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Tracking characterization of Mycobacterium strains in Tanzania and some sub-Saharan African Countries: An overview on genotyping studies, implication and trends in advancing technology

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ABSTRACT

Tuberculosis (TB) is a devastating chronic debilitating infection that imposes a considerable negative impact on human health. The disease also affects the quality of livestock, wildlife and their products. *Mycobacterium tuberculosis* (*M. tuberculosis*) is the causative agent for human TB, while *Mycobacterium bovis* (*M. bovis*) predominantly causes TB in animals, though is also zoonotic. In Tanzania and other developing countries timely diagnosis of disease is hampered by poor access to appropriate capacity and technology to characterize the pathogen. This review explores the diverse methods available for the diagnosis and characterization of Mycobacterial species and strains isolated from both humans and animals in sub Saharan Africa. The review will identify knowledge gaps and highlight direction for future investigation of the interface TB risk, which could lead to a better regional control strategy. A systematic search of PubMed, Google and Google Scholar retrieved 716 published articles on TB and methods used with the aim of tracking the advancement in technology and to reflect where we are and what can best be done to improve the strategy for best control of the disease. The search terms included but not limited to *"(Whole Genome Sequencing AND sub-Saharan Africa); (Diverse TB strains + Human +Animals) AND Tanzania + Africa + Sub-Saharan Africa; (Diverse TB strains + human+ animals) OR Tanzania + Africa + Sub-Saharan Africa)"*. The review illustrates an advancement of technology from 1950s to 2000s with only 17.7% studies having been done using DNA-based methods and 81.3% being studies that used conventional methods. Most of the molecular studies cluster in the mid- to late 2000s which could be due to lack of expertise, slow adoption of technology or the high cost of running these valuable molecular tests. This overview on genotyping studies and trend in molecular studies highlights the need for more investment in this region of the world. An increased use of molecular methods will help in increasing the chances of detecting new TB strains in circulation and identifying potential risks for cross-transmission at humans-livestock-wildlife interface. A strengthened ability to detect and characterizes disease will better support country and regional control strategies.

Key words: Mycobacterium strains, Trends, Genotyping studies, Advancing technology, Tanzania.

INTRODUCTION

Tuberculosis (TB) is a devastating chronic debilitating infection which negatively impacts on the health and wellbeing of humans, and livestock and wildlife and their products. With potential for cross-transmission of the disease amongst species, an inter-sectoral participatory approach that involve multiple disciplines (One Health

approach) could be relevant as a control and disease management strategy, particularly in developing countries. Whilst *Mycobacterium Tuberculosis* is predominantly the causative agent for human TB, *Mycobacterium bovis* predominantly causes TB in animals, though is also zoonotic. Zoonotic TB risk factors include consumption of animal products, particularly raw milk and uncooked meat. Transmission of bovine tuberculosis (bTB) by *M. bovis* from cattle to humans is well known but little is known of transmission of bTB between animals in Africa and risk of transmission to from humans. Many societies in Africa share shelters with animals, particularly cattle and goats. It is therefore important to explore the disease dynamics in these interactions. Understanding the habitual practices, and being able to identify the prevalent TB strains in circulation in sub-Saharan Africa are important to design suitable interventions to improve health in both humans and animals. In this review, we explore the diversity of Mycobacterial species and strains isolated from both humans and animals in sub-Saharan Africa in order to identify knowledge gaps to drive further research and support an evidence based regional control strategy.

TB occurs worldwide with about 10 million cases and 1.6 million deaths in 2017 (WHO). TB is amongst the top causes of deaths in women aged 15 to 44 years[1] and is even more prevalent in males. According to the World Health Organization (WHO) reports the largest number of new TB cases occurred in Asia, (62% of new cases globally in the last 2 years) and then sub-Saharan African (25% of all new cases in 2017)[2]. The global impact of TB is exacerbated by co-infection with HIV[8] and drug resistance[3-10]. Extensive drug resistant TB (XDR-TB) has been widely reported in many TB endemic countries[8,11-17]. Tanzania is among the African countries with highest burden of TB in the world; having approximately 295 TB cases per 100,000 adults. In addition, Tanzania has a national HIV prevalence of 5%, which increases risk of TB infection by over 30 times[1].

Existing economic and social obstacles challenge global TB elimination[5]. Treatment and prevention strategies are hampered by socio-economic problems and limited political will. Such strategies rely on better diagnostics to increase case detection[5], and to reduce the risks of exposure. The implementation of disease control policies, including case management, require adequate and consistent funding[5]. Part of the strategy should bring in the concept of 'One Health' with medical, veterinary and other stakeholders such as environmentalists which could be a key control strategy[18].

Animal tuberculosis, zoonotic and epizoonotic implications

Bovine TB (bTB) in humans is a well-documented zoonosis and is thought to be acquired mainly from cattle through the consumption of infected meat or milk, or via the respiratory route. However, humans can also be a source of infection to livestock, for example there are reports of the isolation of *M. tuberculosis* in goats and sheep in Ethiopia[19] and even a wild elephants in South Africa[20]. The epizoonotic and zoonotic nature of TB would benefit from a One Health approach to control. For example, the use of genotyping and whole genome sequencing methods, can help to identify sources and transmission routes within and across species and geographic regions[21], which may identify points for intervention. These advances in technology, however, need committed and coordinated efforts from both within and outside the health sector[22]. In addition, it will require willingness from governments in endemic areas to invest in TB disease control programs[18] and not entirely depend on donor-funded programs.

There is insufficient information on the distribution, epidemiology patterns and zoonotic potential of bTB in all environments, but perhaps most pertinently in traditional rural environments[23]. Putting in place diagnostic programs focusing on isolation, culture and pattern discrimination of mycobacteria from human and animal TB cases is important for pinpointing the mechanisms and routes of disease transmission, spread and zoonotic impact. Undiagnosed cases contribute towards the force of infection and disease recurrence[24]. This is particularly so in resource-poor countries (mostly disease endemic African countries) where rare resource sharing could be critically important. In such resource sharing fashion, mycobacterial culture as well as isolation facilities could be done at reduced cost, consequently, enhancing effective and efficient diagnosis[23]. However, diagnosis is only the first step towards control and unfortunately, the test and slaughter strategy for TB positive animals as a control measure is rarely used in African countries, for a variety of reasons including cultural backlash and the lack of funds for adequate compensation. Thus, without change, developing countries (and particularly African countries) will remain focal points for a number of infections in both humans and animals, adversely affecting their economy. The eradication of TB will not be possible without good cooperation and collaboration between medical and veterinary personnel for effective and cost-efficient investigation, prevention, control and management of the disease[23]. This is well supported by the East African Community which has so far conducted a series of curriculum validation meetings to advocate regional pandemic preparedness with a 'One Health' approach. This is an important step because, although it targets pandemics other than TB, it builds the capacity of practitioners for the development of a common approach for the control of zoonotic infections. A missing link previously identified by Mbugi et al[25].

The epidemiology of tuberculosis has been extensively studied for decades, and considerable technological advancements have increased our ability to explore the disease and its causative agent. In more recent times pathogens such as *M. tuberculosis* and other bacteria have been characterized using their nucleic acids (DNA and RNA) and proteins to study factors such as lineage or strain diversity, drug resistance as well as other physico-chemical properties important for pathogen survival in the host. These methods have provided a particularly suitable tool to investigate the epidemiology of bacterial diseases, including sub-Saharan Africa[26]. Several molecular tools, including polymerase chain reaction (PCR), restriction fragment length polymorphisms (RFLP) analysis, spacer oligonucleotide typing (spoligotyping), mycobacterium interspaced repetitive units variable number tandem repeats (MIRU-VNTR) and sequencing have gradually been adopted in many countries to study the epidemiology of tuberculosis. The advantage of these molecular methods in tuberculosis studies rests on their ability to not only discriminate between isolates and identify strains but also to distinguish antibiotic sensitive from resistant strains in *M. tuberculosis* complex infection. These methods have been used successfully to identify chains of transmission and to trace the origins of infection (or pathogen)[27,28].

Although various genotyping tools have been instrumental in advancing our understanding of TB dynamics, it is becoming clear that whole genome sequencing is superior to conventional genotyping for *M. tuberculosis* studies, particularly to provide a better understanding of *M. tuberculosis* genome evolution over time in its natural host context. Thus evaluation of the trend of advancement in using these new and modern technologies over time is necessary to get a clear picture on stage of our diagnostic capacity. Diagnostic approaches range from conventional genotyping to sequencing, allowing for diverse species and strains of *M. tuberculosis* complex (MTBC) to be discerned. This approach has been useful in tracing the evolution of strains over time[29], the geospatial distribution of strains[30] as well as the strains of *M. tuberculosis* that seem to be independently acquiring drug resistance[31,32]. Proper diagnosis is important, since complimentary studies on non-tuberculous mycobacteria (NTM) have shown the potential of diverse NTM species for causing disease in both animals and humans [33]. Despite the availability of these genotyping methods, very few studies tracing TB strains in human and animals in sub-Saharan Africa have been conducted.

Literature Search Strategy

A literature search was conducted using PubMed with search teams "Whole Genome Sequencing AND sub-Saharan Africa". To ensure that the retrieved articles are relevant, PRISM guidelines (http://www.prisma-statement.org/) were used, and the search included sufficient terms to extract maximum number of informative publications.

The search, initially included only studies done in Tanzania, then some sub-Saharan Africa. The initial search retrieved 40 published article ***(Table 1)***, three[34-36] done in Tanzania and the rest (37 articles) done in other areas. Most of these articles were from South Africa and Ethiopia with little coming from Djibouti (1), Mali (1), Nigeria (1), Equatorial Guinea (1), Malawi (1) and Uganda (1). The value of whole genome sequencing has been important in tracking evolutional changes in mycobacterial genome that gradually resulted into development of TB drug resistance.

RESULTS AND DISCUSSION

Search results and implication

A total of 716 publications from search were eligible for inclusion in this review 442 (61.7%) of which, originated from Tanzanian studies and 274 (30.3%) publications originated from other countries ***(Table 2)***. The geographic spread of these latter publications were variably and regionally distributed along South Africa, the Horn of Africa, West Africa, East and Central African countries. In the general research results, both conventional and molecular studies were unevenly distributed. The uneven distribution of these studies is a reflection of country and regional variability in adoption of modern technology in disease screening and diagnosis.

In Tanzania there were just 16 studies from 2001 to 2018 which have characterized TB in both humans and animals to genetic level ***(Table 1)***. All of those studies used genotyping methods, of which 6 focused on characterization of the infective bacteria (*M. bovis*) in cattle[37-41] and rodents [42], only nine[34,35,43-50] dealt with infection in humans while the rest were mainly studies that involved conventional methods both in humans and animals. The 16 genetic studies include those carried out at human-animal interface. One study (Katale et al)[33] explored species diversity of non-tuberculous mycobacteria isolated from humans, livestock and wildlife at their interface.

In Tanzania between 2014 and 2018, there was a gradual increase of genotyping studies resulting in 66 publications (retrieved from PubMed). Similarly, 15 publications have been published that use the rapid GeneXpert test (Cepheid, USA), a molecular test for TB detecting the presence of TB bacteria, as well as testing for rifampicin resistance (Table 2). Despite the approximately 4-fold increase in genetic based studies over the previous 4 years compared to those prior to 2014, only four (4/66, 6.1%) of the studies[33,39,48,49], have focused on the animal-human interface in tuberculosis. The rest focused on human TB alone or animal TB alone or were simply diagnostic, leaving a large gap in our

understanding of the dynamics of tuberculosis at the interface.

Genotyping and phylogeographical analyses done in Tanzania have highlighted the predominance of the CAS, T, EAI, and LAM MTBC lineages in Tanzania with the most frequent Spoligotype International Types (SITs) being SIT21/CAS1-Kili, SIT59/LAM11-ZWE and SIT126/EAI5. Other circulating lineages include Haalem, Beijing, LAM, S, T, and X and Manu. Studies done in the past four years while mapping the MTBC genetic diversity in Tanzania, neighbouring East African and other several African countries[49], have described a new lineage (designated EAI3-TZA) that seems to be specific to Tanzania[48,49]. The studies collectively highlighted the absence of clear evidence for recent cross-species transmission of either *M. tuberculosis* or *M. bovis* between humans, livestock and wild animals[33,39,48,49] as well as identifying a novel *M. bovis* strain which have not been previously reported in the Serengeti ecosystem [48]. This information is important when evaluating prevailing strains in an area where humans and animals are in intense contact with potential for cross-transmission.

Those studies focusing exclusively on animal to animal transmission have also highlighted that, despite wild animals being at risk of acquiring *M. bovis* infection from livestock due to occasional interactions in sharing of pasture and water sources, no *M. bovis* was isolated from hunted wild animals. It should be noted that such studies may have been biased to low endemic areas and have not sampled large numbers of animals, nor targeted animals that were visibly unhealthy and therefore the presence of *M. bovis* cannot be ruled out and this does not rule out a call for integrated efforts by all stakeholders for effective control of spread of tuberculosis[41].

In Uganda both *M. tuberculosis* and *M. bovis* have been studied in humans and animals. Characterization of modern *M. tuberculosis* strains has indicated spoligotypes and drug susceptibility patterns of isolates from tuberculosis patients to consist of strains mainly belonging to the Uganda genotype and with a low anti-TB drug resistance rate[51]. A wide diversity of strains had previously been reported[52] with the majority of the TB cases supposedly due to reactivation rather than re-infection. Studies have also shown that despite TB epidemic genotypes being predominantly localized[53,54], strain types were not associated with drug resistance nor HIV sero-status[53]. Despite having little impact on the clinical course for individual patients, infection with multiple MTBC strains has been shown to occur in patients with a first episode of pulmonary disease, in settings with high TB incidence[55].

Characterization of *M. bovis* isolates in Uganda have indicated some spoligotype patterns that had not been previously reported[56], indicating the lack of comprehensive studies in this country. It was reported that infected carcasses, even with multiple sites of infection, are not routinely condemned as unfit for human consumption, and even if this should be done, people may illegally obtain meat from the carcasses for consumption, particularly in rural areas [56]. This situation occurs in most of the traditional African communities and societies where cattle are kept for prestige rather than as a source of wealth and food. This cultural difference between so-called developed and developing countries necessitates the need for specific and relevant approaches in control strategies[57].

TABLE 1. ***Published Tuberculosis research in humans and animals performed in Tanzania from the year 2000 to 2018*.***

Infection category	Target host	Method	Year	Author
Bovine tuberculosis	Cattle	Tuberculin test	2001	Kazwala et al [63]
M. bovis infection	humans	Culture and biochemical typing	2001	Kazwala et al [64]
Diseases of humans and their domestic mammals	Humans and domestic mammals	Species database construction	2001	Cleaveland et al [67]
M. tuberculosis and M. bovis	humans	Interview	2003a	Mfinanga et al [68]
M. tuberculosis and M. bovis	humans	Interview	2003b	Mfinanga et al [69]
Bovine tuberculosis and non-specific infections	Cattle	Tuberculin test	2003	Shirima et al [65]
M. tuberculosis, M. bovis, non-tuberculous mycobacterium and HIV	Humans	Culture and comparison of isolates	2004	Mfinanga et al [70]
Bovine tuberculosis	Wildlife	Enzyme Immunoassay	2005	Cleaveland et al [66]

M. bovis	Cattle	PCR, RFLP and spoligotyping	2006	Kazwala et al [45]
M. tuberculosis	Humans	Spoligotyping	2006	Eldholm et al [48]
Human tuberculosis	Humans	Culture	2007	Søborg et al [71]
M. tuberculosis in HIV infection	Humans	Spoligotyping	2007	Kibiki et al [51]
Human tuberculosis	Human	Microscopy, culture, drug susceptibility testing, Chest X-ray and CD4+T cells count (blood)	2008	Ngowi et al [72]
Common zoonoses	Humans	Structured questionnaire	2008	John et al [73]
Bovine tuberculosis and atypical mycobacterioses	Cattle	Tuberculin test, milk culture, RNA sequencing and PCR	2009	Durnez et al [43]
Various zoonoses	Human, animal and environment	Review	2009	Mazet et al [74]
TB infection	Humans	TB detection using rats from sputum samples	2009	Weetjens et al [75]
TB infection	Humans	TB detection using rats from sputum samples	2010	Poling et al [76]
Bovine tuberculosis	Cattle	Spoligotyping, VNTR typing, microarray analysis, deletion typing and IS*6110* RFLP typing	2011	Berg et al[#] [42]
Non-specific mycobacteria	Small mammals (rodents)	Culture of isolates and PCR	2011	Durnez et al [47]
Bovine tuberculosis	Cattle and wildlife	Deletion typing and spoligotyping	2013	Mwakapuja et al [46]
TB infection	Humnas	*M. tuberculosis* DNA sequencing for resistance mutations in rpoB	2013	Mpagama et al [77]
Non-tuberculous mycobacteria	Cattle-human interface	Culture, PCR and sequencing	2014	Katale et al [41]
TB infection and drug resistance	Humans	Culture, PCR, spoligotyping	2014	Kidenya et al [78]
TB infection	Humans-animal interface	Culture, PCR, spoligotyping, MIRU-VNTR	2015	Mbugi et al [53]
Bovine tuberculosis	Human-animal interface	Culture, PCR, spoligotyping, MIRU-VNTR	2015	Katale et al [44]
Disseminated TB in HIV-infected patients	Humans	GeneXpert	2015	Gamell et al [79]
TB infection	Humans	Microarrays-based spoligotyping, MIRU-VNTR	2016	Hoza et al [49]
TB infection	Human-animal interface	Spoligotyping and molecular data mining	2016	Mbugi et al [54]
TB infection	Humans	GenoType®	2016	Hoza AS et al [50]
Tuberculous spondylitis	Humans	GeneXpert MTB/RIF	2016	Sikalengo et al [80]
TB infection	Humans	GeneXpert MTB/RIF	2017	Sariko et al [81]
TB infection	Humans	GeneXpert®	2017	Mnyambwa et al [82]
TB infection	Humans	GeneXpert (MTB/RIF) assay and culture on the Lowenstein Jensen (LJ) media	2017	Kidenya et al [52]
TB infection	Humans	Xpert MTB/RIF assay	2017	Mbelele et al 2017 [83]
TB infection	Humans	GeneXpert GxAlert platform	2018	Mnyambwa et al [84]
TB infection	Humans	Fluorescent smear microscopy, GeneXpert MTB/RIF and Löwenstein-Jensen (LJ) culture	2018	Beyanga et al [85]
TB/HIV Co-infection	Humans	GeneXpert® MTB/RIF assay and the MPT64 test	2018	Jørstad et al [86]
TB infection	Humans	Culture, spoligotyping and WGS	2018	Kidenya et al [4]

TB Infection	Humans	Whole genome shotgun sequencing and comparative microbial genomic analyses	2018	Mnyambwa et al [87]

**** The survey (Table 1) was based on research published in internationally reputable journals retrievable from PubMed and other electronic databases (Google and Google Scholar)***

[#] Samples were obtained from cattle in Tanzania, and laboratory work done elsewhere

Disease dynamics and risk of resurgence

Major factors potentiating TB resurgence include poverty, consequently poor health facilities, which lead to failures in the treatment systems as well as immigration[80]. In addition, a more recent risk is co-morbidity with HIV infection, which increases risk of progression to active disease and latent TB reactivation[81-83]. There is also an alarming rise in drug resistant TB cases. In most localities, it is not known whether the drug resistant cases result from treatment failures, i.e. acquisition, or are from transmission. Genotyping and sequencing studies are ideal tools to establish the major driver of these epidemics and are crucial for design of appropriate intervention and treatment [84-89]. However, this is only possible where there is adequate funding and health care facilities. Therefore, studies using advanced tools have been sporadic and limited in scope while their implementation for routine screening has been impossible in most countries. For example only three published studies[35,36,47] investigated *M. tuberculosis* at the whole genome sequencing level in Tanzania, while another one (Katale et al, Bacterial Zoonozes Community of Practice under SACIDS, Katale, personal communication) has been done but is not yet published. However, evidence suggests regional TB epidemics in Africa, characterized by genetically distinct lineages of *M. tuberculosis*. *M. tuberculosis* in these regions may have been introduced from either Europe or Asia and has spread through pastoralism, mining and war[30].

Emergence of migrating, highly virulent strains of *M. tuberculosis*, sometimes in association with multidrug resistance, is a warning sign of a serious threat to TB control[90]. This necessitates the identification of major driving forces for the transmission dynamics within specific populations, which in turn may have significant impact on disease control and vaccine development strategies[30,91]. Such goal cannot be achieved without use of advanced molecular methods for screening, diagnosis and comparative molecular epidemiological studies. Advancement in genotyping methods for examples, can provide sufficient information that can determine the approach for planning control strategies. In African settings where there is close interaction and intense contact between humans and animals (including wild animals), the risk of possible cross-transmission of antibiotic resistant TB strains between species is high. This may be an emerging problem, as suggested by the increasing isolation of *M. tuberculosis* from animals, including pigs[92]. In such circumstances, the increase in cross-transmission would consequently mean cross-transmission of resistant TB (*M. bovis* or *M. tuberculosis*) strains, identification of which, needs advanced diagnostic tools. Improved diagnostic facilities as well as the use of isoniazid prophylactic therapy in endemic areas [5] may be the best global TB control strategy particularly in this era where the focus can as well be on better management of HIV and TB co-infections. All these require early screening and diagnosis at point of care, which can be ensued via advancement in technology.

TB strain diversity in animals

A variety of molecular methods ranging from simple techniques (Polymerase Chain Reaction, PCR) to Next Generation Sequencing, have variably been adopted to characterize TB strains in diverse clonal complexes both in humans and animals. Similar to TB strain distribution in humans, studies carried in bTB, have found a degree of regional and geographic distribution. While a clonal complex of *M. bovis* (Af2) was isolated at high frequency from cattle in Uganda, Burundi, Tanzania, and Ethiopia and was identified as an East African strain[37]**,** another clonal complex (Af1) has been found to be a predominantly West African strain[93] with high frequencies of isolation from Mali, Nigeria, Cameroon, and Chad**.** Similar to clonal complexes that have been identified and destined in different regional foci in Africa are the European *M. bovis* clone complexes Eu1[21] and Eu2[94] which, within Europe, are of high frequency in certain countries and low in others, but have also been identified in Africa, most likely imported with cattle originating from Europe. Within the clonal complexes, there are specific families and strains[95], allowing further discrimination and which will allow contact tracing, infection route identification and design of interventions. All these groupings of TB strains and their distribution country-wide and regionally, has been made possible via advancement in molecular-based mycobacterial studies.

Contribution of MOTT in TB infection

Mycobacteria other than *M. tuberculosis* (MOTT), commonly known as NTMs (non-tuberculous mycobacteria), are mostly ubiquitous environmental mycobacteria found in soil, water, dust as well as in food[96-99]. They do not usually cause infection or illness in healthy

animals or humans, however many can cause life threatening illness under situations when the immune response becomes weakened, for example[100-102]. Included in this group are *Mycobacterium avium complex (MAC), Mycobacterium intracellulare, Mycobacterium kansasii, Mycobacterium marinum, Mycobacterium ulcerans* which all habitually cause opportunistic infections. People with critically compromised immune response such as those with HIV/AIDS or chronic lung diseases are very vulnerable to infection by these organisms and symptomatically the disease manifests similarly to TB infection (primarily in lungs, disseminating to other body tissues), however antibiotic susceptibility is often quite different from *M tuberculosis*[103,104]. As a result, these opportunistic infections may often be confused with TB infection. As such, these mycobacteria have also been referred to as atypical mycobacteria to differentiate them from the typical mycobacterium causing tuberculosis. Contradicting views are available on the spread of atypical mycobacteria in man. Despite atypical mycobacteria living on human skin or in the nose, there is no or little evidence of the mechanism of dissemination. Reports propose the idea of atypical mycobacterial infections as being not spread from person to person but through direct contact with the bacteria in the environment[111] including drinking water[105]. Direct transmission from animals to humans has again been questioned and considered unimportant for human infection[106] and infected patients are not necessarily isolated as a protective measure to limit human to human transmission[103]. Regardless of their mode of transmission, the impact of MOTT should receive more attention. At present there are no reliable clinical predictors for the identification of MOTT infection that have been identified for adults and in children, or animals[107]. Furthermore, identification of MOTT infection in resource-limited countries is sorely lacking[98]. A recent study in South Africa[108] has revealed the diversity of NTM species in cattle and African buffaloes ascribed to at least eight clusters, with a possible eight different NTM species. This report is particularly important as it suggests that inclusion of NTM in investigative and control strategies incorporated in various TB control programs could be important. In so doing any chances of spread of infection both in humans and animals might, to a greater extent, be minimized. The presence of NTM species in all likelihood negatively affects the host immune response to tuberculosis and thus progression to TB disease and will possibly complicate immune based diagnostics[108].

Trends in Molecular based Mycobacterial studies

We found published work on tuberculosis in Africa ranging from 7 articles in the 1950s to 600 publications in the 2000s as retrieved from PubMed which is over 80 folds higher in the 2000s than in the 1950s (Search strategy: *(Diverse TB strains + Human +Animals) AND Tanzania + Africa + Sub-Saharan Africa;* (*Diverse TB strains + human+ animals) OR Tanzania + Africa + Sub-Saharan Africa)*. The search was also complemented by Google and Google Scholar search to widen the chance of capturing more articles. Microsoft Excel sheet was used to add up the number of articles in each category and for calculating proportions where needed to. Where specific tests were needed, we included in the search term e.g. DNA sequencing, GeneXpert accordingly. A total of 716 articles ***(Table 2)*** were obtained and manually categorized by EVM into different categories namely molecular, GeneXpert or others (all other approaches than molecular-based). The contribution of published molecular work in TB to the general research work is 17.7% of the total retrieved published work from 1950-2018. Among these, 15.1% are typical genotyping emanating publications and about 2.6% from GeneXpert ***(Table 2)***. In the Table we also note that the move from conventional to genotyping molecular methods has been increasing with time with larger proportions of molecular work clustering in the years 2000 – 2009. The trend however is not uniform which could be a reflection of donor dependence for funding such projects and thus periodicity. Too much dependence on donors may have a detrimental effect (boom and bust cycles) and since donors may not prioritize on developing country problems, instead being rather biased to donor priorities. Abuzeid[109] highlights problems with foreign aid flows from developed to developing countries. This paper further emphasizes the need for examination of such aids for potential detrimental effects, instead of providing solutions to poverty as envisaged. The study also uncovers that most of GeneXpert-based studies falls in the years 2010 onwards, an indication that the technique is relatively new in terms of implementation in Africa.

Table 2: ***Track record of published work in TB done in Tanzania and other sub-Saharan countries indicating the contribution of molecular methods approach in general research profile.***

SN	No. of Manuscripts	Year of publication	Molecular	Xpert MTB/RIF	Other areas	Tanzania	Others
1	39	2018	11	3	25	20	19
2	79	2017	21	5	53	42	37
3	50	2016	13	3	34	22	28
4	71	2015	12	2	57	25	46

5	56	2014	9	2	45	38	18
6	46	2013	5	2	39	34	12
7	53	2012	5	1	47	42	11
8	33	2011	1	1	31	24	9
9	32	2010	4	0	28	24	8
10	26	2009	6	0	20	14	12
11	22	2008	2	0	20	16	6
12	22	2007	4	0	18	19	3
13	19	2006	3	0	16	12	7
14	11	2005	1	0	10	7	4
15	6	2004	1	0	5	5	1
16	11	2003	1	0	10	7	4
17	4	2002	1	0	3	1	3
18	11	2001	0	0	11	9	2
19	9	2000	3	0	6	5	4
20	7	1999	0	0	7	3	4
21	5	1998	1	0	4	3	2
22	6	1997	0	0	6	4	2
23	10	1996	1	0	9	8	2
24	18	1995	1	0	17	12	6
25	12	1994	2	0	10	8	4
26	6	1993	0	0	6	4	2
27	3	1992	0	0	3	3	0
28	10	1991	0	0	10	4	6
29	6	1990	0	0	6	4	2
30	2	1989	0	0	2	2	0
31	1	1985	0	0	1	1	0
32	2	1984	0	0	2	2	0
33	1	1983	0	0	1	1	0
34	2	1982	0	0	2	2	0
35	1	1978	0	0	1	0	1
36	1	1977	0	0	1	1	0
37	2	1976	0	0	2	1	1
38	1	1975	0	0	1	1	0
39	1	1974	0	0	1	1	0
40	1	1973	0	0	1	1	0
41	1	1972	0	0	1	1	0
42	3	1971	0	0	3	1	2
43	1	1970	0	0	1	0	1
44	2	1968	0	0	2	2	0
45	1	1967	0	0	1	0	1
46	1	1962	0	0	1	1	0
47	1	1961	0	0	1	1	0

48	1	1960	0	0	1	0	1
49	1	1957	0	0	1	0	1
50	1	1953	0	0	1	0	1
51	3	1952	0	0	3	3	0
52	1	1951	0	0	1	1	0
53	1	1950	0	0	1	0	1
Total	**716**		**108**	**19**	**589**	**442**	**274**

CONCLUSION AND RECOMMENDATIONS

In Africa, the occurrence of zoonosis is common, in the main part from the close proximity of humans and animals. Exploring the disease dynamics at these interfaces could provide the key for developing successful interventions to improve health in both humans and animals. We now have molecular tools which can examine the transmission dynamics of Mycobacterial disease in these settings. In order to do this, a strategy that involves medical and veterinary professions as well as other stakeholders will be needed. The Southern African Centre for Infectious Disease Surveillance (SACIDS), as well as other consortia working under the One Health concept including CORDS (Coordinating Organizations for Regional Disease Surveillance) may be a good starting point for such an approach. From this review we found an increase in published work in retrievable papers of over 80-fold in late years compared to earlier years, which is an indicator of trend-wise development in research. Most of molecular studies, however, cluster in the mid- to late 2000s, with little developments since, suggesting a slow adoption of technology and a high cost of running molecular tests. This overview of genotyping studies and trend in molecular studies should therefore provide an insight at regional level for more dedicated efforts to invest in this area. This review lays the foundation for a more robust investigation of TB particularly at human-animal interface to reduce the potential risk of cross-species transmission. This can be achieved by using molecular screening methods to assess the disease dynamics at this interface for early detection to provoke deployment of best control strategies.

Limitations

Possibility of missing of some papers and reports that were not published is potential limitation that we think might have been the case for our review. However, the 716 published articles in the area we focused are believably, sufficient to reflect the trend in which technological advancement has been growing. We encourage researchers to publish their research work so that can be kept tracked. Our future plans are to further do a systematic review and meta-analysis that could cover the whole of Africa with an improved search strategy.

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