(75%) ($p < 0.001$). RAPD analysis showed no relationship from different cases.
Conclusions: NPRGM could be responsible of clinical syndromes in 1/4 of the cases, and this association was different for the species studied. Non-respiratory isolates were significant in 3/4 of the cases. The predominant species in our environment is *M. fortuitum* (57.1% of cases), although the most significant one was *M. abscessus*. No relationships among isolates from independent cases were detected, suggesting transmission routes other than interhuman.
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O451 *Mycobacterium szulgai* causes tuberculosis-like disease in Zambia

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Introduction: In Africa, the diagnosis of tuberculosis is almost invariably based on the microscopic examination of Ziehl–Neelsen stained clinical material. However, not only *Mycobacterium tuberculosis*, but also non-tuberculous mycobacteria (NTM) yield a positive result in microscopic examination for acid-fast bacilli (AFB). Furthermore, a significant part of the patients, especially HIV-positives, may represent AFB-negative, but culture-positive mycobacteriosis.
Objective: To investigate the clinical relevance of isolation of NTM in Africa, in the light of the increasing prevalence of HIV.
Methods: In Sesheke, Zambia, 64 (HIV positive and negative) patients, who were chronically ill for more than two weeks, were included in the study. Sputum was collected and cultured for mycobacteria using Mycobacteria Growth Indicator tubes. The isolated Mycobacterium cultures were identified by 16S rRNA gene sequencing.
Results: Thirty out of 64 (47%) patients yielded positive Mycobacterium cultures that were identified as *M. tuberculosis* (8 times), *M. szulgai* (7), *M. avium-intracellulare* (3), *M. simiae* (1) and *M. terrae* (1). Ten isolates were not suitable for identification due to contamination, re-culture problems, etc. Thirteen of the 30 culture-positive patients (43%) were also positive in microscopic examination, including four patients with NTM infections. Especially the patients infected by *M. szulgai* manifested symptoms highly similar to regular tuberculosis caused by *M. tuberculosis*. DNA fingerprinting analysis revealed four different patterns among the seven *M. szulgai* isolates, excluding the possibility of a laboratory cross-contamination or a common source of infection. Only two out of seven patients with a *M. szulgai* infection responded well to treatment by tuberculostatics.
Conclusion: The contribution of NTM, and especially of *M. szulgai*, to tuberculosis-like diseases in both HIV positive and negative patients in Africa may be underestimated.

O452 Administration of TNF-α did not inhibit *Mycobacterium avium* infection in the mouse lung

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Objective: *Mycobacterium avium* causes chronic and progressive respiratory infection. TNF-α plays a central role in innate immunity against mycobacteriosis. Although administration of TNF-antibody has been reported to cause mycobacteriosis including non-tuberculous mycobacteriosis, study concerning administration of TNF-α in non-tuberculous mycobacteriosis remains insufficient. In this study, we investigated the effect of TNF-α administration in *M. avium* infection in mice.
Methods: Clinically isolated strains of *M. avium* were used. Proliferation of *M. avium* within peritoneal macrophages in the existence of recombinant human TNF-α were examined. Wild type of C57Bl/6 mice, perforin-deficient mice or TNF-α overexpression mice were administered *M. avium* (1×10^7 cfu/body) intratracheally. Recombinant human TNF-α (10 ug/body) was injected into wild mice or perforin-deficient mice on day 0 and day 7 after *M. avium* administration. Mice were sacrificed on day 21, 60 after *M. avium* administration. The lung homogenates were inoculated on Middlebrook 7H10 agar plates for counting the number of colonies. Tissue sections of the lungs were stained by hematoxylin and eosin or Ziehl–Neelsen methods.
Results: *In vitro study*: administration of recombinant human TNF-α inhibited proliferation until day 3, but not day 7. *In vivo study*: administration of recombinant human TNF-α did not inhibit *M. avium* infection based on the lung histology and bacterial number in the lungs of perforin-deficient mice. In TNF-α transgenic mice, *M. avium* induced several lymphoid tissues proliferation. Bacterial proliferation in the lung was not inhibited compared to wild type mice.
Conclusion: Both exogenous and endogenous TNF-α administration did not attenuate *M. avium* infection in vivo. The present data indicate that the role of TNF-α against *M. avium* is different from that of *M. tuberculosis*.

New antimicrobial compounds overcoming common resistance mechanisms

O453 Mercapto-phosphonate compounds as broad-spectrum inhibitors of the metallo-β-lactamases

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Objectives: One of the emergent factors for the β-lactam antibiotic resistance of pathogenic bacteria is the production of metallo-β-lactamases (MBLs), which are able to hydrolyse the β-lactam ring in a broad spectrum of substrates, particularly the carbapenems. MBLs have been divided into three different sub-classes B1, B2 and B3 based on sequence similarities [1]. In this report, we investigated the inhibitory effect of mercapto-phosphonate derivatives against MBLs.
Methods: The laboratory of P Metzner (University of Caen, France) synthesized 12 different mercapto-phosphonate compounds with the ability to inhibit the subclass B1 VIM-4, the subclass B2 CphA and the subclass B3 L1 MBLs respectively. Consequently, we determined the competitive inhibition constant (Ki) as described by DeMester et al. [2]. We also measured the minimal inhibition concentration (MIC) for *Escherichia coli* recombinant strains producing VIM-4, CphA or L1, for ampicillin or imipenem in the presence or absence of mercapto-phosphonate compounds.
Results: In the present study, we show that all the mercapto-phosphonates, with the exception of compound 1a, behaved as good competitive inhibitors ($Ki < 15\,\mu M$) for CphA. Their activities against the sub-classes B1 and B3 enzymes were more contrasted. In addition, the presence of free Zn++ abolished the inhibitory activity of compound 2b. The compound behaving as zinc chelator could explain this phenomenon. Nevertheless, the (2-sulfanylphenyl) phosphonic acid, the (4-bromophenyl)(sulfanyl)methyl phosphonic acid and the [(2,4-dichlorophenyl)(sulfanyl)methyl] phosphonic acid were good inhibitors ($Ki < 15\,\mu M$) against the different studied enzymes and can be used as leads to the synthesis of new MBL inhibitors. Our tests indicated that the presence of compounds 2b, 4a and mgfg decreased the MIC value for imipenem.
Conclusion: In this study, we show that members of the phosphonates group are able to enhance the inhibition of Zn β-lactamases. This is the first report of new inhibitors possessing a strong activity against the different sub-classes of MBLs.

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