**Nioella aestuarii** sp. nov., of the family *Rhodobacteraceae*, isolated from tidal flat

In-Tae Cha,† Eui-Sang Cho,‡ Jung-Min Park,† Jung-Yong Yeh† and Myung-Ji Seo†,‡,*

**Abstract**

A bacterium, designated strain MME-018\textsuperscript{T}, was isolated from a tidal flat of the Muui-do in the Republic of Korea and identified within the family *Rhodobacteraceae*. The 16\textsubscript{S} rRNA gene sequence of the isolate showed the highest similarity to that of *Nioella sediminis* JS7-11\textsuperscript{T} (98.9\%), followed by *Nioella nitratireducens* SSW136\textsuperscript{T} (97.1\%). In phylogenetic analyses, these taxa formed a clade at neighbour-joining, maximum-likelihood, and maximum-parsimony algorithms, in which it was separated from other genus belonging to the family *Rhodobacteraceae*. Ubiquinone-10 (Q-10) was the major respiratory quinone. Major polar lipids included phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, two unidentified phospholipids, and an unidentified lipid. Major fatty acids were summed feature 8 (C\textsubscript{18:1}ω7c and/or C\textsubscript{18:1}ω6c) C\textsubscript{16:0}, cyclo C\textsubscript{19:1}ω8c, and 11-methyl C\textsubscript{18:1}ω7c. Genomic DNA G+C content was 61 mol%. Cells were Gram-stain negative, non-motile, aerobic, and rod-shaped. This strain grew in 1–4\% (w/v) NaCl, at 4–40 °C and pH 6.0–8.0, with optimal growth in 2\% (w/v) NaCl, at 25–30 °C and pH 7.0. DNA–DNA hybridization values between strain MME-018\textsuperscript{T} and *Nioella sediminis* KCTC 42144\textsuperscript{T} and *Nioella nitratireducens* KCTC 32417\textsuperscript{T} were 17±3 and 13±1\%, respectively. On the basis of polyphasic taxonomic analysis, strain MME-018\textsuperscript{T} is proposed to represent a novel species of the genus *Nioella*, for which the name *Nioella aestuarii* sp. nov. The type strain of *Nioella aestuarii* is MME-018\textsuperscript{T} (=KCCM 43135\textsuperscript{T}=JCM 30752\textsuperscript{T}).

The *Roseobacter* clade within the family *Rhodobacteraceae* is known to originate from marine ecosystems. Most members of this family have been isolated from saline or hypersaline environments [1, 2]. Among them, some species are moderately halophilic or halotolerant, requiring sodium ions for growth [3, 4]. The genus *Nioella* that belongs to the *Roseobacter* clade was first proposed by Rajasabapathy et al. [5] with the description of one species, *Nioella nitratireducens*. Recently, *Nioella sediminis* has been isolated from the surface sediment of the Jinulong River and emended to the genus *Nioella* [6]. Features of this genus are known to be Gram-stain negative, aerobic, non-spor forming, and rod shaped bacterium that requires sodium ions for growth [5, 6]. From a tidal flat of the Muui-do in Republic of Korea, we isolated a bacterium that has similar characteristics with the members of the genus *Nioella* requiring sodium ions for growth. In this study, we investigated the taxonomic position of this strain, designated MME-018\textsuperscript{T}, using a polyphasic approach.

Sample was collected from a tidal flat of the Muui-do near Incheon in the Republic of Korea, (37°24′ 17″ N 126°24′ 49″ E) in September of 2014. The sample was serially diluted with 3\% (w/v) NaCl. An aliquot (200 µl) of the diluted solution was spread onto the natural seawater agarose medium that contained 0.5 g yeast extract, 1.5\% (w/v) agarose, 1 ml of trace element solution SL-6 (DSM medium no. 27), and 1 ml of vitamin solution [7] per 1 l of natural seawater. The plates were incubated at 30 °C for 2 weeks. Colonies were streaked onto the same medium at least three times to obtain single and pure colonies. The pure colony designated MME-018\textsuperscript{T} was transferred onto marine agar 2216 (BD; MA) and routinely cultivated on MA at 30 °C.

To amplify the 16\textsubscript{S} rRNA gene sequence of strain MME-018\textsuperscript{T}, its genomic DNA was extracted by using G-spin Total DNA Extraction Kit (IntRON Biotechnology) and QuickGene DNA tissue kit S (Kurabo) according to the manufacturer’s instructions. The extracted genomic DNA was PCR amplified using the universal primers 27F and 1492R [8]. The PCR products were then sequenced by CosmogeneTech.

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**Keywords:** *Rhodobacteraceae*; *Nioella aestuarii*; tidal flat; polyphasic taxonomy.

**Abbreviations:** CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid.

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16\textsubscript{S} rRNA gene sequences of strain MME-018\textsuperscript{T} is KP410676. Two supplementary figures are available with the online version of this article.
Co. Ltd. using the bacterial primers 27F, 337F, 518R, 785F and 1492R [9]. These sequences were assembled using the SeqMan software (DNASTar) according to the methods of Roh et al. [10]. To find closely related taxa, the 16S rRNA gene sequence of strain MME-018T was aligned by using SILVA (http://www.arb-silva.de/aligner) [11]. To analyze a phylogenetic tree, based on the 16S rRNA gene sequence, the nearest phylogenetic neighbours of the isolated strain were identified using the BLAST (http://www.ncbi.nlm.nih.gov/blast/) and the EzBioCloud database (http://www.ezbiocloud.net/) [12]. The sequences of related taxa were acquired from the same websites. The Kimura two-parameter model [13] was used to calculate evolutionary distances between the isolated strain and reference taxa. The phylogenetic tree of the 16S rRNA gene sequences of the strain and related taxa was constructed using MEGA 6 program [14] and three algorithms, neighbour-joining [15], maximum-likelihood [16], and maximum-parsimony [17]. In this study, the bootstrap values were calculated based on 1000 random replications. The 16S rRNA gene sequence of strain MME-018T was determined to be 1392 bp in length. Levels of 16S rRNA gene sequence similarity between the isolated strain and reference taxa were identified using the SILVA (http://www.arb-silva.de/aligner) [11]. The sequences of related taxa were obtained from the following databases: GenBank (http://www.ncbi.nlm.nih. gov/blast/) and the EzBioCloud database (http://www.ezbiocloud.net/) [12]. The sequences of related taxa were acquired from the same websites. The Kimura two-parameter model [13] was used to calculate evolutionary distances between the isolated strain and reference taxa. The phylogenetic tree of the 16S rRNA gene sequences of the strain and related taxa was constructed using MEGA 6 program [14] and three algorithms, neighbour-joining [15], maximum-likelihood [16], and maximum-parsimony [17]. In this study, the bootstrap values were calculated based on 1000 random replications. The 16S rRNA gene sequence of strain MME-018T was determined to be 1392 bp in length. Levels of 16S rRNA gene sequence similarity between the isolated strain and reference taxa were identified using the SILVA (http://www.arb-silva.de/aligner) [11]. The sequences of related taxa were obtained from the following databases: GenBank (http://www.ncbi.nlm.nih.gov/blast/) and the EzBioCloud database (http://www.ezbiocloud.net/) [12]. The sequences of related taxa were acquired from the same websites.

The respiratory quinone of strain MME-018T was ubiquinone-10 (Q-10), which was related to the family Rhodobacteraceae. In this study, the major common polar lipids of the isolated strain MME-018T and the related taxa Niella sediminis KCTC 42144T and Niella nitratireducens KCTC 32417T were phosphatidylglycerol (PG), phosphatidycholine (PC), phosphatidylethanolamine (PE), two unidentified phospholipids, and an unidentified lipid (Fig. S1, available in the online version of this article). Although two unidentified aminolipids had been slightly detected from Niella nitratireducens SSW136T [5], they were not detected along with strain MME-018T in the current study. The predominant (>5 %) cellular fatty acids of strain MME-018T were summed feature 8 (C18:1ω7c and/or C18:1ω6c), C16:0, cyclo C19:1ω8c, and 11-methyl C18:1ω7c. The cellular fatty acid composition was similar to that of the related taxa Niella sediminis KCTC 42144T and Niella nitratireducens KCTC 32417T (Table 1). The genomic DNA G+C content of the isolated strain was 61.5 mol%.

To investigate the morphological, physiological and phenotypic properties, the strains MME-018T, Niella sediminis KCTC 42144T and Niella nitratireducens KCTC 32417T were routinely cultivated on MA at 30 °C, but conditions were changed slightly to fit the experimental needs. Cell motility was determined as described by Tittsler and Sandholzer [24] using semi-solid agar medium (containing 0.3 % agarose). To observe the flagella and the cell morphology, microscopic test was performed using phase contrast microscopy (Primo Star; Carl Zeiss) and transmission electron microscopy (JEM-1010; JEOL). Gram staining was carried out using Gram staining kit (Bioworld), according to the manufacturer’s instructions. Catalase- and oxidase-tests were performed according to the procedures previously described by Kovacs [25] and Smibert and Krieg [26]. Growth at temperature ranges of 0, 5, 10, 15, 20, 25, 30, 37, 40, 45, and 50 °C was assessed on MA for 4 weeks. The NaCl concentration for growth of the strain MME-018T was investigated on artificial seawater (ASW) agar medium [27] of which the range of NaCl concentrations was 0–10 % (w/v) with 1 % increments. The pH for growth was investigated at pH 5.0–11.0 with 1.0 pH unit increment by using 10 mM MES (pH 5.0 and 6.0), 10 mM bis-Tris propane (pH 7.0–9.0), or 10 mM CAPS (pH 10.0 and 11.0). Anaerobic growth was determined by incubation on MA with 10 mM nitrate, 10 mM FeCl3, and 10 mM thiosulfate as electron acceptors in a GasPak EZ anaerobic gas-generating pouch system with indicator (BD) at 30 °C for 4 weeks. Starch and casein hydrolyses were tested as per the methods provided by Benson [28], while hydrolysis of Tween 20, 40, and 80 was determined as described by Gonzáles et al. [29]. Gelatin and l-tyrosine hydrolysis were evaluated according to the methods of Smibert and Krieg [26]. Substrate utilization was assessed using the modified MA according to methods previously described by Park et al. [30] supplemented with followings at 1 % (w/v): acetate, L-arabinose, benzoate, cellobiose, citrate, formate, D-fructose, D-galactose, D-glucose, D-glutamate, glyceral, inositol, lactose, malate, maltose, D-
mannitol, D-mannose, melibiose, L-ornithine, pyruvate, raffinose, L-rhamnose, D-ribose, D-sorbitol, succinate, sucrose, trehalose and D-xylene. Acid production from carbohydrates were detected as described by Park et al. [30] and Leifson [31] supplemented with 1% (w/v) cellobiose, D-galactose, D-mannose, melibiose, L-rhamnose, and trehalose. The enzyme activities of strain MME-018T were determined using the API 20NE and API ZYM strips.

After 3 days on MA at 30°C, colonies of strain MME-018T were circular, smooth, convex, white in colour, and 0.5-1.0 mm in diameter. These isolates were Gram-negative and rod-shaped bacteria 0.5-0.8 μm wide and 1.6-2.0 μm long (Fig. S2). The flagella were not observed. Catalase- and oxidase-reactions were positive. Strain MME-018T grew in the presence of 1-4% (w/v) NaCl at 4-40°C and pH 6.0-8.0, and grew optimally in the presence of 2% (w/v) NaCl, at 25-30°C and pH 7.0. Strain MME-018T required sodium ions for growth, which is consistent with Nioella nitratireducens KCTC 32417T [5]. Anaerobic growth was not observed. Hydrolysis of starch, casein, L-tyrosine, gelatin, and Tween 20, 40, and 80 was not detected in this study. Nitrate was aerobically reduced to nitrite, but reduction of nitrite to nitrogen was not observed. In the API 20NE, arginine dihydrolase and β-galactosidase were positive, whereas indole production, glucose fermentation, and activities of urease, β-glucosidase (aesculin), and protease (gelatin) were negative. In API ZYM tests, activities of alkaline phosphatase, esterase (C8), -galactosidase, and β-glucosidase, and β-galactosidase were positive. Other characteristics are provided in Table 2 along with the species description.

To investigate the requirement of cationic ions for the growth of strain MME-018T, each of 0.2% (w/v) CaCl2, 2H2O, 0.05% (w/v) KCl and 0.6% (w/v) MgCl2.6H2O was added to modified ASW containing 5 g yeast extract, 1 g peptone, 25 g NaCl and 0.01 g FePO4 per litre of distilled water as described by Seo et al. [32], followed by the
incubation at 30 °C for 7 days. The strain MME-018\textsuperscript{T}
required Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ions for growth. 
*As indicated by Montero-Calasanz et al. [37] summed features are
groups of two or three fatty acids that are treated together for the
purpose of evaluation in the MIDI system and include both peaks with
discrete ECLs as well as those where the ECLs are not reported sepa-
rate. Summed feature 3 comprises C\textsubscript{16:1}, \omega\textsubscript{7c} and/or C\textsubscript{16:1}, \omega\textsubscript{6c};
summed feature 8 comprises C\textsubscript{18:1}, \omega\textsubscript{7c} and/or C\textsubscript{18:1}, \omega\textsubscript{6c}.

To test antibiotic susceptibility, strain MME-018\textsuperscript{T} was inco-
culated onto MA plates containing discs with the following antibiotics
(μg ml\textsuperscript{-1} unless indicated): ampicillin (10), car-
benicillin (100), cephalothin (30), ciprofloxacin (10), eryth-
romycin (25), gentamicin (30), kanamycin (30), lincomycin
(15), neomycin (30), norfloxacin (20), novobiocin (10), peni-
cillin G (20 UI), polymyxin B (100 UI), streptomycin (50)
and tetracycline (30). Strain MME-018\textsuperscript{T} was resistant to
gentamicin, but susceptible to other antibiotics.

DNA–DNA hybridization (DDH) was conducted fluoro-
metrically by the membrane filter method using a DIG High
Prime DNA Labelling and Detection Starter kit II (Roche
Applied Science) to identify the genetic relationship of
strain MME-018\textsuperscript{T} and reference strains, *Nioella sediminis*
KCTC 42144\textsuperscript{T} and *Nioella nitratireducens* KCTC 32417\textsuperscript{T}
[33, 34]. The DDH values of strain MME-018\textsuperscript{T} with *Nioella sediminis*
KCTC 42144\textsuperscript{T} and *Nioella nitratireducens* KCTC 32417\textsuperscript{T}
were 17±3 and 13±1 %, respectively. According to
current prokaryotic systematics defining DDH values of
<70% as indicative of a distinct species [35, 36], the deter-
mined DDH values indicated that the strain MME-018\textsuperscript{T}
were distinguished from *Nioella sediminis* KCTC 42144\textsuperscript{T}
and *Nioella nitratireducens* KCTC 32417\textsuperscript{T}.

The results of phenotypic, genotypic, and chemotaxonomic
analyses revealed that the strain MME-018\textsuperscript{T} shares common
taxonomic properties with the members of the genus

### Table 1. Cellular fatty acid compositions (%) of strain MME-018\textsuperscript{T} and related taxa

<table>
<thead>
<tr>
<th>Fatty acid (%)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{10:0} 3-OH</td>
<td>1.8</td>
<td>2.2</td>
<td>2.4</td>
</tr>
<tr>
<td>C\textsubscript{12:0} 3-OH</td>
<td>1.9</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>C\textsubscript{14:0}</td>
<td>10.4</td>
<td>10.5</td>
<td>14.4</td>
</tr>
<tr>
<td>C\textsubscript{16:0} 2-OH</td>
<td>4.9</td>
<td>4.3</td>
<td>4.5</td>
</tr>
<tr>
<td>C\textsubscript{18:0}</td>
<td>2.4</td>
<td>4.2</td>
<td>2.3</td>
</tr>
<tr>
<td>11-methyl C\textsubscript{18:1}, \omega\textsubscript{7c}</td>
<td>8.9</td>
<td>9.2</td>
<td>4.9</td>
</tr>
<tr>
<td>C\textsubscript{18:1} 2-OH</td>
<td>TR</td>
<td>TR</td>
<td>1.3</td>
</tr>
<tr>
<td>Cycle C\textsubscript{19:0}, \omega\textsubscript{8c}</td>
<td>10.1</td>
<td>5.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Summed features*</td>
<td>3</td>
<td>1.5</td>
<td>TR</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>54.7</td>
<td>58.0</td>
</tr>
</tbody>
</table>

*Data from this study.

### Table 2. Differential phenotypic characteristics of strain MME-018\textsuperscript{T} and related taxa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at/in</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>40 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1 % (w/v) NaCl</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>11 % (w/v) NaCl</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Optimal growth pH</td>
<td>7.0</td>
<td>7.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Hydrolysis of*</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Geratin</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>-Tyrrosine</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activity of*</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Arginine dihydrolyase</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Utilization of*</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Glycerol</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

*Requirement of cations:
| Ca\textsuperscript{2+} | + | + | − |
| K\textsuperscript{+} | − | + | + |
| Mg\textsuperscript{2+} | + | − | − |

*Data from this study.*
Nioella. According to our phylogenetic analysis, the strain MME-018\(^T\) was the most closely related to Nioella sediminis, but some aspects of the polyphasic taxonomic properties of strain MME-018\(^T\) were different from those of the reference species. Therefore, strain MME-018\(^T\) is considered to represent a novel species of the genus Nioella, for which the name Nioella aestuarii sp. nov. is proposed.

**DESCRIPTION OF NIOELLA AESTUARII SP. NOV.**

Nioella aestuarii (aes.tu.a’ri.i. L. gen. n. aestuarii of a tidal flat).

Cells are Gram-stain negative, non-motile, aerobic, rod-shaped and 0.8–1.0 \(\mu\)m in width by 1.8–2.0 \(\mu\)m in length. Colonies on MA are circular, smooth, convex, and white in colour and 0.5–1.0 mm in diameter after 3 days incubation at 30°C. Growth occurs at 4–40°C (optimum 25–30°C) in presence of 1–4% NaCl (optimum 2%, w/v) and at pH 6.0–8.0 (optimum pH 7.0). Requires Na\(^+\), Ca\(^2+\), or Mg\(^2+\) ions for growth. Nitrate, Fe\(\text{Cl}_3\), and thiosulfate are not reduced under anaerobic conditions. Catalase and oxidase are positive. Hydrolysis of starch, casein, L-tyrosine, gelatin, and Tween 20, 40, and 80 does not occur. Nitrate is aerobically reduced to nitrite in API 20NE kit. Arginine dihydrolyase, cystine arylamidase, lipase (C\(\text{a}\)-glycerol), -glucosidase (aesculin), and protease (gelatin) are negative. Indole is not produced. Glucose is not fermented. In the presence of 1% NaCl (optimum 2%, w/v) and at pH 7.0, Nioella aestuarii can reduce nitrite in API ZYM strips, alkaline phosphatase, esterase (C\(\text{b}\)-glucuronidase, -galactosidase, -mannosidase, and -glucosaminidase, and -acetyl- \(\beta\)-glucosidase (aesculin), and protease (gelatin) are negative. Indole is not produced. Glucose is not fermented. In the API ZYM strips, alkaline phosphatase, esterase (C\(\text{a}\)), esterase lipase (C\(\text{b}\)), leucine arylamidase, valine arylamidase, acid phosphatase, \(\alpha\)-glucosidase, naphthol-AS-BI-phosphohydrolase, and \(\alpha\)-galactosidase activities are positive, whereas cystine arylamidase, lipase (C\(\text{a}\)), trypsin, \(\alpha\)-chymotrypsin, \(\alpha\)-galactosidase, \(\beta\)-glucuronidase, \(\beta\)-glucosidase, N-acetyl-\(\beta\)-glucosaminidase, \(\alpha\)-mannosidase, and \(\alpha\)-fucosidase activities are negative. Acetate, cellobiose, citrate, D-galactose, D-gluconate, glycerol, inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, L-ornithine, pyruvate, raffinose, L-rhamnose, D-sorbitol, succinate, and trehalose are utilized, while L-arabinose, benzoate, formate, D-fuctose, D-glucose, maltose, D-ribose, sucrose, and D-xylene are not utilized. Acid is produced from D-galactose, D-mannose, L-rhamnose, but not cellobiose, melibiose, raffinose, and trehalose. The dominant respiratory quinone is Q-10. The major polar lipids are PC, PG PE, two unidentified phospholipids, and an unidentified lipid. The major fatty acids are summed feature 8 (C\(18:\text{ω7c}\) and/or C\(18:\text{ω6c}\)), C\(16:\text{ω0}\)-cyclo C\(19:\text{ω8c}\), and 11-methyl C\(18:\text{ω7c}\).

The type strain, MME-018\(^T\) (=KCCM 43135\(^T\)=JCM 30752\(^T\)) was isolated from the tidal flat sediment of Muui-do in Incheon, the Republic of Korea. The genomic DNA G+C content is 61.6 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


