Analysis of distribution and antibiotic resistance of pathogens isolated from the paediatric population in Shenmu Hospital from 2011–2015

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Abstract

Objective: This study aimed to investigate the epidemiology and changes in antibacterial susceptibility of children in Shenmu City, northern Shaanxi, and provide a basis for rational drug use.

Methods: The distribution and drug resistance pattern of pathogenic bacteria isolated from children were retrospectively analysed.

Results: A total of 573 strains of pathogens were cultivated. A total of 201 (35.07%) strains of Gram-positive cocci and 183 (31.93%) strains of Gram-negative cocci were detected. A total of 189 (32.98%) strains of fungi were detected. The resistance rate of Staphylococcus to penicillin was 100% and that to erythromycin was 90.69%. There were varying degrees of resistance to other drugs, but no single strain had vancomycin resistance. Gram-negative bacilli were generally resistant to ampicillin, but had low resistance to the combined preparation of enzyme inhibitors, quinolones, and aminoglycosides, and were highly sensitive to imipenem and meropenem.

Conclusion: Gram-negative bacilli are the main pathogens of bacterial infection in the paediatric ward. Strengthening clinical monitoring of bacterial distribution in paediatric clinical isolates and understanding changes in drug resistance are important for guiding the rational use of antibiotics. These measures could also prevent emergence and spreading of resistant strains.

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Introduction

Antimicrobial resistance is the ability of a microorganism (e.g., bacteria, viruses, and some parasites) to stop an antimicrobial (e.g., antibiotics, antivirals, and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist, and infections may become severe and can also spread to others. Antimicrobial resistance is an internationally documented threat to health. Bacterial infections that are resistant to antibiotics can limit the availability of effective treatment options, rendering some commonly encountered bacterial infections challenging to treat. Antibiotic-resistant infections are also twice as likely to be associated with greater morbidity and mortality and are associated with increased healthcare costs. Antibiotics are a keystone for treating bacterial infections and children receive these drugs more frequently than any other class of medication. However, inappropriate and unnecessary use of antibiotics in the past decades has increased the emergence of resistant bacterial strains. Children receive a lot of primary healthcare services, and as such, receive an excessively high number of antibiotics compared with middle-aged populations. Children are also major drivers of infection within communities and can contribute to the spread of bacteria from person to person. Nearly 80% of childhood cases of urinary tract infection in poorer countries are resistant to amoxicillin, and 60% are resistant to co-amoxiclav. More than a quarter of children are resistant to ciprofloxacin (Cipro), and 17% to nitrofurantoin. All of the above-mentioned factors require clinicians to be responsible for a correct diagnosis and treatment for affected children as soon as possible to avoid unnecessary disability and death. Despite this, little research has been published describing the microbial spectrum and antimicrobial resistance profile of bacteria from paediatric patients in China. Therefore, an understanding of the pathogens and antibiotic resistance associated with paediatric infections at our hospital and in nearby regions is necessary. In this study, we selected clinical data from patients who were admitted to the Paediatric Department during January 2011 to December 2015 for retrospective analysis of the distribution of bacterial species and changes in drug resistance. This study aimed to determine the profile and susceptibility patterns of bacterial pathogens associated with infections in paediatric patients during 5 years.

Materials and methods

Source of strains

Pathogens were isolated from specimens that were collected from all children who were admitted to Shenmu Hospital during January 2011 to December 2015. A total of 573 strains were isolated from 419 male patients and 154 female patients.

Ethics statement

Non-routine procedures were used, and only data regarding the results from culture and sensitivity of specimens were analysed retrospectively. Therefore, no consent was required from the patients’ caregivers.
Bacteriological identification

Isolation and culture of bacteria were carried out according to the “National Clinical Practice Guidelines”\(^9\). Bacteria were identified by the automatic bacteria analyser VITEK - 2 identification system and a manual method.

Drug sensitivity test

The fully automatic bacterial identification instrument VITEK - 2 MIC and the K-B (Kirby Bauer) Paper Slice diffusion method were used to test drug sensitivity. Antimicrobial impregnated absorbent paper discs were provided by Oxoid Company (UK) and Kangtai Company (China). Test methods and criteria were performed according to the latest standards of the American CLSI (Clinical & Laboratory Standards Institute) Standards.\(^10\) The choice of antimicrobials was as follows: penicillin (10 U), ampicillin (10 µg), piperacillin (100 µg), oxacillin (1 µg), ampicillin/subactam (10 µg/10 µg), amoxicillin (20 µg/10 µg), cefoperazone/subactam (75 µg/30 µg), cefazolin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), amikacin (30 µg), gentamicin (10 µg), a high concentration of gentamicin (120 µg), ciprofloxacin (5 µg), vancomycin (30 µg), erythromycin (15 µg), clindamycin (15 µg), meropenem (10 µg), imipenem (10 µg), sulfamethoxazole/trimethoprim (1.25 µg/23.75 µg), and aztreonam (30 µg). Mueller–Hinton medium was used for drug sensitivity tests. M-H agar, supplemented with 5% defibrinated sheep blood, was used for determining sensitivities of Streptococcus pneumoniae, Streptococcus pyogenes and other streptococcus spp. Haemophilus spp. were used to supplement the basic medium for culture of Haemophilus influenzae. The above-mentioned antimicrobial sensitivity paper discs and culture medium were provided by Oxoid and Kang Tai Companies.

Extended-spectrum beta-lactamase strains were confirmed by the double-disc method. The antimicrobials used were cefotaxime/clavulanic acid (30 µg/10 µg) and ceftazidime/clavulanic acid (30 µg/10 µg), both provided by Oxoid Company.

Quality control strains

Escherichia coli ATCC25922, Staphylococcus aureus ATCC29213, and Pseudomonas aeruginosa ATCC27853 were purchased from the Shaanxi Provincial Clinical Laboratory.

Statistical analysis

VITEK - 2 identification system software was used for analysis and processing of all of the original test data. IBM Statistics SPSS 21 Premium (Chicago, IL, USA) and GraphPad Prism 6 (La Jolla, CA, USA) statistical analysis software were used for the statistical analysis.

Results

Distribution of specimens and strains

During January 2011 to December 2015, 573 strains of pathogens were isolated from 73.2% (419) male patients and 26.8% (154) female patients. The Fisher–Irwin test showed a significant difference in the number of isolated strains between sexes (\(\chi^2 = 246.514, P < 0.0001\)). When we divided the time period into three quantum zones, spanning 20 months each, we found that the portion of male children steadily increased over time (Kruskal–Wallis \(\chi^2 = 25.20, P < 0.01\)). A total of 73 (12.8%) children were aged less than 28 days, 254 (44.4%) were between 29 days and 1 year, 124 (21.6%) were between 1 and 3 years, 67 (11.7%) were between 3 and 6 years, and 55 (9.6%) were older than 6 years. The average length of stay in hospital was 6 days and the mean ± standard deviation was 7.8 ± 4.0 days (Table 1).
The distribution of pathogens included 285 respiratory specimens, 68 blood specimens, 26 cerebrospinal fluid specimens, 24 urine specimens, and 11 faecal specimens (Table 2). A total of 201 (35.07%) strains of Gram-positive cocci and 183 (31.93%) strains of Gram-negative cocci were detected. The main five bacteria were Escherichia coli (9.94%), Klebsiella pneumoniae (7.32%), coagulase-negative staphylococci (9.94%), Staphylococcus aureus (2.79%), and Enterobacter cloacae (2.79%).

A total of 189 (32.98%) strains of fungi were detected. A significantly higher number of pathogens were isolated from respiratory secretion and blood compared with other types of secretions. The yearly (2011–2015) distribution ratio of pathogens is shown in Table 3. The distribution of pathogens according to variety of specimens is shown in Table 4.

### Drug-resistant rate

Among common Gram-positive bacteria, 16 strains of Staphylococcus aureus and 27 strains of Staphylococcus haemolyticus were isolated. The average resistance rate of Staphylococcus aureus and Staphylococcus haemolyticus against penicillin was 100%. Among macrolides, the resistance rate of erythromycin was >80%, but no vancomycin-resistant staphylococci were found (Table 5). Among Gram-negative bacilli, the Escherichia coli resistance rate for ampicillin and piperacillin was >80% while its resistance rate against first, second, third, and fourth generation cephalosporins was nearly 70%. The Pseudomonas aeruginosa resistance rate to ampicillin was 100%.

### Table 1. Demographic representation of the isolated pathogens.

| Specimens       | Jan 2011 to Aug 2012 | Sept 2012 to April 2014 | May 2014 to Dec 2015 | Total |
|-----------------|----------------------|-------------------------|----------------------|-------|
| Children, n (%) | 82 (14.3)            | 194 (33.8)              | 297 (51.8)           | 573 (100) |
| Males, n (%)    | 58 (70.7)            | 140 (72.3)              | 221 (74.5)           | 419 (73.2)* |
| Hospital stay (days) | Median (interquartile range) | 6 (7–9)               | 5 (7–8)              | 5 (7–8)            | 6 (4–8) |
|                 | Mean ± standard deviation | (7.8 ± 4.0)           | (7.2 ± 3.5)          | (2.5 ± 3.2)        | (6.8 ± 3.8) |

* = Kruskal–Wallis $\chi^2 = 25.20$, $P < 0.01$.

n = number.

### Table 2. Composition ratio of pathogen specimens.

| Specimens           | Strains, n | %  |
|---------------------|------------|----|
| Respiratory         | 285        | 49.80* |
| Blood               | 68         | 11.90 |
| Cerebrospinal fluid | 26         | 4.50 |
| Urine               | 24         | 4.20 |
| Faeces              | 11         | 1.90 |
| Wound secretions    | 14         | 2.40 |
| Eye exudates        | 17         | 2.97 |
| Other               | 128        | 22.20 |
| Total               | 573        | 100.00 |

*Fisher–Irwin test: $\chi^2 = 65.980$, $P < 0.0001$. 
### Table 3. Yearly distribution of pathogens from 2011–2015.

| Pathogens                     | 2011 Strains, n | 2011 % | 2012 Strains, n | 2012 % | 2013 Strains, n | 2013 % | 2014 Strains, n | 2014 % | 2015 Strains, n | 2015 % |
|-------------------------------|-----------------|--------|-----------------|--------|-----------------|--------|-----------------|--------|-----------------|--------|
| *Escherichia coli*            | 6 (3.77)        | 9 (7.75)| 11 (9.09)       | 16 (18.82)| 15 (16.30)      |
| *Klebsiella pneumoniae*       | 2 (1.25)        | 8 (6.89)| 15 (12.39)      | 7 (8.23) | 10 (10.86)      |
| *Acinetobacter* spp.         | 1 (0.62)        | 0 (0.00)| 2 (1.62)        | 5 (5.88) | 4 (4.34)        |
| *Pseudomonas aeruginosa*      | 3 (1.88)        | 2 (1.72)| 4 (3.30)        | 0 (0.00) | 3 (3.26)        |
| *Enterobacter cloacae*        | 0 (0.00)        | 2 (1.72)| 8 (6.61)        | 3 (3.52) | 3 (3.26)        |
| *Staphylococcus aureus*       | 12 (0.50)       | 1 (0.06)| 4 (3.30)        | 4 (4.70) | 3 (3.26)        |
| *Streptococcus pyogenes*      | 13 (8.17)       | 3 (2.58)| 34 (28.09)      | 12 (14.11)| 9 (9.78)        |
| *Enterococcus*                | 5 (3.14)        | 1 (0.86)| 4 (3.30)        | 4 (4.70) | 3 (3.26)        |
| *Streptococcus pneumonia*     | 3 (1.88)        | 3 (2.58)| 7 (5.78)        | 4 (4.70) | 10 (10.86)      |
| *Streptococcus*               | 1 (0.00)        | 1 (0.06)| 4 (3.30)        | 4 (4.70) | 3 (3.26)        |
| *Candida albicans*            | 66 (41.50)      | 19 (16.37)| 5 (4.13)       | 7 (24.70) | 8 (15.22)       |
| Others, including CoNS and non-fermenting bacteria | 13 (8.17) | 22 (18.96)| 24 (19.38)      | 21 (24.70)| 14 (15.22)     |
| Total                         | 159 (100)       | 116 (100)| 121 (100)       | 85 (100) | 92 (100)        |

CoNS = coagulase-negative staphylococci.

### Table 4. Distribution of various common pathogens in specimens.

| Pathogens                              | Total n | Respiratory tract n (% | Urine n (% | Blood n (% | CSF n (% | Stool n (% | Others n (% |
|----------------------------------------|---------|------------------------|------------|------------|---------|-----------|-------------|
| *Escherichia coli*                     | 57      | 29 (50.8)              | 8 (14.0)   | 10 (17.5)  | 2 (3.5) | 3 (5.2)  | 5 (8.7)     |
| *Klebsiella pneumoniae*                | 42      | 32 (76.2)              | 1 (2.3)    | 4 (9.5)    | –       | 3 (7.1)  | 2 (4.7)     |
| *Acinetobacter* spp.                   | 12      | 10 (83.3)              | –          | 1 (8.3)    | –       | –         | 1 (8.3)     |
| *Pseudomonas aeruginosa*               | 12      | 6 (50)                 | –          | 2 (16.6)   | –       | –         | 4 (33.3)    |
| *Enterobacter cloacae*                 | 16      | 14 (87.5)              | –          | –          | –       | 2 (12.5) | –           |
| *Staphylococcus aureus*                | 16      | 5 (31.2)               | –          | 3 (18.7)   | –       | –         | 8 (50)      |
| CoNS                                   | 30      | 8 (26.6)               | 2 (6.6)    | 9 (30)     | 7 (23.3) | –         | 4 (13.3)    |
| *Streptococcus haemolyticus*           | 27      | 8 (29.5)               | 5 (18.5)   | 8 (29.6)   | –       | –         | 6 (22.2)    |
| *Streptococcus pneumoniae*             | 9       | 4 (44.4)               | –          | 1 (11.1)   | 3 (33.3) | –         | 1 (11.1)    |
| *Streptococcus pyogenes*               | 11      | 10 (90.9)              | –          | –          | –       | –         | 1 (9.1)     |
| Other streptococci                     | 71      | 43 (60.5)              | –          | 19 (26.7)  | –       | –         | 9 (12.6)    |
| Enteroococcus                          | 17      | 4 (23.5)               | 2 (11.7)   | 5 (29.4)   | 4 (23.5) | –         | 2 (11.7)    |
| Fungus (yeast-like fungi + Candida albicans) | 189      | 73 (38.6)              | –          | 5 (2.6)    | 1 (0.5) | 110 (58.2)     |
| Other non-fermenting bacteria          | 24      | 17 (70.8)              | 3 (12.5)   | 2 (8.3)    | –       | 2 (8.3)  | –           |
| Others                                 | 40      | 22 (55)                | 3 (7.5)    | 4 (10)     | 5 (12.5) | –         | 6 (15)      |
| Total                                  | 573     | 285                    | 24 (8.6)   | 68 (26)    | 26 (11) | 11 (6)   | 159         |

Lower 95% CI of the mean

Upper 95% CI of the mean

*Pathogens from respiratory secretion and blood showed a significant probability of isolation compared with pathogens extracted from other secretions.

CSF = cerebrospinal fluid, CoNS = coagulase-negative staphylococci, CI = confidence interval.
Additionally, except for the low resistance rate of *Pseudomonas aeruginosa* against the third generation cephalosporin ceftazidime and fourth generation cephalosporin cefepime, its resistance rate was higher than 90% against all other cephalosporins. The resistance rate of *Pseudomonas aeruginosa* to levofloxacin, gentamicin, amikacin, imipenem, and meropenem was <10%, while its resistance to piperacillin/tazobactam was 30%. *Escherichia coli* was not resistant to imipenem and meropenem (Table 6).

**Discussion**

Shenmu City coal mining has resulted in serious smoky air pollution, rendering the air quality poor. This has caused an enormous increase in infections of the respiratory tract in hospitalized children.11 There is an upward trend of yearly detection of pathogens, but the overall number of pathogens that are detected annually has decreased.12

Our study showed that the main pathogens of paediatric infection were Gram-positive bacteria, accounting for 35.07%, while Gram-negative bacteria accounted for 31.93% and fungi accounted for 32.98%. These numbers of infected pathogens are consistent with most domestic reports.13

The predominance of Gram-negative bacteria in hospital-acquired infections is probably due to the fact that patients are treated with antimicrobial agents before admission. Additionally, many individual clinics do not perform allergy tests, and thus use macrolides of which the main antimicrobial spectrum is of Gram-positive bacteria.14

This results in a low detection rate of Gram-positive bacteria, while Gram-negative bacteria have become predominant in examinations of pathogens in hospitalized

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**Table 5. Resistance of Gram-positive bacteria to commonly used antimicrobial agents.**

| Antibiotics | Staphylococcus aureus (NT = 16) | Staphylococcus haemolyticus (NT = 27) | Streptococcus pyogenes (NT = 11) | Streptococcus pneumoniae (NT = 9) |
|-------------|---------------------------------|--------------------------------------|---------------------------------|---------------------------------|
|             | NA | R/R (%) | NA | R/R (%) | NA | R/R (%) | NA | R/R (%) |
| Penicillin  | 15 | 100     | 25 | 100     | 9  | 77.78   | 6  | 33.33   |
| Erythromycin| 16 | 81.25   | 27 | 96.30   | 11 | 100     | 8  | 100     |
| Clindamycin | 16 | 81.25   | 27 | 88.89   | 8  | 87.5    | 6  | 83.33   |
| Rifampicin  | 16 | 56.25   | 27 | 77.78   | 9  | 11.11   | 5  | 0.00    |
| Vancomycin  | 16 | 100     | 27 | 100     | 10 | 0.00    | 7  | 0.00    |
| Tetracycline| 15 | 80.00   | 25 | 80.00   | –  | –       | –  | –       |
| Oxacillin   | 15 | 33.33   | 25 | 100     | –  | –       | –  | –       |
| Cefoxitin   | 11 | 27.27   | 21 | 100     | –  | –       | –  | –       |
| Linezolid   | 14 | 0.00    | 18 | 100     | –  | –       | –  | –       |
| Cotrimoxazole| 12 | 50.00  | 25 | 20.00   | 4  | 50.00   | –  | –       |
| Nitrofurantoin| 16 | 0.00   | 25 | 0.00    | –  | –       | –  | –       |
| Levofloxacin| 16 | 25.00   | 27 | 74.07   | 7  | 14.29   | 5  | 0.00    |
| Moxifloxacin| 15 | 6.67    | 25 | 56.00   | –  | –       | –  | –       |
| Gentamicin  | 16 | 43.75   | 27 | 85.19   | –  | –       | –  | –       |
| Piperacillin/tazobactam | 10 | 20.00 | 20 | 100     | –  | –       | –  | –       |
| Cefuroxime  | –  | –       | –  | –       | 9  | 33.33   | 7  | 14.29   |

_N_T = Total number of specimens isolated
NA = Number of specimens on which Antibiotic was applied
R/R = Resistance rate = Number of resistant specimens / NA *100
patients. Our study showed that detection of coagulase-negative staphylococci infection was highest among all hospital infections and its detection rate was much higher than that of other bacteria. This finding is in accordance with previous studies. The pathogenicity of coagulase-negative staphylococci is less than that of Staphylococcus aureus. However, in the case of immunocompromised patients, infection may still occur, especially an increasingly growing resistance of these strains (e.g., methicillin-resistant coagulase-negative staphylococci), suggesting that coagulase-negative staphylococci is an important nosocomial infection pathogen. Monitoring in children shows that 33.33% of Streptococcus pneumoniae is resistant to penicillin, while 100% of Streptococcus pneumoniae is sensitive to vancomycin and rifampicin, which has important implications for treating penicillin-resistant Streptococcus pneumoniae. Our 5-year study of paediatric infections also showed that the main five Gram-negative bacteria were Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Acinetobacter baumannii, and Pseudomonas aeruginosa. This finding is consistent with the international literature. Among Gram-positive bacteria, Staphylococcus haemolyticus is the most common, followed by Staphylococcus aureus. Fungal infection also affects a large proportion of children, with the main cause being Candida albicans.

Common Gram-negative bacilli show general resistance against ampicillin and

Table 6. Resistance of Gram-negative bacteria to commonly used antimicrobial agents.

| Antibiotics used per specimen | Escherichia coli (NT = 57) | Klebsiella pneumoniae (NT = 42) | Acinetobacter spp. (NT = 12) | Enterobacter cloacae (NT = 16) | Pseudomonas aeruginosa (NT = 12) |
|------------------------------|---------------------------|-------------------------------|----------------------------|-------------------------------|----------------------------------|
| Levofoxacin                  | 49                        | 37 5.41                        | 12 0.00                    | 14 0.00                      | 11 0.00                          |
| Gentamicin                   | 54                        | 42 35.71                       | 12 0.00                    | 14 14.29                     | 10 0.00                          |
| Imipenem                     | 51                        | 38 0.00                        | 12 0.00                    | 14 0.00                      | 11 0.00                          |
| Meropenem                    | 51                        | 42 7.14                        | — —                       | 14 0.00                      | 11 0.00                          |
| Ampicillin                   | 48                        | 38 97.37                       | 12 100                     | 4 100                        | 11 100                           |
| Cefazolin                    | 52                        | 42 61.90                       | 12 100                     | 14 100                       | 11 90.91                         |
| Cefuroxime                   | 48                        | 38 57.89                       | 12 91.67                   | 14 71.43                     | 11 90.91                         |
| Ceftazidime                  | 49                        | 38 52.63                       | 12 8.33                    | 14 35.71                     | 10 0.00                          |
| Cefotaxime                   | 36                        | 26 38.46                       | 10 100                     | 10 10.00                     | 5 100                            |
| Ceftriaxone                  | 53                        | 42 57.14                       | 12 91.67                   | 14 35.71                     | 10 90.00                         |
| Cefepime                     | 52                        | 42 54.76                       | 12 0.00                    | 14 35.71                     | 11 0.00                          |
| Amikacin                     | 48                        | 38 5.26                        | — —                       | 14 7.14                      | 11 0.00                          |
| Piperacillin/tazobactam      | 54                        | 38 7.14                        | 12 8.33                    | 14 14.29                     | 10 30.00                         |
| Aztreonam                    | 53                        | 42 57.14                       | 12 91.67                   | 14 35.71                     | 5 0.00                           |
| Nitrofurantoin              | 50                        | 37 75.68                       | 12 100                     | 14 85.71                     | 11 90.91                         |
| Cefotetan                    | 48                        | 38 26.32                       | 12 8.33                    | 14 100                       | 11 90.91                         |
| Piperacillin                 | 50                        | 38 97.37                       | 12 8.33                    | 14 42.86                     | 11 27.27                         |
| Cefoperazone/sulbactam       | 50                        | 38 11.23                       | — —                       | 8 100                        | —                               |

NT = Total number of specimens isolated
NA = Number of specimens on which Antibiotic was applied
R/R = Resistance rate = Number of resistant specimens / NA *100
piperacillin, while they also show cross-resistance against aminoglycosides and fluoroquinolones. Because these two drugs show adverse reactions in children, their use in children is already limited. Our study showed that sensitivity of *Escherichia coli* for amikacin, cefoperazone/sulbactam, and nitrofurantoin was >90.0%. Sensitivity of *Klebsiella pneumoniae* to fluoroquinolones and amikacin was >92.0%. Pseudomonas aeruginosa was 100% sensitive to ceftazidime, cefepime, amikacin, fluoroquinolone, and aztreonam. *Enterobacter cloacae* was also highly sensitive to fluoroquinolones, cefoperazone/sulbactam, cefotaxime, and amikacin. The resistance of Gram-negative bacilli to imipenem and meropenem is lower in Shenmu City than that of other regions in China, which may be related to the strict control of antimicrobial drug use in our hospital.

Overall, the situation in Shenmu City regarding resistant paediatric pathogens is serious. There is an urgent need to strengthen monitoring of the distribution of pathogens and bacterial resistance. Strict implementation of antimicrobial management, and good use of consultation and an approval system resulting in the rational use of antibiotics are also required. At the same time, food and drug supervision agencies and health administrative departments should strengthen supervision of antimicrobial drug purchase and strictly supervise prescription drugs, especially in private clinics and drug retail outlets. Publicity on antibacterial drug health education should be increased through print and social media to avoid pre-hospital, non-prescription, irrational use of antibiotics. In short, prevention and control of bacterial drug resistance in standard paediatric treatment is an important task.

**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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