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endoscopic retrograd colangiopancreatography; peptostreptococcus micros.

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Abstract: Parvimonas micra is an anaerobic, Gram-positive coccus belonging to oral, gastrointestinal and genital flora, rarely causing infections in humans. It was mainly deemed to cause bacteremia, septic bone and cerebral infections in patients which have undergone dental procedures or with suboptimal dental hygiene. We report the first case of Parvimonas micra bacteremia following endoscopic retrograde colangiopancreatography performed due to choledocholithiasis in a patient with good oral health. Identification of P. micra was finally performed by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (VITEK MS system, bioMérieux, Marcy l'Étoile, France). All cases reported in english language of Parvimonas micra infections after medical procedure are reviewed in order to alert clinicians about new possible routes of infection of this emerging pathogen.

Anaerobe

To the Editor

14<sup>th</sup> May, 2018

Dear Sir,

We submit our manuscript "*Parvimonas micra* bacteremia following endoscopic retrograde cholangiopancreatography: a new route of infection" for publication as case report in Anaerobe.

We report the first case of *Parvimonas micra* bacteraemia following endoscopic retrograd colangiopancreatography together with a comprehensive review of *Parvimonas micra* infections after medical procedure in order to alert clinicians about new possible routes of infection of this emerging pathogen.

We think that this kind of paper could contribute to the Anaerobe mission.

All the authors have seen and agree with the contents of the manuscript.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

We also certify that this submission is not currently under review at any other publication and there is no ghost writing by anyone not named on the author list.

Waiting to hear from you at your earliest convenience,

Sincerely,

Matteo Boattini, Gabriele Bianco, Rossana Cavallo, Cristina Costa

Anaerobe

To the Editor

3<sup>rd</sup> September, 2018

Dear Sir,

We hereby resubmit our manuscript "*Parvimonas micra* bacteremia following endoscopic retrograde cholangiopancreatography: a new route of infection" for peer review to consider eventual publication as case report in Anaerobe.

We thank the Reviewers for their accurate appraisals. We are sure the paper has now been improved after the Reviewers' suggestions. We have revised the manuscript in accordance with the comments and suggestions raised. We enclose below a point-by-point response to the issues raised by the Reviewers specifying the changes made to the revised version of the manuscript.

We hope the revised version will now be suitable for publication in Anaerobe.

Waiting to hear from you at your earliest convenience,

Sincerely,

Matteo Boattini, Gabriele Bianco, Rossana Cavallo, Cristina Costa

**Reviewer #2:**

Dear Editor

Thank you to give me the opportunity for reviewing this interesting case report.

This case report is interesting, well-written adding a comprehensive review of the literature.

As I think very important to publish new clinical conditions in great journals, I consider that this case report could be accepted for a publication in Anaerobe.

*We thank the Reviewer for his/her comment.*

**Reviewer #3:**

Boattini et al. presented case regarding Parvimonas micra bacteriemia following ERCP procedure as a new route of infection. The case is interesting but the abstract and introduction do not achieve enough quality for Anaerobe. Nevertheless the discussion and conclusion are quite good and case seems to be important to present it to the readers. Hence I propose to improve abstract, introduction and also add important information in case description.

Moreover I need some more information about this patient. After these improvement another assessment must be done.

Examples:

1).

Abstract: Parvimonas micra is an anaerobic, Gram-positive coccus belonging to oral, gastrointestinal and genital flora, mainly causing bacteremia, septic bone and cerebral infections in patients which have undergone dental procedures or with suboptimal dental hygiene.

I do not agree that this pathogen mainly is a cause of bacteriemia etc.

Mainly - is isolated form dental plaque, rarely is cause of infection

*We thank the Reviewer for these relevant comments.*

*In agreement with his/her comment we changed the text (lines 30-33) as “Parvimonas micra is an anaerobic, Gram-positive coccus belonging to oral, gastrointestinal and genital flora, rarely causing infections in humans. It was mainly deemed to cause bacteremia, septic bone and cerebral infections in patients which have undergone dental procedures or with suboptimal dental hygiene”.*

2).

#### INTRODUCTION:

46 Endogenous microbiota translocation into the bloodstream is deemed to contribute to  
47 high morbidity, disability and mortality rates, mainly causing endocarditis,  
48 spondylodiscitis and central nervous system infections. It is considered to be result of  
49 several mechanisms entailing mucosal or deeper injury. Suboptimal dental hygiene,  
50 dental treatment but also routine daily activities as brushing and flossing teeth, using  
51 toothpicks have been described to be associated to important endogenous microbiota  
52 bacteremia rates [1].

This statement is too general. Pathogenesis of neuroinfections, endocarditis is very complex. the reference number 1 it is a endocarditis guidelines from 2007. It would be better to cite newer guidelines and I doubt that this guidelines are describing problem of nervous system epidemiology/microbiology.

*We thank the Reviewer for highlighting this point.*

*Endogenous microbiota bacteremia can be associated with endocarditis, spondylodiscitis and central nervous system infections. We cited the endocarditis guidelines in order to highlight*

*pathogenic mechanisms of endogenous microbiota translocation into the bloodstream in developing primarily heart valve infection and, eventually, bone and CNS infections.*

*In agreement with the Reviewer's suggestion, we have now cited the newer guidelines (lines 173-178): "Suboptimal dental hygiene, dental treatment but also routine daily activities as brushing and flossing teeth, using toothpicks have been described to be associated to important endogenous microbiota bacteremia rates [Habib G, Lancellotti P, Antunes MJ, Bongiorni MG, Casalta JP, Del Zotti F, et al, 2015 ESC Guidelines for the management of infective endocarditis: The Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC).*

*Endorsed by: European Association for Cardio-Thoracic Surgery (EACTS), the European Association of Nuclear Medicine (EANM). Eur Heart J 2015;36:3075-128. doi: 10.1093/eurheartj/ehv319]."*

3) case report

-what was the WBC count, neutrophil percentage, value of CRP, value of procalcitonin

-Did you perform USG of abdomen ?

*We thank the Reviewer for highlighting this point. Accordingly, we changed the text (lines 85-88) as: "Biochemistry showed white blood cell count 15,500 [4,500-11,000 per mm<sup>3</sup>], neutrophilia of 90%, C-reactive protein 160 mg/L [<5.0] and procalcitonin 2 ng/mL. Chest-X ray showed no lung infiltration. Abdominal ultrasound revealed no significative alterations."*

-You mentioned that "two pairs of aerobic and anaerobic blood culture were drawn". Did both pairs were taken before antibiotic?

*We thank the Reviewer for these remarks.*

*Both pairs of blood culture were drawn before antibiotic. We changed the text accordingly (line 90):*

*"Two pairs of aerobic and anaerobic blood cultures were drawn peripherally and Ciprofloxacin (500 mg every 12h) was subsequently started".*

- Did pathogen was confirmed in one or both blood cultures?
- Did you perform blood culturing which was negative after or even in the middle of antibiotic therapy

*We thank the Reviewer for these remarks.*

*P. micra was isolated in both blood cultures. No more blood cultures were performed.*

*The text was changed as (Line 91): "After 30 hours, Gram staining showed gram positive cocci in both anaerobic blood cultures".*

- How long patient had fever.
- When (in which day) you switched ciprofloxacin to penicilin.
- have you observed improvement after ciprofloxacin?
- Have you checked WBC, CRP, procalcitonin at the end of treatment

*We thank the Reviewer for these relevant comments.*

*Patient had fever during 4 days, once a day, late in the afternoon. Penicilin was started on hospital day 5 (4<sup>th</sup> day of fever). No improvement was observed on Ciprofloxacin. WBC, CRP, procalcitonin were checked at the end of treatment reaching normal range.*

*In agreement with Reviewer's suggestions we changed the text (line 100-103) as "On hospital day 5, patient still had fever. In agreement with the results of susceptibility testing, antimicrobial therapy was switched to intravenous Penicillin G (2 millions units every 4 hours) and it was continued for 14 days obtaining clinical and laboratorial improvement".*

1    **Highlights**

2    • A case report on bacteremia following endoscopic retrograd  
3    cholangiopancreatography caused by rare anaerobic gram-positive coccus, *Parvimonas*  
4    *micra*

5    • A new route of *Parvimonas micra* infection is highlighted through this case

6    • *Parvimonas micra* translocation mechanism into the bloodstream highlighted in this  
7    case is imperceptible mucosal injury during endoscopy

8    • Review of previous cases of *Parvimonas micra* infections after medical procedure are  
9    also discussed in order to alert clinicians about new possible routes of infection of this  
10   emerging pathogen

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1 PARVIMONAS MICRA BACTEREMIA FOLLOWING ENDOSCOPIC  
2 RETROGRADE CHOLANGIOPANCREATOGRAPHY: A NEW ROUTE OF  
3 INFECTION

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29 ABSTRACT:

30 *Parvimonas micra* is an anaerobic, Gram-positive coccus belonging to oral,  
31 gastrointestinal and genital flora, rarely causing infections in humans. It was mainly  
32 deemed to cause bacteremia, septic bone and cerebral infections in patients which have  
33 undergone dental procedures or with suboptimal dental hygiene. We report the first case  
34 of *Parvimonas micra* bacteremia following endoscopic retrograde  
35 colangiopancreatography performed due to choledocholithiasis in a patient with good  
36 oral health. Identification of *P. micra* was finally performed by Matrix-assisted laser  
37 desorption ionization–time of flight mass spectrometry (VITEK MS system,  
38 bioMérieux, Marcy l'Étoile, France). All cases reported in english language of  
39 *Parvimonas micra* infections after medical procedure are reviewed in order to alert  
40 clinicians about new possible routes of infection of this emerging pathogen.

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44 KEYWORDS: *Parvimonas micra*; bacteraemia; anaerobe; gram-positive coccus;  
45 endoscopic retrograd colangiopancreatography; peptostreptococcus micros.

46 INTRODUCTION:

47 Endogenous microbiota translocation into the bloodstream is deemed to contribute to  
48 high morbidity, disability and mortality rates, mainly causing endocarditis,  
49 spondylodiscitis and central nervous system infections. It is considered to be result of  
50 several mechanisms entailing mucosal or deeper injury. Suboptimal dental hygiene,  
51 dental treatment but also routine daily activities as brushing and flossing teeth, using  
52 toothpicks have been described to be associated to important endogenous microbiota  
53 bacteremia rates [1]. Medical procedures such as gastrointestinal endoscopy (GIE) can  
54 also implicate technique-related mucosal trauma, sterile space or tissue local infections  
55 and endogenous microbiota translocation [2]. However, oral and gastrointestinal  
56 commensal flora bacteremia rates following GIE are reported to be lower than in routine  
57 daily activities [2] and antibiotic prophylaxis recommendations before endoscopic  
58 procedures have been widely debated. When recommended, prior GIE antimicrobial  
59 prophylaxis should be provided to aim *Enterobacteriaceae*, enterococci, alpha-  
60 hemolytic streptococci, *Bacteroides fragilis* and *Clostridium spp* [3] in order to prevent  
61 infectious consequences [3]. Anaerobic commensal flora is an important part of oral and  
62 gastrointestinal microbiota and anaerobes other than Gram-negative bacilli can be  
63 involved in endogenous translocation. *Parvimonas micra*, previously known as  
64 *Peptostreptococcus micros* or *Micromonas micros* [4], is a non-spore forming, strictly  
65 anaerobic, slow-growing, occurring in pairs and short chains, Gram-positive coccus,  
66 belonging to commensal flora of oral cavity, gastrointestinal and genital tracts. It was  
67 deemed to cause bacteremia [5,6], endocarditis [7], pleural effusion [8], septic  
68 pulmonary embolism [9], bone [10-13] and cerebral infections, mainly in patients which  
69 have undergone dental medical procedures (DMP) [11,12-19] or with suboptimal oral  
70 hygiene [20-23]. Few data about clinical features and management of *P. micra*  
71 infections after medical procedures, above all in cases of infections after non-dental

72 medical procedures (NDMP), are reported in literature [8,19,22,24-28]. A case of a  
73 patient with *P. micra* bacteremia following endoscopic retrograd  
74 colangiopancreatography (ERCP) for choledocholithiasis together with a  
75 comprehensive review of *P. micra* infections after NDMP in patients with good oral  
76 health and after DMP is presented in order to compare epidemiological and clinical  
77 features and to alert clinicians about new possible routes of infection of this emerging  
78 pathogen.

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#### 80 CASE REPORT:

81 In January 2018, a 85-year-old man underwent ERCP due to choledocholithiasis with  
82 mild obstructive jaundice. Patient had a medical history of hypertension. ERCP  
83 achieved complete biliar drainage with no complications and patient was transferred to  
84 the medical ward. On hospital day 2 patient presented with fever (Temperature 39.4°C)  
85 and shivering. The physical examination was unremarkable. Biochemistry showed  
86 white blood cell count 15,500 [4,500-11,000 per mm<sup>3</sup>], neutrophilia of 90%, C-reactive  
87 protein 160 mg/L [<5.0] and procalcitonin 2,1 ng/mL. Chest-X ray showed no lung  
88 infiltration. Abdominal ultrasound revealed no significant alterations. Two pairs of aerobic  
89 and anaerobic blood cultures were drawn peripherally and Ciprofloxacin (500 mg every  
90 12h) was subsequently started. After 30 hours, Gram staining showed gram positive  
91 cocci in both anaerobic blood cultures. After 48 hours incubation, 1 mm diameter,  
92 white, glistening and domed colonies grew on anerobic blood agar plates. Matrix-  
93 assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry  
94 (VITEK MS system, bioMérieux, Marcy l'Étoile, France) provided identification of *P.*  
95 *micra* (confidence values of 99.9%). The *in vitro* susceptibility of the isolate was  
96 assessed with E-test. According to 2018 EUCAST, *P. micra* showed suceptibility for

97 Penicillin G (MIC: 0.002 µg/mL), Amoxicillin/Clavulanic Acid (MIC: 0.016 µg/mL),  
98 Imipenem (MIC: 0.002 µg/mL), Meropenem (MIC: 0.002 µg/mL), Ertapenem (MIC:  
99 0.002 µg/mL), Clindamycin (MIC: 0.19 µg/mL), Vancomycin (MIC: 0.175 µg/mL) and  
100 Metronidazole (MIC: 0.032 µg/mL). On hospital day 5, patient still had fever. In  
101 agreement with the results of susceptibility testing, antimicrobial therapy was switched  
102 to intravenous Penicillin G (2 millions units every 4 hours) and it was continued for 14  
103 days obtaining clinical and laboratorial improvement. Patient was discharged in good  
104 clinical condition and he remains under follow-up at the Gastroenterology out-patient  
105 clinic.

106

#### 107 DISCUSSION:

108 Anaerobic microbiota translocation predisposing factors are described to include  
109 suboptimal dental hygiene, dental treatment, undrained abscesses, surgery such as oral,  
110 gastrointestinal, gynecologic or transplantation, cancer, hematologic malignant disease,  
111 immunodeficiency, chemotherapy and steroid treatment [29]. This case shows as  
112 NDMP such as ERCP should be considered a new possible route of *P. micra* infection.  
113 In our case, patient showed no dental alterations nor periodontal disease and *P. micra*  
114 probably translocated into the bloodstream due to an imperceptible mucosal injury  
115 during endoscopy. Equally, medical procedures other than dental treatment should be  
116 also contemplated as possible risk factors for anaerobic infections. For this reason, *P.*  
117 *micra* infections after NDMP in patients with good oral health and *P. micra* infections  
118 after DMP have been compared and results are reported in the Table. *P. micra* has been  
119 mainly identified in males, middle-aged people or older (45-81 years) and with co-  
120 morbidities such as diabetes mellitus, brain tumor, chronic B hepatitis and others  
121 widespread non-communicable diseases. *P. micra* infection seems to be also associated

122 with common conditions such as joint osteoarthritis, spondylolisthesis, hip and heart  
123 valve prosthesis and recurrent pneumothorax. These conditions may probably represent  
124 a *locus minoris resistentiae* which predispose to *P. micra* mechanism of translocation  
125 into the bloodstream or where *P. micra* is able to settle, irrespective of medical  
126 procedure is dental or non-dental related. Tooth extraction has been described to be  
127 main DMP. NDMP have included neuro, spinal, retropharyngeal and heart valve  
128 surgery, pleurectomy, transurethral resection of the prostate, spinal instrumentation and  
129 joint corticosteroid injection. *P. micra* infections diagnosis after NDMP and DMP have  
130 not been reported to be so different, and consist mainly of central nervous system, bone,  
131 hip and heart valve prosthesis infections.

132 Variable time between medical procedure and *P. micra* infection diagnosis has been  
133 observed. However, if not considering shorter and longer time values, *P. micra*  
134 infections after DMP have been reported to be diagnosed in a shorter time lapse than in  
135 NDMP (2-6 months vs 2 days-many years).

136 *P. micra* translocation mechanism, spread and infection site in NDMP have been  
137 supposed to include retropharyngeal surgery/contiguous spread/meningoencephalitis,  
138 transurethral procedure-related genital mucosa injury/hematogenous  
139 spread/spondylodiscitis, endotracheal intubation/aspiration/pleural effusion and  
140 pleurectomy causing esophageal pleural fistula/hematogenous spread/central nervous  
141 system abscesses.

142 Nevertheless, the incidence of *P. micra* infections may be underreported due to  
143 difficulty in culturing and identifying anaerobic bacteria. Recent introduction of  
144 MALDI-TOF mass spectrometry helped to improve the possibilities to phenotypically  
145 identify this unusual pathogen, also reducing time of identification. Moreover, 16S

146 rRNA gene sequencing continues to be a reliable identification system but it is rarely  
147 performed in clinical practice due to time and cost reasons.

148 Despite of causing severe infections, *P. micra* has shown wide antimicrobial  
149 susceptibility with only few cases of Metronidazole [30], Penicillin and Clindamycin  
150 resistance [31].

151 We highlight the considerable need to report *P. micra* infections above all after NDMP  
152 in order to follow new possible routes of anaerobic infections. Equally, we believe that  
153 suboptimal dental hygiene as well as report of medical procedure are probably  
154 underestimated and it is not always possible to completely understand cause and effect  
155 relationship between NDMP and *P. micra* translocation mechanism, above all in bone  
156 infections.

157 Checking oral cavity before medical procedures, considering medical procedure-related  
158 trauma in patients with fragile oral, gastrointestinal and genital mucosa, prescribing  
159 antibiotic prophylaxis covering anaerobes and monitoring rigorously fever after medical  
160 procedure should be suitable measures in order to avoid anaerobic infections, above all  
161 in the elderly.

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1 Table. Literature review of *Parvimonas micra* infections after medical procedures  
2

Reference	Age/ Gender	Co-morbidity	Medical procedure	Diagnosis	Culture sample	Time lapse between medical procedure and diagnosis	Supposed translocat ion mechanis m and route of infection
<i>Parvimonas micra</i> infections after non-dental medical procedures in patients with good oral health							
Brook <i>et al.</i> 1999	N/R (6 cases)	N/R	Spinal surgery	Spinal fusion surgery infection	Vertebral biopsy and pus specimens	4-25 days	N/R
Frat <i>et al.</i> 2004	61/M	Diabetes mellitus	Retropharyngeal surgery	Meningoencephalitis, epidural abscess	Cerebrospinal fluid	1 month	Surgery, contiguous spread
Uemura <i>et al.</i> 2014	70/M	N/R	Transurethral resection of the prostate	Spondylodiscitis	Paravertebral mass biopsy specimen	N/R	Procedure-related genital mucosa injury, hematogenous spread
Jones <i>et al.</i> 2015	72/F	Hip and cervical spine osteoarthritis	Spinal corticosteroid injections	Paravertebral abscess, spondylodiscitis	Paravertebral abscess pus	Many years	N/R
Cobo <i>et al.</i> 2017	75/F	Diabetes mellitus, hypercholesterolemia	Heart valves replacement, mechanical ventilation	Pleural effusion	Pleural fluid	1-8 days	Endotracheal intubation, aspiration
Shtaya <i>et al.</i> 2017	65/M	Asthma, recurrent pneumothorax	Pleurectomy	Brain and cervical epidural abscesses	Epidural abscesses pus	48 years	Esophageal pleural fistula, hematogenous spread
Sultan <i>et al.</i> 2018	73/M	Diabetes mellitus, obesity, hypertension, knee osteoarthritis	Intra-articular corticosteroid injection	Septic knee arthritis	Knee joint surgical irrigation fluid	2 days	N/R
Lee <i>et al.</i> 2018	49/F	Brain tumor, with hydrocephalus	Neurosurgery	Brain abscess, bacteremia	Blood	N/R	N/R
Boattini <i>et al.</i> 2018	81/M	Hypertension	ERCP	Bacteremia	Blood	1 day	Endoscopy-related oral/gastrointestinal mucosal injury, hematogenous spread
<i>Parvimonas micra</i> infections after dental procedures							
Bartz <i>et al.</i> 2005	63/F	Hip prosthesis	Tooth extraction	Hip arthroplasty infection	Prosthetic joint surgical infected tissue and swab	6 months	
Bassa Malondra <i>et al.</i> 2008	74/M	Prosthetic mitral valve	Tooth extraction	Prosthetic Endocarditis	Blood	6 months	

Ko <i>et al.</i> 2015	61/M	Chronic hepatitis B, dyslipidemia, periodontal disease	Tooth extraction	Meningitis, bacteremia	Blood	14 days	Dental procedure, hematogenous spread
Jones <i>et al.</i> 2015	72/M	N/R	Tooth extraction	Spondylodiscitis	Vertebral biopsy specimens	2 months	
George <i>et al.</i> 2015*	45/M	Spondylolisthesis	Tooth extraction	Osteomyelitis, epidural abscess	Spinal surgical tissue and bone specimens	2 months	
Endo <i>et al.</i> 2015	55/F	N/R	Dental treatment	Epidural abscess, spondylodiscitis	Vertebral and abscess surgical tissue specimens	> 2 months	
Baghban <i>et al.</i> 2016	65/M	Diabetes mellitus, knee osteoarthritis, periodontal disease	Dental treatment	Knee arthritis	Knee joint synovial fluid	2 months	
Dietvorst <i>et al.</i> 2016	68/F	None	Dental treatment	Knee arthritis	Knee joint synovial fluid	2 months	
Cleaver <i>et al.</i> 2017**	45/F	Smoker	Dental treatment	Spondylodiscitis	Vertebral surgical tissue and pus specimens	Some years	

3 \* Doubtful case, also with medical history of spinal instrumentation 6 months before;

4 \*\* Doubtful case, also with medical history of intra-uterine device insertion 2 months before;

5 N/R: Not reported;

## \*Conflict of Interest

- 1 CONFLICT OF INTEREST:
- 2 All authors report no conflicts of interest.