Ureaplasma parvum as a Cause of Sternal Wound Infection

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Few reports in the literature have documented the isolation of *Ureaplasma* species from sternal wounds. A case of sternal wound infection likely due to *U. parvum* is described. When routine bacterial cultures from a sternal wound infection fail to yield a pathogen, diagnostic testing for mycoplasmas and ureaplasmas should be considered.
Case

A 66 year-old Aboriginal male presented to a tertiary care hospital in Winnipeg, Manitoba, Canada with an episode of chest pain that was unrelieved by nitroglycerin. His past medical history was significant for known ischemic heart disease, hyperlipidemia, hypertension, type 2 Diabetes Mellitus, remote tuberculosis, and bilateral knee arthroplasty. Laboratory investigations performed at the time of arrival (and repeated 18 hours later) demonstrated a troponin T of <0.01 µg/L. The patient was admitted under the cardiology service with a diagnosis of unstable angina. Therapy with heparin was initiated.

A coronary angiogram performed 2 days post admission demonstrated 70% stenosis of the left anterior descending coronary artery, complete occlusion of the right coronary artery, and an ejection fraction of 44%. Following consultation with a cardiac surgeon, the patient underwent 3 vessel coronary artery bypass grafting. Cefazolin was administered before the procedure and continued for 5 days post-operatively. The patient was transferred out of the surgical intensive care unit to the general cardiac surgery ward on post-operative day 1. His post-operative course was complicated by ileus that spontaneously resolved over several days. On post-operative day 5, he developed a new onset fever of 38.6°C and increasing shortness of breath. He was also having runs of sustained ventricular tachycardia. On post-operative day 6, he was re-admitted to the surgical intensive care unit with a diagnosis of possible pneumonia. Empiric antimicrobial therapy with piperacillin/tazobactam was initiated. His condition stabilized over the next few days. Blood cultures were negative.
On post-operative day 10, purulent drainage from the sternal wound was observed. The sternum was also found to be unstable on clinical examination. A chest CT scan demonstrated soft tissue stranding and fluid around the sternum, consistent with expected post-operative changes. No definite evidence of osteomyelitis was noted. A wound swab of the sternal drainage was sent to the microbiology laboratory on post-operative day 10. A Gram stain demonstrated 1+ PMNs. No bacteria were isolated on culture. A second wound swab submitted on post-operative day 11 also did not yield bacterial growth.

On post-operative day 15, due to sternal instability and ongoing purulent drainage from the sternal wound, the patient was taken back to the operating room. The sternal incision was opened and a substantial amount of pus was drained. The edges of the sternum were debrided and the sternal wires were removed. The wound was closed again with stainless steel wires. Given the lack of positive cultures to date, a wound swab for mycoplasma/ureaplasma was obtained.

For the isolation of mycoplasma and ureaplasma, wound swabs were inoculated into bromothymol blue broth (B broth), prepared as described by Robertson without lincomycin (13). In a further modification to the broth described by Robertson, arginine was added to allow for the detection of *Mycoplasma hominis* in addition to *Ureaplasma* species. Current laboratory protocol at our institution is for B broth to be incubated for 5 days at 37ºC. Broth that undergoes a color change to green is submitted for further evaluation by PCR assays.

Molecular detection was performed initially using 3 separate PCR reactions to test for *M. hominis*, *Ureaplasma* species, and members of the *Mycoplasma* group with
primers previously described by Abele-Horn et al. and Van Kuppeveld et al. (1,15,16). Briefly, a small aliquot of B broth (0.5 mL) was placed in a sterile microfuge tube and spun at 5000 rpm for 5 min at 4ºC. A 2.5 µL aliquot of supernatant was then added to 47.5 µL of master mix (consisting of: 0.5 µM of each primer, 2.5 units of Taq polymerase, 0.5 units of uracil DNA glycolase, 0.2 mM of dATP, dCTP, dGTP, 0.6 mM of dUTP, 0.1 mM of dTTP, 2.5 mM MgCl2, 5 µL of 1XPCR buffer, and sterile distilled water). Amplification was performed with an Eppendorf thermocycler (Eppendorf AG, Hamburg), involving 40 cycles of 94ºC for 30 seconds, 55ºC for 30 seconds, and 72ºC for 60 seconds. Amplified products were visualized under ultraviolet light after being run on a 1.5% (w/v) agarose gel containing 25 µg of ethidium bromide. Control organisms run with each PCR include *Ureaplasma* ATCC 27618, *M. hominis* ATCC 23114, and *E. coli* ATCC 25922 (negative control).

The patient’s wound swab yielded *Ureaplasma* species, demonstrated by the presence of PCR product with both the Mycoplasma group primers and the *Ureaplasma* species primers. The B broth subculture was shipped frozen on dry ice to the University of Alabama at Birmingham Diagnostic Mycoplasma Laboratory for further genotypic characterization and antimicrobial susceptibility testing.

Genomic DNA from the *Ureaplasma* subculture was extracted by centrifuging 200 µl of culture at 14,000 g for 20 minutes at 4ºC; pellet was digested with 200 µL proteinase K (1 mg/ml) lysis buffer for 1 hour at 60ºC. Proteinase K was then inactivated by incubation at 95ºC for 5 minutes. Speciation of the isolate was performed by a multiplex real-time PCR based on the hypothetical gene OFR00127 and UU063 as described previously (8). The PCR assay demonstrated that the organism was *U. parvum*.  


Following species determination, four real-time PCR assays were performed as described by Xiao et al. (19) which determined the organism to be serovar 6. Minimum inhibitory concentrations determined by microbroth dilution as described by Waites et al. (17) were: doxycycline 0.5, tetracycline 1.0, levofloxacin 1.0, and erythromycin 1.0 µg/ml.

On post-operative day 17, ciprofloxacin 500 mg po given twice daily was initiated in response to the detection of *Ureaplasma*. Piperacillin/tazobactam was discontinued on post-operative day 21. At the same time, doxycycline 100 mg po given twice daily was added to the ciprofloxacin to provide additional *Ureaplasma* coverage. By post-operative day 28, the patient’s sternal wound was clinically improving. The patient was discharged from hospital on a combination of ciprofloxacin and doxycycline. Given the possibility of sternal bone involvement based on clinical presentation, a decision was made to continue therapy for at least 3 months. The patient was subsequently evaluated in follow-up one month after discharge. At this time, his sternal wound appeared to be healing well.
Ureaplasma urealyticum belongs to the class Mollicutes (18). Ureaplasma spp. appear as coccoid cells, ranging from 0.2 to 0.3 µm in diameter (18). Like other members of the class Mollicutes, Ureaplasma spp. lack a cell wall and consequently, cannot be visualized by Gram staining. Culture may be used to identify Ureaplasma spp. in the clinical microbiology laboratory. The presence of sterols is required for growth. Media that can be used for isolation include Shepard’s 10 B broth and A 8 agar (18). The generation time for Ureaplasma spp. is approximately 1 hour and most cultures will be positive in 2 to 4 days. On agar plates, colonies of Ureaplasma spp. are small (15 to 60 µm) and low power microscopic magnification is required for visualization (18). Ureaplasma spp. may be distinguished from other human mycoplasmas by their ability to hydrolyze urea (18). Identification may also be achieved through molecular methods, as described in the case presented here. The most active antimicrobials in vitro against Ureaplasma spp. are macrolides, fluoroquinolones, and tetracyclines (18).

Ureaplasma spp. colonize the lower genitourinary tract in 40 to 80% of sexually active women (11). These organisms have been implicated as etiologic agents of nongonococcal urethritis, stillbirth/premature delivery, postpartum bacteremia, and wound infection post caesarean section (3,7,12,14). They have also been associated with the development of neonatal pneumonia, meningitis, and chronic lung disease (8,11,18). There are case reports of Ureaplasma spp. as a cause of meningitis, aortic graft infection, and intrarenal abscess in adults (4,6,9).

To date there have been few reports of Ureaplasma spp. as a cause of sternal wound infection/mediastinitis. A review of the English Language literature yielded 3 additional cases beyond the one presented here (2,5,10). Pigrau et al. described a 63
year-old male who developed a sternal wound infection 7 days after coronary bypass surgery (10). Both *M. hominis* and *Ureaplasma* spp. were isolated from cultures taken at the time of surgical wound drainage. The patient responded to combination therapy with clindamycin, gentamicin, and doxycycline (10). Garcia-de-la-Fuente et al. published a case of a 77 year-old male who developed sternal wound dehiscence, mediastinitis, pleural effusion, and pericardial effusion following aortic valve replacement (5). Cultures demonstrated both *M. hominis* and *Ureaplasma* spp. This patient improved slightly in response to therapy with clindamycin and doxycycline. However, he subsequently developed a gastrointestinal bleed and expired (5). Finally, Boyle et al. have reported the case of a 48 year-old male who developed an unstable sternum with associated fever and leukocytosis 30 days post heart-lung transplantation (2). Mediastinitis was diagnosed at the time of sternal debridement. Wound cultures demonstrated heavy growth of *M. hominis* and moderate growth of *Ureaplasma* spp. The infection was successfully treated with a combination of doxycycline and erythromycin (2). In all 3 of these cases, *Ureaplasma* spp. were isolated in association with *M. hominis*. The case described in this report is unique in the respect that *U. parvum* was the sole organism detected.

All of the reports of *Ureaplasma* infections published prior to 2003 used the designation of *Ureaplasma urealyticum*, but since that time the genus has been subdivided into two separate species: *U. urealyticum*, which includes serovars 2, 4, 5, 7, 8, 9, 10, 11, 12, 13 and *U. parvum* which includes serovars 1, 3, 6, and 14 (18). There is debate as to whether there is differential pathogenicity between the two species or among the various serovars, but this has not been settled (18). *U. parvum* tends to be the more
common of the two species in most clinical specimens (19). The only method to
distinguish the two species from one another is through the use of the PCR assay. Due to
the limited availability of PCR assays and the uncertainty of the significance of
differentiating the two species and their respective serovars, species determination is not
performed routinely for diagnostic purposes but may be valuable to characterize invasive
isolates and for epidemiological purposes (18).

There are several limitations to the case presented here. First of all, the patient
did not have a urethral swab submitted for *Ureaplasma* spp. culture, so the source of the
organism in his wound remains unclear. Secondly, antimicrobial therapy was initiated
prior to sternal wound swabs being sent for routine bacterial culture. This may have
interfered with our ability to identify bacteria more commonly associated with sternal
wound infection. Finally, routine bacterial cultures were not sent at the time of sternal
debridement, once again limiting our ability to attribute the current infection entirely to
*Ureaplasma*. It is not known whether *Ureaplasma* spp. ever colonize sternal wounds.
The frequency of ureaplasm as pathogens in sternal wound infections alone or in
association with other more frequently recognized pathogens is also unknown since these
organisms are seldom sought except as in this case when infection persists without an
apparent etiology and shows no response to beta lactam antibiotics. In our institution we
do not routinely perform diagnostic testing for either *M. hominis* or *Ureaplasma* spp. in
the setting of sternal wound infection.

In summary, this report describes a case of sternal wound infection likely due to
*U. parvum*. For patients with a sternal wound infection/mediastinitis where routine
bacterial cultures are negative, consideration should be given to pursuing diagnostic testing
for *M. hominis* and *Ureaplasma* spp. Further research is required to determine whether *Ureaplasma* spp. ever occur as a colonizer of uninfected sternal wounds or wounds infected with other more common bacterial pathogens.

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References:


