Infective endocarditis due to *Abiotrophia defectiva* and *Granulicatella* spp. complicated by infectious intracranial cerebral aneurysms: a report of three cases and review of the literature

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Nutritionally variant streptococci, now classified as *Abiotrophia defectiva* or *Granulicatella* spp., are thought to account for 2% of all infective endocarditis cases but estimates of their frequency are complicated by changes in nomenclature and difficulties in obtaining positive microbiology cultures. Their growth characteristics and difficulty undertaking antibiotic susceptibility testing may impede optimal antibiotic treatment decisions. We describe three patients with definite infective endocarditis due to these organisms seen at our hospital between 2005 and 2010, all of whom presented with neurological symptoms due to infectious intracranial cerebral aneurysms. We recommend that, for patients with left-sided infective endocarditis due to *A. defectiva* and *Granulicatella* spp., clinicians should consider imaging the central nervous system.

**INTRODUCTION**

Nutritionally variant streptococci were first described in 1961 in the setting of endocarditis (Frenkel & Hirsch, 1961). *Streptococcus adiacens* and *Streptococcus defectiva* were identified by Bouvet & colleagues in 1989, and in 1991 a new genus — *Abiotrophia* — was proposed following the use of 16S rRNA gene sequencing (Bouvet et al., 1989; Weisburg et al., 1991). This genus has subsequently been divided into the genera *Abiotrophia* and *Granulicatella* on the basis of additional phylogenetic analysis using 16S rRNA gene sequencing. Collins & Lawson (2000) proposed that three species members of the genus *Abiotrophia* be reclassified in a new genus *Granulicatella* encompassing the species *G. adiacens*, *G. elegans* and *G. balaenopterae*. *A. defectiva* is the sole remaining member of the genus *Abiotrophia*.

Infective endocarditis (IE) due to these organisms is infrequent, and specific estimates are compromised by changes in nomenclature. Nutritionally variant streptococci have been estimated to cause between 5 and 6% of all streptococcal endocarditis, although this is often over-reported as 5% of all endocarditis cases (Brouqui & Roult, 2001; Roberts et al., 1979; Facklam, 2002; Rouff, 1991; Wijetumga et al., 2002). It is estimated that 2% of IE cases are caused by these organisms, leading to under-reporting of their true incidence and may contribute to their higher rates of complications compared with viridans streptococci (Roberts et al., 1979).

Neurological symptoms occur in between 20 and 40% of all cases of IE (Baddour et al., 2005; Habib et al., 2009;
Garcia-Cabrera, 2013). In a prospective neuroimaging and neurochemical marker study of cerebrovascular complications of left-sided IE in 60 subjects, 35% had symptomatic and another 30% had silent neurological findings. No specific data on infectious intracranial cerebral aneurysms (IICAs) were reported (Snygg-Martin et al., 2008). Intracerebral aneurysms complicating IE have a reported incidence of 2 to 4%, but since aneurysms may be silent, their true incidence is unknown (Baddour et al., 2005; Peters et al., 2006; Corr et al., 1995). Although IE due to Staphylococcus aureus is an independent risk factor associated with all neurological complications (Garcia-Cabrera et al., 2013), the microbiology of IICAs complicating IE is a subject of debate (Peters et al., 2006), with several authors noting the preponderance of a streptococcal aetiology (Salgado et al., 1987; Baddour et al., 2005). Aneurysms are often found as a result of neurological imaging studies in patients presenting with focal symptoms or signs, or acutely with central nervous system (CNS) haemorrhage from aneurysmal rupture.

We report three patients with definite left-sided IE, two due to A. defectiva and one due to G. adiacens, seen at Hennepin County Medical Center (HCMC) between 2005 and 2014. All cases observed at HCMC had co-existing neurological symptoms and signs and were found to have IICA on radiological evaluation. We reviewed all published reports of Abiotrophia and Granulicatella IE complicated by IICA, and they are described herein.

**CASE REPORTS**

The clinical, echocardiographical, radiological, microbiological and treatment characteristics of the three HCMC patients and the four additional cases of IE complicated by IICA identified following literature review are summarized in Table 1. All three HCMC patients had pre-existing mitral valve disease, one (Case 3) had prior mitral valve repair and two (Cases 1 and 2) had documented antecedent dental infection resulting in tooth extractions. All had focal neurological symptoms and signs; two patients developed aphasia and right-sided weakness (Cases 1 and 3) in the context of a mild insidious illness over many weeks, while the remaining patient (Case 2) presented suddenly with aphasia. This patient had serial cerebral angiograms over the subsequent 2 months, which showed gradual aneurysmal enlargement and culminated in endoneurological vascular surgery and subsequent mitral valve replacement. The remaining two patients (Cases 1 and 3) had mitral valve replacement (bioprothestic) and repair at 6 and 1 month, respectively, following completion of a two-drug antibiotic treatment regimen.

Despite publication of over 100 reports of IE due to Abiotrophia and Granulicatella, we were only able to identify reports of four additional adult patients with IICAs. Two patients had A. defectiva endocarditis, both complicated by subarachnoid haemorrhage (Cases 4 and 5, Table 1); one patient (Case 4) (Yang et al., 2010) underwent successful clipping of a cerebral aneurysm, while the other patient (Case 5) died of a massive cerebral bleed (Kohok et al., 2011). The remaining two patients, both infected with G. adiacens, presented with acute neurological findings. One required neurosurgical intervention (aneurysmal clipping) and both required mitral valve surgery (valvuloplasty and repair, respectively). Both patients survived (Chang et al., 2008; Lin et al., 2007). Of note, all seven cases had IE of the mitral valve. An additional patient, included in a series with nutritionally variant streptococcal infection, had IE due to G. elegans complicated by a ruptured IICA and an intracranial bleed (Liao et al., 2004). This case is not included in Table 1 because detailed clinical, laboratory and treatment information was not available.

**METHODS**

HCMC is a 455 bed inner city teaching hospital in Minneapolis, MN. We identified two patients with A. defectiva and one with G. adiacens IE hospitalized between 2005 and 2014 by retrospective review of the Infectious Diseases patient registry and the microbiology laboratory’s blood culture data bank. All patients had definite endocarditis based on modified Duke criteria (Li et al., 2000) and IE was complicated by neurological findings. We undertook a Medline and PubMed literature search of the English language literature between 1966 and 2015, using the key phrases: ‘nutritionally variant streptococci’; ‘Abiotrophia defectiva’; ‘Granulicatella spp.; ‘infective endocarditis’ and ‘intracranial cerebral aneurysms’.

All organisms were recovered in BacT/Alert FAN (bioMérieux) blood culture medium. Identification to the species level was initially achieved based on traditional microbiological characteristics, including pleomorphic Gram stain, differential growth patterns on chocolate agar versus 5% sheep red blood cell agar, and confirmation of pyridoxal and vitamin B6 pathways. Vitek 2 (bioMérieux) Gram-positive identification test cards containing a number of dehydrated biochemical substrates were initially used to identify A. defectiva and Granulicatella sp. for the three cases. Definitive species identification was achieved using 16S rRNA sequencing. The suspended organism was inoculated into 200 µl SeeGene extraction solution, vortexed, heated at 100°C for 20 min and then centrifuged for 5 min at 15,000 g. Bacterial DNA was extracted using automated easyMAG (bioMérieux) extraction. After initial PCR amplification, with primers 5'-TGGAGAGTTT-GATCCTGGCTCAG-3' and 5'-GTATTACCGCGCTGCTGG-3' (Chow & Claridge, 2014) the PCR products were purified using Exo-SAP-IT (Affymetrix) according to the manufacturer’s instructions. Sequencing of both strands was carried out, with the same primers as in the PCR, using an ABI Prism BigDye Terminator Cycle Sequencing kit (Applied Biosystems). The sequence cycling products were analysed by capillary electrophoresis and fluorescence detection with an Applied Biosystems ABI 3500 Genetic Analyzer. The sequences were analysed and compared with GenBank databases and the 16S Centroid database available through the SmartGene® software program. Similar reference and published sequences were directly aligned and nucleotide sequences were compared to known ATTC strains and quality published sequences. The ~300 bp nucleotide sequences of the 16S rRNA gene of A. defectiva strains and Granulicatella spp. have been deposited in GenBank: Data with accession numbers KU242743–KU242745. In our series, the two strains of A. defectiva isolated from two different patients were identical and displayed 4 bp mismatches for a 512 bp region with the 16S rRNA gene to a known sequence published by Senn et al. (2006) (Genbank accession number AY879307). The strain
<table>
<thead>
<tr>
<th>Ptno. (ref)</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Pre-existing heart disease</th>
<th>Echocardiographic findings</th>
<th>Cerebral angiography/ CT head</th>
<th>Microbiological and sensitivity data</th>
<th>Blood culture (BC) data</th>
<th>Treatment/surgeries outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 HCMC case (this series)</td>
<td>50 M</td>
<td>Myxomatous MV, mitral insufficiency</td>
<td>Ruptured chordae of the posterior MV leaflet, myxomatous MV with prolapse, MV vegetation, MV insufficiency (TEE)</td>
<td>L cortical infarct, fusiform dilatation of distal M2 segment of L MCA (5 × 3 mm aneurysm)</td>
<td>Abiotrophia defectiva PEN MIC=0.25 mg µl⁻¹ (intermediate)</td>
<td>8/8 BC positive 4 days to BC positivity BC sterile on days 1, 3 and 5 following antibiotic initiation</td>
<td>VAN, GEN for 6 weeks Bioprosthetic MV, dual chamber pacemaker for 3º heartblock 6 months after treatment Cerebral angiography at 3, 6 and 12 months revealed a stable fusiform 5 × 2.9 mm dilatation of the left MCA Patient alive and well at 4 year follow-up</td>
<td></td>
</tr>
<tr>
<td>2 HCMC case (this series)</td>
<td>25 F</td>
<td>MVP, mitral insufficiency</td>
<td>Ruptured chordae of MV, myxomatous MV, MV vegetation (2 × 0.7 cm and 1 × 0.6 cm) on posterior leaflet, MV insufficiency 4+, LAE, small pericardial effusion</td>
<td>L acute tempoparietal infarct, L MCA mycotic aneurysm (4 × 3 mm) at distal M2-proximal M3</td>
<td>Abiotrophia defectiva PEN MIC=0.125 mg µl⁻¹ (susceptible) CRO MIC=0.064 mg µl⁻¹ (susceptible)</td>
<td>4/4 BC positive 4 days to BC positivity BC sterile on days 1, 2, 3, 4, 5 following antibiotic initiation</td>
<td>CRO and RIF for 6 weeks; GEN was added for 2 weeks following neurologic event On serial cerebral angiograms the aneurysm grew to 10 × 7.3 mm over the two months following diagnosis. Aneurysmal resection, microvascular bypass, MV repair, plication of the posterior leaflet, insertion of new chordae at 2 months Patient alive and well at 6 month follow-up</td>
<td></td>
</tr>
<tr>
<td>3 HCMC case (this series)</td>
<td>48 M</td>
<td>Previous MV repair, Maze procedure for atrial fibrillation, prior L MCA stroke</td>
<td>LVEF 60–65 %, no WMA, myxomatous MV with prolapse of posterior leaflet, small mobile vegetation on posterior annulus of MV</td>
<td>New R MCA mycotic aneurysm, thrombosed Old L posterior, frontal, parietal encephalomalacia</td>
<td>Granulicatella adiacens. PEN MIC=0.5 mg µl⁻¹ (intermediate)</td>
<td>4/4 BC positive 1 day to BC positivity BC sterile on days 1, 2, 12 following antibiotic initiation</td>
<td>PEN, GEN for 6 weeks Patient’s mental status returned to baseline Mitral Valve repair; 1 month following treatment</td>
<td></td>
</tr>
<tr>
<td>4 (39)</td>
<td>60 M</td>
<td>Native MV with severe mitral insufficiency</td>
<td>TTE: MV vegetation and severe regurgitation</td>
<td>SAH/hydrocephalus us, 3.8 mm aneurysm, ruptured distal branch of R MCA</td>
<td>Abiotrophia defectiva No further data</td>
<td>2 sets of BC positive after 2 days Final identification at 7 days “Positive BC” No further data</td>
<td>PEN, GEN × 6 weeks External ventricular drainage, aneurysm clipped Patient died</td>
<td></td>
</tr>
<tr>
<td>5 (19)</td>
<td>89 F</td>
<td>History of rheumatic fever, MV regurgitation</td>
<td>TTE: Mobile vegetation 0.8 × 0.5 cm on posterior MV leaflet with severe MR</td>
<td>Head CT: SAH with extensive intracerebral hemorrhage and hydrocephalus</td>
<td>Abiotrophia defectiva No further data</td>
<td></td>
<td>PEN, GEN Patient died within 24 h of neurological symptom onset</td>
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**Table 1. cont.**

<table>
<thead>
<tr>
<th>Pt.no. (ref)</th>
<th>Age (years)</th>
<th>Pre-existing heart disease</th>
<th>Echocardiographic findings</th>
<th>Cerebral angiography/ CT head</th>
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<th>Treatment/surgeries outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (5)</td>
<td>31 M</td>
<td>No known heart disease</td>
<td>TTE: Ruptured chordae tendineae and moderate to severe MR. Later TTE: 1 x 1 cm mass on MV posterior leaflet</td>
<td>Head CT: haemorrhage in the R frontal area, multiple aneurysms (6-8 mm) in the R and L MCA and L ACA detected on cerebral angiography</td>
<td><em>Granulicatella adiacens</em> isolated after second CNS event</td>
<td>No further data</td>
<td>SAM, 4 months later OXA + GEN R MCA clipped, mitral valvuloplasty Patient survived</td>
</tr>
<tr>
<td>7 (21)</td>
<td>30 M</td>
<td>No known heart disease</td>
<td>MV vegetation (11 x 11 mm), severe mitral regurgitation</td>
<td>Ruptured mycotic aneurysm in the L frontal, parietal area</td>
<td><em>Granulicatella adiacens</em> *PEN sensitive</td>
<td>No further data</td>
<td>PEN (rash) 4–8 weeks, then CRO and GEN MV repair 2 months after treatment Patient survived</td>
</tr>
</tbody>
</table>

ACA, Anterior cerebral artery; AMP, ampicillin; SAM, ampicillin-sulbactam; CRO, ceftriaxone; GEN, gentamicin; L, left; LAE, left atrial enlargement; LVEF, left ventricular ejection fraction; MCA, middle cerebral artery; MV, mitral valve; MVP, mitral valve prolapse; OXA, oxacillin; PEN, penicillin; R, right; RIF, rifampin; SAH, subarachnoid haemorrhage; TEE, trans-esophageal echocardiography; TTE, transthoracic echocardiogram; VAN, vancomycin; WMA, wall motion abnormality.

**DISCUSSION**

*Granulicatella* and *Abiotrophia* species are coccoid-negative, facultative anaerobic, Gram-positive cocci, and belong to the family *Carnobacteriaceae*. Granulicatella (Collins & La yaşan, 2000; Ludwig et al., 2008; Abiotrophia (Collins & La yaşan, 2006) and *Granulicatella* are part of the normal oral flora, and in two of the HCMC patients there was a history of dental infection and procedures.

Two of our patients had an indolent clinical course, which has been identified as an important factor in the increased morbidity and mortality with this group of organisms. *A. defectiva* and *G. adiacens* have been associated with intracranial aneurysm development, but the association of specific bacteria and subsequent bacterial aneurysm site, and leading to the hypothesis that TLRs are actively involved in intracellular signalling cascades essential to promotion of inflammatory and cytokine responses. It is interesting to note that 100% of patients with IE involving *A. defectiva* and *G. adiacens* in our series had IE involving an area that was actively involved in intracellular signalling cascades that TLRs are known to be involved in.

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**References**

- Okada, et al. (2009) examined intracranial aneurysm specimens collected from 70 patients, demonstrating that bacterial Toll-like receptor 2 (TLR2) and TLR4 receptors were actively expressed at the ruptured aneurysm site, and leading to the hypothesis that TLRs are actively involved in intracellular signalling cascades essential to promotion of inflammatory and cytokine responses. It is interesting to note that 100% of patients with IE involving *A. defectiva* and *G. adiacens* in our series had IE involving an area that was actively involved in intracellular signalling cascades that TLRs are known to be involved in.
species-specific but rather a finding attributable to IE involving both \textit{A. defectiva} and \textit{G. adiacens}.

Historically, there have been difficulties in growing \textit{Granulicatella} and \textit{Abiotrophia} following detection in blood cultures. These organisms were known as nutritionally variant streptococci because of their requirement for pyridoxal hydrochloride or supplementation of L-cysteine into standard media for successful laboratory isolation; the use of Schaedler agar containing haemin and vitamin K-supplemented horse blood has been recommended. Contemporary experience with members of the genera \textit{Abiotrophia} and \textit{Granulicatella} has shown that improvements made to current blood culture media enable routine isolation of the organisms, and visible colonies may appear on subculture from positive blood culture bottles at 48 h using \textit{Brucella} and chocolate subculture media. Additional subcultures from positive blood culture media to \textit{Brucella} agar supplemented with 5% horse blood may further enhance laboratory isolation of the organisms (Rouff, 1991; Jorgen & Hindler, 2007). Our patients’ blood cultures became positive within 1 to 4 days of incubation, which is likely the result of advances in media formulation discussed above. In cases involving IE with \textit{A. defectiva} and \textit{Granulicatella} spp., anaerobic blood cultures typically become positive earlier than aerobic cultures (3.56 h, vs 8.49 h), supporting the role of anaerobic blood culture collection in cases of suspected IE (Cargill et al., 2012).

Because of its infrequent isolation, laboratory personnel may be unfamiliar with \textit{Abiotrophia} and \textit{Granulicatella} species’ pleomorphic appearance, satellite growth patterns, and growth requirements. Standard biochemical techniques and algorithms can often fail to provide speciation (Radcliffe et al., 2013). Clinical microbiology laboratories must be alert to the fact that pleomorphic, variable Gram-staining organisms that selectively grow only from enriched (i.e. chocolate) media are key diagnostic indicators of \textit{Abiotrophia} and \textit{Granulicatella} spp.

Newer diagnostics, including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), have been reported in the detection of \textit{Granulicatella} spp. and \textit{A. defectiva} (Holler et al., 2011; Schulthess et al., 2013). However, use of MALDI-TOF technology for accurate detection may require further study. Neville and colleagues reported that identification of \textit{G. adiacens} to species level could be achieved in only one-third of cases (Neville et al., 2011). Recently, Radcliffe et al. (2013) reported that successful speciation of both \textit{A. defectiva} and \textit{G. adiacens} using MALDI-TOF may require formic acid protein extraction prior to MALDI-TOF MS analysis. In cases where definitive identification is sought, we recommend the use of the 16S rRNA sequencing.

Antibiotic susceptibility testing by disc diffusion is not recommended for \textit{Abiotrophia} or \textit{Granulicatella} isolates (Zheng et al., 2004); the Clinical Laboratory Standards Institute (CLSI) suggests broth microdilution MIC testing in cation-adjusted Mueller–Hinton broth with 2.5–5% lysed horse blood and 0.001% pyridoxine hydrochloride (NCCLS, 2010).

Resistance to beta-lactam antibiotics has been reported in both \textit{G. adiacens} (susceptibility rates of 55–67% to penicillin, 0–63% to ceftriaxone, 81% to amoxicillin and 96% to meropenem) and \textit{G. elegans} (susceptibility rates of 100% to penicillin, 0% to cefuroxime and 33% to ceftriaxone) (Zheng et al., 2004; Tuohy et al., 2000). Species-specific differences in susceptibility profiles have also been reported. \textit{G. adiacens} is more susceptible to penicillin than \textit{A. defectiva} (55 vs 8%), and up to 60 and 47% of the strains may be resistant to ceftriaxone and cefepime, respectively, emphasizing the importance of isolation, identification and appropriate sensitivity testing (Tuohy et al., 2000; Murray et al., 2001). Resistance has also been reported to clindamycin, tetracycline, erythromycin and ciprofloxacin but not to vancomycin or rifampicin (Zheng et al., 2004; Tuohy et al., 2000). E-test results using Schaedler agar have been shown to be similar to broth dilution for penicillin, although there is a higher rate of intermediate susceptibility reported (Douglas et al., 1994). In our isolates, penicillin MICs ranged from 1.25 to 5 µg/ml and were susceptible by MIC to benzylpenicillin using both the European Committee on Antimicrobial Susceptibility Testing (EUCAST eucast.org/clinical_breakpoints) and CLSI streptococcal breakpoints (EUCAST, 2011; CLSI, 2014).

A longstanding concern in the treatment of \textit{Abiotrophia} and \textit{Granulicatella} infections is the significant tolerance to penicillin demonstrated by some isolates (Liao et al., 2004). For tolerant strains, the minimum bactericidal concentration of penicillin greatly exceeds the MIC, usually by 32-fold. These strains are killed more slowly by penicillin in animal models of endocarditis and may result in higher relapse rates (Wilson et al., 1985).

The treatment of \textit{Abiotrophia} and \textit{Granulicatella} infection is difficult because in vitro susceptibility does not always correlate well with the clinical response (Brouqui & Roult, 2001). There are recommendations that even cases of IE may be managed by following standard treatment recommendations without the benefit of susceptibility testing (Jorgensen et al., 2007). There are some differences in the treatment recommendations for IE due to \textit{Abiotrophia} and \textit{Granulicatella} between standard guidelines, but typically treatment is with penicillin or another cell wall agent for 6 weeks, together with an aminoglycoside for at least the first 2 weeks and ideally as long as 4 to 6 weeks (Habib et al., 2009; Baddour et al., 2005; Gould et al., 2012).

Treatment failures have been reported in 40% of cases of IE caused by nutritionally variant streptococci and up to 50% of patients require prosthetic valve replacement for congestive heart failure, major systemic emboli or other major complications (Stein & Nelson, 1987; Adam et al., 2015). Such treatment failures may be due to the indolent nature of the infections as well as delays in the initiation of appropriate antimicrobial therapy because of the increased time required for isolation and identification of these
organisms (Stein & Nelson, 1987; Perkins et al., 2003). Furthermore, the slower generation time for nutritionally variant streptococci (2–3 h) versus viridans streptococci (40–50 min) may attenuate the ability of beta-lactam antibiotics to kill organisms that do not maintain predictable log phase growth kinetics (Perkins et al., 2003; Jeng et al., 2005).

A number of predictors of embolic events for patients with left-sided IE have been reported, including the length or size of the vegetation (greater than 10 mm or 15 mm) and mobility described on echocardiography, mitral valve location (especially the anterior leaflet) and infection due to Staphylococcus aureus (Baddour et al., 2015; Di Salvo et al., 2001). While Staphylococcus aureus is now the commonest cause of IE in most settings and the most likely etiology of neurological events (Garcia-Cabrera et al., 2013), for IICAs streptococcal organisms have been the most frequently implicated organisms (Saldago et al., 1987; Baddour et al., 2005). Our report reinforces this association, which applies to both Abiotrophia and Granulicatella species. While there are no clear-cut predictors of IICA, a low threshold for neuroradiological imaging studies in patients with left-sided IE due to these organisms is recommended, including patients with vague complaints such as severe localized headaches or mild confusion. Management of IICAs is challenging as patients may be asymptomatic, present symptomatically with focal neurologic symptoms and signs, or acutely with rupture of a previously unsuspected lesion. Many aneurysms will resolve with appropriate antibiotic therapy and the risk of rupture diminishes with time on antibiotic therapy, typically with reduction in aneurysmal size over the first 1 to 2 weeks.

Abiotrophia and Granulicatella IE is an infrequent occurrence and accounts for approximately 2% of all patients with IE. Isolation and susceptibility testing may be challenging due to the unique growth requirements of the organisms. In our experience, 100% of all our cases of IE involving these organisms occurred with significant neurosurgical events (Garcia-Cabrera et al., 2015; Di Salvo et al., 2000). The genus Abiotrophia (Kawamura et al.) is not monophyletic: Proposal of Granulicatella gen. nov., Granulicatella adiacens comb. nov., comb. nov. and Granulicatella balaenopterae comb. nov. Int J Syst Evol Microbiol 50, 365–369.


the European Society of Cardiology (ESC). Eur Heart J 30, 2369–2413.

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