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Methylobacterium and Its Role in Health Care-Associated Infection

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Methylobacterium **species are a cause of health care-associated infection, including infections in immunocompromised hosts. The ability of** ***Methylobacterium*** **species to form biofilms and to develop resistance to high temperatures, drying, and disinfecting agents may explain the colonization of** ***Methylobacterium*** **in the hospital environment in, e.g., endoscopes. Due to its slow growth, it can be easily missed during microbiological surveillance of endoscope reprocessing. The purpose of this minireview is to present an overview of documented infections and cross-contaminations with** ***Methylobacterium*** **related to endoscopic procedures and to illustrate the health care-associated relevance of this slow-growing bacterium.**

Methylobacterium species are fastidious, Gram-negative bacilli which have been reported to be opportunistic pathogens in immunocompromised patients. These species form pink-pigmented colonies on agar plates and have been frequently isolated from tap water in hospitals. The ability to form biofilms and to develop tolerance to disinfecting agents, high temperatures, and drying may explain the frequent occurrence and colonization of *Methylobacterium* in the hospital environment. Here we review the microbiology and health care-associated relevance of this slow-growing bacterium with particular attention to biofilm formation in medical devices and transmission of *Methylobacterium* during endoscopic procedures.

MICROBIOLOGY, LABORATORY IDENTIFICATION, AND ANTIBIOTIC SUSCEPTIBILITY

The genus *Methylobacterium*, of the family *Methylobacteriaceae* (class *Alphaproteobacteria*), was described as a new genus of facultative methylotrophic bacteria by Patt et al. in 1976 (1). This genus, including the first *Methylobacterium* organophilum and 3 species from the *Pseudomonas* genus (e.g., *Pseudomonas mesophilica*, *Pseudomonas radiora*, and *Pseudomonas rhodos*), currently consists of 49 different species (http://www.bacterio.net/methylobacterium.html; last accessed 20 December 2013). *Methylobacterium* spp. are commonly isolated from various natural environments (i.e., leaf surfaces, soil, dust, and fresh water) (2, 3).

Methylobacterium spp. are strictly aerobic, facultatively methylotrophic, fastidious, slow-growing bacteria. They form small (1 to 2 mm in diameter), pink-pigmented colonies on ordinary solid media such as tripticase soy agar, sheep blood agar, nutrient agar, and Mueller-Hinton agar and on plate count agar and R2A agar, two media used for plate count analysis in drinking water (4). Optimum growth occurs between 25 and 30°C after 5 to 7 days of incubation, with moderate growth at 35°C and no growth at 42°C (4–6). The best growth was observed on Sabouraud dextrose agar and buffered charcoal yeast extract agar. The species grow as Gram-negative or gram-variable, pleomorphic, non-spore-forming, vacuolated, rod-shaped cells and have one polar flagellum (2). *Methylobacterium* spp. are nonfermenting, β-galactosidase- and nitrate reductase-negative, and trypsin- and urease-positive bacteria and are resistant to desferrioxamine (7). These biochemical tests are helpful to differentiate methylobacteria from other aerobically growing Gram-negative bacteria which form pink-pigmented colonies on blood agar (i.e., *Serratia*, *Azospirillum*, *Roseomonas*, and *Asaia*). *Methylobacterium* spp. were reported to be catalase and oxidase positive (1) but were oxidase negative in tests with the dimethyl-paraphenylenediamine reagent (2).

Identification of *Methylobacterium* is performed using commercially available manual-identification test strips (8, 9). However, determination to the species level by these systems can be difficult. 16S rRNA gene sequence analysis can differentiate *Methylobacterium* isolates to the species level with pairwise similarity of 16S rRNA gene sequences of between 97% and 99.6% (10, 11). Recent developments in mass spectrometry (MS) have shed light on rapid and precise identification of *Methylobacterium* spp. Tani et al. (10) applied the whole-cell matrix-assisted laser desorption ionization–time of flight (MALDI) mass spectrometry (WC-MS) technique to identify *Methylobacterium* spp. collected from plant samples. A total of 213 *Methylobacterium* isolates were analyzed with WC-MS using MALDI Biotyper software (Bruker Daltonics), and this identification was confirmed by 16S rRNA gene sequencing. The WC-MS technique demonstrated high effectiveness in the identification of known and novel species of *Methylobacterium*.

Methylobacterium spp. are susceptible to amikacin, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole and have various levels of susceptibility to the β-lactam antibiotics (due to β-lactamase production), with high sensitivity to ceftriaxone, ceftizoxime, and imipenem (6, 8, 12). Discordant carbapenem susceptibilities, with high sensitivity to imipenem (MIC = 0.25 to 1 mg/liter) and resistance to meropenem (MIC > 32 mg/liter), seem to be a distinctive feature of *Methylobacterium* spp. (13).

HEALTH CARE-ASSOCIATED TRANSMISSION OF METHYLOBACTERIUM

Members of the genus *Methylobacterium* are major inhabitants of aqueous environments, including potable water supplies and hospital tap water (4). Transmission of *Methylobacterium* spp. in the hospital environment has been related to contaminated tap water.

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These species have also been isolated from water in dental and blood bank purification units (14, 15) and from endoscopes and automated endoscope reprocessors (AERs), which are probably contaminated by the use of nonsterile water for rinsing (16–18). High resistance to dehydration, chlorination, and elevated temperatures and slow growth and the ability to form biofilms can explain the frequent occurrence and colonization of *Methylobacterium* in the hospital environment (12, 18, 19). Since methylobacteria have been isolated from tap water in hospital units, Hornei et al. (5) suggested monitoring water for the occurrence of methylobacteria in hospital units in which immunocompromised patients are admitted.

Despite low virulence, *Methylobacterium* is able to cause colonization and infections in immunocompromised patients (3, 8). *Methylobacterium mesophilicum*, *Methylobacterium zatmanii*, and *Methylobacterium extorquens* are the three most commonly reported species isolated from normally sterile body sites, i.e., blood, liquor cerebrospinalis, bone marrow, synovia, and ascitic and peritoneal fluids (3, 5). An underlying state of immunosuppression, including that corresponding to solid or hematologic malignancy, organ transplant, renal failure, HIV infection, tuberculosis, or alcoholism, may predispose subjects to a systemic infection caused by methylobacteria (3, 8). In general, *Methylobacterium* spp. cause mild clinical symptoms, such as fever, but severe infections, including bloodstream infections, peritonitis, and pneumonia, have also been reported (3, 8). Central catheter infections are the most common portal of infection in these situations.

Most reported *Methylobacterium* infections have been nosocomial. Two cases of bloodstream infections due to *M. mesophilica* were attributed to tap water used for oral irrigation for patients with mucositis as a complication after bone marrow transplantation (20). Contaminated preservative fluid used for bone marrow harvesting was a possible source of *Methylobacterium* bacteremia in a patient receiving hematopoietic stem cell transplantation (21). A patient receiving continuous ambulatory peritoneal dialysis developed recurrent peritonitis due to *M. mesophilicum*, which was also isolated from contaminated stagnant water in the bathroom (22). Several *Methylobacterium* infections in immunocompromised patients have been associated with environmental exposure (ingestion of raw vegetables, gardening, swimming in a river, and exposure to soil, leaves, and flowers) (3) just before development of infection.

ENDOSCOPE REPROCESSING AND METHYLOBACTERIUM TRANSMISSION RELATED TO ENDOSCOPIC PROCEDURES

Contaminated endoscopes are medical devices frequently associated with outbreaks of health care-associated infections (19, 23). These instruments are difficult to disinfect and easy to damage because of their complex design with multiple internal channels and narrow lumina. Most flexible endoscopes belong to semicritical devices which come into contact with mucous membranes during use and may be either sterilized or disinfected. Flexible endoscopes for therapeutic procedures (i.e., bronchoscopy and gastrointestinal endoscopic procedures) used in sterile body cavities are classified as critical devices and require sterilization after each procedure. Due to their material composition, most flexible endoscopes cannot be steam sterilized but tolerate ethylene oxide and hydrogen peroxide plasma sterilization.

Accurate reprocessing of flexible endoscopes involves cleaning and high-level disinfection followed by rinsing with bacterium-free water and drying before storage (19). Glutaraldehyde and peracetic acid are disinfecting agents frequently used for decontamination of flexible endoscopes. They are active against viruses, fungi, mycobacteria, and all vegetative bacteria, including *Methylobacterium*. However, resistance of *M. mesophilicum* to 2% glutaraldehyde has been reported (24). A recent study demonstrated high efficacy of 1% peracetic acid against *M. extorquens* in the planktonic state, but the disinfectant was less efficient in biofilms (18).

According to Furuhata and Koike (25), 70% (70/100) of examined samples from chlorinated drinking water were positive for *Methylobacterium* spp. and 25% to 93% of methylobacterial strains isolated from tap water were highly resistant to chlorine and survived contact at 0.1 mg/liter concentration of free residual chlorine for 5 min (11, 25).

Accurate endoscope drying is crucial, whereas a humid environment facilitates microbial growth during storage (19). The final drying steps greatly reduce the risk posed by remaining pathogens, as well as the possibility of recontamination of the endoscope by waterborne microorganisms such as *Pseudomonas* spp., *Acinetobacter* spp., and *Methylobacterium* spp. (19, 23). Members of the genus *Methylobacterium* have been reported to be highly resistant to dehydration and high temperatures (18). Hence, they can survive in endoscope channels during inadequate or insufficient drying, resulting in recontamination of endoscopes.

Methylobacterium outbreaks after endoscopic procedures have been related to contaminated tap water (6, 24), AERs containing biofilm (16), and contaminated endoscope channels (17, 18).

Cross-contaminations with *Methylobacterium* in 7 patients during bronchoscopy have, despite the usual disinfection procedure, been related to contamination by tap water of endoscope channels (6). Growth of pink-pigmented bacteria, later identified as *M. mesophilicum*, was observed in mycological surveillance cultures of samples obtained from a bronchoscope which was used for a diagnostic procedure in a patient with an atypical pneumonia. Growth of *Methylobacterium* was discovered as a consequence of the extended incubation time for fungal culture. In the next 3 months, *M. mesophilicum* was isolated from 6 other patients after bronchoscopy. Cultures obtained from bronchoscopes, biopsy forceps, AERs, tubing, glutaraldehyde disinfectant, and environmental samples from the bronchoscopy unit were negative for this bacterium. *M. mesophilicum* was isolated from tap water collected from the sink faucet in the bronchoscopy room. It was considered a colonizer because none of the patients developed a postbronchoscopy infection from this organism.

Cross-contaminations with bronchoscopy-associated *Methylobacterium* in 18 patients have been documented by Kressel and Kidd (16). *M. mesophilicum* and *Mycobacterium chelonae* were isolated from deep respiratory specimens obtained from ventilated patients during bronchoscopy. None of the patients manifested postendoscopic infection with this bacterium. *M. mesophilicum* grew in the cultures obtained from AERs and from 2% glutaraldehyde used during the automated disinfection procedure and did not grow from bronchoscopes, tap water, or unopened glutaraldehyde containers or from the containers used for collecting the clinical samples. The presence of *M. mesophilicum* biofilm on the tubing from one of the AERs was confirmed. Contaminated endoscope disinfectors were replaced by new AERs that use peracetic acid instead of glutaraldehyde for disinfection procedures.

Nosocomial *M. mesophilicum* transmission was related to contaminated 2% glutaraldehyde solution used to manually disinfect the bronchoscopes (24). Environmental cultures from the AER, bronchoscopes, gastroscopes, and brushes were positive for this pink-pigment-forming bacterium. The procedure of reprocessing endoscopes was investigated and showed no shortcomings in technique. Endoscopes were sent for ethylene oxide sterilization, but *M. mesophilicum* from endoscope channels was identified again 2 months later. The water supply was assumed to be the source of contamination, and a submicron filter was installed to get filtered tap water for rinsing of endoscopes after cleaning and disinfection procedures. After disinfection and sterilization of the reprocessing equipment, subsequent cultures were negative for 4 months. *M. mesophilicum* caused no infections in patients after endoscopic procedures with contaminated bronchoscopes.

Repeated contaminations of flexible bronchoscopes with *Methylobacterium* spp. have been detected at the University Medical Center Groningen (UMCG), Groningen, The Netherlands (18). Growth of the bacterium, later identified as *M. extorquens*, was accidentally observed on Sabouraud dextrose agar used for culturing of surveillance samples from endoscopes. Because of the slow growth and unclear significance of this bacterium, it was necessary to prolong the incubation time for 7 days to recover these bacteria from the surveillance samples. Cultures were positive for *Methylobacterium* from 2009 to 2011 from endoscope channels, particularly from bronchoscopes. *Methylobacterium* was also isolated from bronchoalveolar lavage fluid samples from the patients after bronchoscopy. We considered *Methylobacterium* to be a contaminating nonpathogen causing the colonization, because no patient manifested true infection with this bacterium. The procedures of endoscope reprocessing revealed no recurrence. Environmental cultures from the endoscopy unit, including AERs and rinsing water, were negative for *Methylobacterium*. Biofilm formation inside bronchoscope channels was suspected. Strains of *M. extorquens* isolated from the contaminated flexible bronchoscope were investigated for the ability to form biofilms, and the effects of peracetic acid disinfection and drying on *M. extorquens* biofilm formation were studied (see the section discussing the impact of biofilm formation by *Methylobacterium* below).

To date, only one case of *Methylobacterium* bacteremia in a patient following endoscopy has been published (17). A 77-year-old patient with biliary lithiasis underwent a biliary sphincterotomy and implantation of a prosthesis in the biliary tract via endoscopic retrograde cholangiopancreatography. The prosthesis was removed by means of an endoscopic procedure 10 days later. The next day, the patient developed fever, and after 5 days, bacterial growth was detected in one aerobic blood culture bottle. *M. mesophilicum* was isolated from a positive blood culture, and the determination was confirmed by 16S rRNA gene sequencing. The procedures of peracetic-acid-based decontamination and endoscope washer maintenance appeared to be effective because no recurrence of *Methylobacterium* was seen. Water samples obtained for culture from the tap water points in the endoscopy room and from the AER, before and after rinsing, were negative. The inner endoscope channels were found to be the source of contamination with *M. mesophilicum.* The endoscope was sent to the manufacturer for replacement of the inner channel sheath.

IMPACT OF BIOFILM FORMATION BY METHYLOBACTERIUM

A biofilm is an assemblage of microbial cells that is attached to a surface and enclosed in a matrix of exopolymeric substances (26). Biofilms may form on different surfaces, including medical devices, water supply systems, or endoscope channels (16, 18, 26). They are extremely difficult to remove and allow microorganisms to survive under conditions of drying and chemical and antibiotic exposure. Settlement of biofilm-producing species inside endoscope channels can result in failure of the endoscope reprocessing and is an important factor in the pathogenesis of endoscopy-related infections (19, 23). The reduced sensitivity of bacteria to disinfectants within a biofilm can be explained by poor penetration of a disinfectant into the underlying cells, chemical interaction between the biofilm itself and the disinfectant, and the low growth rate and nutrient limitation of microorganisms in biofilms (27).

The presence of biofilm on the tubing from one of the AERs with growth of *M. mesophilicum* was the source of an outbreak described in patients following bronchoscopy (16). Also, biofilm formation inside endoscope channels was suspected to be the cause of repeated cross-contaminations of flexible bronchoscopes with *M. extorquens* at the UMCG (18). Mimicking biofilm formation in an *in vitro* study, *M. extorquens* isolated from a contaminated bronchoscope was tested in 96-well microtiter plates (18). In this model, the effects of the 1% peracetic acid disinfectant (10 min incubation at 25°C) without and with the additional drying (2 h at 50°C followed by 7 days drying at room temperature) on *M. extorquens* biofilm formation were studied to imitate the procedures used for reprocessing of flexible endoscopes.

M. extorquens had a strong biofilm-producing ability, with the highest biofilm amount and the maximum metabolic activity after 7 days incubation in R2A broth (18). The use of 1% peracetic acid disinfectant caused a marked inhibition of *M. extorquens* growth in 2-, 5-, and 7-day biofilms directly after treatment. Regrowth of *M. extorquens* biofilms occurred following 7 days of incubation with R2A broth directly after the disinfection procedure. Regrowth of *M. extorquens* biofilms was observed in wells after disinfection when the drying procedure was skipped. No biofilm regrowth was observed after a drying procedure. This study demonstrated insufficient efficacy of the peracetic acid against *M. extorquens* biofilms and high efficacy of the drying procedure after the disinfection step against *Methylobacterium* in biofilms.

According to the literature, *Methylobacterium* has a strong biofilm-producing ability (28–30). Simões et al. (29) tested the effects of sodium hypochlorite (liquid bleach) on the activity and culturability of *Methylobacterium* biofilms. *Methylobacterium* biofilms recovered their mass, activity, and culturability after 1 h of treatment with 0.01% sodium hypochlorite; a concentration of only 0.1% completely inactivated this bacterium in biofilms after 1 h of incubation. *Methylobacterium* in biofilms survived after contact with other cleaning agents, including 1% benzalkonium chloride for 24 h (28). The strains demonstrated a high tolerance to drying. Ten days after drying, the reduction in the survival of *Methylobacterium* was less than 1 log. Some strains of *Methylobacterium* in biofilms survived and exhibited a potential to grow after 4 weeks of desiccation without any nutrients.

CONCLUSION

Methylobacterium spp. are fastidious microorganisms that have been described as a cause of cross-contaminations related to en-

doscopes and reprocessing equipment and have been reported as a cause of infections in immunocompromised patients. Due to its slow growth, the bacterium can be easily missed during surveillance of endoscope reprocessing. The ability to form biofilms and to exhibit tolerance to cleaning and disinfecting agents and to high temperatures and drying is probably the cause of their predominance in the hospital environment, particularly in tap water and endoscope channels.

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