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Original article

Diagnostic approach and epidemiology of Microbial Keratitis: findings from an Italian Tertiary Care center.

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ABSTRACT

Rapid identification of causative microorganisms of microbial keratitis (MK) and knowledge of the most common local pathogens are prerequisites for rational antimicrobial therapy. We retrospectively reviewed the characteristics of MK diagnosed at the IRCCS Arcispedale Santa Maria Nuova of Reggio Emilia (Italy) in a 5-years period, where the Ophthalmologist Unit is a reference center for corneal infections.
During the study period, 183 MK were evaluated through corneal scrapings cultures. The positivity rate was 54,1%. A total of 107 microorganisms have been isolated: *Acanthamoeba* species was the etiologic agent in 19 cases. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were more frequently isolated in bacterial keratitis, while *Fusarium* spp., *Candida albicans,* and *Alternaria alternata* were predominant among the fungal isolates. Strict cooperation between ophthalmologists and clinical microbiologists is advisable to allow the best diagnostic approach for MK.

1. Introduction

Microbial keratitis (MK) is defined as an infectious disease of the cornea, characterized by ocular pain, conjunctival injection, stromal inflammatory infiltrate, and, frequently, corneal ulceration. MK is the fourth leading cause of blindness worldwide, and 1.5–2.0 million new cases occur every year in developing countries (Austin et al., 2017, Pascolini and Mariotti, 2012, Whitcher et al., 2001). Instead, MK incidence within developed countries is markedly lower, ranging from 3,6 to 40,3 per 100,000 person-years (Ibrahim et al., 2009, Jeng et al., 2010, Lam et al., 2002, Seal et al., 1999). MK should be considered a significant public health problem and an ophthalmic emergency: prompt diagnosis and treatment are crucial to avoid corneal perforation and subsequent vision loss. A high range of pathogens (fungi, bacteria, protozoa, and viruses) can cause MK, and infectious etiology is related to climatic, socio-economic and predisposing risk factors (Bharathi et al., 2007, Stapleton and Carnt, 2012, Wong et al., 2012).

Bacterial keratitis (BK) accounts for 90% of all MK cases (Musa et al., 2010). Contact lens wearing, trauma, topical steroidal medications, and ocular surgery are the most prevalent risk factors associated with BK (Green et al., 2008). *S. aureus, P. aeruginosa, S. pneumoniae*, and *Serratia* species are the most frequently involved microorganisms in BK (Hemavathi et al., 2014, Iwalokun et al., 2011, Karsten et al., 2012, Orlans et al., 2011, Ubani, 2009).

Mycotic keratitis (MYK) is a fungal infection with global distribution. Its etiology is geographic related: filamentous fungi are predominant in tropical and subtropical regions, whereas yeasts are in temperate countries. *Fusarium, Aspergillus, Phaeohyphomycetes, Curvularia, Paecilomyces, Scedosporium*, and *Candida* spp. are frequently isolated from corneal scraping (Sengupta et al., 2012, Shigeyasu et al., 2012, Theoulakis et al., 2009, Thomas, 2007, Thomas and Kaliamurthy, 2013).

Acanthamoeba keratitis (AK) is caused by ubiquitous free-living amoebae and is considered a rare cause of MK if compared with bacterial and fungal keratitis (Neelam and Niederkorn, 2017). However, in the last decade, an increasing number of AK cases have been reported, being contact lens wearing the leading risk factor (90% of all the AK cases) (Sengor et al., 2015).

The correct diagnosis of microbial keratitis can be achieved using microbiological procedures that are therefore mandatory. The specimens obtained from the base and the corneal lesion edges are evaluated through microscopic examination and cultures, representing the diagnostic gold standard. A corneal biopsy should be performed in all the cases of negative cultures in a patient with a high clinical suspicion (Alexandrakis et al., 2000). Ophthalmologists should be aware of the proper sampling techniques as a crucial step in identifying MK's

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etiologic agent. According to a paper by Bhadange et al., although the treatment outcome is similar in both culture-positive (CP) and culture-negative (CN) keratitis, the absence of etiological diagnosis is associated with prolonged duration of topical medication; moreover, the number of major surgical interventions in CN keratitis is significantly less compared with CP keratitis (Bhadange et al., 2015).

Polymerase chain reaction (PCR) is a molecular diagnostic tool coul be useful in microbial keratitis diagnosis if viruses are suspected and may be considered in case of high clinical suspicion with negative corneal scraping cultures.

The present paper aims to retrospectively review the epidemiology of all the microbial keratitis cases diagnosed at the IRCCS Arcispedale Santa Maria Nuova of Reggio Emilia (Italy) between January 2015 and December 2019.

2. Material and methods

Setting and case definition – The study was performed in the IRCCS Arcispedale Santa Maria Nuova of Reggio Emilia, Italy, a 923 acute care beds tertiary hospital located in Reggio Emilia, the fourth largest city in the region Emilia-Romagna, in a 5-year period (from January 2015 to December 2019). The Ophthalmology Unit of the hospital is a national reference center for corneal infections.

MK was defined on a clinical basis as a suppurative corneal infiltrate with an overlying epithelial defect. All the diagnostic procedures described below did not change during the study period.

Diagnostic procedures: corneal scraping – In all the patients complying with the case definition, the ulcer was scraped using 21-gauge needles or sterile Kimura platinum spatulas at the base and the infiltrate's leading edge. The corneal scrapings are transferred on the solid media in multiple "C" shaped streaks, as previously described (Gupta and Tandon, 2008; Alkatan and Al-Essa, 2019) (Fig. 1): all the procedures were performed in the Ophthalmology Unit after training the medical personnel and strict cooperation between the clinical microbiologists and the ophthalmologists. The advantage of using this streaking technique is that, independently of the device used for the sample collection (needles or spatulas), it allows the best release of the sample on the agar plate (particularly useful for *Acanthamoeba* cultures). In all the suspected bacterial or mycotic keratitis, the specimen was directly inoculated on Columbia blood agar (sheep, 5%) (BA)

Fig. 1. Growth of *Fusarium* species on Sabouraud agar after multiple "C" shaped streaks.

and chocolate agar (CA). Trypticase soy broth (TSB) was also inoculated with corneal tissue to increase the cultural method's sensitivity.

If *Acanthamoeba* keratitis was suspected, the BA plates were replaced with an agar (NNM), which is based on the formula of the non-nutrient agar (NN-agar) modified with the addition of 0.4% of malt extract and 0.4% of yeast extract, making, therefore, the medium suitable also for the growth of mold and yeast. In all the cases, corneal material was also directly spread on one or more sterile slides. The NNM formulation performances were previously validated using spiked samples (multiple "C" shaped streaks of a suspension containing 10^3 CFU/ml *Fusarium* species; multiple "C" shaped streaks with one 10-μL loop suspension scraped from the agar surface of an NN-agar culture positive for *Acanthamoeba* species).

Once collected, the slides and the inoculated agar plates were sent to the Laboratory within a maximum of one hour. If the patient used contact lenses, he was invited to send them to the Microbiology Laboratory.

Diagnostic procedures: microbiology – The specimens were processed after their arrival in the Laboratory immediately. The NNM plates were inoculated with a strain of *Escherichia coli* (the ATCC-25922^T) and then incubated at 30°C in aerobic atmosphere for ten days. A daily evaluation was performed starting on the 3rd day. BA and TSB were set at 37°C in aerobic atmosphere for seven days, whereas CA was incubated at 37°C in microaerophilic conditions. The slides were stained using the Gram or Giemsa stains based on clinical suspicion (BK or MYK versus AK, respectively). For microbiological analyses of contact lenses, the lens was cut in a sterile Petri dish, and a small portion was inoculated on BA, CA, Sabouroud dextrose agar, Mannitol Salt Agar, McConkey agar, NNM and TSB. A small amount of the lens was also used for the direct microscopic examination (Gram and Giemsa stains).

The inoculated BA, CA, and TSB plates were examined daily and discarded on the 7th day if growth was not documented. For BK, cultural positivities were considered significant if the same microorganism was grown on two or more inoculated media or on a single agar with a previously positivity of a compatible finding at direct microscopy. The cultures yielding coagulase-negative staphylococci (except for those positive for *Staphylococcus lugdunensis*) were discarded, considered sample contamination, likely during the collecting procedure.

Bacterial and yeast identification was performed using the MALDI-TOF Biotyper system (Bruker Daltonics, Bremen, Germany), according to the manufacturer's protocols. The protein profiles were obtained and analyzed using the most updated software version available at the time of microbial isolation (mainly, FlexControl™ 3.4 and FlexAnalysis™ 3.4, Bruker Daltonics).

For bacteria, antimicrobial susceptibility testing was performed using the automated system Phoenix 100™ (Becton Dickinson, USA), together with the disk diffusion method - according to the EUCAST rules (https://www.eucast.org/ast_of_bacteria) – in case of isolation of *Pseudomonas* species. For these isolates, a third method, the gradient MIC using the MIC Test Strip™ (Liofilchem, Roseto degli Abruzzi, Italy) was used to define the result in case of discrepancies. The MIC results were then categorized using the most updated EUCAST clinical breakpoints for systemic isolates available at the time of microbial isolation.

Molds identification was achieved through macroscopic and microscopic morphology as per the evaluation of well-trained microbiologists. In some instances, when the identification was not sure or in case of unusual pathogens, the isolate was referred to a reference laboratory, which identified the microorganisms using the MicroSEQ™ D2 rDNA Fungal PCR Kit and MicroSEQ™ D2 rDNA Fungal Sequencing Kit (Applied Biosystems, ThermoFisher Scientific, USA) according to the manufacturer's instructions.

For AK, the presence of trophozoites and/or cysts was evaluated daily by observing the presence of lytic zones on NNM plates (Fig. 2). If such zones are present, a 10 μL loop suspension scraped from the agar surface was mixed with lactophenol-cotton blue and observed

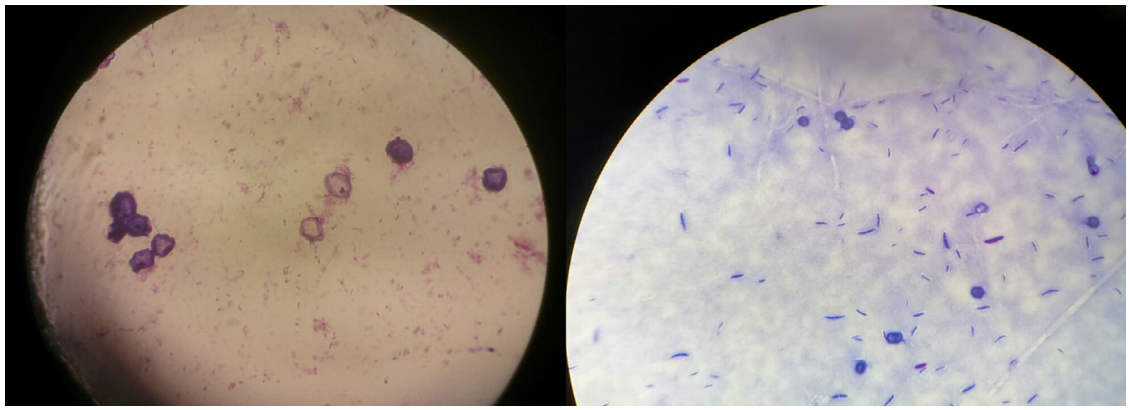

Fig. 2. Left: Gram staining of cysts of *Acanthamoeba* species grown on NNM agar. Right: Giemsa staining of *Fusarium* species and *Acanthamoeba* species grown on NNM agar.

Table 1
Microorganisms isolated from corneal scraping cultures (n = 107).

Gram-negative		Gram-positive		Fungi		Protozoa	
Pseudomonas aeruginosa	18 (16,8%)	*S. aureus*	15 (14,0%)	*Fusarium* species *	8 (7,5%)	*Acanthamoeba* species	19 (17,8%)
Moraxella species	8 (7,5%)	*S. pneumoniae*	3 (2,8%)	*Candida albicans*	7 (6,5%)		
Serratia species	6 (5,6%)	*S. pyogenes*	1 (0,9%)	*Alternaria alternata*	5 (4,7%)		
Pseudomonas species	1 (0,9%)	*S. agalactiae*	1 (0,9%)	*Aspergillus flavus*	2 (1,9%)		
Morganella morganii	1 (0,9%)	*E. faecalis*	1 (0,9%)	*Tintelnotia destructans* *	1 (0,9%)		
Escherichia coli	1 (0,9%)	*Actinomyces* spp	1 (0,9%)	*Purpureocillum lilacinum* *	1 (0,9%)		
Enterobacter cloacae	1 (0,9%)			*Penicillium glabrum* *	1 (0,9%)		
Acinetobacter species	1 (0,9%)			*Scopulariopsis brevicaulis* *	1 (0,9%)		
Achromobacter species	1 (0,9%)			*Aspergillus niger*	1 (0,9%)		
				Beauveria bassiana *	1 (0,9%)		
TOTAL	38		22		28		19

* For these microorganisms, identification to the species level was confirmed through molecular methods. See in text for details.

at direct microscopy. The plates without lytic zones were discarded on the 10th day if no growth was observed.

3. Results

During the study period, 227 patients were admitted to the Ophthalmologic Ward with MK. Among them, 183 MK from 183 different patients were analyzed through corneal scrapings. All the cases that underwent microbiological diagnosis presented a severe disease, a nontypical presentation, or had risk factors such as contact lens wearing, ocular surgery, trauma, dry eye, diabetes, previous treatment with steroids or antibiotics.

Eighty-four out of the 183 samples (45,9%) were negative, whereas 99 (54,1%) were positive. A total of 107 organisms have been isolated (Table 1): among them, 60 (56,1%) were bacteria, 28 (26,2%) fungi, and 19 (17,7%) *Acanthamoeba* species (Fig. 2). *Pseudomonas aeruginosa* (18 isolates) and *Staphylococcus aureus* (15) were the microorganisms more frequently isolated in BK, while *Fusarium* spp. (8), *Candida albicans* (7), and *Alternaria alternata* (5) were predominant among the fungal isolates. Among the 8 *Fusarium* spp., molecular analyses allowed to distinguish 5 *Fusarium solani* and one each of *F. proliferatum, F. delphinoides* and *Plectosporium tabacinum*. Other occasionally isolated fungi (one isolate each) were: *Tintelnotia destructans, Purpureocillum lilacinum* (previously known as *Paecilomyces lilacinus*), *Penicillium glabrum, Scopulariopsis brevicaulis* and *Beauveria bassiana* (for all these molds, the identification was confirmed using molecular techniques). Finally, 3 *Aspergillus* species were isolated (2 *A. flavus* and 1 *A. niger*).

Seventeen out of the 18 *Pseudomonas aeruginosa* isolates were susceptible to many common drugs active against this microorganism, whereas a strain was multi-drug resistant, producing metallo-β-lactamases, as determined phenotypically through the evaluation of the *in vitro* synergy using disks of meropenem and meropenem added with dipicolinic acid (KPC/MBL/OXA48 kit, ROSCO Diagnostica, Denmark). The isolate harbored the bla_{VIM} gene, as verified by using the Xpert Carba-R assay (Cepheid, USA) (Traczewski et al., 2018). Two isolates were resistant to tobramycin and levofloxacin, which were used as a topical treatment.

Only one *Staphylococcus aureus* strain was resistant to methicillin.

Ten out of the 183 specimens (5.5%) had mixed cultures: in 5 cases, bacteria and fungi were isolated, three had mixed bacterial infections, and two patients presented *Acanthamoeba* and fungi (Table 2).

For 18 patients, it was possible to culture both the contact lenses and corneal tissue (Table 3). In 7 out of the 18 cases (38,9%), the results of the two cultures were fully concordant. In four cases, the culture of corneal scraping was negative, while contact lens cultures were positive. In one case, only the corneal scraping culture was positive (*Alternaria alternata*). In two cases, the cultures were in disagreement: in one patient, S. *aureus* grew on corneal scraping and *P. aeruginosa* on contact lenses, whereas in another, *S. aureus* grew on corneal scraping

Table 2
List of polymicrobial infections

Moraxella species + *Penicillium glabrum*
Staphylococcus aureus + *Fusarium* species
Staphylococcus aureus + *Alternaria alternata*
Moraxella species + *Candida albicans*
Staphylococcus aureus + *Achromobacter* species
Pseudomonas aeruginosa + *Serratia liquefaciens*
Staphylococcus aureus + *S. pyogenes*
Scopulariopsis brevicaulis + *Acanthamoeba species*
Aspergillus flavus + *Acanthamoeba species*
Acinetobacter baumannii + *Fusarium* species

Table 3
Comparison between corneal scraping cultures versus contact lens cultures.

	Corneal craping culture	Contact lens culture
1	negative	*Beauveria bassiana*
2	*Candida albicans*	*Candida albicans*
3	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
4	negative	*Achromobacter xylosoxidans*
5	*Staphylococcus aureus*	*Achromobacter xylosoxidans + Klebsiella oxytoca*
6	*Serratia marcescens*	*Serratia marcescens + Klebsiella oxytoca*
7	*Staphylooccus aureus*	*Pseudomonas aeruginosa*
8	*Alternaria alternata*	negative
9	*Aspergillus flavus + Acanthamoeba* species	*Aspergillus flavus + Acanthamoeba* species
10	*Serratia marcescens*	*Serratia marcescens + Alcaligenes faecalis + Klebsiella oxytoca*
11	*Fusarium* species	*Fusarium* species
12	*Moraxella* species	*Moraxella* species
13	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa + Enterobacter cloacae + Achromobacter species*
14	negative	*Pseudomonas aeruginosa + Serratia marcescens*
15	*Serratia liquefaciens + Pseudomonas aeruginosa*	*Serratia liquefaciens + Pseudomonas aeruginosa + Alcaligenes faecalis + Proteus rettgeri*
16	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
17	negative	*Klebsiella oxytoca + Stenotrophomonas maltophilia + Achromobacter xylosoxidans*
18	negative	negative

and *Achromobacter xylosoxidans* and *Klebsiella oxytoca* on the contact lenses. In the remaining cases, the contact lens culture yielded more microorganisms compared with the culture of the corneal scrapings,

4. Discussion

In our settings, 80,6% of the cases of MH (183/227) required corneal scraping for the proper microbiological diagnosis.

MK's diagnosis is considered a challenge, and often the microbiological procedures fail in identifying the etiologic agent of the disease. Different factors may contribute to this pitfall: the relatively low sensitivity of the cultural methods can be due to prior antibiotic use, technical difficulties in performing a correct corneal scraping, the small amount of corneal tissue scraped and therefore available for the culture, or the presence of pathogens requiring different growth conditions (Kaye et al., 2003; Gupta and Tandon, 2008).

Therefore, a strong synergism between ophthalmologists and microbiologist is mandatory to achieve the diagnostic procedures' best performances. Every clinical suspicion of MK should be carefully evaluated, and the most appropriate microbiological methods should be chosen through this close cooperation among all healthcare workers involved in the procedure. In our experience, the best results can be achieved when the media used for the cultures are inoculated directly by the Ophthalmology Unit personnel in the surgical room, immediately after the scraping, thus avoiding any possible delay in transport or sample manipulation. Following this approach, in our experience, the corneal scraping cultures' positivity rate was 54,1%. This result is comparable with previous reports demonstrating a culture positivity rate ranging from 32,6% to 79,4% (Ung et al., 2019). However, the positivity rate obtained should be considered satisfactory since most patients came to our attention with subchronic or chronic forms of the diseases, previously misdiagnosed and often overtreated. Many patients were also under antibiotic treatment at the moment of the diagnosis. According to other experiences, these patients have a culture-positive rate of 41.3%, and performing microbial cultures in this group is recommended since positive culture results provide valuable suggestions toward a therapy change (Rodman et al., 1997, van der Meulen et al., 2008).

Previous studies demonstrated that the use of the E-Swab (Copan Group, Brescia, Italy) provides comparable results to the standard multi-sampling method for the diagnosis of MK (Pakzad-Vaezi et al., 2015). This approach has great importance for all the centers that do not have quick access to the Microbiology laboratory and is certainly efficient, since a single sample collection can be put in a liquid broth medium that guarantees the possibility of processing the specimens for the following 48 hours. However, the E-Swab's performances in diagnosing fungal and *Acanthamoeba* infections are still uncertain (Pakzad-Vaezi et al., 2015). The main problem in collecting samples from MK is that specimens probably yield a low number of microbial agents. For this reason, in our center, we prefer to work directly in the surgical room, where the sample is immediately seeded in agar plates, avoiding its dilution in transport medium and further manipulation, and immediately cultured for all the pathogens, including fungi and *Acanthamoeba*. The only disadvantage of this approach is that multiple sampling is required. A further study comparing this approach with the one-touch sampling with E-swabs would be desirable.

Although different reports highlight a higher portion of infections due to Gram-positive (Orlans et al., 2011, Reddy et al., 2010), 63,3% (38 of 60) of the BK in our experience were due to Gram-negative microorganisms. This finding is consistent because Gram-negative pathogens are frequently found among contact lens wearers (van der Meulen et al., 2008).

The present study does not report the isolation of coagulase-negative staphylococci, which were considered potential contaminants. In our opinion, except for *Staphylococcus lugdunensis*, CNS isolated from corneal scraping should be carefully reviewed according to the clinical suspicion, and the isolation should be confirmed by repeating a second sample yielding the same microorganism (i.e., same bacterial species and susceptibility pattern). This approach allows a reduction in unnecessary treatments and contributes to preventing the spread of antimicrobial resistance. In the present study, no one of the patients with CNS isolation required a second scraping.

The most common Gram-positive microorganism associated with BK is *S. aureus*, which was involved in 25% of episodes (15 of 60), and it was second only to *P. aeruginosa*, isolated in 30% of BK (18 of 60).

All the *P. aeruginosa* infections were reported in patients using contact lenses, as previously demonstrated in other studies (Hemavathi et al., 2014, Karsten et al., 2012, Orlans et al., 2011). Among other less common bacterial isolates, *Moraxella* spp. and *Serratia* spp. were the third and four causes of BK (13,3% and 10%, respectively).

Polymicrobial keratitis remains challenging to be diagnosed. Several studies have shown that these infections' prevalence ranges from 2% to 10,4% (Bourcier et al., 2003, Lim et al., 2013, Tena et al., 2019). In the present study, the percentage of polymicrobial infections was 5,5% (10 of 183), and also in this case, wearing contact lenses appears to be critical since all the patients with a polymicrobial infection belonged to this category of patients.

Fungal infections are relatively rare in temperate regions and developed countries (less than 10%) (Shah et al., 2011). Reggio Emilia

is a city of 171,944 inhabitants in Northern Italy with a wide rural neighborhood and a temperate climate. Our study found that the overall yield of fungal-positive cultures was 26,2% (28of 107 cases). *Fusarium* spp. (8 isolates, 5 of them *F. solani*, 7,5%), *C. albicans* (7, 6,5%) and *Alternaria alternata* (5, 4,7%) were the predominant pathogens. Considering the pathogens isolated in the study, *Fusarium* spp. was the fourth most prevalent microorganism.

Finally, *Acanthamoeba* keratitis is still considered a rare sight-threatening corneal infection, but in recent years the incidence of this infection has shown a remarkable increase. Our data highlight a percentage of positivity for amebic infections of 17,8% (19/107): *Acanthamoeba* species were overall the microorganism more frequently isolated. All 19 patients with Acanthamoeba isolates were contact lens users.

It was difficult, in our experience, to link the pathogens unequivocally to specific risk factors, since often different conditions occurred together. For sure, contact lens wearing was the most important risk factor since all the patients with AK and *Pseudomonas aeruginosa* infections belonged to this risk group. In our experience, fungal infections were often present in subjects with preexisting trauma with possible exposures to the soil, whereas staphylococcal infections were often subsequent to other ocular diseases (such as blepharitis).

Regarding the outcome of the infections, for 75 cases a therapeutic keratoplasty was needed, with a cure rate of 100%. A restitutio ad integrum after local therapy with antibiotics was obtained in 14 patients, whereas 15 patients showed a MK resolution with minor sequelae (mainly corneal leukomas not requiring further interventions). One patient with *Fusarium* keratitis underwent evisceration bulbi, whereas other two patients experienced unresponsive MK.

In 18 cases, we could compare the different growth between corneal scraping and contact lens culture. Many contact lens cultures yielded more than a single microorganism, while the corresponding corneal culture showed a monomicrobial growth. Therefore, the discrepancies between scraping culture and contact lenses culture were always challenging to be analyzed. It could be true or false positivity, and it should be interpreted based on the clinical suspicion and etiology of the pathogen grown on culture.

The present study did not consider viral infections. The diagnosis of HSV keratitis is mainly clinical, based on slit-lamp examination, and laboratory diagnosis is not needed if clinical signs are highly characteristics. Therefore, the molecular techniques used for this diagnostic are not part of our MK routine evaluation and are considered only for selected cases. As a possible limitation of our study, we did not use molecular techniques for MK with negative cultures. Ferrer et al. found that PCR had positive results in 92.6% of the cases compared to 66.7% by stains and 59.3% by culture in 20 corneal samples of patients with proven mycotic keratitis (Ferrer and Alió, 2011). PCR, and especially real-time PCR, also provides a rapid and sensitive method for diagnosing AK (Alkatan and Al-Essa, 2019).

Our study revealed a predominance in our setting of AK. In Italy, few data are reporting the incidence of MK, being the majority of the reports focused on the molecular epidemiology of *Acanthamoeba* (Antonelli et al., 2018, Di Cave et al., 2014, Gatti et al., 2010).

The list of microorganisms most frequently involved (*Acanthamoeba, P. aeruginosa, S. aureus,* and *Fusarium* spp.) is consistent with the fact that many of the patients were contact lens users. Although the microbiological analysis of the contact lenses may be useful to address the empirical therapy (more than 60% of the positive cultures were in agreement with the cultural result of the corneal scraping), microscopic examination and culture of the cornea remain the gold standard.

The strict cooperation between the ophthalmologists and the clinical microbiologists is essential to maximize the results, allowing them to handle all the cases with the best approach.

Author contributions

RG carried out the microbiological diagnoses and wrote the manuscript draft; FL and MA managed the patients clinically, discussed the findings, and revised the manuscript; VL carried out the microbiological diagnosis; FC performed the molecular identification of the molds; CE reviewed, edited, finalized the manuscript for the submission, and revised the manuscript after reviewers' suggestions. All authors have read and agreed to the submitted version of the manuscript.

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Institutional review board statement and informed consent statement

Our investigations were carried out following the Declaration of Helsinki's rules of 1975, revised in 2013. Ethical Approval and the informed consent statement collection are not required since the study is retrospective, and no personal data about the patients were collected.

Declaration of competing interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.diagmicrobio.2021.115470.

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