Peritonitis due to *ralstonia mannitolilytica* in a pediatric peritoneal dialysis patient

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SUMMARY

Ralstonia mannitolilytica constitutes a rare isolate in clinical specimens and to date very few infections with this Gram-negative bacillus have been reported. The first case of peritonitis in a pediatric patient due to *R. mannitolilytica* in the setting of peritoneal dialysis is described. It is very important to view this organism as a pathogen rather than contaminant when isolated in children with peritonitis.

KEY WORDS: Ralstonia mannitolilytica, Peritonitis, Peritoneal dialysis.

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INTRODUCTION

Ralstonia mannitolilytica is being identified as an opportunist human pathogen although it is widely distributed in nature as a frequent contaminant in water supplies (Daxboeck *et al.*, 2005). Specifically, it is identified as an opportunistic pathogen in nosocomial infections and it has also been implicated in common source of nosocomial outbreaks due to the addition of contaminated water to intravenous solutions and to oxygen-delivery devices (Gröbner *et al.*, 2007; Jhung *et al.*, 2007). Herein, we describe the first case of *R.mannitolilytica* peritonitis in a pediatric peritoneal dialysis (PD) patient.

CASE REPORT

A 6-year old girl who had end stage renal disease (ESRD) due to hemolytic uremic syndrome was treated with automated PD (APD). Her course on dialysis was complicated by numerous episodes of peritonitis, all due to Gram-positive bacteria. During the most recent 12-month period, there were a total of five episodes of peritonitis, two due to *Staphyloccocus epidermidis*, two due to *Corynobacterium* spp. and one due *Staphyloccocus capitis*. In the recent episode, she was admitted to the hospital with complaints of non localized abdominal pain with cloudy effluent and was brought in for consultation. Her abdomen was tender, with guarding and rebound pain and bowel sounds were diminished. The PD fluid was slightly opalescent and was sent for microbiological investigations. Empirical treatment with intraperitoneal vancomycin (30 mg/L) plus ceftazidime (125 mg/L) was initiated due to our department protocol. The PD fluid white cell count was 3500 cells/μL, with neutrophils cell count of 84% and Gram-negative bacilli were detected. Peripheral blood showed 9410 leukocytes/mm^3, with 74% neutrophils, erythrocyte sedimentation rate (ESR) 84 mm/h and C-reactive protein (CRP) 11.5 mg/L. There was no infection at the exit site and tunnel of the PD "Tenkhoff" catheter. Within 76 hours, PD fluid was clear and cell count was 0/μL. A sample of peritoneal effluent was collected for culture on conventional media and after incubation at 37°C for 24 h in aerobic environment, grew non-lactose fermenting, nonpigmented, smooth, convex and round

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colonies of Gram-negative rods that were catalase and oxidase positive (tablets; Rosco, Taastrup, Denmark) and esculin negative. Morphologically the rods were straight or slightly curved and motile with a single polar flagellum. They were resistant to desferrioxamine and colistin. Acid was oxidatively produced from glucose, lactose, mannitol, maltose, D-arabitol, D-xylose and L-arabinose but not with sucrose. Alkalization occurred on minimal mineral agar with acetate, butyrate, citrate, formate, fumarate, lactate, malate, malonate, serine, succinate, and β-alanine but not with acetamide, allantoin, maleate, tartarate, L-arginine and L-ornithine. No acid was produced from ethylene glycol with the nitrate and nitrite reduction tests being negative. In last, urease, pyrrolidonyl arrylamidase (Rosco), Tween esterase and phenylalanine deaminase were positive. The isolate was identified as *R. mannitolilytica* by a VITEK 2 system (bioMérieux, Marcy l'Etoile, France) and by conventional tests. Unfortunately, genome-based confirmation of *R. mannitolilytica* was not available. In vitro susceptibility testing was performed by Kirby-Bauer disc diffusion test on Mueller-Hinton agar according to NCCLS guidelines. From the antimicrobial agents tested, the required diameters for resistance, intermediate susceptibility, and susceptibility are given in parentheses for each one; aztreonam 30 μg (<16, 17-49, >50), ceftazidime 30 μg (<16, -, >16), cefepime 30 μg (<18, -, >18), ciprofloxacin 5 μg (<22, 23-24, >25), colistin 10 μg (<10, 11-12, >13), imipenem 10 μg (<17, 18-19, >20), levofloxacin 5 μg (<17, 18-19, >20) and meropenem 10 μg (<18, 19-23, >24). The isolate was found to be resistant to aztreonam, colistin and meropenem and sensitive to all other agents tested. Treatment with intraperitoneal ceftazidime (125 mg/L) was continued for a total of 21 days and the patient responded favorably without catheter replacement. However, the catheter adapter was removed and sent for culture with negative results. In addition, cultures of the patient taken from nose and hands shown negative result for the nose sample, but the hands sample was positive for *R. mannitolilytica*. After intensive hand hygiene with the use of a disinfection solution containing equal amounts of propranol and isopropanol (Sterillium® classic pure, BODE Chemie GmbH, Germany), hand culture became negative and remained negative. During peritonitis the APD program was changed to continuous ambulatory PD (CAPD) temporarily and at the end of peritonitis episode switched again to APD. Throughout the peritonitis course, CAPD was continued without ultrafiltration problems. When reviewed one week and one month after the end of treatment, she was well without any signs or symptoms of peritonitis.

DISCUSSION

Despite the wide acceptability of PD as a dialytic modality of choice for pediatric patients with ESRD, peritonitis remains a major complication and one of the most common causes of morbidity and treatment failure in such patients (Li *et al*., 2010). In children, as in adults, the most frequently encountered pathogens are Gram-positive organisms (40 to 60%), including coagulase-negative *Staphylococcus* followed by *Staphylococcus aureus* and *Streptococcus* spp. Gram-negative organisms, with the main representative being *Pseudomonas aeruginosa*, constitute the second most common cause of peritonitis (20 to 30%). Generally, the incidence of fungi is less than 5% (Li *et al*., 2010). Very rare cases have been reported of peritonitis due to *Ralstonia* spp. which are Gram-negative bacilli.

Species of the genus *Ralstonia* can be separated clearly into two genotypically and phenotypically distinct groups. Specifically, sequence analysis of the 16S rRNA gene indicates that two distinct sublineages are present within the genus *Ralstonia sensu lato* (Vaneechoutte *et al*., 2004). The *Ralstonia eutropha* lineage comprises *Ralstonia basilensis*, *Ralstonia campinensis*, *R. eutropha*, *Ralstonia gilardii*, *Ralstonia metallidurans*, *Ralstonia oxalatica*, *Ralstonia paucula*, *Ralstonia respiraculi* and *Ralstonia taiwanensis*. The other lineage, called *Ralstonia pickettii*, comprises *Ralstonia insidiosa*, *R. mannitolilytica*, *R. pickettii*, *Ralstonia solanacearum* and *Ralstonia syzygii* (Daxboeck *et al.,* 2005; Vaneechoutte *et al*., 2004). This genotypic distinction is supported by a number of phenotypic differences presented in Table 1. *R. mannitolilytica*, previously named *R. pickettii* biovar 3/"*thomassii*", infections in humans are reported to be infrequent. *R. mannitolilytica* has often being isolated from the respiratory tract of patients with cystic fibrosis (Coenye *et al*., 2002).

TABLE 1 - *Main differential phenotypic characteristics for Ralstonia mannitolilytica and other Ralstonia spp.*

Characteristic	*R. mannitolilytica*	*R. basilensis*	*R. campinensis*	*R. eutropha*	*R. gilardii*	*R. insidiosa*	*R. metallidurans*	*R. oxalatica*	*R. paucula*	*R. pickettii*	*R. respiraculi*	*R. solanacearum*	*R. syzygii*	*R. taiwanensis*
Flagellation	Polar, single	Peritrichous	Peritrichous	Peritrichous	Polar, single	Polar, single	Peritrichous	Peritrichous	Peritrichous	Polar, single	Peritrichous	None	None	Peritrichous
Susceptibility to:														
Colistin	R	S	S	S	S	R	S	S	S	R	S	R	R	S
Desferrioxamine	R	S	NK	R	R	R	NK	NK	R	S	NK	S	S	R
Production of acid from:														
Glucose	+	–	–	–	–	+	–	–	–	+	–	+	+	–
Mannitol	+	–	–	–	–	NK	–	–	–	–	–	–	–	–
D-Arabitol	+	–	–	–	NK	NK	–	–	–	–	–	–	–	–
Production of:														
Alkaline phosphatase (Rosco)	–	+	+	+	+	+	+	NK	–	+	+	–	–	+
Pyrrolidonyl arylamidase	+	–	+	+	–/+	+	+	NK	+	+	–	–	–	+
Reduction of:														
Nitrate	–	–/+	+	V	V	+	–/+	+	–	+	+	+	+	+
Assimilation of:														
3-Hydroxybenzoate	–	NK	NK	+	NK	–	–	NK	–	–	NK	–	–	NK
Propionate	+	NK	NK	+	NK	+	+	NK	+	+	NK	–	–	NK

S, sensitive; R, resistant; NK, not known; –/+, majority of strains negative; V, variable.

Additionally, it has been associated with catheter-related bacteremia (Gröbner *et al*., 2007), eye infections (Daroy *et al*., 2011), and very rare as a cause of urinary tract infection (Daxboeck *et al.*, 2005), recurrent meningitis (Vaneechoutte *et al*., 2001), infection of a hemoperitoneum (Vaneechoutte *et al*., 2001), post-renal transplant infection (Mukhopadhyay *et al*., 2003) and infection in chronic obstructive pulmonary disease (Zong & Peng, 2011). Hospital outbreaks of *R. mannitolilytica* due to contamination of water, saline solutions or oxygen-delivery devices have also been reported (Gröbner *et al*., 2007). To our knowledge, this is the first case of *R. mannitolilytica* peritonitis in a pediatric PD patient in the English medical literature and confirms the fact that this opportunistic pathogen can cause infections.

The probable source of infection and the portal of entry in our patient seem to be obvious. The fact that hands culture was positive for *R. mannitolilytica* indicates that contamination occurred via the hands. A previous study found that contamination of a respiratory gas humidification device had caused a national outbreak in the U.S. due to *R. mannitolilytica* in pediatric patients (Jhung *et al*., 2007). In another study, monoclonal outbreak of catheter-related bacteremia by *R. mannitolilytica* on two hemato-oncology wards was presented (Gröbner *et al*., 2007). These studies confirm that *R. mannitolilytica*, as already known for other *Ralstonia* spp., exhibit biofilm formation in plastic devices. In addition, the response of Gram-negative peritonitis in pediatric PD patients treated with empiric intraperitoneal ceftazidime seems to be often suboptimal (Li *et al*., 2010). However, in our patient after the appropriate treatment culture became negative and remains negative without the need for catheter removal, which is a common procedure after peritonitis due to Gram-negative pathogens (Li *et al*., 2010).

In summary, *R. mannitolilytica* peritonitis asso-

ciated with CAPD in pediatric patients is rare. Nevertheless, it requires prompt treatment and aggressive care to avoid catheter replacement and result in a favorable outcome.

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