# Monitoring changes in respiratory pathogen carriage in Swiss Armed Forces Personnel deployed in Kosovo using pre- and post-deployment nasal swabs

## Surveillance de l'évolution du nombre d'agents pathogènes respiratoires chez le personnel de l'armée suisse déployé au Kosovo à l'aide d'écouvillons nasaux pré et post-déploiement

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## Abstract

**Background**
Airborne transmission of pathogens can be a major cause of morbidity in deployed military.
*Objectives*
In this study, conducted prior to the COVID-19 pandemic, we sought to monitor changes in respiratory pathogen carriage in Swiss military personnel deployed in Kosovo.
**Methods**
The study was designed as a prospective evaluation using paired nasal swabs. The swabs were taken immediately prior to departure and on the return flight from Kosovo. Short questionnaires were administered at the time of swabbing to capture data on the occurrence of respiratory symptoms and influenza like illness. The paired swabs were tested for 34 respiratory viruses and bacteria using Real Time PCR. Differences in proportions were tested by McNemar's chi-square which considers the pair structure of questionnaires and PCR results.
**Results**
We evaluated 204 participants. The PCR results showed changes in colonization and pathogen carriage pre- and post-deployment particularly with respect to rhinovirus, common coronavirus and the bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae*. We found concordance between symptom questionnaires and PCR results.
**Conclusions**
The study showed that nasal swabs can be successfully used for deployed military to monitor circulating respiratory pathogens and to may be effective in recognising regional emergence of new pathogens. This will be of added value for military preparedness and allow for tailored planning of medication needs and vaccines.

## Résumé

**Contexte**
La transmission par voie aérienne d'agents pathogènes peut être une cause majeure de morbidité chez les militaires déployés.
Objectifs de l'étude
Dans cette étude, menée avant la pandémie de COVID-19, nous avons cherché à surveiller les changements dans le portage de pathogènes respiratoires par le personnel militaire suisse déployé au Kosovo.
**Méthodes**
L'étude a été conçue comme une évaluation prospective utilisant des écouvillons nasaux appariés. Les prélèvements ont été effectués immédiatement avant le départ et sur le vol de retour du Kosovo. Des questionnaires succincts ont été administrés au moment du prélèvement pour recueillir des données sur l'apparition de symptômes respiratoires et d'affections pseudo-grippales. Les écouvillons appariés ont été testés pour 34 virus respiratoires et bactéries par PCR en temps réel. Les différences de proportions ont été testées par le chi-carré de McNemar, qui tient compte de la structure en paires des questionnaires et des résultats de la PCR.
**Résultats**
Nous avons évalué 204 participants. Les résultats de la PCR ont montré des changements dans la colonisation et le portage d'agents pathogènes avant et après le déploiement, en particulier en ce qui concerne le rhinovirus, le coronavirus commun et les bactéries Staphylococcus aureus et Klebsiella pneumoniae. Nous avons constaté une concordance entre les questionnaires sur les symptômes et les résultats de la PCR.

**Conclusions**
L'étude a montré que les écouvillons nasaux peuvent être utilisés avec succès par les militaires déployés pour surveiller les agents pathogènes respiratoires en circulation et qu'ils peuvent être efficaces pour reconnaître l'émergence régionale de nouveaux agents pathogènes. Cela apportera une valeur ajoutée à la préparation militaire et permettra une planification personnalisée des besoins en médicaments et en vaccins.

## Background

Air borne transmission of pathogens in military populations is important for many reasons: Military personnel are often confined to close living quarters that facilitate the spread of respiratory infections. Personnel may be subject to psychological stress and have difficulty in maintaining protective, personal hygiene under strict training conditions. Interactions between personnel from other military units and local inhabitants may lead to exposure to respiratory pathogens from diverse sources. Respiratory infections and influenza-like illness (ILI) can be debilitating and may decrease deployment capability. A previous study of symptomatic Polish soldiers who sought medical advice for ILI showed a wide spectrum of respiratory pathogens (Kocik J et al. 2014). Travel within Europe is associated with respiratory illness (Schlagenhauf 2015). Near real-time surveillance may be effective in recognizing regional, emergence of new respiratory pathogens. Furthermore, some infectious disease caused by biological warfare agents may initially resemble ILI at first presentation and early identification of unusual or unexpected pathogens is imperative.
The investigation of nasal carriage of respiratory pathogens using non-invasive sampling has been used in recent studies of French pilgrims to the Hajj. These studies showed rapid acquisition of respiratory viruses and bacteria carriage, particularly rhinovirus and *Streptococcus pneumonia* (Benkouiten S et al. 2013, Gautret P et al 2014, Memish et al 2015) during travel. The approach of using non-invasive nasal swabs and then detecting carriage and acquisition of pathogens using real-Time PCR methodology has been shown in the afore-mentioned studies to be a safe and successful methodology that has high participant acceptance due to the non-invasive sample collection. The Marseille group form a centre of excellence in the diagnosis of respiratory pathogens using advanced PCR methodology.
Prior to the COVID-19 pandemic we aimed to assess the changes in the spectrum of respiratory pathogen carriage in Swiss Armed Forces deployed to Kosovo. In a prospective paired cohort study, we specifically aimed to

1. To investigate the panel of respiratory pathogen encountered by Swiss forces deployed in Kosovo
2. To document changes in colonization of pathogens
3. To target pre-travel advice regarding preventive measures for respiratory illness such as the use of hand sanitizers and personal protection measures including appropriate vaccinations.

## Methods

The study was approved by the regional Swiss Ethics Committee (EKNZ 2015-279)
Participants needed to satisfy the following inclusion criteria in order to participate

- deployment in Kosovo in the period September 2015 to March 2016 for a stay longer than one week and up to six months (Figure 1)
- aged 18 or over
- written informed consent
- willingness to provide nasal swab samples before and after travel
- willingness to answer a short questionnaire before and after travel

Upon completion of a written informed consent, available in the three national languages of Switzerland (German, French and Italian), the volunteers provided the first nasal swab sample and completed a short *baseline* questionnaire. On return from Kosovo the study participants provided a second nasal swab and will completed a brief questionnaire on respiratory illness (Figure 1 a) and b). Nasal sampling procedure, from the anterior nares followed the same protocol (figure 1 c) on both occasions. Each study participant thus provided pre- and post- travel nasal swab samples and the following data:
Demographic data such as initials, day of birth, sex.
History of previous respiratory illness and travel history (in the past 6 months)
Concomitant illness and information on smoking Concomitant medications.
Nasal swabs were taken immediately prior to departure to Kosovo and after return (anterior nares) using commercial rigid cotton tipped swab applicators (Remel, USA) that was placed in universal transport medium (Remel) at the time of collection and stored in a freezer at -80°C within 48 hours

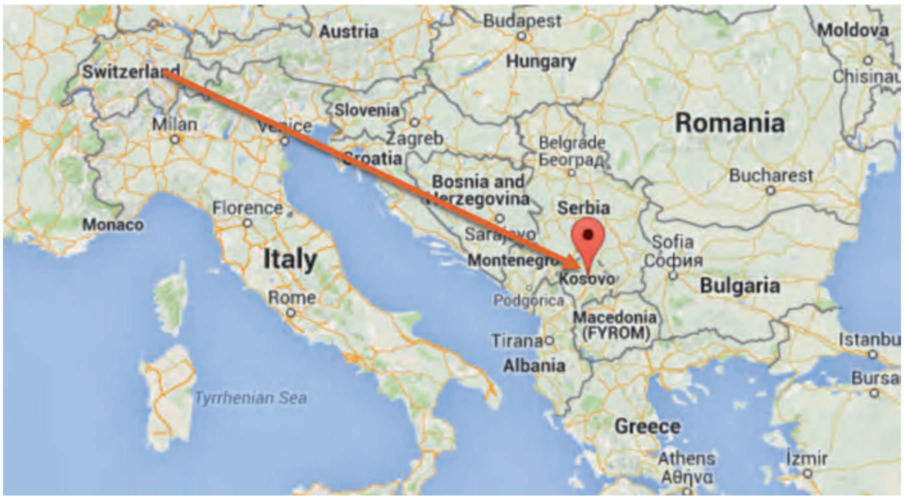


Figure 1a. Deployment of Swiss Armed Forces in Kosovo

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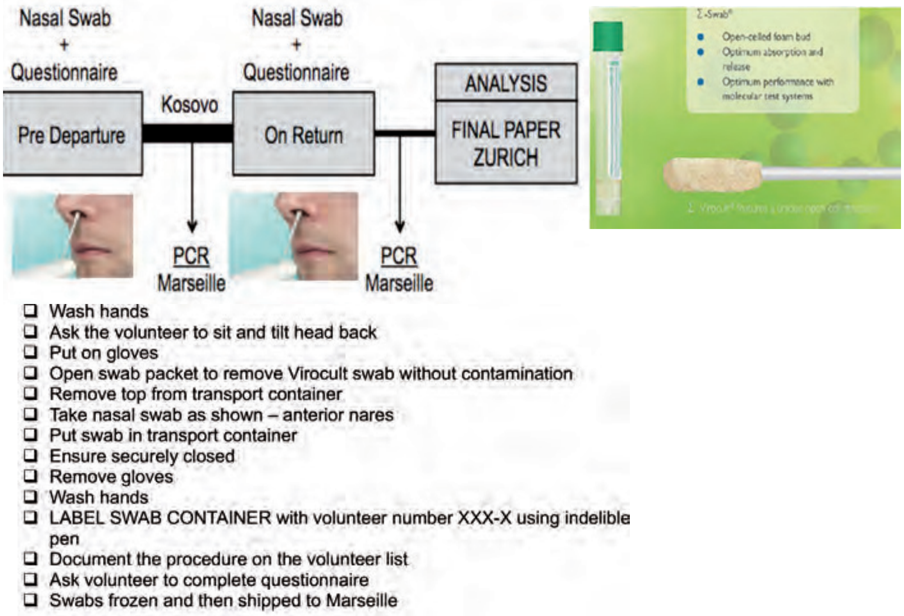


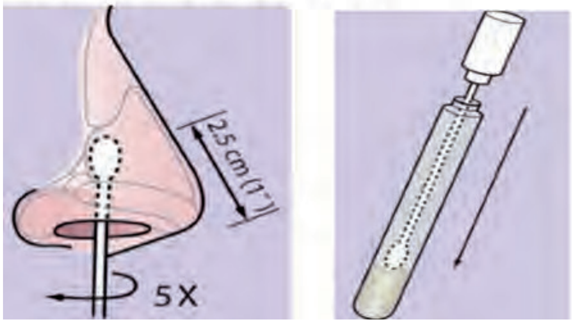


Figure 1b) and Figure 1c) nasal Swab sampling and questionnaire administration
Figure 1c) SOP for taking nasal swabs
Figure 1c – Protocol for nasal swab sampling

of collection and thereafter transported in dry ice for analysis to Marseille, France. Each swab was processed manually. One millilitre of medium will be pipetted automatically (Beckman NX automate) to prepare 96-well microplates for total (DNA+RNA) nucleic acid extraction and log-term storage. To avoid unnecessary testing, a dual aliquot was prepared from 100mL of nasal swab origin. The total nucleic acids were purified using the Macherey-Nagel Nucleospin-96 kit. The extract was split for the testing for the presence of viruses and bacteria. Real-time PCR was performed using FTD Respiratory Pathogens 21-plus kit for multiple respiratory viruses (Memish ZA et al 2015). Respiratory bacteria were investigated using a 7900HT-thermocycler (Applied Biosystems, USA) and QuantiTect-Probe PCR Kit (Qiagen, France). Table 1 shows the range of tested pathogens.

Differences in proportions were tested by McNemar's chi-square which considers the pair structure of questionnaires and PCR results.

Table 1. Panel of respiratory pathogens for inclusion in the analysis of the paired cohort

| **Type** | **Respiratory Pathogen** | |
|---|---|---|
| Virus | Influenza virus A/H3N2 | Human cytomegalovirus |
| | Influenza virus 2009 A (H1N1) | Human enterovirus |
| | Influenza virus B | Human metapneumovirus |
| | Influenza virus C | Human respiratory syncytial virus |
| | Human parainfluenza viruses 1 | Human rhinovirus |
| | Human parainfluenza viruses 2 | Human adenovirus |
| | Human parainfluenza viruses 3 | Human bocavirus |
| | Human parainfluenza viruses 4 | Parechovirus |
| | MERS coronavirus | |
| | Human coronavirus 229E | |
| | Human coronavirus NL63 | |
| | Human coronavirus HKU1 | |
| | Human coronavirus OC43 | |
| Bacteria | *Bordetella pertussis* | *Legionella pneumophila* |
| | *Mycoplasma pneumonia* | *Salmonella* spp |
| | *Neisseria meningitidis* | *Staphylococcus aureus* |
| | *Streptococcus pneumoniae* | *Coxiella burnetii* |
| | *Streptococcus pyogenes* | *Chlamydophila pneumoniae* |
| | *Klebsiella pneumonia* | *Haemophilus influenzae* |
| | *Francisella tularensis* | |
| Other | *Pneumocystis jirovecii* | |

## Results

### Sociodemographic Characteristics

The study cohort consisted of 202 Swiss military personnel deployed in Kosovo. Participants ranged in age from 22 to 68 years, with a median age of 28.5 years (mean 31.2 years, SD 8.36). The gender distribution was predominantly male, with 168 males (83%) and 34 females (17%), and this distribution remained unchanged post-deployment. Smoking was reported by 30% of the soldiers before deployment and increased slightly to 32% after deployment. Alcohol consumption remained stable, with 91% of the participants reporting alcohol use both before and after deployment. The quality of the data collected was high, with 99% of participants completing both the pre- and post-deployment questionnaires. There were minimal data omissions due to technical issues, and all PCR tests were successfully conducted.

### Symptom Questionnaire Analysis

The prevalence of various respiratory and systemic symptoms was assessed using questionnaires administered before and after deployment. A significant reduction in the number of soldiers reporting cold symptoms was observed post-deployment, with 86 soldiers reporting cold symptoms before deployment compared to 43 soldiers after deployment ($p = 0.01$). Similarly, the incidence of throat symptoms decreased significantly from 33 soldiers pre-deployment to 21 soldiers post-deployment ($p = 0.02$). Reports of wet cough symptoms also showed a significant reduction, from 26 soldiers before deployment to 18 soldiers after deployment ($p = 0.04$). However, other symptoms, including flu, fever, headache, limb pain, sneezing, breathing difficulties, earache, eye irritation, dry cough, diarrhea, vomiting, and

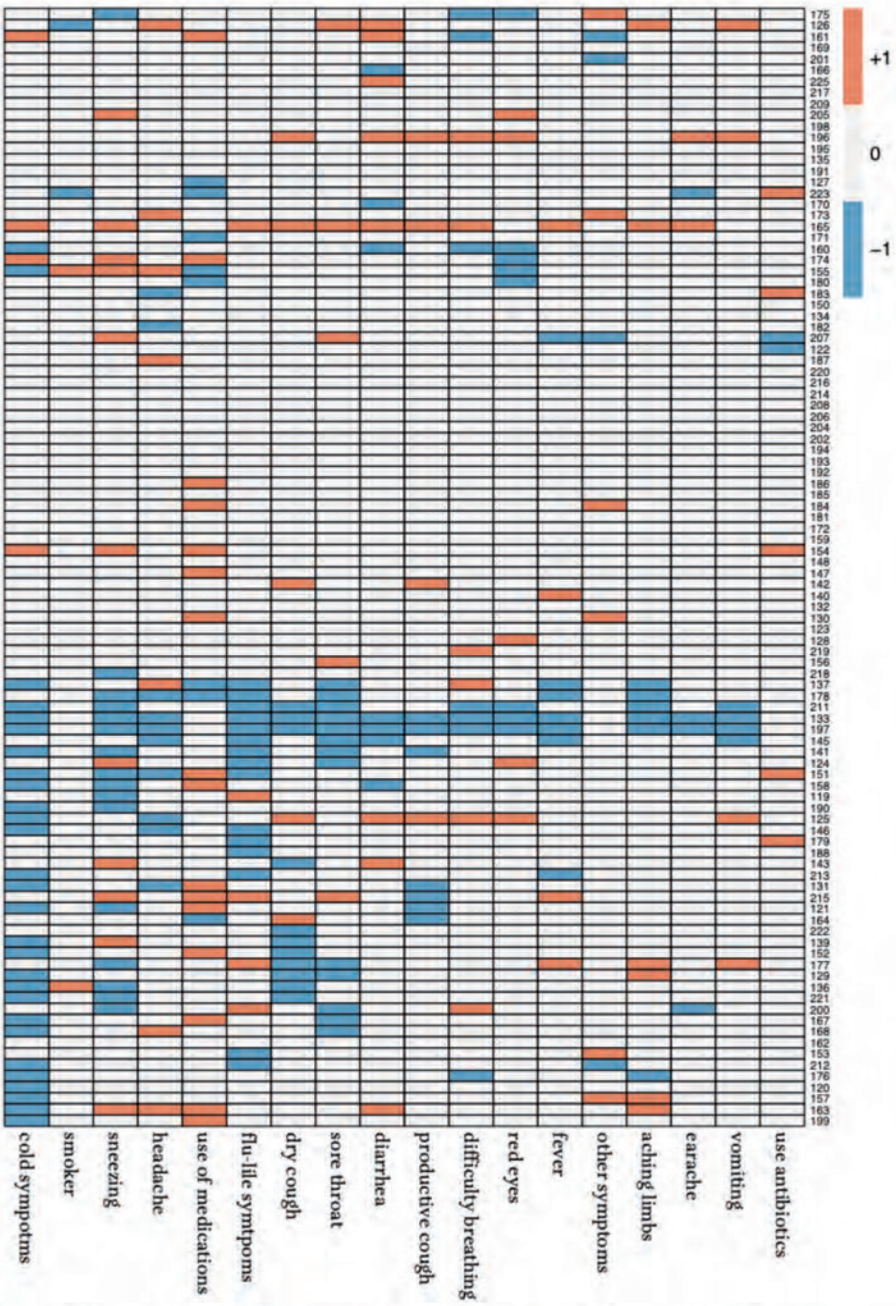


Figure 2: Heatmap of answer differences before and after Kosovo. Each row corresponds to one soldier. Colors indicate direction of changes (-1: only before Kosovo, 0: same before and after Kosovo, +1: only after Kosovo).

antibiotic use, did not show statistically significant changes between the pre- and post-deployment periods. (Figure 2)

**Pathogen Carriage Analysis**

Paired nasal swabs were analyzed using Real-Time PCR to detect a panel of respiratory pathogens before and after deployment. The analysis revealed significant changes in the carriage of specific pathogens. For *Staphylococcus aureus*, the positive rate decreased from 20.3% (41/202) pre-deployment to 15.3% (31/202) post-deployment, with an acquisition rate of 3.7% (6/161) among those who were initially negative. *Klebsiella pneumoniae* exhibited a reduction in the positive rate from 2.0% (4/202) pre-deployment to 0.5% (1/202) post-deployment, with no new cases detected, resulting in an acquisition rate of 0.0%. In contrast, Rhinovirus showed an increase in the positive rate from 1.5% (3/202) pre-deployment to 4.0% (8/202) post-deployment, with an acquisition rate of 3.5% (7/199). Moraxella had a positive rate that decreased from 1.5% (3/202) pre-deployment to 0.5% (1/202) post-deployment, with an acquisition rate of 0.5% (1/199). The positive rate for MPV (*Metapneumovirus*) declined from 1.0% (2/202) pre-deployment to 0.5% (1/202) post-deployment, yielding an acquisition rate of 0.5% (1/200). Lastly, hCMV (Human Cytomegalovirus) maintained a consistent positive rate of 0.5% (1/202) both pre- and post-deployment, resulting in an acquisition rate of 0.5% (1/201). (Figure 3)

Other respiratory pathogens included in the analysis but with no positive detections before or after deployment were Influenza viruses (A/H3N2, 2009 A (H1N1), B, C), Human parainfluenza viruses (1, 2, 3, 4), MERS coronavirus, Human coronaviruses (229E, NL63, HKU1, OC43), bacteria (*Bordetella pertussis, Mycoplasma pneumoniae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus pyogenes, Legionella pneumophila, Salmonella spp., Coxiella burnetii, Chlamydophila pneumoniae, Haemophilus influenzae, Francisella tularensis*), and *Pneumocystis jirovecii*.

## Discussion

In the peri and post-pandemic periods, respiratory tract infections (RTI) have come increasingly under scrutiny, not least because of their potential to cause outbreaks and to be the causative agents of pandemics. These RTI infections can be caused by viruses, bacteria or fungi but are mainly caused by viruses. Studies on respiratory infections in travellers show that respiratory symptoms and infections represent a significant burden for travellers (Schlagenhauf et al 2015, Lovey et al 2023). Settings where persons are in close proximity to each other such as mass gathering events, sporting events and concerts are particularly conducive to the spread of airborne infections (Gautret 2015, Hoang et al 2019). Respiratory infections in mobile populations such as deployed military personnel can have a major impact on performance and well-being. In addition, the concentration of persons in a confined space such as deployed soldiers constitutes a risk factor for the rapid spread of airborne infections. Kocic et al evaluated the epidemiology of influence like illness in deployed military but to date, this is the most comprehensive evaluation of respiratory pathogen carriage in military personnel. We evaluated 202 participants using a methodology that was previously used for Hajj pilgrims (Memish et al). The PCR results showed changes in colonization and pathogen carriage pre- and post-deployment with some acquisition and some loss of pathogens. We found concordance between symptom questionnaires and PCR results. As in other studies, viruses were the most common pathogens reported. The carriage of some pathogens decreased during the stay in Kosovo and there were

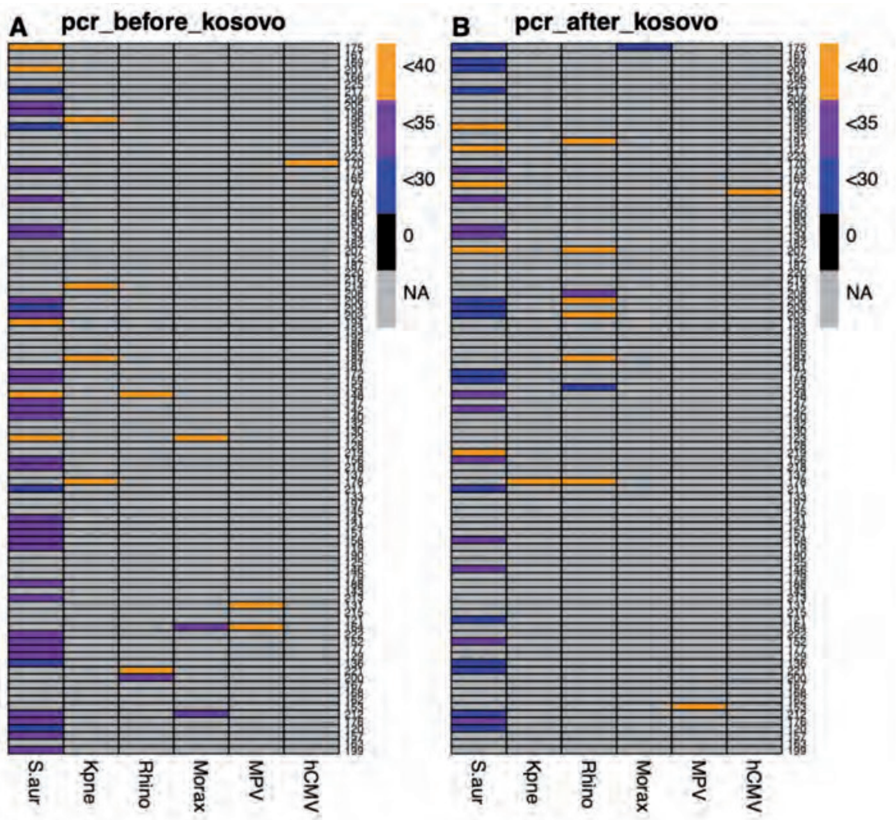


**Figure 3: Heatmap of PCR results before (A) and after (B) Kosovo. Each row corresponds to one soldier. Colors represent PCR values. S.aur stands for Staphylococcus aureus, K.pne stands for Klebsiella pneumoniae, Rhino stands for Rhinovirus, Morax stands for Moraxella, MPV stands for Human metapneumovirus, and hCMV stands for Human cytomegalovirus.**

changes in the overall spectrum of colonization. Evaluation of the epidemiology of RTIs in military groups allows for "preparedness" with respect to medication needs and tailored prevention and vaccines based on local infection epidemiology. In certain circumstances monitoring respiratory infections can identify outbreaks, bioterrorism, unusual signals or the emergence of a novel pathogen. The respiratory symptoms experienced in Kosovo, included cough, sore throat and general flu-like symptoms which required an increase of antibiotic use (4 persons prior to deployment and 10 during the stay in Kosovo). The study has some limitations, personnel were deployed for varying lengths of stay in Kosovo, preventive measures and hygiene may differ between individuals and there may be confounding due to varied interactions with other groups deployed in the area and with local populations. There is also a seasonal element as most of the deployed personnel left Switzerland in winter and were thus deployed to a warmer climate.

## Conclusions

The study showed that nasal swabs can be successfully used for deployed military to monitor circulating respiratory pathogens and to may be effective in recognising regional emergence of new pathogens. This will be of added value for military preparedness and allow for tailored planning of medication needs and vaccines.

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### Funding and Acknowledgements

This study was funded by the Swiss Armed Forces.

Thank you to all the deployed personnel who took part and to Murielle Goy, Chief Nurse

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