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Case of *Shewanella putrefaciens* Gastroenteritis in Bulgaria – an Evaluation of *Shewanella* Role in Infectious Diarrhea

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Abstract

To emphasize the need of precise microbiological diagnosis of *Shewanella* gastroenteritis as well as the identification of both species - *Shewanella algae* and *Shewanella putrifaciens*.

Sixty-one years aged woman with diarrheal syndrome after fish consumption abroad attended the

Clinic of Infectious Diseases at the University Hospital in Plovdiv. The patient complained of watery stools, mixed with mucus; nausea with vomiting, stomach discomfort and low grade fever. A stool specimen was collected for microbiological examination. The isolate identification was performed using API-20NE (BioMerieux) and automated Vitek 2 system. The biotype detection was based on carbohydrate utilization of sucrose, maltose and arabinose. The antimicrobial susceptibility was determined by disk-diffusion test and minimal inhibitory concentrations.

Shewanella putrifaciens biotype I in pure culture was isolated from patient's stool specimen. It showed good susceptibility to tested antimicrobials except to tetracycline, cefalotin and ciprofloxacin. The latter was included in the therapy together with Enterol, Smecta, aqueus-saline and glucose solutions. No increased leukocyte count was observed. However the sedimentation rate was slightly increased and ketones were detected in urine. The patient was dehospitalized in reconvalescence after 4 days with diagnosis acute gastroenteritis.

This case is the first in Europe of proven *Shewanella putrifaciens* gastroenteritis. With this we aim to focus microbiological and clinical attention towards active looking for *Shewanella* participation in gastrointestinal infections. Species identification with routine and automated methods is mandatory since these bacteria possess different pathogenic potential and antimicrobial susceptibility.

Key words: *Shewanella,* gastroenteritis, antimicrobial susceptibility

Introduction

Infectious diarrhea primarily affects children in both the developed and developing countries. While in children the leading causative agents are rotavirues, gastroenteritis in adults has various etiology – noroviruses and *Campylobacter jejuni* are more common than other bacteria (*Salmonella, Shigella, Escherichia coli, Clostridium difficile*) and parasites (most commonly *Giardia lamblia*, but *Entamoeba histolytica* and *Cryptosporidium* spp. have also been implicated) [1,2]. Infectious diarrhea results in several billion cases worldwide and causes more than a million deaths a year. Antibiotics are generally not recommended. Two cases of acute gastroenteritis with bloody diarrhea have been reported in India, in both of which *Shewanella algae* was isolated. The cases were initially diagnosed as acute bacillary dysentery but later the isolates were identified by 16S r-RNA gene sequence analysis [3].

Shewanella spp. is a Gram negative motile rod, defined as saprophytic species whose natural

habitat are soil and water [4]. It was first isolated from tainted butter by Derby and Hammer in 1931 and was classified as *Achromobacter putrefaciens* [5]. Later, based on phylogenetic studies, these microorganisms have been reclassified in the *Vibrionaceae* family. In 1985 MacDonell and Colwell describe the *Shewanella* genus, named after James Shewan for his work in fisheries microbiology. After DNA hybridization analyzes in 2004 the new family *Shewanellaceae* was formed, including 30 species [6,7]. These species are to be found mainly in warm waters and seafood, but they have been as well isolated from fresh drinking water and other foods [8,9]. *Shewanella* infections have been reported mainly in countries with warm climate in USA, Africa, Asia and southern Europe [9,10,11]. *Shewanella spp*. is the only oxidase-positive non-fermenter microorganism producing hydrogen sulfide. It is cultivated in the usual nutrient media for intestinal bacteria - MacConkey, Eosin-methylene blue (EMB) and Deoxycholate agar media, at 37°C. After 24 hours of cultivation it grows in yellow pigmented colonies in most cases. The species most frequently isolated from clinical specimens are *Shewanella putrefaciens* and *Shewanella algae.* Both species, although very similar, differ in some biochemical and culture characteristics. *Shewanella algae* has the ability to grow at a temperature of 42° C and 6,5% NaCl the medium, but can not proliferate at a temperature of 4° C. On blood agar β-hemolytic zone is formed around the colonies [12,13]. Unlike *Shewanella putrefaciens* the other species - *S. algae*, hydrolyzed ribose but does not hydrolyze the sugars arabinose, maltose and sucrose. A major difference in the pathogenic arsenal is the production by Shewanella algae of tetrodotoxin (TTX) – a powerful neurotoxin, making it the cause of food toxic infections, especially in the consumption of poorly processed seafood [14,15]. Other pathogenic factors are hemolysines, siderophores and some exo-enzymes [9,12]. *Shewanella spp*. rarely cause diseases in humans, but recently an increased incidence of infections caused by these bacteria has been reported. Cases of cellulitis, abscesses, ear inflammation, wound infections, food poisoning and even bacteraemia have been described [9,15,16]. In most cases it was wrongly identified as *Pseudomonas spp*. instead of *Shewanella spp*., given that members of this genus are also Gram negative oxidase positive non-fermentative rods [17]. The major risk factors of *S. putrefaciens* infection are hepatobiliary disease, peripheral vascular disease with

chronic leg ulcer, poor hygiene, and socioeconomic status. *Shewanella* species are resistant to penicillin and most of the first and second generation cephalosporins, but there are poly-resistant isolates, which is a reason for caution towards the representatives of this genus. More than 80% of the reported isolates were identified as *Shewanella algae* [9].

The objective of this paper is to emphasize the etiological role of *Shewanella putrefaciens* in the development of infectious gastroenteritis by presenting a clinical case.

Clinical case

In the summer of 2014 the patient (R.S., 61-year old) with complaints of sore throat visited her general practitioner. He diagnosed her with catarrhal angina and prescribed antibiotic treatment with Levofloxacin. On the third treatment day the patient develops diarrhea syndrome characterized by watery stools mixed with mucus, nausea and single vomiting with a feeling of epigastric heaviness. After consultation in the emergency room of Infectious Diseases Clinic at the University Hospital "St. George "in Plovdiv, antibiotic treatment is changed with Ciprofloxacin 500 mg po every 8 hours in combination with the anti-diarrheal absorbent Smecta and the probiotic Enterol, containing *Saccharomyces boulardii*. Despite the three-day antibiotic therapy the diarrhea lasts with 4 to 8 defecations per day and low-grade fever of 37°C. After 4 days the patient was admitted to the Infectious Diseases Clinic with the diagnosis acute gastroenteritis, she was in mildly morbid general condition, had second degree dehydration, general weakness and intoxication. During history taking the patient shared that several weeks before she has visited a neighboring country (Turkey) and has consumed seafood. The other family member who was with her in Turkey, did not consume the same food and was healthy. Clinical tests did not reveal leukocytosis - WBC - 5.7×10^9/l (reference range 3.5 - 10.5×10^9/l), but there was slightly increased ESR - 26mm/h (up to 20mm/h in women over 60 years). Ketones were detected in urine as a result of dehydration; Hb - 121 g/l (reference range 120 -160 g/l); RBC - 3.7×10^{12}/l (reference range 3.9 – 5.3×10^{12}/l); Ht - 0.33 (reference range 0.40 – 0.54); PLT - 309×10^9/l (reference range 140 - 400×10^9/l); DBC (differential blood count): St 0% (

reference range 3-5%), Sg 35% (reference range 50- 62%), Eo 1% (reference range 0-3%), Ba 0% (reference range 0-1%), Mo 8% (reference range 3-7%), Ly 56% (reference range 25-40%); urea - 2.2 mmol/l (reference range 2.2 – 7.0 mmol/l), creatinine – 82 mmol/l (reference range 58-96 mmol/l), glucose - 4.5 mmol/l (reference range 2.78 – 6.2 mmol/l), urine – acetone (+) positive. The therapy continued with Ciprofloxacin, Enterol, Smecta, Mezym forte, Hidrasec, aqueous-salt and glucose solutions. Stool culture was taken for microbiological testing, from which pure culture of *Shewanella putrefaciens* was isolated. After 5 days of treatment the patient was discharged clinically healthy. Control microbiological tests subsequently proved the existence of the normal intestinal flora of *E.coli*.

Clinical samples were cultured on EMB, Apocholate citrate agar, and Selenite broth blood agar. Culture plates were incubated at 37°C for 24 h. Bacterial pure culture was detected, with lactose-negative colonies lacking dark center, normally associated with the production of hydrogen sulfide. Staining of microscopic preparation after Gram visualized gram negative rods. These data initially shifted diagnostic thinking to possible shigellosis. After positive oxidase test this hypothesis was rejected and thinking was oriented to *Pseudomonas, Aeromonas* or *NAG Vibrio* as other possible causes of infectious diarrhea. Biochemical tests from single colonies were made for identification of the isolate - Kligler-iron agar, tests for indole and urease production, Methyl Red and Voges-Proskauer on Clark and Lubs media for detection of acidic metabolic byproducts following fermentation, and Simmons' citrate test for the ability of bacteria to use citrate as a sole carbon source. Tests results showed that the isolate is non-fermenter glucose and lactose with abundant production of H_2S. We have hused the manual system API-20NE (BioMerieux) for the identification of non-fermenter bacteria and the automated system VITEK-2 (BioMerieux), both of which have proved the microorganism to be *Shewanella putrefaciens* (99.9%). The species was distinguished from *Shewanella algae* based of additional biochemical and culture features offered by other authors [8,9,11] and summarized in Table 1.

Cultivated on blood agar our isolate did not perform beta hemolysis. There was no growth in

Table 1. Biochemical differentiation between *Shewanella putrefaciens* and *Shewanella algae*

Tests	*Shewanella putrefaciens*	*Shewanella algae*
Maltose	+ d	-
Sucrose	+ d	-
Ribose	(+)	+
Arabinose	+ d	-
Hemolysis on Blood agar	-	+
Growth at 6,5%NaCL	-	+
Growth at 42˚C	-	+

∗ delayed positive reactions are indicated in brackets; d shows results which can vary

saline- milk agar, as well as upon cultivating at 42˚C. Based on the carbolytical profile to maltose, sucrose and arabinose sugars Khashe and Janda distinguish three biotypes *Shewanella putrefaciens:* biotype1- degrading all three sugars, biotype 2 - positive to maltose and arabinose, and biotype 3 - positive only to maltose [11]. Our isolate showed positive saccharolytic activity to maltose, sucrose and arabinose, which in combination with other microbiological data defined it as *Shewanella putrefaciens* biotype 1. Maltose and arabinose were positive after 24 hours. Sucrose test was positive 72 hours after the beginning of the study.

Another significant difference between the two types of Shewanella is their sensitivity to polymyxins (Colistin). It has been found that *Shewanella algae* is resistant to Colistin unlike *Shewanella putrefaciens* - a feature that can also successfully be used to differentiate between them [9]. The antimicrobial sensitivity of our isolate was determined by disk-diffusion test (DDT) of Bauer-Cirby, as well as by the minimal inhibitory concentrations (MIC) with VITEK-2 (Table 2).

Table 2 Antimicrobial sensitivity of *Shewanella putrefaciens*, isolated from the patient

No	Antimicrobial agent	DDT interpretation	MIC interpretation
1	Ampicillin	R*	ND
2	Cefalotin	R	ND
3	Ampicillin/Clavulanic acid	R	ND
4	Ticarcillin	ND	S
5	Ticarcillin/Clavulanic acid	ND	S
6	Piperacillin	ND	S
7	Piperacillin/Tazobactam	ND	S
8	Ceftazidime	ND	S
9	Cefepime	ND	S
10	Aztreonam	ND	S
11	Amikacin	S	S
12	Gentamicin	S	S
13	Tobramycin	ND	S
14	Imipenem	S	S
15	Meropenem	S	S
16	Ciprofloxacin	R	R
17	Pefloxacin	ND	R
18	Levofloxacin	S	ND
19	Colistin	S	S
20	Minocycline	ND	S
21	Rifampicin	ND	S
22	Trimetoprim/Sulfamethoxazole	S	S

23	Erythromycin	S	ND
24	Clindamycin	I	ND
25	Chloramphenicol	S	ND
26	Tetracycline	R	ND
27	Tigecycline	S	ND

*R – resistant, S - sensitive, I – intermediate, ND – no data

The isolated *Shewanella* showed sensitivity to a wide range of antimicrobial agents but it was resistant to the quinolone family, incl. ciprofloxacin used in treatment regimen of the patient.
Based on the epidemiological and clinical data of the patient (consumption of marine food in a warm country, severe diarrhea) and microbiological results (pure culture of non-lactose-fermenting colonies, biochemical tests, sensitivity to Colistin and confirmation of the preliminary microbiological diagnosis by the National Reference Laboratory at the National Centre for Infectious and Parasitic Diseases in Sofia) we resumed that the isolated *Shewanella putrefaciens*, biotype 1, was the causative agent of the infectious gastroenteritis of this case.

Discussion

Shewanella infections are unusual, especially for countries with mild climate, such as Bulgaria.
They can be presented as otitis media, skin ulcers of lower extremities and hands, infective endocarditis, arthritis, peritonitis and rarely as systemic infections [8,13]. Most of these patients have predisposing factors such as hepatobiliary disease, neutropenia, malignancy or prematurity. Even more rare are the cases of gastroenteritis, probably hypo-diagnosed due to lack of knowledge of these rare microorganisms and their identification as etiologic agents [8,14,15]. This is partially explained by the difficulties in the microbiological diagnosis of these bacteria. In differential diagnosis *Shewanellas* should be distinguished from members of the genus *Salmonella*, which also give lactose-negative colonies with a dark center, the genus *Shigella* (in case of non-manifested production of hydrogen sulfide), and genus *Pseudomonas*, *Aeromonas*

and *NAG Vibrio*, which are also oxidase positive Most often errors occur with the *Pseudomonas* genus, since after reading lactose-negative and oxidase positive colonies, most laboratories stop identification at this point, considering that the isolate is Pseudomonas. On the other hand, the clinical picture of *Shewanella* gastroenteritis more closely resembles diarrheal syndromes caused by Aeromonas and NAG Vibrio, but without blood in the stool [8]. Unlike reported cases of bloody diarrhea caused by *Schewanella algae* [3], our case of diarrhea *Schewanella putrefaciens* had no presence of blood [16]. *Shewanella* intestinal infections occur through direct contact with sea water or consumption of seafood, especially uncooked. The history of the patient reported no contact with sea water, but prior to hospital stay she had visited Istanbul where she had eaten freshly cooked fish, which probably was the cause of infection.

Some automated identification systems fail to differentiate between *S. putrefaciens* and *S. algae* because *S. algae* may not be included in the databases of these systems [8]. For this reason, it is believed that most *Shewanella* infections reported during recent years have been attributed to *S. putrefaciens*. This requires further identifying by different biochemical tests and culture features. The reasons for the shortcomings in diagnosis of the microbiological identification between the two types should be sought also in the delayed saccharolytic activity of *Shewanella putrefaciens*, which sometimes is positive on the seventh day.

Antibiotics are not usually used for gastroenteritis, although they are sometimes recommended if symptoms are particularly severe or if a susceptible bacterial cause is isolated or suspected. In our country the quinolones are among the most commonly used chemotherapeutic agents in the empiric treatment of severe infectious diarrhea in adults. Therefore the resistance of *Shewanella* to them raises serious concerns. Regarding antibiotic sensitivity, our isolate appeared resistant to Ampicillin, Cefalotin, Amoxicillin/clavulanic acid, Tetracyclin and Ciprofloxacin, used in the treatment regimen of the patient. Polyresistant strains of Shewanella spp, incl. resistance to Imipenem, have been rarely reported in the literature [18,19,20]. In the described clinical case early empirical antibiotic therapy with levofloxacin and ciprofloxacin diverged from the

antibiogram results. It has started earlier, prior to receiving data on the sensitivity of the isolate. As a result, we can conclude that the infection has proceeded as self-limiting, because after the 5-day stay in the clinic the patient was discharged as clinically healthy. We rule out the possibe catarrhal angina debut of the infection. Tonsillitis was probably of viral etiology from CMV, which is supported by DBC data for lymphocytosis with neutropenia on arrival of the patient for inpatient treatment, as well as positive anti-CMV-IgG two weeks after discharge. We believe that the Shewanella infection has developed against the temporarily weakened by virus infection immune system, and therefore the characteristic leukocytosis was not observed. Patient history, however, gives proof of an immunocompetent patients without evidence of frequent and recurrent infections.

This clinical case study outlines several features of *Shewanella* gastroenteritis: 1/ difficulties in the interpretation of the isolate as the etiologic agent, if medical history and epidemiological data of the patient are not taken into account; 2/ challenges for the bacteriological identification due to delayed saccharolytic activity of the bacterium; 3/ resistance to quinolones, necessitating a change in the empirical therapy, if it was applied; 4/ opportunity for self-limiting of the infection in immunocompetent patients.

Cases of *Shewanella* infections described in available literature report a trend of benign course in immunocompetent persons even in the presence of bacteremia [21,22]. Until recently, the pathogenic potential of *Shewanella* was disputable due to the fact that in most cases they have been isolated together with other bacteria, most commonly with E. coli [9,22]. Still the question remains open why *Shewanella algae* is reported as the dominant isolate in *Shewanella* infections in humans compared with other members of the *Shewanella* genus.

Conclusion

This case report emphasizes the necessity for further identification of the oxidase-positive, H_2S-producing non-fermenter gram-negative rods from stool specimen in patients with diarrhea. We

conclude that *Shewanella putrefaciens* although rarely considered as a human pathogen, can cause infectious diarrhea. To our knowledge this is the first reported case of severe *S. putrefaciens* biotype I gastroenteritis infection not only in Bulgaria but in Europe. It is aimed at focusing the attention of clinicians and microbiologists in the active search for *Shewanella* participation in infections of the gastrointestinal tract specifically in negative microbiological data for the most common bacterial pathogens - *Salmonella*, *Shigella* and *Campylobacter*. In the overall clinical and microbiological interpretation of intestinal infections thinking should be geared to *Shewanella* etiology, to assessment of patient immunocompetence and to searching history and epidemiological data related to residence in risky countries. Differentiation between the the two types *Shewanella* with routine or automated methods is imperative, as they have different pathogenic potential and antimicrobial susceptibility.

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