without scolex. The strobila was approximately 1.5 m long. There were 748 segments and the proglottids measured 2.0–2.5 mm long × 5.0–7.5 mm wide after fixation. Close up view of the proglottids showed rosette-shaped uterus in the centre of each proglottid (Fig. 1B). Eggs measured approximately 55 μm × 90 μm and had an apical knob at the abopercular end (Fig. 1C).

Both patients regularly consumed *sashimi* and *sushi*, including raw salmon. The patients were treated with the anthelminthic praziquantel. Both patients did not report further passing of identifiable worm segments per rectum after praziquantel administration.

While broad fish tapeworms are generally identified to a genus level based on morphological characteristics, accurate determination of the species is only possible by molecular analysis. It is probable that most diphyllobothriosis cases originally attributed to *D. latum* may have been caused by *D. nihonkaiensis* tapeworms when morphology alone was used. DNA was extracted from the proglottids using DNeasy Blood and Tissue kit (Qiagen, Germany). Polymerase chain reaction (PCR) was performed using Qiagen multiplex PCR kit with forward primer 5′-ACAGTGGGTTTAGATGTAAAGACGGC-3′ and reverse primer 5′-AGCTACAACAAACCAAGTATCATG-3′ for the amplification of a 249 bp fragment of the *cox1* gene as previously published.[8] As the tapeworm samples were stored in 10% neutral buffered formalin, a protocol yielding a short amplicon was chosen to minimise the effect of DNA fragmentation due to formalin. DNA sequences from both samples most closely matched that of *D. nihonkaiensis* (GenBank accession number LC070678).

Diphyllobothriosis is a rare infection in Singapore. These cases demonstrate the global spread of a food-borne pathogen as a result of global food trade and the increasing popularity of *sashimi* and *sushi*. Although species identification is not essential for the effective treatment of diphyllobothriosis, it is important for the purpose of epidemiology and food safety. Due to the worldwide transport of raw fish including salmon, various unreported broad fish tapeworm species can be introduced into Singapore. Species level identification will aid in the determination of the possible sources of plerocercoids. This in turn will help local food safety surveillance and practices.

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Yersinia pseudotuberculosis bacteraemia: a diagnostic dilemma in the era of MALDI-TOF mass spectrometry

Sir,

A 73-year-old woman presented febrile, hypotensive and tachycardic with profuse diarrhoea whilst receiving oxaliplatin chemotherapy for metastatic colorectal carcinoma and letrozole therapy for synchronous breast and vulval carcinoma. Additional history included type 2 diabetes mellitus. She had not recently travelled, had no regular animal contact and had an absence of flea bites, although a mouse plague was contemporaneously afflicting the local region.

After 28 hours incubation, peripheral and PICC anaerobic blood cultures (BACTEC; BD, USA) flagged with Gram-negative bacilli with bipolar staining. Identification by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Australia) on early growth from the chocolate-agar (bioMérieux, Australia) incubated in 5% carbon dioxide at 37°C was performed using the Bruker Standard Taxonomy (version 7.0) and Security Relevant databases. A reliable identification was not obtained with acceptable scores for both *Yersinia pestis* (2.24) and *Yersinia pseudotuberculosis* (2.23), with scores greater than 2.0 considered consistent with species-level identification.

Given the public health and biosecurity significance of *Y. pestis*, a definitive identification was urgently sought. A positive urease test was suggestive for *Y. pseudotuberculosis*; however, the 2 hour peptone broth motility at 25°C was negative. The VITEK 2 (bioMérieux) supported an identification of *Y. pseudotuberculosis* using phenotypic characteristics with a 99% probability (Bionumber 4005711300100210). 16S ribosomal RNA gene sequencing obtained a 1, 147 base pair sequence; however, it was unable to distinguish between the two species as the obtained sequence was almost identical to both the *Y. pestis* (GenBank CP019708.1) and *Y. pseudotuberculosis* (Genbank HG326181.1) reference sequences.

Bacteriophage lysis testing was performed by the *Yersinia* Reference Laboratory for the Australian Public Health Laboratory Network (PHLN) (Public Health Microbiology, Forensic and Scientific Services, Queensland Department of Health) and interestingly, the isolate did not exhibit lysis at either 25°C or 37°C. The lack of lysis at both temperatures excluded *Y. pestis*; however, it was inconsistent for *Y. pseudotuberculosis* which typically demonstrates lysis at 37°C but not 25°C. Nucleic acid amplification testing (NAAT) using an assay provided by the Laboratory Response Network (LRN), Centres for Disease Control and Prevention (CDC), Atlanta, did not detect any of the three *Y. pestis* targets. The specific details of the targets used in the *Y. pestis* LRN NAAT are not publicly available; however, NAAT assays typically target the plasminogen activator (*pla*) gene, murine toxin (*mlt*) gene, or *caf1* gene.[1] Whole genome sequencing (WGS) was subsequently undertaken using the NextSeq500 workflow (Illumina, USA) to obtain a definitive identification. Bioinformatics analysis using k-mer (KmerFinder 2.5 tool and Kraken v0.10.5) based methods on SPAdes v3.10.1 assembled contigs and a mapping based method performed on the sequence reads (Clinical Pathoscope 1.0) were performed to assist in identification due to the high genetic homogeneity of *Y. pestis* and *Y. pseudotuberculosis*. All three databases determined that the sequence assemblies and reads showed the most similarity to *Y. pseudotuberculosis*. Interrogation of the *Yersinia* species pubmlst database (https://pubmlst.org/yersinia/) identified that the isolate belonged to sequence type (ST) 93. ST93 has previously been reported as a ST associated with *Y. pseudotuberculosis*.[2]

The patient received 7 days of intravenous ceftriaxone and ciprofloxacin, followed by 14 days of oral ciprofloxacin. She experienced prompt symptom resolution; however, died approximately 1 month later due to complications of her multiple malignancies.

Yersinia pseudotuberculosis is typically a zoonosis,[3] with infection in humans being uncommon and typically acquired through ingestion of contaminated food or water. When infection does occur, *Y. pseudotuberculosis* typically manifests as a self-limiting gastroenteritis or mesenteric adenitis. Bacteraemia is a rare but described occurrence, most often in patients with impaired immunity.[4]

In comparison, *Y. pestis* represents a serious international public health biothreat with a high case mortality rate and the capability to 'trigger severe epidemics'.[5] As such, it is a Tier 1 security sensitive biological agent (SSBA, http://www.health.gov.au/ssba#list) and accordingly, there are regulations on its storage, possession and transport. Laboratory handling requirements also differ between the species: *Y. pestis* is potentially acquired via aerosolisation and as a Risk Group 3 organism requires a Physical Containment (PC) Level 3 Laboratory for handling. Clinically, *Y. pestis* manifests as three disease forms, bubonic, septicaemic or pneumonic, with pneumonic plague being the most severe and having the capability for person-to-person[1] transmission. Although there has been no locally acquired *Y. pestis* cases in Australia since the early 1900s[1] or imported cases notified on the National Notifiable Diseases Surveillance System (http://www9.health.gov.au/cda/source/cda-index.cfm) since 1991, plague remains endemic in several African, Asian and South American countries.[5] Consequently, healthcare services globally need to remain alert for its presentation in returning travellers.

Given the public health, laboratory and clinical implications, there is a clear imperative to rapidly differentiate the two species and phenotypic characteristics can provide a preliminary indication. *Yersinia pseudotuberculosis* and *Y. enterocolitica* are both urease positive and motile at 25°C, whereas *Y. pestis* is biochemically more inert being both non-motile and urease negative. Ideally, for laboratory safety reasons motility should be evaluated by an agar method rather than peptone water and slide microscopy as performed in this case. Automated biochemical identifications systems such as the API 20E (bioMérieux) and the VITEK 2 (bioMérieux) may also be useful in differentiating the species.

MALDI-TOF MS is rapidly becoming the dominant identification tool in the modern microbiology laboratory. It has the major advantages of being rapid, sensitive, cost-effective and without complex specimen preparation requirements. However, the utility and validation of mass spectrometry in the identification of high-risk pathogens remains unclear. There are two major challenges: firstly, the adequacy of reference databases; and secondly, the laboratory safety of using mass spectrometry with high-risk pathogens and potential isolate inactivation methods.

Although phenotypically distinct, the successful differentiation of *Y. pestis* and *Y. pseudotuberculosis* by MALDI-TOF MS is complicated by their high degree of genomic similarity.[6] Furthermore, identification accuracy depends upon a comprehensive database against which to compare the generated protein profile. Standard databases for both the MALDI BioTyper CA System (Bruker Daltonics) and Vitek MS (bioMérieux) include spectra for *Y. enterocolitica* and *Y. pseudotuberculosis* but not *Y. pestis.* Most frequently *Y. pestis* is misidentified as *Y. pseudotuberculosis*,[7,8] creating the opportunity for a highly significant pathogen to be overlooked. Diagnostic accuracy is improved if 'Security Relevant' or in-house research databases are used, with accurate identification of *Y. pestis* in up to 82.4% of isolates.[7] However, these databases are not routinely available to most clinical laboratories. Furthermore, frequently these databases result in *Y. pseudotuberculosis* being misidentified as its near-neighbour *Y. pestis*,[7,9] resulting in utilisation of substantial resources to perform confirmatory testing as observed in our case.

In most microbiology laboratories, the MALDI-TOF MS is located within a PC-2 laboratory. This creates laboratory safety challenges for its use with Risk Group 3 organisms such as *Y. pestis* which require handling in a PC-3 laboratory. Routine procedures of spot drying and matrix use with formic acid has insufficient inhibitory effect to allow safe handling of these high-risk organisms outside a PC-3 laboratory, with up to 68% of bioterrorism agents remaining viable after direct matrix application.[7] A variety of inactivation methods have been developed to render highly-pathogenic organisms non-viable and permit handling in a PC-2 laboratory. This requires a high degree of suspicion by the laboratory scientist prior to isolate testing. Ethanol and tube-based trifluoroacetic (TFA) based methods are the most effective at inactivation,[9–11] with the exception of *B. anthracis* which requires a post-extraction centrifugal filtration step to remove spores.[9,10] Ethanol-based inactivation also yields the highest spectral qualities as measured by the number of peaks and signal-to-noise ratio, with a lower impact on the proteomic composition of the isolate.[10] In practice, it is important to utilise the same extraction method as that used to obtain the database against which the spectra is compared, as differing methods impact the identification power.

Other emerging technologies include the BioFire Diagnostics FilmArray Biothreat Panel (bioMérieux) which is a multiplexed, hemi-nested polymerase chain reaction (PCR) within a single pouch which includes two targets for *Y. pestis*. To date, there is only one published study evaluating the role of the FilmArray in the detection of *Y. pestis.*[12] Nevertheless, results are encouraging with sensitivity studies demonstrating detection down to 25 genome equivalents.[12] With minimal sample preparation and all reactions within the single pouch, the FilmArray may have utility as a supplementary test along with the MALDI-TOF MS in reference laboratories.

In conclusion, advances in organism databases and safe extraction techniques have allowed mass spectrometry to have a promising role in the identification of high-risk pathogens such as *Y. pestis*. However, the inability of mass spectrometry to reliably identify *Y. pestis* and other SSBA pathogens to a species level demands that further confirmatory testing remains necessary. It is likely that bacteriophage lysis testing, NAAT, and increasingly, WGS will continue to have a key role in definitively identifying *Y. pestis*, all of which can only be performed in Australia through the PHLN. Confirmatory testing for *Y. pseudotuberculosis* identified by MALDI-TOF MS should also be pursued, as even the enhanced 'Security Relevant' databases can miscall *Y. pestis*. Mass spectrometry will likely have an ongoing utility in ruling out rather than ruling in an SSBA pathogen.[7,9]

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Transthyretin Val122Ile amyloidosis associated with isolated gastrointestinal disease and bowel rupture in a Caucasian woman

Sir,

An 84-year-old Caucasian woman was referred for gastroenterology review in December 2016 to investigate 6 months of diarrhoea. Her medical history included type 2 diabetes mellitus with nephropathy, chronic obstructive airways disease secondary to smoking, gout, depression, peptic ulcer disease, hysterectomy, right hip arthroplasty, spinal laminectomy and carpel tunnel release in August 2013. Her medications were glimpiride, allopurinol, ipratropium bromide, fluticasone, cholecalciferol, furosemide and duloxetine.

Shortly after the referral, the patient's diarrhoea and abdominal pain became worse. A computed tomography (CT) scan of the abdomen and pelvis was unrevealing. Bloods showed a raised C-reactive protein of 131.1 mg/L (normal <3.0 mg/L), normocytic anaemia (Hb 90 g/L, MCV 90 fL) with normal iron, B12 and folate levels and stable chronic renal impairment (creatinine 145 μmol/L, eGFR of 38 mL/min). Her albumin was 34 g/L and liver function tests were normal apart from a mildly raised ALP. Physical examination was unremarkable with the exception of nail dystrophy and carpal tunnel release scars.

A repeat colonoscopy was notable only for lax anal tone; colonic mucosa appeared normal which was confirmed on histology. Subsequently, the patient suffered a rectal prolapse that required surgical correction. Biopsies of the rectal mucosa demonstrated prominent eosinophilic deposits within muscularis blood vessel walls and nodular deposits surrounding perivascular tissues. These infiltrates were Congo red positive, consistent with amyloid. The patient was referred to the Victorian and Tasmanian Amyloidosis Service for evaluation.

At this review, the patient complained of inconsistent neuropathic pain in her hands and feet, and postural symptoms. On examination, however, there was no objective evidence of peripheral neuropathy or postural hypotension. Apart from nail dystrophy and carpal tunnel release scars, there were no other soft-tissue signs of amyloidosis, nor evidence of hepatomegaly or cardiac failure.

Investigations to determine the amyloid subtype and extent of systemic involvement were undertaken. There