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Isolation and identification of *Sphingomonas paucimobilis 503* from water: *A* case report

Noha Awwad[1], Dalia Talat[1], Hany F. Ellkany[2], Madiha Salah Ibrahim[1*]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Damanhour University, Egypt
[2]Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Damanhour University, Egypt

Abstract

In this study, the bacterium *Sphingomonas sphingomonas* 503 was isolated from water samples. One ml of water sample was cultured on MH broth for enriching bacterial growth then subcultured on MH agar plates till obtaining pure separate deep yellow-pigmented colonies (after four subcultures). The bacteria were cultured on blood agar and chromogenic agar, giving white hemolytic and green colonies, respectively. The bacterial isolates showed Gram-negative, polymorphic rods without special arrangement. The bacteria was identified by the VITEK Compaq® 2 system as *Sphingomonas paucimobilis 503* and assayed for antibiotic susceptibility using Ampicillin, Ampicillin/sulbactam, Piperacillin/tazobactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Meropenem, Amikacin, Gentamycin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Trimethoprim/Sulfamethazole. This bacterium was sensitive to all tested antibiotics except Ceftazidime, which showed the highest MIC ≥ 64, while the lowest MIC was that of Levofloxacin ≤0.12and Ciprofloxacin ≤ 0.25.

Keywords: *S. paucimobilis* 503; Biochemical identification; Antimicrobial susceptibility

*Correspondence: Madiha Salah Ibrahim
Department of Microbiology, Faculty of Veterinary Medicine, Damanhour University, Egypt
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1. Introduction

Sphingomonas paucimobilis is a Gram-negative bacillus extensively dispersed in natural and nosocomial environments, obligate aerobic, oligotrophic opportunistic yellow-pigmented, glucose non-fermenting, capsuled, non-spore-forming Gram-negative bacillus with a single polar flagellum (Lin et al., 2010). *Sphingomonas* has been detected in water and soil (e.g., pipelines, bathtubs, distilled water, and hemodialysis fluid) (Vaz et al., 2011). *Sphingomonads* are found in diverse natural environments playing an important role in nutrient cycling, especially in oligotrophic settings in particular (Aylward et al., 2013). Some have been found in plant and animal ecosystems and are linked to an increase in health-care-associated infection (Aylward et al., 2013; Narciso-da-Rocha et al., 2014). *Sphingomonads* are able to co-aggregate and create biofilms while surviving chlorination in tap water. This group has a large number of phenotypically and phylogenetically similar strains. According to Takeuchi et al (2001), the genus *Sphingomonas* was separated into four clusters and three new genera based on phylogenetic analysis of 16S rRNA gene sequences as well as some chemotaxonomic and phenotypic distinctions. *Novosphingobium*, *Sphingobium*, *Sphingomonas*, *Sphingopyxis*, *Sphingosinicella*, *Sphingomicrobium*, *Sphingorhabdus*, and *Parasphingopyxis* are the eight genera of *sphingomonads* that exist today (Stolz, 2013). This study aimed to isolate *S. paucimobilis* from drinking water and investigate its morphological and cultural characteristics using gram staining and various media, respectively. The VITEK Compaq® 2 system was used to identify and characterize the isolate for antibiotic suceptibility.

2. Materials and Methods

2.1. Samples and Culture media

Water samples were collected from drinking water that had been kept in the refrigerator for a long time. A 1 mL sample of water was cultured on Muller Hinton (MH) broth and agar (Sigma Aldrich) according to Krishna et al (2011), Chromogenic agar medium (Conda lab) according to André et al (2010), blood agar medium and MacConkey agar medium (HIMEDIA) and incubated at 37°C for 24-72 hr.

2.2. Gram staining

Bacteria were subjected to gram staining according to Kulkarni et al (2020).

2.3. Identification with VITEK Compaq® 2system

VITEK Compaq® 2 system was used for biochemical identification and detection of antimicrobial susceptibility of the isolated bacteria, according to Cengiz et al (2015).

3. Results

3.1. Bacterial identification

After 48 hours incubation, the bacterial growth was shown as deep turbidity, unlike the control nagative tubes that were clear without any turbidity on Muller Hinton (MH) broth. In contrast, on Muller Hinton agar, the bacteria showed deep yellow rounded pigment colonies of medium size, moist surface, and mucoid in appearance (**Figure 1**). Unlike on blood agar medium, where the bacteria showed a white opaque hemolytic colonies of medium size, moist mucoid like appearance (**Figure 2**). Furthermore, the bacteria showed deep green pigmented medium sized colonies on Chromogenic agar medium (**Figure 3**) and the bacteria showed no growth on Macconkey agar medium.

3.2. Gram staining

Revealed a gram negative polymorphic bacilli (rods) without special arrangment (**Figure 4**).

3.3. Biochemical identification of the isolated bacteria

The bactria was subjected to VITEK Compaq® 2 system analysis. Results revealed that the bacterial isolate was identified as *S. paucimobilis* 503 (**Table 1**).

3.4. Antimicrobial susceptibility test

S. paucimobilis was tested for antibiotic susceptibility by the automated method in VITEK Compaq® 2 system. The bacteria showed sensitivity to all tested antibiotics except Ceftazidime which showed the highest MIC (≥ 64 µg) while the lowest MIC was with Levofloxacin (≤ 0.12) and Ciprofloxacin (≤ 0.25) (**Table 2**).

Figure 1. a) Deep yellow rounded pigmented colonies of *S. paucimobilis 503* on MH agar; b) negative control plate of MH agar.

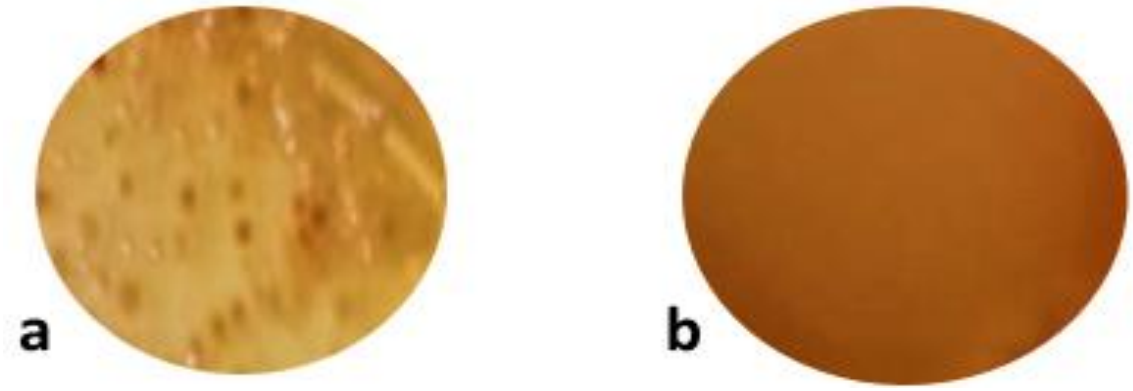

Figure 2. a) White to off-white colonies of *S.paucimobilis* on blood agar; b) control Blood agar plate.

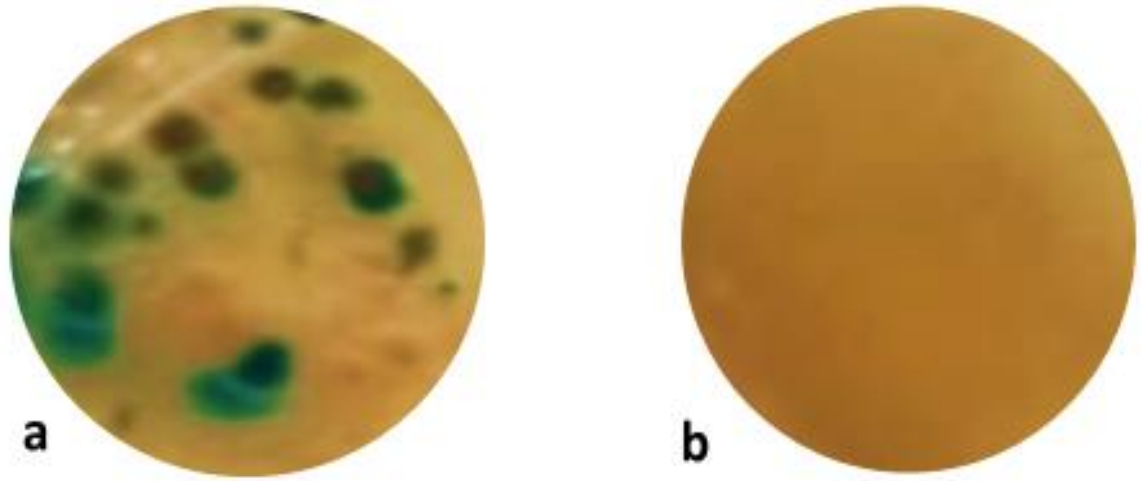

Figure 3. a) Deep green colonies of *S.paucimobilis* on chromogenic agar; b)control negative plates of chromogenic agar without culture.

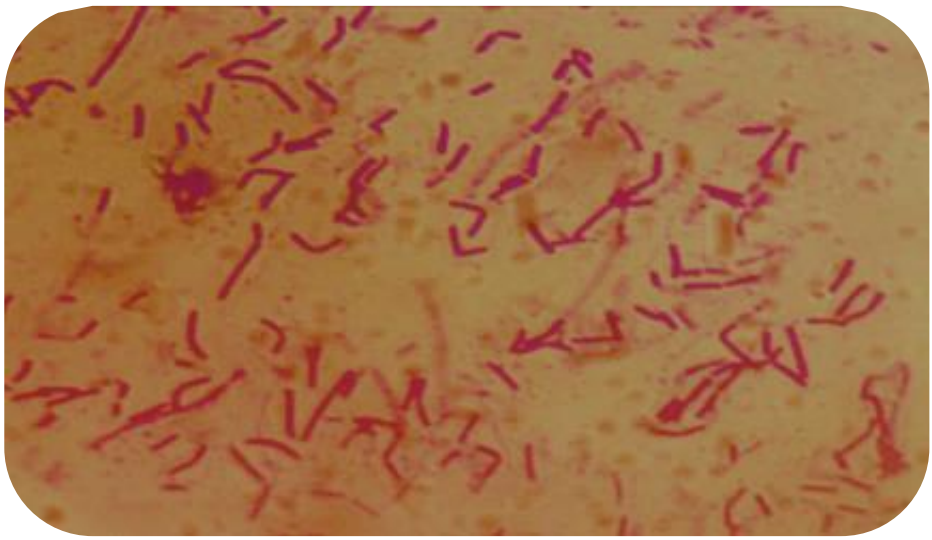

Figure 4. Gram staining of *S. paucimobilis* revealing gram negative polymorphic bacilli (red rods).

Table 1. Biochemical identification of *S. paucimobilis* 503

Biochemical analysis											
APPA	+	*ADO*	-	*PyrA*	+	*IARL*	-	*dCEL*	-	*BGAL*	-
H2S	+	*BNAG*	+	*AGALTp*	-	*dGLU*	+	*GGT*	-	*OFF*	-
BGLU	-	dMAL	+	dMAN	-	dMNE	-	BXYL	–	BAIap	–
ProA	-	LIP	–	PLE	–	TyrA	-	URE	–	dSOR	–
SAC	-	dTAG	–	dTRE	+	CIT	–	MNT	–	5KG	–
ILATk	+	AGLU	+	SUCT	-	NAGA	-	AGAL	-	PHOS	+
GlyA	-	ODC	-	LDC	-	IHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	–	IMLTa	–	ELLM	–	ILATa	–	*BGAL*	-

Biochemical profiles created with the Vitek 2 compact system helped to identify *S. paucimobilis* 503 (bioMerieux, France).

Table 2. The antimicrobial susceptibility of *S.paucimobilis* 503

Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
ESBL			Cefepime	8	S
Ampicillin			Meropenem	1	S
Ampicillin/suibactam			Amikacin	≤ 2	s
Pipracillin /tazobactam	≤ 4	S	Gentamycin	≤ 1	s
Cefazolin	≤ 4	S	Tobramycin	≤ 1	S
Ceftazidime	≥ 64	R	Nitrofurantoin		
Levofloxacin	≤0.12	S	Trimethoprim /Sulfamethazole	≤ 20	S
Ceftriaxone	8	S			

Antibiotic susceptibility test created by Vitek 2 system (bioMerieux, France).

4. Discussion

Sphingomonas paucimobilis is an opportunistic human pathogen that is thought to be infrequent. This organism can be abundant in natural world (particularly in water and soil). *Sphingomonas* spp. is a Gram-negative rod that is aerobic, non-fermentative, and oxidase positive. Despite its lack of familiarity, the bacterium is highly pathogenic and causes serious clinical symptoms (Ryan and Adley 2010). Bacteria can be found in animal and human tissues and can cause diseases, particularly in the respiratory system, due to qualities such as bacterial growth temperature, widespread distribution in the environment, and the ability to cling on air by catching dust (Koskinen et al., 2000, Tokajian et al., 2008). Another study on the identification of the bacteria has revealed its high pathogenicity, demonstrating that it causes severe illnesses with various clinical symptoms in numerous body systems (Maragakis et al., 2009).

Sphingomonas species are found worldwide and have been isolated from soil, subterranean sediments, and plants in the past (Kawahara et al., 1994; Fredrickson et al., 1995; Takeuchi et al., 1993; Mueller et al., 1997). *Sphingomonas* has also been found in seawater (Cavicchioli et al., 1999), sea ice (Bowman et al., 1997), river water (Tabata et al. 1999), wastewater (Neef et al., 1999), polluted groundwater (Mannisto et al., 1999), mineral water (Ferreira et al., 1996; Vachee et al., 1997, and Geldreich, 1996). *Sphingomonas*' ability to live and develop in low-oxygen environments explains their worldwide distribution.

Sphingomonas organisms have been found in drinking water on rare occasions, according to reports (Fredrickson et al., 1999). Using the VITEK Compaq® 2 system, *S. paucimobilis* 503 was isolated and characterized from drinking water.

The cultural characteristics of *S. paucimobilis* was investigated by culturing on different medium under strict aerobic conditions where colonies were observed; yellow-pigmented, flat or raised with entire margins 2 to 3 mm colonies following a 24-hour incubation period in an aerobic environment at 37°C on MH agar, few colonies appeared deep yellow or mustard colored according to Paul et al (2007) and Ryan and Adley (2010). After 48-73 hours of aerobic incubation at 37°C on blood agar, white to off-white colonies (dew drop-like colonies) raised with whole edges colonies were noticed. However, after 48 hours of aerobic incubation at 37°C on chromogenic agar medium, deep greenish colored, flat, or elevated colonies with whole edges were noticed, unlike in case of MacConkey agar, there was not any growth of *S. paucimobilis* (Kulkarni et al., 2020)**.** Morphology was examined by gram staining of a fresh cultured colony of *S. paucimobilis* revealing that it is a gram-negative bacillus (red rods), polymorphic without special arrangement in accordance with Paul et al (2007), who investigated the morphological characteristics of *S. paucimobilis*, discovering that it is 0.3-0.8 x 1.0-2.7 m in size, nonsporting, and capsulated. Even though the cells have a single polar flagellum, only a small percentage are actively motile. As a result, *paucimobilis* was coined. Motility occurs at temperatures ranging from 18 to 22 °C but not at 37°C. Using an automated bacterial identification system, VITEK Compaq® 2 system according to Cengiz et al (2015) for biochemical identification of the bacterium, the biochemical profile revealed that the bacterial isolate was identified as *S. paucimobilis* 503 Also, the bacteria were tested for the antimicrobial susceptibility using VITEK Compaq® 2 system against the following antibiotics: Ampicillin, Ampicillin/sulbactam, Piperacillin /tazobactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Meropenem, Amikacin, Gentamycin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Trimethoprim/Sulfamethoxazole. The bacteria were susceptible (sensitive) to all tested antibiotics except Ceftazidime, which showed the highest MIC (≥ 64) while the lowest MIC was that of Levofloxacin (≤ 0.12) and Ciprofloxacin (≤ 0.25).

5. Conclusion

S. paucimobilis was isolated from water samples. It was identified based on the morphological and cultural characteristics as gram-negative polymorphic bacilli, aerobic, generating deep yellow colored colonies on MH agar, white colonies on blood agar, and green colonies on chromogenic agar. The automated Vitek 2 system identified it as a *S. paucimobilis* 503 species. The bacteria were further analyzed for antibiotic susceptibility using the VITEK Compaq® 2 automated system, which revealed that all antibiotics tested, except Ceftazidime, were highly sensitive.

Conflict of interest: None.

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