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Review Article

Bleached Sphingomonas Paucimobilis

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Abstract

Sphingomonas are ubiquitous bacteria, widely distributed in the nature, soil and water including the water sources in the hospital environment, contains at least more than 30 species, which of only *paucimobilis* is an occasional pathogen.[1] It is one of the non-fermenting Gram-negative bacilli that is emerging as an opportunistic pathogen [7]. *S.paucimobilis* is considered to be the organism of low virulence likely owing to absence of endotoxin and typical polysaccharide and thus responsible for less mortality and morbidity but sometimes can lead to septic shock. To support this, retrospective study conducted at our hospital on total 1580 inpatient (IP) blood samples for culture yielding positivity of 0.44% for the growth of *S.paucimobilis*, did favour the survival of the all the patients. All the blood isolates produced distinct white or off-white coloured colonies on blood and chocolate agar hence referred as "Bleached *Sphingomonas paucimobilis."*

Keywords: *Sphingomonas paucimobilis*, blood culture, white or off white or bleach coloured colonies, susceptibility.

INTRODUCTION

The genus Sphingomonas widely distributed in the nature, soil and water including the water sources in the hospital environment, contains at least more than 30 species, which of only paucimobilis is an occasional pathogen. Currently, there are more than 30 species with validly published names in the genus S. sensustricto [1-12].

Sphingomonas are ubiquitous bacteria that have been reported to be found in water (i.e. the pipes, bathtubs, distilled water, sea water, sea ice, river water, mineral water, haemodialysis fluid, ultrapure distilled water systems) the space shuttles and dental irrigation systems, nebulizers and hospital water systems and have colonised invertebrates collected from these water distribution systems [1-12]. Sphingomonas species have been linked to the death of coral reefs off the coast of Florida and some species are phytopathogenic such as Sphingomonas meloni [7].

It also has the ability to form the biofilms in the plumbing systems thus causing biological accumulation and spreading in the industrial and drinking water systems. Moreover, it can pass through 0.2 um filters that are traditionally used for the terminal sterilization of several medicinal products [7].

One of the best-known species of the genus, S. paucimobilis (type strain ATCC 29837), was regarded originally as the only representative of clinical importance. Recently, two novel species Sphingomonas mucosissima and Sphingomonas adhesiva have been described that are of moderate clinical importance. However, S. paucimobilis is still regarded as the main pathogenic species of this genus [7]. It is thought to be opportunistic pathogen and rarely reported in the clinical settings [1]. Nowadays, most cases of Sphingomonas paucimobilis are health care associated, majority in immunocompromised hosts [1]. It is one of the non-fermenting Gram-negative bacilli that is emerging as an opportunistic pathogen [7]. S.paucimobilis is considered to be the organism of low virulence, likely owing to the absence of endotoxin and typical polysaccharide but sometimes can lead to septic shock [1-12].

History

Sphingomonas paucimobilis was first reported to cause infection in humans in 1979 when it was isolated from a pure culture specimen of a sailor who had developed a leg ulcer; and at this time, it was known as Pseudomonas paucimobilis. It was renamed Sphingomonas paucimobilis in 1990 based on the phylogenetic data. The genus Sphingomonas was first proposed by Yabuuchi et al., and was amended by Takeuchi et al and divided into four genera:

Sphingomonas sensustricto, Sphingobium, Novosphingobium and Sphingopyxis. Currently,there are more than 30 species with validly published names in the genus S. sensustricto [7].

Morphology and Cultural Characteristics

Sphingomonas paucimobilis is strictly aerobic, oligotrophic, yellow pigmented, catalase positive, glucose non-fermenting, nonsporing, capsulated, polymorphic, 0.3-0.8 x 1.0-2.7µm size Gram-negative bacillus with a single polar flagellum. Though weakly positive, occasional strains (10%) may be oxidase negative. They are strong esculin hydrolysis positive. It does grow on blood agar and chocolate agar but not on MacConkey agar and usually produce yellow pigmented or off-white, 1 to 2 mm, circular, opaque, flat or raised with entire margins colonies after 48 hours of aerobic incubation at 37 ^{o}C. Few colonies do appear after 24 hours of aerobic incubation. Old colonies do show deep yellow or mustard colour [5-7, 13].

Despite presence of the single polar flagellum, only low percentage of the cells are actively motile. Hence the name paucimobilis. Motility occurs at 18 to 22^{0}C but not at 37^{0}C.They produce a zone of inhibition around vancomycin disc (30 µg), aids in identification [13].

Most of the cultural and biochemical characteristics of S.paucimobilis and S.parapaucimobilis are similar, they do differ as depicted in the Table-1.

Table-1: Display of difference between S.paucimobilis and S.parapaucimobilis

S. No	Characteritistics	S.paucimobilis	S.parapaucimobilis
	Gram stain	Gram negative bacilli	Gram negative bacilli
	Catalase	+	+
	Oxidase	+	+
	Indole	_	_
	Citrate	_	+
	Esculin hydrolysis	+	+
	H_2S in lead paper	_	+
	Growth on MacConkey agar	_	_
	Polymyxin B susceptibility	Susceptible	Variable
	Vancomycin susceptibility	Susceptible	Susceptible
	Yellow pigment	+	+
	Motility at 18 to 22 ^{0} C	+	+

Experimental Design

Retrospective study done at our hospital from 02/02/2017 to 02/02/2019 on total adult 1580 inpatient (IP) blood cultures, did support the growth Sphingomonas paucimobilis in seven cases (0.44 %) only whereas one each of wound swab, CSF and urine culture was positive for the growth of Sphingomonas paucimobilis during this specified time.

All the clinical samples were inoculated on blood agar, chocolate agar, MacConkey agar and urichrome agar (chromogenic media) and incubated for more than 7 days at 37^{0} C. All the ten isolates did grow only on blood agar and chocolate agar. On blood agar, all the isolates produced 2 to 3 mm white or off-white coloured resembling white bleach, opaque, flat with entire margins, nonhemolytic colonies at 37^{0} C after 2 days of subculture as shown in figure 1. Equivalently, similar colonies were seen on Chocolate agar as shown in Figure-2.

Figure 1
Bleached *Spingomonas paucimobilis*
Nonhemolytic white or off white coloured colonies on blood agar

Figure 2

Bleached *Spingomonas paucimobilis*
White or off white coloured colonies on Chocolate agar

None of the colonies produced classic yellow pigment after prolonged incubation (>10 days) at 37^0C and room temperature. Hence the colonies were referred as "Bleached Sphingomonas paucimobilis". All the isolates were catalase and weakly oxidase positive and nonmotile. Gram stain done from all the colonies showed noncapsulated, nonsporing, 1x1.5 µm size, short gram negative bacilli or coccobacillary forms. All of them were identified up to the species level by automated Vitek 2 (bacterial identification system.) Antimicrobial susceptibility testing was done by Kirby Baur disc diffusion method and the organism was tested against ampicillin, ceftazidime, cefotaxime, ceftriaxone, cefadroxil, cefoperazone, cefoxitin, cefepime, ampicillin+sulbactum, amoxicillin+clavulanic acid, piperacillin+tazobactum, ceftazidime+tazobactum, cefoperazone +sulbactum, ticarcillin+clavulanic acid, aztronam, ciprofloxacin, ofloxacin, gentamicin, tobramycin, amikacin, imipenem,meropenem, ertapenem, doripenem, colistin, tigecycline, polymyxin b, cotrimoxazole, chloramphenicol, tetracycline and azithromycin. All Spingomonas paucimobilis recovered from blood and urine were susceptible to all antibiotics except three blood isolates, one urinary and CSF isolate displayed varied resistance pattern as shown in Table-2.

Table-2: Showing number of isolates resistant to the antibiotics.

SAMPLE	TOTAL ISOLATES	CAZ	TCC	AT	AMP	CPM	COT	CFR	IPM	CFS
BLOOD	7	3	3	2	3	1	1	2	2	2
WS	1	-	1	-	1	-	-	-	-	-
CSF	1	-	-	-	1	-	-	-	-	-
URINE	1	-	1	-	-	-	-	-	-	-

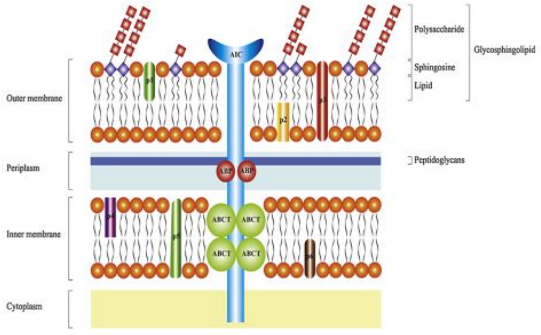
Note: Ceftazidime(CAZ), Ticarcillin+clavulanicacid(TCC), Aztreonam(AT), Ampicillin(Amp), Cefepime(CPM), Cotrimoxazole(COT), Cefadroxil(CFR), Imipenem(IPM), Cefoperazone+sulbactum(CFS.)

Structure

The bacterial wall of Sphingomonas paucimobilis is characterized by the presence of an inner cytoplasmic membrane, separated from the outer membrane by the periplasm, a structure that is composed of a thin layer of peptidoglycans in gram-negative bacteria. It expresses glycosphingolipids on its external surface. Despite the presence of several membranous and transmembraneous proteins, this bacterium carries an alginate import complex (AIC), associated with periplasmic alginate-binding proteins (ABP) and cytoplasmic membrane-linked ATP-binding cassette transporters (ABCT), to assist in alginate incorporation into the cytoplasm, preventing bacterial phagocytosis as represented in Figure-3 [5].

Figure 3

Structure of *Sphingomonas paucimobilis* bacterial wall.



Unlike other gram-negative bacteria, this organism lacks the distinctive frequently encountered lipopolysaccharide membrane and its related endotoxin activity, but expresses several different glycosphingolipids on its outer membrane. Besides the presence of a unique Sphingoglycolipid, the bacteria do possess the long-chain base dihydrosphingosin, ubiquinone10(Q-10), and 2-hydroxymyristic acid(2-OHC14:0) and the absence of 3-hydroxyfattyacids [7]. By maintaining the bacterial wall immunogenicity and protecting it from antimicrobial agents, glycosphingolipids have been suggested to function as a substitute for lipopolysaccharide in Sphingomonas paucimobilis.Sphingomonas deposit high strength and adhesive exopolymers that promote bacterial cell bridging, thus producing and reinforcing the biofilm scaffold over colonized surfaces [5].

Such experiments highlight the role of glycosphingolipids and exopolymers in enhancing the adhesive properties of the Sphingomonas genus through altering the hydrophobicity of the underlying bacterium-surface interface. The presence of glucuronic acid among the exopolymers synthesized and deposited by Sphingomonas paucimobilis corroborates these findings, since this molecule is negatively charged, and may hence help in connecting surfaces to foulants. Through their glycosphingolipid units, Sphingomonas species also contribute in promoting a favourable, conditional and rigid scaffolding core to recruit other lipopolysaccharide-positive gram-negative organisms to dual-species biofilms. An evolutionary analysis revealed that Sphingomonas paucimobilis expresses several virulence associated genes. Genes pertaining to cellular survival were shared with Brucella species, whereas adhesion and motility genes were mutually expressed with Legionella species. Many virulence-related genes were also co-expressed by Sphingomonas paucimobilis and either Pseudomonas or Bordetella sp [5, 6].

The enzyme profile of S. paucimobilis has several different aspects of interest. Several enzymes were detected which may contribute to the virulence of S. paucimobilis. For example, in earlier studies S. paucimobilis strains showed evidence of deoxyribonuclease activity and the present study confirmed the activity of esterases and phosphatases which may be involved in the virulence of these bacteria. Those enzymes identified included esterase (C4), esterase lipase (C8), alkaline phosphatase, and acid phosphatase as shown in Table-3. The tabular display also presents the comparing results of S.paucimobilis with in F. multivorum (group IIK biotype 2) in which it was previously included [4].

Table-3: Display of enzymatic reactions of S.paucimobolis and F. multivorum

Enzymes	S.paucimobolis	F. multivorum
Alkaline phosphatase	+	+
Acid phosphatase	+	+
Phosphoamidase	+	+
β-Galactosidase	+	+
α-Glucosidase	+	+
N-Acetyl-3-glucosaminidase	+	+
Leucine AP	+	+
Valine AP	+	+
Esterase lipase (C8)	+	+
Esterase (C4)	+	-
Lipase (C14)	+	-
α-Galactosidase	+	-
β-Glucosidase	+	-
Cystine AP	+	-
β-Glucuronidase	-	-
α-Mannosidase	-	-
α-Fucosidase	-	-

Note: AP, Aminopeptidase.

Endotoxin-lipid A fraction of this bacterium has a sphingolipid-structure that stimulates mononuclear cells 105 times less than lipid A and causes secretion of TNF-α, interleukin-1 and interleukin-6. That explains the indistinct clinical symptoms and less mortality and morbidity and better outcome [12].

Risk Factors

IV drug users, malignancy (57.1%) like gall bladder carcinoma, diabetes (11.9%), alcoholism (9.5%), immunosuppressed states, immunosuppressive drugs (40.5%), liver cirrhosis (9.5%), ESRD (7.1%), COPD (4.8%), burn injury (2.4%), and acquired immunodeficiency syndrome (2.4%) and catheter-related infections act as mitigating factors for acquisition of Sphingomonas infection. The figures in

the parentheses do represent the percentage of risk factors responsible for infection as per one school of thought [1-12].

Pathogenicity

This organism is found to have a significantly different enzyme profile from other bacteria, which may contribute to its pathogenesis.

The presence of atypical lipopolysaccharide constituents of the outer cellular membrane of S. paucimobilis that may correspond to the lipid A present in other Gram-negative bacteria, with the accompanying deficiency in endotoxin activity, has been proposed to explain the low virulence of S. paucimobilis. Although S. paucimobilis may be considered to have low virulence, it can still cause life-threatening septic shock, especially in immunocompromised hosts [2].

The mean age has been reported to be 48 years with range from 5 months to 87 year. A wide variety of community-acquired and hospital-acquired infections including bacteremia, catheter-related infections, meningitis, peritonitis, osteomyelitis, endophthalmitis, septic arthritis, urinary tract infections, biliary tract infections, lung empyema, cutaneous infections, diarrhoea, and pneumonia have been associated with Sphingomonas [1].

Sphingomonas paucimobilis presenting as acute phlebitis in heroine drug user has been documented [1].

In majority of the reported cases, Sphingomonas did cause community acquired bacteraemia. As per 2017 study, a total of 40 patients with S. paucimobilis bacteremia were identified. Among them, 10 (25.0%) were community-acquired. Primary bacteraemia was the most frequent (37.5%), followed by pneumonia (27.5%), gastrointestinal infection (25.0%). Only 2.5% presented with catheter-related infection. This could be reflecting the changing scenario of clinical manifestations of Sphingomonas infection [10]. Similar results are documented by another comparing study, primary S. paucimobilis bacteremia (35.7%), Catheter-related bloodstream infection (33.3%), skin and soft tissue infection (9.5%,) pneumonia (9.5%), urinary tract infection (4.8%), biliary tract infection (4.8%), and meningitis (2.4%) [2].

Besides adult, S.paucimobilis do cause healthcare associated infections in paediatric patients specially in hemato/onchology wards ranging from 1.08 to 13.3%, most frequent being bacteremia. But it did not result in considerable mortality and morbidity rates [3]. On the contrary, Bayram et al., has reported 24 cases of Sphingomonas infection in paediatric patients, 3.13 % of them had community acquired infection with a clinical presentation of primary bacteremia in 12 cases and in only one case patient had UTI. Among the 24 cases, he had reported 16 patients did not have any underlying disease [6].

As per another study, Sphingomonas paucimobilis isolates were recovered from 24 paediatric blood samples. The median age was 4 years (ranging from 3 days infant to 15 years) and 58.3% were male. Eight (33.3%) of the patients were under 1 months of age.

Lemaitre et al reported tracheal colonization with S.paucimobilis in mechanically ventilated neonates due to contaminated ventilator temperature probes and Mutlu et al., reported an outbreak of S.paucimobilis septicemia in a neonatal intensive care unit. Eleven of 24 patients had underlying diseases and co-morbidities including 4 acute lymphocytic leukaemia with neutropenia, 2 surgical co-morbidities (duodenal atresia and imperforated anus), 1 Down syndrome, 1 steroid induced immunosuppression due to post streptococcal acute glomerulonephritis, 1 burn injury, and 2 history of prematurity. Among the patients; 13 (54.2%) infections were community related however 11(45.8%) infections were health care associated infections. The median duration of hospital stay was 7 days (ranging from 4 to 22 days). The most effective antibiotics were fluoroquinolones (ciprofloxacin), carbapenems(imipenem) and trimethoprim/sulfamethoxazole (8.3%) [11] S.paucimobiis do cause bone and soft tissue infections, frequently encountered are osteomyelitis, cellulitis, and arthritis [5].

It has also been rarely known to cause PD-associated peritonitis and is notorious for its resistance to the commonly used antibiotics. In half of the cases reported so far, the peritonitis was refractory to treatment, necessitating PD catheter removal. Peritonitis due to this organism tends to have a variable outcome, as can be seen from the cases reported so far which is contradictory to the usual findings due to its low virulence. As per one case report of peritonitis due to CAPD since 28 months, initially noncompliance to combination of intraperitoneal vancomycin and IV ciprofloxacin, responded well to intraperitoneal tobramycin (4 mg/L) and intravenous meropenem for the duration of three weeks. Repeat culture of PD fluid after the completion of treatment was sterile. No recurrence of peritonitis occurred during the subsequent three months of follow-up and PD catheter was salvaged [8].

Lab Diagnosis

It is identified by cultural characteristics and biochemical reactions. Nowadays, it correctly identified by rapid kits API ID32GN system (bioMerieurx, Marcy L Etoile, France), automated Vitek2 and arbitrarily primed polymerase chain reaction. The susceptibility testing is done by KB method and E test [1-14].

It is correctly identified by MALDI-TOF mass spectrometry.

Treatment

Usually, they show susceptibility to wide range of antibiotics such as tetracycline, chloramphenicol, cotrimoxazole, aminoglycosides, fluroquinolones and levofloxacin. Besides this, they respond to cefotaxime, ceftazidime, imipenem, meropenem, piperacillin/tazobactum, carbenicillin, cefepime but do appear resistant to ampicillin, colistin, cephalothin and streptomycin. It is considered to have limited virulence, probably related to the lack of lipopolysaccharide A in its cell wall. These findings do explain the relatively favourable clinical outcome of the patients with S paucimobilis infections [1-3].

Survival has been reported to be favourable, reportedly as high as 95%, despite initial usage of inappropriate antimicrobials. The mortality rate is about 6% [1].

There are no standardized and recommended for therapies of S.paucimobilis infections. Thus, antibiotic treatment rests on clinical experience. As per one study, the duration of the treatment in paediatric patients was 7–13 days according to the clinical response of the patients [11]. Therapeutic failure to oral quinolone may be associated with lower bioavailability of drug or irregular oral therapy [1].

Surveillance

Whenever there is outbreak of S.paucimobilis, medical devices, tap water, bathtubs, aerators, IV fluids, hands of HCW, filters of tap water, patients room, bedside equipment, bathroom surfaces, sinks, beds should be screened [3].

To trace the outbreak, biotypying, morphotyping, antibiogram and PFGE typing can be done [3]. The clonal relatedness of the outbreak isolates from patients and from ventilator temperature probes was documented by fingerprinting with the arbitrarily primed polymerase chain reaction as per one study done on ventilated neonates [14].

The source of the infection was not able to determine even after environmental surveillance in some incidences and also in case of meningitis [3, 9].

CONCLUSION

Various infections in humans due to S.paucimobilis have been reported, but most have been limited to sporadic case reports which is congruent with present work revealing low positivity of growth of S.paucimobilis (0.44%) [2]. The retrospective study conducted at our hospital did reveal the uncommon “white or off white coloured” characteristic colony hue of all blood and exudate isolates. Being nonpigmented, all the strains were susceptible to most of the classes of antibiotics invitro and in vivo which correlated with theme of “more the pigmentation, more the invasiveness or virulence of the organism”.

Due to its widespread habitat and ability to survive in stress conditions, it could be a potential future threat as emerging pathogen in the era of increasing antimicrobial resistance globally**.**

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