID-19

Characteristic	Pre-COVID (n = 108)	During-COVID (n = 68)	U	P
Sperm count (millions/ml)				
Mean \pm SD.	38.1 \pm 37.5	37.8 \pm 38		
Median (Min. – Max.)	28.5 (0 – 160)	27 (0 – 160)	3642.50	0.929
Rapid Progression %				
Mean \pm SD.	14.5 \pm 18.1	20.9 \pm 20.1		
Median (Min. – Max.)	5 (0 – 80)	20 (0 – 80)	2947.0*	0.026*
**Total motility				
Mean \pm SD.	50.6 \pm 27.4	53.8 \pm 30.9		
Median (Min. – Max.)	55 (0 – 95)	60 (0 – 95)	3223.50	0.263
Vitality by dye %				
Mean \pm SD.	62.1 \pm 27.4	62.8 \pm 30.4		
Median (Min. – Max.)	70 (0 – 98)	72.5 (0 – 98)	3449.0	0.496
Abnormal forms %				
Mean \pm SD.	56.1 \pm 22.9	51.7 \pm 21.9		
Median (Min. – Max.)	50 (10 – 97)	45 (10 – 97)	3282.50	0.235

Table 3 depicts various semen parameters', the only parameter that shows significant differences was the rapid progression ($P = 0.026$), while as, the other

parameters like sperm count, total movement and vitality were insignificant pre- and during COVID-19 period.

Table 4: Comparison between pre-COVID and during-COVID regarding agglutination, pus cell million, and smoking

Characteristic	Pre-COVID (n = 108)	during-COVID (n = 68)	χ^2	P value
Type of infertility				
Primary	77 (71.3%)	49 (72.1%)		
Secondary	31 (28.7%)	19 (27.9%)	0.012	0.913
**Agglutination				
No agglutination	59 (54.6%)	31 (45.6%)	1.365	0.243
+	24 (22.2%)	16 (23.5%)	0.041	0.840
++	18 (16.7%)	17 (25.0%)	1.819	0.177
+++	7 (6.5%)	4 (5.9%)	0.026	1.000
Pus cell million /ml				
<1 million	40 (37.0%)	11 (16.2%)	8.823*	0.003*
\geq 1 million	68 (63.0%)	57 (83.8%)		
Smoking				
Non-smoker	40 (37.0%)	29 (42.6%)		
Smoker	52 (48.1%)	26 (38.2%)	1.729	0.421
Ex-smoker	16 (14.8%)	13 (19.1%)		

SD: Standard deviation; U: Mann Whitney test; *: Statistically significant at $P < 0.05$; **All motile sperms in the specimen, normal range (%) = 42 (40–43)

Pus cell per ml of semen (< 1 million) and (\geq 1 million) showed significant ($P = 0.003$) differences in pre-and during COVID-19 period. Rest of the parameters were insignificant. (Table 4)

χ^2 = Chi-square test; *: Statistically significant at $P < 0.05$; **Agglutination= + (Scant agglutination), ++ (Moderate agglutination), +++ (Severe agglutination)

DISCUSSION

Reproductive health has been significantly impacted, among other aspects of daily life, by the COVID-19 pandemic. A crucial component of successful reproduction is male fertility, and the pandemic has sparked concern about the pandemic's possible effect on male reproductive function. This study's goal was to determine AMR among bacteria causing semen infection during COVID-19 infection among men with primary or secondary infertility.

Our study showed a statistically significant surge in the presence of pus cells, and a surge in the rapid progression movement of sperms during-COVID compared with the pre-COVID group, while sperm count, total movement, and vitality by dye in the post-COVID group did not show any significant difference.

The report of Guo et al.¹⁹ showed that first and second samples from 41 males recovered from COVID-19 were analyzed based on sperm and sex hormone parameters (the first sampling was taken 56 days following hospital release, and the second sample was taken after a median time interval of 29 days from first sampling). The first sampling showed a significant decrease in total sperm count, sperm concentration, and percentage of motile sperm, but sperm viability and morphology did not change. According to Donders et al. study²⁰, 37% of men tested had a decreased mean sperm count and 60% of men tested with mean progressive motility lowered after two months, a study by He et al.²¹ suggests that COVID-19 could potentially lead to spermatogenic dysfunction. The 2nd sample from 22 of 41 males showed that percentage of morphologically abnormal sperm was reduced, and the total sperm count, sperm concentration, and number of motile spermatozoa per ejaculate were all significantly increased²¹.

Impaired sperm quality with moderate illness, and the loss in sperm quality may be linked to fever, inflammation, and disease severity. Additionally, Holtmann et al.²² found that, in comparison to patients with a mild infection (N=14) and the healthy control group (N=14), patients with a moderate infection (N=4) had a statistically significant impairment of sperm quality (sperm concentration, total number of sperm per ejaculate, total number of progressive motility, and total number of complete motility). The five semen samples from the twelve male COVID-19 patients in a different investigation by Ma et al.²³ showed reduced sperm motility. In a cohort research conducted by Ruan et al.²⁴, the sperm density, total sperm count, and total motility of 70 sperm samples all significantly decreased when compared to the healthy control group. Numerous studies examined the possibility that a COVID-19 infection could result in testicular damage²⁵. These studies confirm that COVID-19 disease affects the male reproductive system making semen quality poor. The mechanisms behind this is not fully understood yet, but

most likely, the virus can directly affect the testes and disrupt normal spermatogenesis. Alternatively, the impact on male fertility may be related to the inflammatory response triggered by the virus, which can lead to oxidative stress and damage to the testes. It is also possible that the psychological stress of the pandemic may contribute to male fertility problems.

The discrepancy between our study and these studies may be due to several causes. First, these studies varied in design and methodology, which may have affected the consistency of the findings. Second, many of these studies had small sample sizes, which limits the generalizability of the results. Finally, other confounding factors, such as age, comorbidities, and medication use may have influenced the results. One of the challenges faced by healthcare providers during the COVID-19 pandemic was managing bacterial infections in patients with COVID-19. The extensive use of antibiotics to treat these infections raises concerns about the development and spread of antibiotic resistance. Antimicrobial prescribing may occur despite the fact that treating COVID-19 with antibiotics is ineffective²⁶⁻²⁸.

Our findings suggest that the COVID-19 outbreak may have influenced the antibiotic resistance profile of microorganisms isolated from semen cultures. These have several explanations. Firstly, the immunocompromised state of COVID-19 patients can make them more susceptible to bacterial infections and increase the likelihood of antibiotic usage. Secondly, the prolonged hospital stays, and invasive procedures associated with severe COVID-19 cases provide opportunities for the acquisition and transmission of antibiotic-resistant bacteria. Additionally, the use of broad-spectrum antibiotics in the management of severe COVID-19 cases disrupts the normal bacterial flora allowing the overgrowth of antibiotic-resistant strains and creating an environment conducive to the spread of resistance.

The observed differences in the prevalence of bacterial species between pre-COVID and during-COVID individuals draw attention to the potential impact of COVID infection on the human microbiota of the genitourinary tract. The decrease in beneficial commensal bacteria and the concurrent rise in opportunistic pathogens suggest a dysbiosis or imbalance in the microbiota composition post-COVID. It is important to note that these changes may not be solely attributed to COVID-19 infection itself but could also be influenced by other factors such as disease severity, antibiotic use, underlying health conditions, or changes in lifestyle and diet during the pandemic.

Several studies have reported high antibiotic resistance during the COVID-19 pandemic. A meta-analysis study done by Langford et al.²⁶ included 148 studies of 362,976 patients in the period between December 2019, and May 2021 found that the most common organisms detected were *S. aureus* (n=2584

isolates [15%]), *Klebsiella spp* (n=2543 [15%]), and *Pseudomonas spp* (n=1830 [10%]). Additionally, secondary infections are prevalent in patients who are admitted to intensive care units. Among all hospitalized patients with COVID-19, bacterial infections, 60.8% (95% CI 38.6–79.3; from 17 studies) had antimicrobial-resistant bacterial infections. Further, 37.5% (26.9–49.5; from 42 studies) of isolates were resistant²⁶.

Another systemic review by Kariyawasam et al.²⁹ conducted during the first 18 months of the pandemic from November 1, 2019 to May 28, 2021 found that among multi-drug resistant organisms, methicillin-resistant *S.aureus*, carbapenem-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and multi-drug resistant *Candida auris* were most commonly reported.

The high levels of antibiotic resistance during COVID-19 were mainly caused by self-medication with antibiotics, empirical antibiotic treatment, and the prescription of antibiotics by general practitioners. A study by Sulayyim et al.³⁰ conducted from December 2019 to May 2022 found that the most commonly reported resistant Gram-negative bacteria was *Acinetobacter baumannii*, followed by *Klebsiella pneumoniae*, *E.coli*, and *Pseudomonas aeruginosa*. *A. baumannii* and *K. pneumoniae* were highly resistant to tested antibiotics compared with *E. coli* and *P. aeruginosa*. Commonly reported Gram-positive bacteria were *S. aureus* and *Enterococcus faecium*. The resistance of *E. faecium* to ampicillin, erythromycin, and Ciprofloxacin was high. The differences in the results of these studies may be because the studies were from different settings, populations, and healthcare systems. In addition, the extracted data for antibiotic resistance profile were measured by different laboratory procedures. Moreover, patient comorbidities might also have affect these results.

Our study has several limitations. Firstly, it is a retrospective analysis of data from a single center, which may limit the generalizability of our findings. Secondly, we did not have access to all clinical data, which may have affected the interpretation of our results. Thirdly, we did not investigate the genetic mechanisms underlying antibiotic resistance, which could provide additional insights into the changes in antibiotic resistance during the pandemic.

CONCLUSION

Several conclusions can be drawn from our study. COVID-19 may have negative effects on semen parameters, but the extent of these effects is not yet fully understood. Antibiotic resistance during COVID-19 infection was relatively high. Further research and follow-up studies with larger sample sizes and control groups are needed to confirm these findings, better understand the effects of COVID-19 on male fertility

and study antibiotic resistance pattern during COVID infection.

Additional information

Authors' contribution

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

Disclosures Human subjects:

The research protocol was approved by the Research Ethics Committee, Faculty of Medicine, Suez Canal University dated 20th December 2022. All methods in this study were used in accordance with the Declaration of Helsinki, and all participants provided their informed consent in writing.

Animal subjects:

All authors have confirmed that this study did not involve animal subjects or tissue.

Conflicts of interest:

In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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