

Non-Tubercle Mycobacteria and Other Contaminants in Metalworking Fluids from Small Turneries

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ABSTRACT

Non-Tuberculous Mycobacteria (NTM) have frequently been isolated from metalworking fluids (MWFs) used in large machining industries. This paper concerns the microbial detection, particularly NTM in MWFs employed in small metalworking shops. A total of 21 samples were collected from different turneries and were tested for several microbiological parameters. A total microbial count above 10^6 CFU ml⁻¹ was observed in 66.6% (14/21) of samples and none of the samples had the count $<10^2$ CFU ml⁻¹. The dominant contaminants were Gram-negative bacteria with 90.5% (19/21) of samples revealing the presence of *Pseudomonas aeruginosa*. Sulfate Reducing Bacteria (SRB) were detected in 52.4% (11/21) of samples, whereas NTM were recovered from 14.3% (3/21) of samples only. Two species of NTM were identified by biochemical reactions as *Mycobacterium chelonae* and *Mycobacterium abscessus*. It was concluded that MWFs used in small turneries are usually contaminated with Gram-negative bacteria and SRB but NTM are not common contaminants of these fluids.

Keywords: metalworking fluids; microbial contamination; small turneries; non-tuberculous mycobacteria.

INTRODUCTION

Metalworking fluids (MWFs) are used in machining industries for boring, tapping, grinding, turnery, and honing of metals (Brinksmeier et al. 2015). Classification and primary functions of these fluids were described elsewhere (Childers 2006). Except for straight oils which are used directly without water, all other types of MWFs contain water and additives, including emulsifiers, biocides, antifoaming agents, and corrosion inhibitors (Passman and Küenzi 2020). These ingredients, in addition to other factors like oxygenation, deoxygenation, pH, and redox potential of the operating fluids, provide certain microorganisms with chemical and physical growth conditions, particularly after the depletion of biocide. MWF concentrates are practically free of bacteria when sold to customers but no matter how hard industries try to keep fluids in operation free from

microorganisms, they eventually become contaminated with a variety of aerobic and anaerobic microorganisms. Circulation of emulsion from the sump to the chip/ tool interface introduces oxygen into it and this practice favors the proliferation of aerobes, while stagnation of emulsion in the sump provides the anaerobic bacteria, particularly Sulfate Reducing Bacteria (SRB), with the opportunity to flourish. (Abu Shaqra and Hill 1984). MWFs can support the growth of microorganisms and microbial numbers as high as 10^9 ml⁻¹ were recovered from these fluids (Sloyer et al. 2002; Van der Gast et al. 2003). Microorganisms, such as species of *Staphylococcus*, *Pseudomonas*, *Alcaligenes*, SRB, and *Acinetobacter* are known to inhabit MWFs (Perkins and Angenent 2010; Van der Gast et al. 2003). Obligatory pathogens and opportunistic pathogens such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* were also found to contaminate MWFs

(Bakalova et al. 2007; Lucchesi et al. 2012). Occupational exposure to MWFs through inhalation of aerosols may irritate the throat, nose, and lung and has been associated with chronic bronchitis, asthma, hypersensitivity pneumonitis (HP), and worsening of pre-existing respiratory problems (Burton et al. 2012). Early findings indicated that the individuals working with MWFs are vulnerable to the infections related to the presence of Non-Tuberculous Mycobacteria (NTM) in these fluids (Muilenberg et al. 1993). *Mycobacterium abscessus*, *Mycobacterium immunogenum* and *Mycobacterium avium* have been detected in MWFs. *M. immunogenum* in particular was identified as an etiologic agent at many metalworking industries where HP cases were diagnosed (Passman and Kuenzi 2020; Kapoor and Yadav 2012; Khan et al. 2005; James et al. 2015).

Detection of NTM in MWFs can be performed using molecular techniques and traditional culture methods (Yadav et al. 2003; Khan et al. 2005). Due to the presence of fast-growing microorganisms in these fluids, they may overgrow the NTM, and thus, their isolation becomes rather difficult (Passman 2008). For the successful recovery of NTM from ecological specimens, the media augmented with antibiotics and the use of certain decontaminating chemicals before culturing to kill fast-growing organisms have been employed. The use of such chemicals in the pretreatment of samples makes it possible to isolate NTM from the most complex of habitats such as soil (Hu et al. 2017). In Jordan, large industries such as automotive factories are not available and most MWFs are used in small metalworking shops. This study aimed to establish for the first time, the microbial diversity of in-use MWFs in small Jordanian turneries and to determine the presence or absence of NTM in these fluids using the culture methods.

MATERIAL AND METHODS

Collection of MWF samples

The MWFs samples were collected in 100 ml sterile screw-capped containers from 21 turneries operating in Amman, Jordan. Two samples were collected from each machine, one from the circulated fluid for the isolation of aerobic contaminants and the other from the sump for the recovery of SRB. The only stipulation observed for the

inclusion of the fluid in this work was the duration of its use and this was specified at 1 to 2 months. The size of the sump was noted and recorded. The pH of each sample collected was also measured.

Isolation of aerobic bacteria and their count

Within 4 hours from sample collection, a loopful of each sample equivalent to 10 μ l was streaked on to the surface of soya bean casein digest (SBCD) agar. An aliquot of 1 ml fluid was also inoculated into 10 ml of selenite broth in tubes. After incubation of plates and tubes at 30°C for 18 h, a loopful (10 μ l) was taken and streaked onto SS and EMB agar plates for the isolation of enteric pathogens. From the SBCD plates, the colonies with apparently different morphologies were isolated, purified by subculture, and then identified using the diagnostic tables described by Barrow and Feltham (2003). The bacterial count was performed for each sample on SBCD agar using 1 ml aliquot of fluid or 1 ml from the appropriate dilution which was made in 10-fold series. The concentration for each microbial type present in the MWF was not determined except for *P. aeruginosa*, as it was the most predominant in the samples tested. The number of this organism in each sample was counted using a selective medium composed of SBCD supplemented with cetrimide.

Fungal isolation

Fungal isolation was performed by inoculating 100 μ l of the respective MWF onto Sabouraud Dextrose Agar plates with antibiotics; 50 mg/l chloramphenicol and 100 mg/l gentamicin. Plates were incubated at 25°C for up to 3 weeks and inspected daily for the appearance of fungal growth. Identification of fungal isolates was performed following the microscopic morphology given by Mycology online (<https://mycology.adelaide.edu.au>, last seen 11.7. 2021). Unless otherwise stated, all media used throughout this investigation were obtained from Difco, Michigan, USA.

Detection of sulfate reducing bacteria

The media used for the isolation of SRB was the modified iron sulfite agar produced by Himedia, India. This medium is recommended by the International Standards Organization (2003) for the detection and enumeration of anaerobic sulfite reducing bacteria. In this case, tubes containing

9 ml of the medium were inoculated with 1 ml of test sample collected from the sump of each machine, while the medium was in the molten state at 45°C. The tubes were shaken using a vortex mixer to homogenize the inoculum with the tube content. All inoculated media were allowed to solidify before 3 ml of the same medium was poured into each tube as an overlay. After solidification of the overlayer, tubes were incubated at 30°C for up to 3 weeks. The appearance of black-colored colonies during the incubation period was considered positive for SRB.

Isolation of non-tuberculous mycobacteria from MWFs and count

Each MWF sample was processed as described by Hu et al. (2017). In brief, an aliquot of 10 ml of the respective MWF collected from the circulated fluid was treated with 1.5 ml of 3% sodium dodecyl sulfate plus 2% NaOH for 30 min. The sample was then homogenized by vortexing before being centrifuged and the supernatant was discarded. An aliquot of 100 µl from the pellet was used to inoculate Middlebrook 7H11 agar supplemented with antibiotics and malachite green (concentration adjusted to 250 mg/l). The antibiotics used in the medium and their concentrations per liter were: Polymyxin B 200,000 units, Carbenicillin 50 mg, Amphotericin B 10 mg, and Trimethoprim Lactate 20 mg. The inoculated plates were incubated for 10 days at 30°C and when colonies appeared, they were counted and acid-fast stained to confirm their affiliation to the genus *Mycobacterium*. Biochemical testing of isolates was performed as given below.

Soil sample processing for NTM isolation

The soil samples were collected from the nearest area to each turnery included in this investigation. Each soil sample was processed for the isolation of NTM as described by Hu et al. (2017). The technique involved the following steps: approximately 5 g of soil were placed in a 50 ml sterile screw-capped container then 15 ml of sterile distilled H₂O were added. After vigorous shaking for 2 min, containers were allowed to stand for another 2 min and 4 ml of the upper one-third of the turbid supernatants was pipetted and resuspended in 4 ml of 3% sodium dodecyl sulfate plus 2% NaOH. This preparation was incubated at room temperature for 30 min.

After incubation, the decontamination solution was removed by centrifugation and the pellet was washed twice with 5 ml of sterile phosphate-buffered saline (PBS) and then resuspended in 1 ml of sterile PBS. The isolation of NTM was achieved by plating 10 µl of the prepared PBS suspension onto modified Middlebrook 7H11 media supplemented with antibiotics and increased concentration of malachite green. Plates were incubated at 30°C for 10 days and developed colonies were identified by the traditional acid-fast staining. The isolated colonies recovered from soil samples collected from the areas close to the turneries where the in-use MWF was found to be contaminated with NTM were subjected to further characterization by biochemical reactions.

Biochemical testing of NTM isolates

The isolates recovered on the modified Middlebrook 7H11 were identified by their biochemical characteristics, including carbohydrate utilization (citrate, sorbitol, inositol, mannitol), 3-d arylsulfatase activity, growth in the presence of 5% NaCl at 30°C, iron uptake, nitrate reduction, pigment production. All tests were performed as described by Bhalla et al. (2018).

Suitability of NTM isolation technique from MWF

The method employed herein for the detection of NTM in MWFs was derived from a technique used for the isolation of these bacteria from soil. Therefore, it was of importance to establish its suitability for the recovery of similar types of bacteria from a different habitat. This was achieved through the separate inoculation of NTM free MWF samples with one *Mycobacterium chelonae* and one *M. abscessus* which were recovered from the tested soil samples. This experiment was repeated several times with the number of inoculated cells being the variable. The number of inoculated NTM into the MWF samples varied from 10² to 10⁶ CFU ml⁻¹ in order to establish the detection limit of NTM from MWFs using the technique employed in this investigation. The period between inoculation and recovery was set at one hour. The samples with added NTM served as positive controls while the samples devoid of NTM were also included to serve as a negative control. The ability to recover these bacteria from the deliberately inoculated MWFs, in addition to

their lack of detection from the negative control, indicated the suitability of the method used.

Isolation of NTM from water

A total of 10 municipality water samples were collected from different turneries in 100 ml sterile containers. These samples represented the type of water used for diluting MWF concentrates. The aliquots equivalent to 50 ml of each sample were centrifuged, pellet collected, resuspended in 10 ml sterile distilled water, and then processed for NTM isolation as described for the recovery of these organisms from MWFs. The identity of the recovered bacteria was established by acid-fast staining and biochemical testing.

RESULTS

All machines investigated were small with a sump located in the lower part of the machine and the MWF was circulated to the chip/ tool interface through the action of a pump. None of the turnery machines studied received fluids from a central system and the sump size never exceeded 50 liter in capacity. The majority of MWFs collected from these turneries were semisynthetic fluids with pH values ranging between 6.6 and 7.8. They were found to be contaminated with microorganisms particularly bacteria. Table 1 shows that 66.6% of samples contained $>10^6$ CFU ml⁻¹, whereas only 7 samples out of 21 contained microbial count between $\geq 10^2$ – $<10^6$. As can be seen

from the same table, none of the samples tested contained less than 10^2 CFU ml⁻¹.

The microbial population detected in the MWFs was dominated by Gram-negative bacteria. Table 2 demonstrates that *P. aeruginosa* and *Pseudomonas olveorans* were the most encountered species in these fluids, whereas other Gram-negatives such as *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp. were also detected but at a lower frequency. The least isolated Gram-negative bacteria was *Citrobacter* spp. which was detected in 2/21 samples only. No *Salmonella* spp. or *Shigella* spp. were found in any of the samples tested. SRB were recovered from 11/21 samples and 7/21 samples harbored the fungi that belonged to *Fusarium* spp. and *Acremonium* spp. The results obtained by counting *P. aeruginosa* on selective culture media indicated that this organism was quantitatively the most prevalent contaminant in the MWFs studied. Only 3 MWF samples revealed the presence of NTM in numbers ranging between 10^{-2} and 10^{-5} CFU ml⁻¹. These organisms were identified using the biochemical tests

Table 1. Ranges of bacterial count detected in MWFs versus the number of samples contaminated and percentages

Microbial count CFU ml ⁻¹	Number of samples	Percentage
$\leq 10^2$	0	0
$\geq 10^2 - <10^4$	2	9.5
$\geq 10^4 - < 10^6$	5	23.9
$\geq 10^6$	14	66.6

Table 2. Frequency and percentage of microbial detection in MWFs collected from Jordanian turneries

Microorganism recovered	Frequency of recovery in MWF samples	Percentage occurrence in MWF samples
<i>Pseudomonas aeruginosa</i>	19	90.5
<i>Pseudomonas alveorans</i>	16	76.2
<i>Escherichia coli</i>	9	42.8
<i>Klebsiella</i> spp	7	33.3
<i>Enterobacter</i> spp	6	28.5
<i>Proteus</i> spp	6	28.5
<i>Citrobacter</i> spp	2	9.5
Coagulase -ve* <i>Staphylococcus</i>	10	47.2
<i>Salmonella</i> and <i>Shigella</i> spp	0	0
Non-Tuberculous Mycobacteria	3	14.3
Sulphate Reducing Bacteria	11	52.4
<i>Fusarium</i> spp	4	19.0
<i>Acremonium</i> spp	3	14.3

*-ve = Negative

as *Mycobacterium chelonae* and *M. abscessus* (table 3). By referring to the biochemical reactions described by Wilson et al. (2001), it was found that the profiles of *M. immunogenum* were not detected among our isolates. Two differences between the biochemical reactions of *M. abscessus* and the *M. chelonae* were noted, the latter was able to utilize citrate as a main source of carbon but could not grow in the presence of 5% NaCl at 35°C. For these two characters, *M. abscessus* exhibited opposite patterns from those that labeled *M. chelonae* (Table 3).

Although the method employed for the detection of NTM was originally used by Hu et al. (2017) for the isolation of these bacteria from soil, it was found in this work to be adequate for the NTM recovery from MWFs. Results of the suitability testing studies demonstrated that when the MWF was inoculated with 10^{-6} CFU ml⁻¹ down to 10^{-2} CFU ml⁻¹ of NTM, the recovery rate was better than 65%. However, when the inoculated number of NTM was below 10^{-2} CFU ml⁻¹, their detection was satisfactory but their enumeration was not reproducible. Therefore, the absence of

NTM from 18 samples of the MWF studied in this work could not be attributed to inadequacy of the isolation technique but due to a genuine absence of these bacteria in the samples investigated. More than 80% of soil samples harbored NTM as established by the acid-fast staining. The soil samples collected from close proxy with the 3 turneries that contained NTM in the MWFs used in their facility were found positive for *M. chelonae* and *M. abscessus*. Testing the municipal water derived from 10 turneries showed that NTM was recovered from 4 samples but none of the isolates fitted the biochemical reaction pattern of either *M. chelonae* or *M. abscessus*. The biochemical reactions of the isolated NTM from the water samples are shown in Table 4. Two water samples harbored the isolates that exhibited the same pattern of biochemical reactions. None of the biochemical reaction patterns of these isolates was found to label any of the *Mycobacterium* recovered from the MWFs investigated.

DISCUSSION

Gilbert et al. (2010) investigated the microbial load of 44 in-use MWFs and detected no difference between the molecular and culture counting methods when the number of contaminants in the fluid was high. Culturable mesophilic aerobic bacteria in their studied samples ranged between being undetectable to 2.36×10^9 CFU ml⁻¹, with a median value of 3.05×10^7 CFU ml⁻¹. The samples that contained 7.53×10^6 copies of 16S rRNA based on real-time PCR assay contained 8.0×10^5 CFU ml⁻¹ culturable mesophilic bacteria. Table 1 shows that 14 out of 21 samples tested herein harbored bacteria above 10^6 CFU ml⁻¹; thus, according to Gilbert et al. (2010) 66.6% of the samples

Table 3. Biochemical reaction results for the NTM (*M. chelonae* and *M. abscessus*) isolated from MWFs

Test Performed	<i>M. chelonae</i>	<i>M. abscessus</i>
Citrate utilization	+	-
sorbitol utilization	-	-
Inositol utilization	-	-
Mannitol utilization	-	-
3-d Arylsulfatase activity	+	+
Growth in 5% NaCl (35 °C)	-	+
Iron uptake	-	-
Nitrate reduction	-	-
Pigment production	-	-

Table 4. Biochemical reaction results for the NTM isolated from water samples

Test performed	Isolate # 1	Isolate #2	Isolate #3 & 4
Citrate utilization	+	-	-
Sorbitol utilization	+	-	-
Inositol utilization	-	+	-
Mannitol utilization	+	+	-
3-d Arylsulfatase activity	-	-	+
Growth in 5% NaCl (35 °C)	+	-	-
Iron uptake	+	-	+
Nitrate reduction	+	-	-
Pigment production	+	+	-

tested in this study, contained bacterial counts indicative of the actual contamination rate. The contaminants of MWFs in this work belonged to a limited number of bacterial genera, dominated by Gram-negative bacteria and this is in agreement with most published literature (Gilbert et al. 2010; Cyprowski et al. 2007; Van der Gast et al. 2003). Dominant contaminants, as shown in table 1 belonged to *Pseudomonas* species, particularly *P. aeruginosa* and *P. oleovorans*. These findings are again consistent with those reported by many other investigators (Gilbert et al. 2010; Cyprowski et al. 2007; Perkins and Angenent 2010). *P. aeruginosa* was the most prevalent not only in their occurrence rate but also in their numbers.

A study by Murat et al. (2012) demonstrated that metal types and the nature of MWF may play an important role in determining the microbial composition of contaminated fluids. They found that Gram-negative rods were predominant in the fluids used in the non-automotive industry, whereas Gram-positive rods were more prevalent in the MWFs used in the automotive industry. The dominance of Gram-negatives- isolates in the MWFs samples studied herein agrees with the findings of Murat et al. (2012) as all of the investigated samples were collected from small turneries and none were obtained from an automotive factory. Metalworking fluid spoilage can be defined as any change in the fluid which adversely affects its utility and this can be brought about by a variety of bacterial genera. However, typical spoilage of these products which is characterized by the development of blue discoloration of the fluid, its loss of stability, and the presence of sulfide which leads to an offensive odor can only be achieved in the presence of SRB (Abu Shaqra and Hill 1986; Bennett 1957). The latter author isolated SRB from 30 out of 33 samples of cutting oil emulsions used in different machines. The occurrence rate of SRB reported by Bennett (1957) was much higher than that detected in the conducted investigation, as only 11/21 samples (52.4%) were found to harbor SRB. The detection rate of these bacteria in this work could have been higher had the swarf or slime of biofilms derived from machine sumps been tested. These bacteria were found by Abu Shaqra and Hill (1984) to be adherent to swarf in higher concentrations than that present in the fluid. Therefore, the recovery of SRB from 52.4% of the samples should be considered as the minimum detectable percentage in the MWF studied.

Veillette et al. (2004) found that the microbial count, particularly NTM by plate culture methods, was lower than that obtained by microscopic means. The media used by the same authors was R2A and Middlebrooks 7H10 supplemented with antibiotics. In the current investigation, Middlebrook 7H11 agar was used, which consisted of Middlebrook 7H10 agar supplemented with pancreatic digest of casein to enhance the growth of fastidious strains of mycobacteria. Middlebrook 7 H11 agar also contains an additional amount of Malachite green which selectively inhibits Gram-positive heterotrophs (Van Schothorst and Renaud, 1985). The inhibitory agents used in the pretreatment step were 3% sodium dodecyl sulfate plus 2% NaOH for 30 min. These chemicals are usually used to lyse Gram-negative bacteria (Wada et al., 2012). These inhibitors are believed to allow the growth of NTM without being suppressed by the other coexisting faster-growing bacteria. NTM were detected in 3 of the 21 collected samples of MWF. This low recovery rate can be interpreted in two different ways. The first is that these organisms were already present in the fluid at a higher frequency but the test method was not efficient enough for their detection. The second possibility was to assume that the results obtained were accurate. The first possibility was excluded as the suitability testing method provided the proof that when NTM were present in the MWFs, they were recovered using the technique employed in this investigation. Therefore, the findings reported herein regarding the low recovery rate of NTM from MWFs collected from small turneries provide the evidence that these organisms were not common contaminants in the fluids used by small metalworking shops, such as turneries.

Two sources of NTM contamination in the MWF were investigated; the first was the water used for diluting the concentrates and the second was soil. Ristola et al. (2015) found that 15% of the water samples collected from domestic and institutional potable water in the United States and Finland were contaminated with mycobacteria. In the present work, 4 out of 10 municipal water samples were found to be to be positive for NTM recovery. None of these bacteria was identified as *M. chelonae* or *M. abscessus*. The slight variation between the obtained results and those of other investigators regarding the recovery rate and types of NTM isolates from water could be attributed to discrepancies in the recovery technique employed. In this investigation, 50 ml of water were

used, whereas others used larger amount of water while employing a filtration method (Thomson et al. 2013; Monde et al. 2018).

Muulinberg et al. (1993) isolated as much as 10^6 to 10^7 CFU ml⁻¹ of NTM from MWFs. These authors did not characterize their mycobacteria isolates other than being acid-fast. In the current research, identification of NTM was performed using the acid-fast staining as well as the biochemical tests proposed by (Wilson et al. 2001). One of the aims of this investigation was to report the presence or absence of NTM from MWFs used in turneries and this was achieved by using simple microbiological techniques without resorting to genotypic identification. Over the years, the NTM isolated from MWFs were identified as members of *M. chelonae* complex including *M. abscessus*, *M. chelonae* and *M. immunogenum*. Phenotypic characters between these three bacteria are almost the same except for a couple of characters. The limited number of biochemical tests used in this investigation were capable of identifying the recovered *Mycobacterium* spp as *M. chelonae* and *M. abscessus* (table 3). Similar findings were observed by Wilson et al. (2001) who tested the mycobacterial isolates recovered from several outbreaks, some involving MWFs, and reported that many of the isolates produced a hybrid pattern of phenotypic and genotypic characteristics that were common to both *M. abscessus* and *M. chelonae*.

Although members of *Mycobacterium chelonae-abscessus* complex are ubiquitous environmental organisms, they have been related to several opportunistic infections in humans, especially pulmonary and skin infections (Whipps et al. 2007). This complex is the most commonly identified mycobacteria causing diseases in humans after the *Mycobacterium tuberculosis* and *Mycobacterium avium* complexes (Sassi and Drancourt 2014). Nowadays, *M. abscessus* is one of the main infectious agents causing respiratory exacerbation in the patients with structural lung disorders such as cystic fibrosis (Bryant et al., 2013; Lopeman et al. 2019). The recovery of *M. chelonae* and *M. abscessus* in this investigation warrants larger scale investigation at a national level, since the inclusion of just 21 samples might not have been sufficient to draw conclusive conclusions about the prevalence of these organisms in metalworking shops operating in Jordan. Although no statistical calculations were performed in this study, it is prudent to say that the

larger the sample size, the more accurate the results would be in terms of statistical significance.

Respiratory and related symptoms of mucosal irritation are common among workers exposed to MWFs, but there are also symptoms potentially related to HP (fever, chills, headache, dry cough, flu-like symptoms, and malaise) and asthma (shortness of breath, chest tightness, and wheeze) (Park 2019). Despite the consensus that the role of NTM in causing respiratory tract diseases among metal mechanists is not clearly understood (Burge 2016), several articles were published during the past decade that have drawn attention to the importance of NTM in MWF (Sastre et al. 2013; Park 2019; Lopeman et al. 2019). Various aspects of lung diseases caused by NTM and the magnitude of this problem worldwide were recently reviewed by Ratnatunga et al. (2020). Cullinan et al. (2014) indicated that the diagnosis of a single case of respiratory tract illness in a working environment should prompt the workplace to review their risk assessment and exposure controls as well as to survey the remaining workforce to identify other affected workers. The health status of the mechanists working with the MWFs investigated herein was not addressed but during sample collection, it was noted that all workers did not complain of any respiratory tract condition. Because disease prevention is better than its treatment, the recovery of NTM from just 3 samples should not be underestimated and should be taken seriously by the health authority of Jordan.

Bernstein et al. (1995) found no evidence of NTM in the MWFs collected from a plant where 6 workers were diagnosed with HP. A similar finding was reiterated by Brookes (2017) who could not recover *Mycobacterium* from MWFs used in a facility where several workers suffered from HP. These observations are of dual importance: it provides the evidence that HP is not always related to the presence of NTM in MWFs and second, these bacteria are not frequent dwellers in this habitat. The results presented herein indicated that NTM cannot be considered as common microbiota of MWFs as they were isolated in this work from just 3 samples. This observation is perhaps the first which indicates the rare encounter of NTM in the MWFs used in small machining shops as opposed to the frequent isolation of these organisms from similar products used in large automotive factories. Burton et al (2011) reviewed 27 outbreaks of respiratory ill health attributed to MWF exposure and found that the majority of cases (81%)

originated in the USA, with the remainder coming from the UK (11%), France (4%), and Croatia (4%). The most commonly affected workplaces were those manufacturing components for the automobile (63%) or the aerospace (15%) industries. Between 1992 and 2002, a total of 250 cases of HP were reported in the United States among the personnel routinely exposed to MWFs used at automotive manufacturing facilities. Since then, many other cases were also reported (Passman and Kuenzi 2020; Tillie-Leblond 2011). In Jordan, there has been no published work related to the microbial contamination of MWFs and no records are available pertinent to the occurrence of HP among the metalworking personnel. Due to the detection of NTM in some of the fluids tested, it is recommended that the health authority in Jordan should establish whether such contamination has any impact on workers' health through a national surveillance and testing program.

Burgess (1995) indicated that when exceptional control programs are applied to MWFs, the level of bacterial concentrations can be decreased to 10^4 CFU ml⁻¹, and for less aggressive programs, microbial contaminants of 10^6 CFU ml⁻¹ may be obtained. According to the results of Burgess (1995), the measures taken by the investigated turneries to control the microbial growth in MWFs were poor, as more than 66.6% of the fluids contained $>10^6$ CFU ml⁻¹. Only two species of filamentous fungi were found to contaminate 33% (7/21) of the samples tested; they belonged to *Fusarium* and *Acremonium* genera. This finding is close to that reported by Van der Gast *et al.* (2001) who isolated mainly *Fusarium solani* and *Acremonium* spp from synthetic oils.

Microbial contaminants are introduced into MWF through make-up water, previous contamination of MWF system, workers, air, microorganisms on incoming parts, and external contamination (Passman and Kuenzi 2020; NIOSH 1998). It is of importance to note that NTM have been frequently isolated from soil (Pereira *et al.* 2020). The turneries in Jordan are small shops that keep their doors open all day. These facilities are not equipped with ventilation systems to prevent the external air from contaminating the internal environment. In hot dry weather the air entering the facility is usually dusty and this dust is derived from the soil which may harbor NTM. This is most probably the source of NTM contamination in the case of the MWF collected from the 3 turneries included in this work. Water is ruled out as a source, because all turneries

in the country use municipal water for diluting the MWF concentrate. Representative water samples collected from 10 turneries were found to be free of *M. chelonae* and *M. abscessus*. Perhaps simple practices to prevent MWF contamination by NTM are to improve ventilation, hygiene, and house-keeping in the workplace.

CONCLUSIONS

This is the first report released from Jordan which deals with the microbial diversity of MWFs used in turneries. Most of the fluids investigated (66.6%) harbored heterotrophs in counts exceeding 10^6 ml⁻¹ with Gram-negative bacteria being the dominant contaminant and SRB were recovered from 11 (52.4%) samples. Only 3 samples (14.3%) contained NTM. The most probable source of this contamination was the dust that infiltrated into the facilities through the air. Due to the lack of records that correlate occupational health with the working environments, it remains to be seen if such contamination poses any health hazards to workers. This work raises many questions which should be answered through prospective research and collection of data by the health authorities in Jordan.

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