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Fecal Carriage of Multi-Drug Coliform among Persons Deprived of Liberty in a Certain Correctional Facility in Negros Occidental, Philippines

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ABSTRACT

Background: *The emergence of multidrug-resistant (MDR) Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumoniae, poses a significant global public health threat. Although these bacteria are common commensals of the human gastrointestinal tract, they may serve as reservoirs of antimicrobial resistance genes that can be transferred to pathogenic strains. Correctional facilities are considered high-risk environments for the transmission of MDR organisms due to overcrowding, limited healthcare access, and close living conditions. This study investigated the fecal carriage of MDR coliforms among persons deprived of liberty (PDLs) in a correctional facility in Negros Occidental, Philippines.*

Methods: *A total of 132 fecal specimens were collected and processed using the modified Landman technique. Isolates were identified through conventional microbiological methods and subjected to antimicrobial susceptibility testing, phenotypic resistance detection, and Multiple Antibiotic Resistance Index (MARI) analysis.*

Results: *Fifty-three coliform isolates were recovered, including 38 E. coli and 15 K. pneumoniae. High rates of resistance were observed to co-amoxiclav and piperacillin/tazobactam, whereas all isolates remained susceptible to carbapenems and amikacin. Four isolates were identified as AmpC producers and three as extended-spectrum β-lactamase (ESBL) producers. The overall MDR prevalence was 7.57%, with E. coli and K. pneumoniae being the predominant MDR organisms. Although the mean MARI was 0.16, ten isolates exhibited MARI values exceeding 0.20, indicating exposure to high-risk antibiotic environments.*

Conclusion: *The presence of MDR coliforms among PDLs highlights correctional facilities as potential reservoirs for antimicrobial resistance. Strengthening antimicrobial stewardship, routine surveillance, infection prevention measures, and policy-driven interventions is essential to limit the emergence and spread of MDR pathogens in correctional settings.*

INTRODUCTION

Multidrug-resistant (MDR) organisms remain a persistent concern worldwide [1], as they are frequently associated with a lack of new antibacterial compounds to counter them. It is a major public health issue that increasingly leads to considerable morbidity and mortality, causing approximately 1.27 million deaths worldwide [2]. Some of the most common multidrug-resistant (MDR) bacteria are *Escherichia coli, Klebsiella pneumoniae,* and Methicillin-Resistant *Staphylococcus aureus,* which colonize the skin, gastrointestinal tract, and respiratory tract. There is already evidence that people colonized with MDR pathogens can later develop MDR infections and can transmit them to susceptible people, not just those in health care facilities, but also in the community [2].

Efforts to combat antimicrobial resistance are widespread among the general population. However, in some closed settings, such as correctional facilities, they are frequently overlooked. Inmates are more likely to contract infectious diseases due to some reasons like overcrowding, poor hygiene conditions, poor ventilated areas, and compromised health status, among others [3,4]. Although prisons and jails were constructed to maximize public safety, minimizing the transmission of infections was generally overlooked, as most prisons lack adequate information technology, clinical information-sharing, or clear jurisdictional oversight of inmate care [5].

Bacterial infections are recurrent in prisons, such as skin and soft tissue infections, respiratory tract infections, urinary tract infections, sexually transmitted infections, tuberculosis, and others. People deprived of liberty or people living in prisons play a crucial role in the spread of antibiotic resistance. This, in turn, leads to the widespread spread of resistant organisms within the prison and the community at large due to the rapid turnover of inmates [6].

Currently, most studies in the scientific literature have focused on Methicillin-Resistant *Staphylococcus aureus* and Multi-Drug-Resistant Tuberculosis. There is a lack of published studies or scarce evidence on the prevalence of the carriage of multidrug coliforms in penitentiary settings. Due to the aforementioned reasons, this study aims to assess the magnitude and distribution of fecal MDR bacterial carriage among people deprived of liberty in a correctional facility in Negros Occidental, as they act as a high-prevalence reservoir, driving the spread of pathogens among inmates and in the community after release. Crowded conditions and potentially limited hygiene practices in these penitentiaries promote transmission, thereby requiring enhanced infection control to manage these risks.

METHODS

This is a descriptive cross-sectional study aimed at determining whether PDLs at a specific correctional facility carry MDR coliforms in their feces. The study was carried out from April 27, 2023, to June 09, 2023. Persons deprived of liberty, or inmates, were considered the participants

in the study. Only correctional facilities that indicated their intent to participate and PDLs who gave their consent were included in this study. Only age, sex, marital status, monthly income, and educational attainment were collected. Each participant was given a unique code instead of their real name to protect their privacy, promote confidentiality, and avoid duplicate samples. Health status was not considered in the criteria due to variability and the difficulty of applying standard, non-incarcerated community health metrics in restricted settings.

Research Environment

The City Jail is located approximately 10.8 to 12.1 km away from the center of Bacolod City, Negros Occidental. The perimeter has an area of 637 m2 with a capacity of 140 PDLs. The facility has 12 cells, 10 for males and 2 for females. Each cell has its own restroom, complete with a handwashing area. Medical programs are also present, like physical recreations, PTB monitoring, mental health, HIV testing, and annual physicals, including dental exams. At the time of the study, the facility housed 174 inmates, well over its maximum capacity. Moreover, the facility has a total of 32 personnel rotating on a 24-hour schedule, working 8 hours per day, 5 days a week.

Collection of Specimens

Before collection, participants were fully informed about the study's methods and protocol and were oriented on proper specimen collection. Before sampling, the participant was made to urinate to prevent or lessen contamination of the specimen. A clean container and a special bedpan were also provided for every participant. Approximately 1-3 grams (3 mL) of stool is placed in a clean container immediately after defecation. Labels are then attached to the container's body, indicating the participant's code, age, and sex. Collected samples were then sent to Colegio San Agustin – Bacolod Laboratory and were processed within an hour. Sample collection was made from April 27, 2023, to May 27, 2023.

Isolation of MDR Coliform from the Stool of PDLs

Isolation of MDR Coliform was based on the Laboratory Protocol of CDC (Modified Landman Technique) for Detection of Carbapenem-Resistant or Carbapenemase-Producing, *Klebsiella spp* and *E. coli* from Rectal Swabs [7]. Freshly submitted stool swabs from PDLs were inoculated on a 5 mL Tryptic Soy Broth with a 10 µg Ertapenem or Meropenem disc and incubated overnight at 35 -37ºC ambient air. After incubation, broth cultures were vortexed and subcultured in MacConkey Agar and again incubated at 35 -37 ºC ambient air overnight. After incubation, representative colonies that were pink to red were purified and streaked on a new MacConkey Agar and again incubated at 35 -37 ºC in ambient air overnight.

Identification of Coliform Bacteria

Isolates were identified by selecting three purified and isolated colonies. They were subjected to a series of biochemical tests, such as Gram stain, catalase, oxidase, sugar fermentation, gas and H2S production, lysine iron agar, sulfide indole motility, Simmon's citrate,

urease, and colonies produced in Eosin Methylene Blue Agar. Identification of isolates was based on the Key Identification characteristics of Enterobacteriaceae [8,9].

In vitro Antibiotic Susceptibility Testing

In vitro susceptibility testing was performed using the Kirby-Bauer disc diffusion assay [10], in accordance with the Clinical Laboratory Standards Institute M02 13th Edition guidelines [10,11]. Three to five colonies from an overnight culture were suspended in Normal Saline Solution adjusted to 0.5 McFarland and mixed vigorously to break up clumps. A sterile cotton swab was moistened in the standardized suspension, and excess moisture was expressed by rotating the swab against the tube wall. The entire surface of Mueller-Hinton Agar (Merck) was then inoculated in different directions to ensure uniform, confluent growth. The plates were then allowed to dry for 3-5 minutes before applying the antibiotics. Certain antibiotics (Liofilchem), Amikacin (30µg), Aztreonam (30µg), Co-Amoxiclav (20/10µg), Cefoxitin (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Cefepime (30µg), Chloramphenicol (30µg), Imipenem (10µg), Meropenem (10µg), Gentamicin (10µg), and piperacillin/tazobactam (100/10µg). Plates were then incubated at 35 -37 ºC for 18-24 hours. The results of disc diffusion tests were then interpreted according to the M100-S33 of the CLSI [11].

Detection of Phenotypic Resistance

Screening and confirmatory tests for Extended Spectrum Beta-Lactamase (ESBL) were determined using the antibiotics discs Aztreonam (30µg), Amoxicillin-Clavulanate (20/10µg), Cefotaxime (30µg), Ceftazidime (30µg), and Cefepime (30µg) as mentioned in the M100 Ed 33 of the CLSI [11]. For ESBL screening, any isolate with zones of inhibition on Aztreonam < 27 mm, Cefotaxime < 27 mm, and Ceftazidime < 27 mm may indicate ESBL production. Any isolates that meet the given criteria will be tested using the double-disc synergistic test with Aztreonam (30 µg), Amoxicillin-Clavulanate (20/10 µg), and Cefepime (30 µg), with a clear distance of 1.5 cm between each disc. Presence of keyhole-like inhibition between Aztreonam and Amoxicillin-Clavulanate Acid or Cefepime and Amoxicillin-Clavulanate Acid indicates the presence of the ESBL [12,13]. AmpC production was screened using Cefoxitin (30µg), Imipenem (10µg), and cefotaxime (30µg) in the same manner as performing the in vitro-susceptibility testing [12]. A zone of inhibition of less than 18 mm or the presence of a flattened edge of the inhibitory zone around cefotaxime adjacent to an imipenem disc indicates its presence.

Quality Control

Reference strains such as ATCC 25922 (*E. coli*), ATCC 35812 (*E. coli),* and ATCC 700603 (*K. pneumoniae*) were used as controls to ensure the quality of the prepared culture media, the biochemical reactions, and the potency of the antibiotic discs. For ESBL production, ATCC 25922 was used as the negative control, and ATCC 700603 as the positive control.

Multiple Antibiotic Resistance Index (MARI)

MARI is commonly used to identify regions or areas with low or high antibiotic use. MARI is calculated as the ratio of the number of antibiotics that were resistant to the total number tested. An index of 0.2 indicates that the organism has originated from an environment where antibiotics are frequently used [14,15].

Data Analysis

All participants' data were stored electronically in a database (WHONET), which was downloaded from the WHO website. Frequency, mean, and prevalence of antimicrobial resistance were estimated as proportions of positive results across the entire sample.

RESULTS

Of the 174 PDLs housed in the City Jail, only 132 submitted their consent and specimens within the allotted time. These PDLs are between 19 and 59 years old. None of them had graduated from tertiary education and had a monthly income of less than Php 10,000.00 before their imprisonment. When grouped by age, the 35 to 39 age group was the most numerous, followed by the 30 to 34 and 40 to 44 age groups. Moreover, out of the 132 specimens provided, only 53 (40.15%) specimens were observed to contain coliforms after the modified Landman technique. Demographics of those who were positive for the Modified Landman Technique are shown in Table 1.

Table 1. Demographics of the Participants and Distribution of Specimens

Age Group	Total Specimens	No of Positive for Landman's Technique	Marital Status			
			Single		Married	
			n	Number of Positive Landman's Technique	n	Number of Positive Landman's Technique
20 – 24	17 (12.88%)	0 (0%)	17 (12.88%)	0 (0%)	0 (0%)	0 (0%)
25 – 29	16 (12.12%)	0 (0%)	15 (11.36%)	0 (0%)	1 (0.76%)	0 (0%)
30 – 34	26 (19.7%)	0 (0%)	20 (15.15%)	0 (0%)	6 (4.55%)	0 (0%)
35 – 39	28 (21.21%)	26 (19.70%)	17 (12.88)	23 (17.42%)	11 (8.33%)	3 (2.28%)
40 – 44	25 (18.94%)	23 (17.42%)	15 (11.36%)	21 (15.91%)	10 (7.58)	2 (1.52%)
45 – 49	12 (9.09%)	4 (3.03%)	4 (3.03%)	3 (2.28%)	8 (6.06%)	1 (0.76%)
50 – 54	5 (3.79%)	0 (0%)	2 (1.52%)	0 (0%)	4 (3.03%)	0 (0%)
55 – 59	3 (2.27%)	0 (0%)	1 (0.76%)	0 (0%)	2 (1.52%)	0 (0%)
Total	132 (100%)	53 (40.15%)	91	47 (35.6%)	41 (31.06%)	6 (4.55%)

Identified Bacterial Isolates

Of the 53 samples, 38 (71.70%) were identified as E. coli and 15 (28.30%) as K. pneumoniae based on colony morphology and a series of biochemical tests. ATCC 25922, ATCC 35218, and

ATCC 700603 were used as reference strains for comparison and assessment of biochemical reactions [13]. The presence of pink, dry, flat, and umbilicated colonies on MacConkey and greenish metallic sheen colonies on EMB confirms the identification of *E. coli. K. pneumoniae* growth, on the other hand, was distinguished by its mucoid growth, appearing as pink color in MacConkey and as mucoid purple colonies on EMB. The list of physicochemical reactions among the isolated organisms is shown in Table 2. This data indicates that the fecal carriage rate of *E. coli* and *K. pneumoniae* among PDLs after Landman's technique was 40.15%.

Table 2. Biochemical Characterization of Isolates

	Reference Strain			Isolate Representatives							
	ATCC 25922	ATCC 700603	ATCC 35218	005A	006A	010A	019A	018A	028A	018B	023B
Gram Reaction	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+
Gas	+	+	+	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+	+
Lysine deaminase	-	-	-	-	-	-	-	-	-	-	-
Methyl Red	+	-	+	+	+	+	+	-	-	-	-
Voges Proskauer	-	+	-	-	-	-	-	+	+	+	+
Motility	+	-	+	+	+	+	+	-	-	-	-
Indole	+	-	+	+	+	+	+	-	-	-	-
Citrate	-	+	-	-	-	-	-	+	+	+	+
Urease	-	+	-	-	-	-	-	+	+	+	+
EMB Agar	GMS	MP	GMS	GMS	GMS	GMS	GMS	MP	MP	MP	MP
MacConkey	DFDP	M	DPC	DPC	DPC	DPC	DPC	M	M	M	M
Identification	ECO	KPN	ECO	ECO	ECO	ECO	ECO	KPN	KPN	KPN	KPN

Legend:
+, positive — DPC, Dry, Pink Colonies — MP, Mucoid Purple
-, negative — M, Mucoid — GMS, Greenish Metallic Sheen
ECO, *E. coli* — KPN, *K. pneumoniae*

Antimicrobial Resistance and Phenotypic Resistance of Isolated Coliforms

Regarding antimicrobial resistance, the majority of the isolated coliforms were found to be resistant to the beta-lactam combination agents co-amoxiclav and piperacillin/tazobactam, followed by chloramphenicol and the tested cephalosporins. All isolated coliforms were susceptible to amikacin and tested carbapenems (Figure 1).

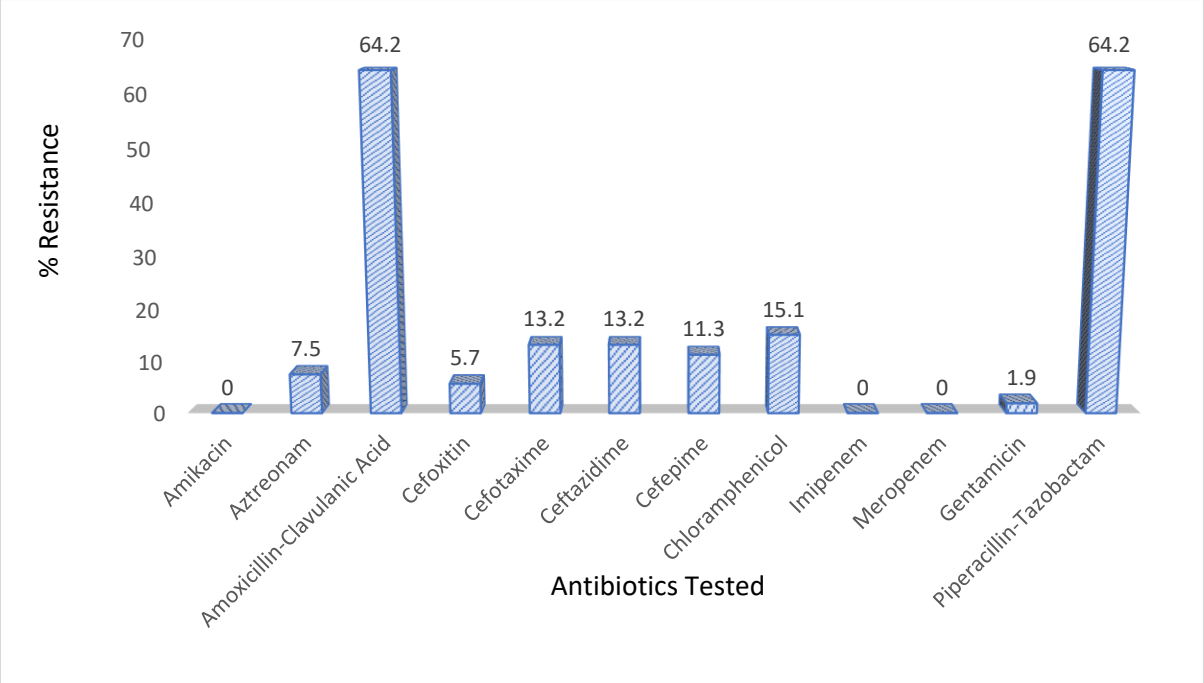

Figure 1. Antibiotic resistance of the isolated coliforms to selected antibiotics.

Additionally, four isolates were observed to produce AmpC, three *E. coli* and one *K. pneumoniae*. Three produce ESBL by disc-synergy assay: two *E. coli* and one *K. pneumoniae.* Whilst seven (13.21%) isolates were noted to be susceptible to all antibiotics tested, 18 (33.97%) isolates were noted to be resistant to only one antibiotic tested, 18 (33.97%) were resistant to two antibiotics, and 10 (18.87%) were considered as MDR as they were resistant to 3 or more antibiotics tested (Figure 2).

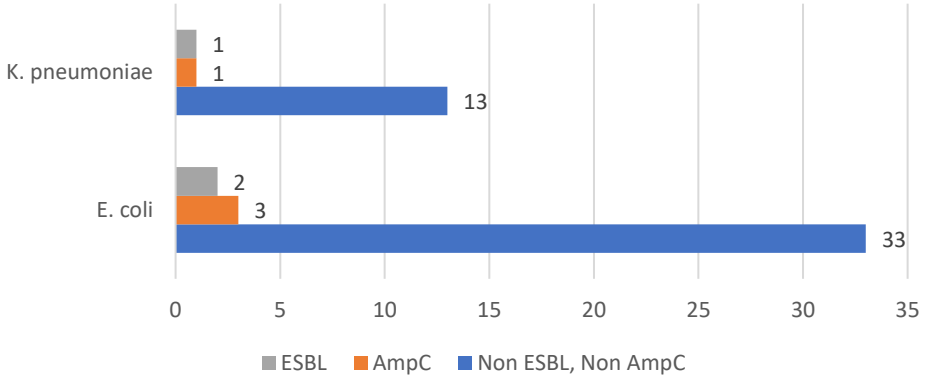

Figure 2. Frequency of ESBL and AmpC-producing Isolates from stool specimens of PDLs

Multiple Antibiotic Resistance Index

The MAR index is a useful tool for health risk assessment when the organism originates from a region with high or low antibiotic use. A MAR index>0.2 indicates a high-risk source of contamination. In this study, 10 isolates had a MAR index > 0.20, indicating they originated from

a high-risk contamination source; thus, the MDR prevalence rate was 7.58%. Overall. The mean MRI is 0.16 (range 0.0-0.58), indicating that the majority were from low-risk sources of contamination. A summary of the MAR index results is shown in Table 3.

Table 3. Biochemical Characterization of Isolates

MAR Index	Frequency Among Isolates
0	7
0.08	18
0.16	18
0.25	3
0.33	1
0.42	0
0.5	4
0.58	2
0.67	0
0.75	0
0.83	0
0.92	0
1.0	0

DISCUSSION

A challenge and a global concern, as the majority of organisms have developed resistance to most known antibiotics [16]. Aside from death and disability, it has a high cost on health care and gross domestic product losses. Antimicrobial resistance is somewhat more problematic in correctional facilities for several reasons, including limited knowledge of microbial resistance, which leads to antibiotic misuse [3].

As observed in this study, carriage of *E. coli* and *K. pneumoniae* is prominent, as these organisms belong to the family Enterobacteriaceae, which are natural inhabitants of the gastrointestinal tracts of healthy humans and animals [17]. They also represent a major reservoir of resistance genes that contribute to treatment failures, acquired through horizontal gene transfer [18]. This means these bacteria can both receive a resistant gene and donate it, as they can also pass it to other bacteria. Possible sources of this carriage include hand-to-hand contamination, occupational risk, travel, spread via inanimate objects, and poor hygiene.

As observed, although the majority of carriage is seen among those aged 30 to 44 and unmarried, the literature generally indicates no association between MDR carriage and age, as it can be acquired at any age, regardless of marital status.

Among the most common pathogens, *E. coli* and *K. pneumoniae* were found to be the most resistant to the most commonly used antibiotic classes in clinical practice [17,19]. As shown in this study, a small number of *E. coli* and *K. pneumoniae* strains harbor ESBL or AmpC genes. These characteristics make these organisms resistant to a broad range of ß-lactam drugs, including

third-generation cephalosporins, limiting treatment options. In addition to β-lactamases, these organisms can produce other enzymes, such as aminoglycoside-modifying enzymes and chloramphenicol aminotransferases. ESBL-producing organisms such as E. coli and K. pneumoniae can be found not only in animals and at infection sites but also in the feces of healthy individuals [18]. This could be one reason for the antimicrobial resistance observed in Fig. 1.

MARI is an important tool for analyzing antibiotic resistance and health risk factors. As shown in Table 3, 10 isolates had a MARI score>0.20. This also suggests the presence of multidrug-resistant genes originating in environments where antibiotics are misused [14]. In this current study, the MARI indicates a mean of 0.16. This indicates that the microorganisms did not originate in areas of high-risk contamination where several antibiotics are used. Although the majority of isolates were non-MDR, preventing their spread in the area or community remains imperative, as it leads to a large increase in the population at risk and, in turn, increases the number of infections caused by MDR isolates.

Antimicrobial resistance is somewhat more problematic in correctional facilities for several reasons, including limited knowledge of microbial resistance, which leads to antibiotic misuse [3]. Although many health programs are in place, those focused on preventing MDR pathogens remain scarce. The carriage of MDR bacteria, especially in the intestinal tract, poses a significant threat to public health, as these bacteria may translocate to other sites and cause opportunistic infections [1]. This simple carriage in healthy individuals can lead to opportunistic infections, which are also common in community-acquired infections and can spread. This study was simple yet showed that some correctional facilities, like those in this study, may pose a potential infectious risk in the near future. It is also recommended that molecular studies be conducted to identify epidemiological markers of these resistances and better understand them.

Given these considerations, the current study was designed to determine the prevalence of MDR carriage among people deprived of liberty, underscoring the need to assess environmental risks to prevent or minimize its spread. Finally, Correctional facility-based health programs, such as but not limited to infectious disease screening and harm-reduction efforts, are essential components in controlling MDR carriage and future infections.

CONCLUSION

This study demonstrated the carriage of MDR pathogens*, E. coli* and *K. pneumoniae*, among PDLs of a certain Correctional Facility in Negros Occidental, Philippines. The organisms exhibit high resistance to ß-lactam combination agents, such as amoxicillin-clavulanic acid and piperacillin/tazobactam. Although resistance was noted, these pathogens remained susceptible to amikacin and carbapenems.

DECLARATIONS

Ethics approval

Before the study commenced, letters of intent were sent to jail officers seeking their approval. Participants' consent was also obtained during specimen collection. Any information that could identify the participant was omitted in accordance with the country's Data Privacy Act of 2012. Biosafety and Ethics clearance was granted by Colegio San Agustin – Bacolod on April 25, 2023, under study protocol code 2023-02 – STU – Sitchon – RPA3 – Multidrug-Resistant Coliform Carriage.

Conflict of interest.

All authors declare that no conflict or competing interest exists.

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