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MYCOBACTERIUM KANSASII, SPECIES OR COMPLEX? BIOMOLECULAR AND EPIDEMIOLOGICAL INSIGHTS

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Mycobacterium kansasii was recognized as new species in 1953[1] and, in the subsequent 30 years, its awareness greatly increased ; this species was in fact the most common non-tuberculous mycobacterium (NTM) causing diseases in USA and in England[2]. In such countries the *M. kansasii* isolations rate was surpassed by *Mycobacterium avium* complex (MAC) in the 1980s[3], while, in the same period, it increased in Japan[4]. In the AIDS era two periods must be considered which include the years preceding, and following, the introduction of highly active antiretroviral treatments. In the first one the infections due to *M. kansasii* increased in HIV-positive patients[5,6], although they remained clearly below the infections due to MAC, while in the second they became very rare while the isolations in non immunocompromised subjects remained unchanged.

Major phenotypic characters of *M. kansasii* include large cross-barred bacilli and predominantly rough colonies which develop an intense yellow pigmentation following the light exposure. The growth is slow and requires 2–3 weeks at temperatures ranging from 30 to 40℃. Most frequently investigated biochemical tests include nitrate reduction, catalase, Tween 80 hydrolysis and urease which are positive, while arylsulfatase and tellurite reduction are negative[7].

The role of *M. kansasii* as significant pathogen is supported by an estimated annual rate of infection ranging from 0.5 to 1 per 100,000 people[8]. It is characterized however by wide geographic variability ranging from a very low frequency in Australia and Japan[9,10] to a very high one in several states of USA, like Louisiana[11], and in central Europe, particularly in Czech Republic[12]. As for other NTM not every *M. kansasii* isolation from human samples should be considered clinically significant. A close adherence to the guidelines proposed by the American Thoracic Society[13], allows in fact to exclude at least 1/3 of pulmonary isolations which reflect colonization rather than infection[14].

In non immunocompromised subjects pulmonary disease is far the most common *M. kansasii* infection ; it is almost always accompanied by predisposing conditions among which stand out various pulmonary disorders like pneumoconiosis[15], chronic obstructive pulmonary disease[16] and emphysema[17]. Other frequent targets of infection are lymph knots, soft tissues, cutis, bone, joints and genitourinary apparatus[18]. Disseminated infections are not very frequent[18].

Other risk factors, in immunocompetent host, include work in dusty conditions, cancer, alcoholism, smoke, systemic illness and exposure to *M. kansasii*-contaminated water[19]. Also to live in hyperendemic regions may be considered a risk factor[19].

Clearly less frequent than several years ago are *M. kansasii* pathologies in immunodeficient patient ; among them the infections limited to the lung and the ones disseminated largely predominate with CD_4 shortage being the main predisposing factor[19].

In absence of standardized methods for antimicrobial susceptibility testing, the treatment follows the recommendations of the literature that consider rifampin as the key drug. With it are almost always associated ethambutol and a third drug chosen among streptomycin, isoniazid or amikacin[13].

The first report of variants within *M. kansasii* dates back to 1962 when Wayne made a distinction between isolates with certain or questionable clinical significance, being the first strong producer of catalase and characterized by high virulence in guinea pig[20].

In the last 20 years the increase of genetic knowledge greatly affected every field of life sciences. The most important targets of genetic studies include the 16S rRNA gene, the 16S-23S internal transcribed spacer (ITS), the 65 kD heat shock protein gene, several repetitive DNA sequences and the

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intein-coding sequence within the gene for the A subunit of gyrase (*gyrA*).

The DNA probe technology was applied to *M. kansasii* investigations since its introduction. Among research tools the pMK1-9 and the p6123, whose target have not been determined, are the best investigated. Great popularity have achieved in diagnostic laboratories the commercial products, the AccuProbe (Gen-Probe, USA), aiming to 16S, and the INNO LiPA (Innogenetics, Belgium), aiming to ITS.

The pMK1-9, which in a first study hybridized with all *M. kansasii* strains tested[21], turned out, on a wider panel of strain to fail hybridization with 20% of the strains[22].

For p6123[23], at present, no hybridization failure has been reported with any isolate of *M. kansasii*. Two different formulations of AccuProbe *M. kansasii* have been developed. The first one, tested in parallel with pMK1-9, hybridized with all the strain pMK1-9-positive and with a part of the negative ones as well[22]. Following the confirmation of the presence a number of *M. kansasii* strains which were AccuProbe-negative[24] a second version was developed; with it also the strains not recognized by the previous probe gave positive results[25].

The LiPA, a reverse hybridization DNA-probe, presents three Line-probes aiming to different *M. kansasii* types; the MKA1 hybridizes with all the strains positive with the first AccuProbe but negative with the second, the MKA2 hybridizes with the strains positive with the second and negative with the first AccuProbe, and the MKA3 reacts with *M. kansasii* which are negative with both AccuProbe[26].

The first sequence alternative to the one previously determined for *M. kansasii*[27] was detected in 1992 in pMK1-9-negative isolates[22]. At present five sequevars are known in the 16S rDNA, differing from 1 to 6 nucleotides[28]. Five sequevars have been detected in the ITS too; they are characterized by extensive diversities involving up to 49 bases[8].

The presence of repetitive DNA sequences has been thoroughly investigated in the last decade. AGC-rich polymorphic repetitive sequence is present, in at least 30 copies, in *M. kansasii*, but also in the *Mycobacterium tuberculosis* complex and in *Mycobacterium szulgai*[29]. The IS*1652* characterized *M. kansasii* pMK1-9-negative only and the number of copies, ranging from 1 to 9, gives rise to extensive polymorphism[30]. The major polymorphic tandem repeat (MPTR) has been in deep investigated by Hermans et al. [31]; it is characterized by tandemly repeated sequences of 10 bp separated by spacers of 5 bases. About 80 different MPTR regions are present in the mycobacterial genome of *M. kansasii*, but also of *M. tuberculosis* complex, *M. gordonae*, *M. gastri* and *M. szulgai*.

A powerful tool for the study of polymorphism is represented by restriction enzyme technology. The restriction fragment length polymorphism (RFLP), produces patterns that are very homogeneous among pMK1-9-positive *M. kansasii*, and very heterogeneous among negative ones[29]. The same technique reveals among *M. kansasii* AccuProbe-positive a 3kb fragment and, among AccuProbe-negative, fragments of variable length[30].

The inteins are protein sequences that are excised from the precursor protein during maturation; the *gyrA* includes, in several species, an intein-coding sequence. *M. kansasii*, along with *Mycobacterium flavescens* and *M. gordonae*, are the only species in which *gyrA* intein, which may or may not be present[32], determines polymorphism.

The mpb70 gene encodes an antigen protein in *Mycobacterium bovis*; the analog gene which is present in *M. kansasii* is characterized by sequence variations which determine further heterogeneity[33].

In *M. kansasii* the ITS-amplification product, far from being reproducible as in other mycobacteria, may be characterized by three different profiles[34].

In a study of ours[35] we investigated the correlations of the genetic variants of *M. kansasii* with several phenotypic characters and with clinical features. A significant correlation emerged of AccuProbe-positive strains with the esterasic activity (Tween 80 hydrolysis) and with the presence of fucosidase enzyme. Even more striking is the significantly higher prevalence of the AccuProbe-negative isolates among HIV-positive patients in comparison with HIV-negative ones.

The paper of Picardeau et al.[36] is a milestones in the knowledge of the heterogeneity characterizing the species *M. kansasii*. In such study two different approaches, the investigation of MPTR, and the PCR-restriction analysis (PRA) agreed in revealing five types within the species *M. kansasii* (Table). Such division was furthermore corroborated by the amplified fragment length polymorphism and the pulsed field gel electrophoresis (PFGE), in which, despite the emergence of numerous patterns, their clustering in five major groups is possible. Only two such types, ii and iii, harbor IS*1652*; in single copy and in 4-6 copies respectively. Type i includes typical *M. kansasii* and, likewise type iv, is AccuProbe-positive. Types ii and iii are the only ones which appear closely related each other.

A substantial confirmation of above findings emerges from the work Alcaide et al.[8] in which the presence of five types (Table), emerging again from PRA and PFGE, is supported by their precise overlapping with the five sequevars present in the ITS. Types i, iv and v differ from the others for the AccuProbe-positivity, the possession of *gyrA* intein-coding sequence and the sharing of a common sequence in the 16S rDNA. Equally confirmed is the polymorphism characterizing the type ii, which contrasts with the very homogeneous type i, which probably reflects a clonal structure. From the epidemiological point of view, the isolation of type i is restricted to clinical samples; type ii is grown both from humans and the environment while types iii, iv and v are environmental only.

The heterogeneity of *M. kansasii* is therefore revealed by a large number of genetic characters, some of which (sequencing, RFLP, PFGE, *gyrA* intein) define, or contribute to the definition of five well separated types (Table).

From the taxonomic point of view, the belonging of the

Table Variability within the species *M. kansasii* as revealed by different genotypic approaches

Type	DNA probe							Sequencing		Molecular typing					
			AccuProbe		INNO LiPA					RFLP					
	p6123	pMK1-9	1st	2nd	MKA1	MKA2	MKA3	16S rDNA	ITS	MPTR	IS*1652*	PRA	PFGE	AFLP[a]	*gyrA* intein
i	+	+	+	+	+	−	−	a	1	I	−	A	i[b]	S	+
ii	+	−	−	+	−	+	−	b	2	II	+	B	ii[c]	M	−
iii	+	−	−	−	−	−	+	b	3	III	+	C	iii[d]	S	−
iv	+	−	−	−	−	−	+	a	4	IV	−	D	iv	S	+
v	+	−	+	+	−	−	+	a	5	V	−	E	v	S	+

[a] amplified fragment length polymorphism; S, single pattern; M, multiple patterns
[b] 4 subgroups
[c] 5 subgroups
[d] 3 subgroups

presently known variants of *M. kansasii* to a single species appears questionable. They are in fact characterized by so extensive divergence that some of them present closer relationships to other species than to other variants within their own species. Striking are the cases of type iii, close to *M. tuberculosis*, and of types i and ii, close to *M. szulgai*[32].

In conclusion, the strains of *M. kansasii* involved in human infections belong almost solely to types i and ii. While however the polymorphism is minimum in type i, it is wide in type ii. The evident clonal structure of type i [8] seems to suggest the adaptation of such strains to the human host with the divergence being restricted by the virulence. On the other hand, the significantly higher involvement of type ii in infections of immunocompromised patients[35] entitles to hypothesize for them a lower ability to overcome natural resistance mechanisms.

A more precise definition of various *M. kansasii* isolates would provide a significant contribution to understanding of its biological and epidemiological key aspects.

Summary

Mycobacterium kansasii is one of the best known nontuberculous mycobacteria and large awareness exists about its involvement in diseases both of immunocompetent and immunocompromised patients. Two phenotypic variants within this species, which differ for the virulence in guinea pig too, have been detected since 1962. It was however following recent progress in genetic studies that a large variability emerged. Major contributions to the disclosure of such findings came from the DNA probes hybridization, the nucleotide sequencing of 16 rDNA and internal transcribed spacer (ITS), and from the analyses of repetitive DNA sequences polymorphism. At present five subtypes of *M. kansasii* are recognized, defined by the ITS sequence and by the polymorphism revealed by different restriction enzyme technologies. Such variants differ from the epidemiological point of view too, with type i being isolated from humans, type ii both from humans and environment, and types iii, iv and v, from the environment only. A revision of the present taxonomic status of *M. kansasii* and its splitting into different species or subspecies seems nowadays necessary.

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第78回総会招請講演

Mycobacterium kansasii は菌種か菌群か，分子生物学的および疫学的洞察

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要旨：*Mycobacterium kansasii* は最もよく知られた一非結核性抗酸菌種であり，“immunocompetent” ならびに “immunocompromised” な患者における疾患の原因菌として注目を惹いている。この菌種にはモルモットに対するビルレンスを異にする2表現型変異株の存在することが1962年に初めて見出された。しかし，極めて多様性のあることが見出されたのは近年の遺伝学的研究の進歩によるものである。これらの知見の解明には，DNAプローブハイブリダイゼーション，16S rDNA塩基配列決定法，内転写スペーサー(ITS)および反復DNAシークエンス多型の諸分析に負うところ大なるものがある。現在 *M. kansasii* にはITSシークエンスおよび異なる制限酵素に基づいた技術により明らかにされた5亜種が認められている。これらのうち，i型はヒトから，ii型は環境ならびにヒトから，他の型 (iii, ivおよびv型) は環境のみから分離され，疫学的見地からも異なる。*M. kansasii* の分類学の現状の改訂，および異種あるいは亜種への分離が今や必要と思われる。 (斎藤　肇　訳)

キーワーズ：*Mycobacterium kansasii*，疫学，系統発生，遺伝学