

# *Salmonella bacilli* negative image recognized on Diff-Quik stain from pleural fluid cytology

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## ABSTRACT

أن فهم آلية الاختبارات الباثولوجية الخلوية في تقييم عمليات الالتهاب المختلفة يعد أساسياً في الوقت الراهن وآمن وسريع وعملية فعالة من حيث التكلفة. نستعرض في هذا التقرير حالة مريض يبلغ من العمر 52 عام مصاب بالسلمونية التيفية حيث تم التعرف على الكائنات المسببة بشكل مبدئي في علم الخلايا وبالزرعة البكتيرية بعد ذلك. أظهرت مسحة ديف كويك عدة عصيات قليلة الدهن، ونحيلة، وعديمة اللون والتي ظهرت في خلفية رمادية سماوية للمسحة. تم التعرف على هذه العصيات داخل الخلية وداخل الخلايا الناسجة وعدلات السيتوبلازم وخارج الخلية في خلفية المسحة. أن هذه الصورة السلبية تمثل الكائن وجفنته والذي ينشأ منطقة لا تمتص ملون ديف كويك. كما تم علاج المريض باستخدام المضادات المناسبة. نستعرض هنا مناقشة موجزة لهذه النتائج المثيرة في مثل هذا الالتهاب النادر مع مراجعة الأدبيات ذات الصلة.

Understanding the significance of cytopathological tests in evaluating various infectious processes have become very essential nowadays, as it is a safe, fast, and cost-effective procedure. We present a case of a 52-year-old male with *Salmonella empyema* where the causative organisms were initially identified on cytology, and subsequently confirmed by microbiological culture. Diff-Quik stained smears showed many colorless, slender, fat short bacilli, which were visualized against the blue-gray background of the smear. These bacilli were identified both intracellularly inside the histiocytes and neutrophils cytoplasm as well as extracellularly in the smear background. We consider that this negative image represents the organism and its capsule creating an area that did not take the Diff-Quik stain. The patient was treated accordingly with suitable antibiotics. A brief discussion of this interesting finding in such a rare infection with pertinent literature review is presented.

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In this era of cost containment, cytopathology plays a pivotal role in the detection and identification of microorganisms in various cytological preparations. Recognition and diagnosis of a wide range of infectious diseases is now amenable from different cytological samples. These diagnoses can be carried out on exfoliative samples, as well as aspirated ones. Actually by utilizing a variety of cytopreparatory and staining methods, cytological examination of different lesions became the first diagnostic tool to make the diagnosis of a long and increasing list of infectious diseases. It is rapid, safe, accurate, and a cost-effective diagnostic procedure. Having said that, and despite the wealth of information that can be gleaned from a stained smears, the specific etiology of an infection often requires a lengthy microbiological culture technique that is still considered the gold standard where the final diagnosis and sensitivity results relies on. Our infectious diseases colleagues consider culture data as essential, and must be considered in the final diagnosis of any infectious etiology.<sup>1</sup> In this short discussion, we are sharing an unusual case of *Salmonella empyema* where the bacilli were initially recognized while examining Diff-Quik (DQ) stained cytological smears of a pleural fluid. Consequently and due to the cytological findings, further culture on the material was pursued where the diagnosis of *Salmonella typhimurium* was confirmed.

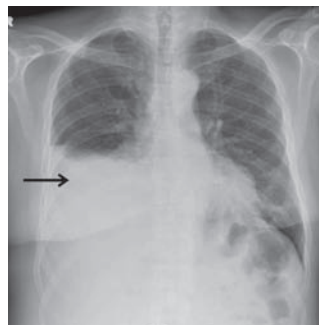
**Case Report.** The patient was a 52-year-old male patient who was diagnosed to have widely metastasizing

and inoperable cholangiocarcinoma of the right hepatic and common bile duct. The diagnosis of metastatic adenocarcinoma of multiple lymph nodes was made after a recent history of weight loss and jaundice with abnormal endoscopic retrograde cholangiopancreatography (ERCP). For that reason, endoscopic ultra sound (EUS) guided fine needle aspiration (FNA) of common bile duct (CBD) was performed and confirmed the diagnosis of an adenocarcinoma. While in the hospital, the patient developed massive right-sided pleural effusion “empyema” detected on chest x-ray (Figure 1) that showed homogenous opacification in the right pleural space. Ultrasonography of the right chest confirmed the presence of pleural effusion where aspiration of the fluid was performed to rule out malignancy. The fluid was sent for cytological examination.

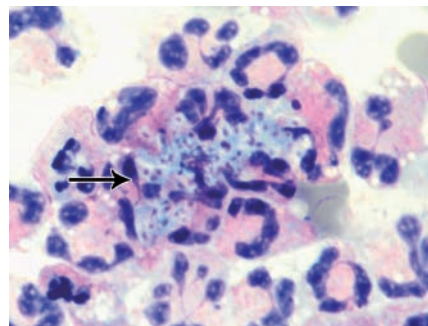
We received 85 ml of yellow turbid fluid. Cytospins were carried out, and stained with both Papanicolaou (PAP) and DQ stains. In addition, a cell block stained with routine Hematoxylin & Eosin was also processed to be evaluated. Cytological examination revealed mainly reactive mesothelial cells, numerous histiocytes and abundant neutrophils. The cell block showed the same purulent exudate. No malignant cells or granuloma was identified. Interestingly, on DQ stained smears, many colorless, slender, fat short bacilli were visualized against the blue- gray background of the smear. These bacilli were identified both intracellularly inside the histiocytes and neutrophil cytoplasm, as well as extracellularly in the smear background (Figures 2 & 3). We consider that this negative image represents the organism and its capsule creating an area that did not take the DQ stain. These features are similar to what was previously published in the literature.<sup>2-4</sup> The negative image was explained as a result of hydrophobic interaction of the water- based DQ stain with the lipid within the cell wall of the bacilli. We believe that the same phenomenon occurs in the polysaccharide capsule of salmonella species, and we would like to add this DQ appearance as one of the characteristic features of these bacteria and similar organisms. Because of this initial cytological findings, the rest of the specimen was sent to the microbiology laboratory for thorough examination. Direct gram stain of the pleural fluid specimen revealed gram-negative rods with rare neutrophils. Cultures became positive within 24 hours. Non-lactose fermenting colonies on MacConkey agar and pink colonies on CHROM agar *Salmonella* was isolated. The organism was identified as *Salmonella* species using the standard biochemical testing and the VITEK2 system (BioMerieux, Marcy L'Etoile, France). The organism was serotyped as group B *Salmonella* using

the Remel Wellcolex Colour *Salmonella* kit (Remel, Kent, UK) as recommended by the manufacturer. In addition, for bacterial identification and speciation, the Microseq 500 16S rDNA bacterial identification kit (Applied Biosystem, Foster City, CA, USA) was used as recommended by the manufacturer.

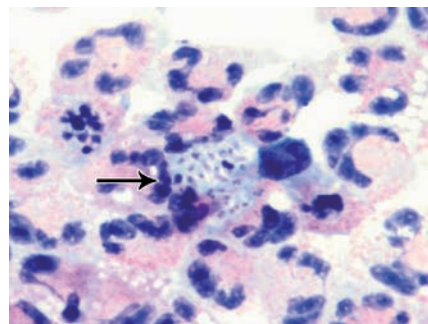
The nucleotide sequence of the polymerase chain reaction (PCR) product revealed that the organism was



**Figure 1** - Chest radiograph (x-ray) showing the right-sided pleural effusion with obliteration of right costo-phrenic angle and homogenous opacification in the right lower and middle lung zones.



**Figure 2** - High power view demonstrating the bacilli (arrow) surrounded by neutrophils (Diff-Quik stain 400x).



**Figure 3** - A high power view demonstrating a collection of bacilli (arrow) surrounded by neutrophils and histiocytes (Diff-Quik stain 400x).

*Salmonella typhimurium*. Antimicrobial susceptibility testing of the isolate was carried out using Etest strips (BioMerieux, Marcy L'Etoile, France). Minimum inhibitory concentrations (MICs) were read after 24 hours of incubation, and were interpreted using Clinical Laboratory Standards Institute (CLSI) guidelines using *Escherichia coli* ATCC 25922 as quality control strain.<sup>3</sup> The organism was susceptible to cefotaxime and ciprofloxacin; and was resistant to ampicillin and trimethoprim/sulfamethoxazole. The advanced expert system of the VITEK 2 interpreted cefoxitin as resistant. No plasmid mediated AmpC beta lactamases were detected by multiplex PCR using primers specific for those genes as recommended in the literature (the multiplex PCR positive controls were kindly offered from the laboratory of Nancy Hanson, Omaha, NE, USA).<sup>5</sup>

The patient was treated accordingly, improved, and was discharged to be followed-up in the oncology clinic for an outpatient chemotherapy treatment. Although rare, but similar cases of pleural fluid empyema due to *Salmonella* species have been published.<sup>6,7</sup>

**Discussion.** Clinically, *Salmonella* infections typically manifest as gastroenteritis, bacteremia, or septicemia. Extra-intestinal complications, such as pleuro-pulmonary infections, secondary to non-typhoid serotypes of *Salmonella* are rare, with only few cases reported in the recent literature.<sup>8</sup> In 1977, one case of *Salmonella* empyema was reported as a complication of a malignant pleural effusion in an immunosuppressed patient. In this case, administration of intra-pleural antimicrobial therapy was found to be more effective than parenteral route. In 1978, 2 cases of *Salmonella empyema* was reported, where the causative organism was found to be *Salmonella* newport. One patient had concomitant sickle cell disease, while the other patient had a splenic abscess. In Italy in 1984, 2 more cases were identified, one was caused by *Salmonella choleraesuis* in a patient with metastatic breast cancer. The other case of an antibiotic-resistant *Salmonella typhimurium empyema* was reported in a patient with underlying alveolar cell carcinoma. Eleven cases of pleuro-pulmonary non-typhoid *Salmonella* infections were described in Spain over a 27-year-period. In

those 11 cases, 8 patients had pneumonia, 2 had a lung abscess, and 1 patient had an empyema. Of these patients, 7 were severely immunocompromised, and 7 had an underlying previous lung disease.<sup>9</sup>

Although most of these cases were reported in immunocompromised patients, few cases of pleural empyema due to non-typhi *Salmonella* occur in a non-immunocompromised patient without previous pleuro-pulmonary disease.

In summary, this short discussion emphasizes the importance of cytology as an initial rapid and cost effective diagnostic tool to recognize infectious agents. It also highlights the usefulness and importance of DQ stain as an "organism stain" even with its "negative image appearance." We would like to draw attention to these organisms and the aforementioned DQ features.

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