Diplomarbeit

Retrospective study of epidemiology, bacteriological background and resistance patterns in chronic ulcers of patients affected by leprosy in India

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Affidavit

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Zusammenfassung

Hintergrund: Lepra, auch Morbus Hansen genannt, ist eine chronisch granulomatöse Infektionskrankheit, die durch *Mycobacterium leprae* oder *Mycobacterium lepromatosis* ausgelöst wird.
Lepra wird zur Gruppe der seltenen Erkrankung gezählt, obschon jährlich fast 215 000 neue Fälle erfasst werden - die Mehrheit davon in Indien, Brasilien und Indonesien.
Die auslösenden Mykobakterien befallen hauptsächlich die peripheren Nerven und die Haut. Es können aber auch die Augen, die Schleimhaut der oberen Atemwege und die Hoden betroffen sein.
Aufgrund des charakteristischen Sensibilitätsverlustes leiden die PatientInnen oft unter trophischen Ulzera- in manchen Fällen verlangt dies eine antibiotische Behandlung, welche an die lokale Resistenzsituation angepasst werden muss.

Ziel: Im Rahmen dieser Diplomarbeit sollen das Keimspektrum und die Resistenzlage in chronischen Wunden bei LeprapatientInnen untersucht werden und die Resultate im Hinblick auf den Einfluss auf eine gegebenenfalls notwendige antimikrobielle Therapie beleuchtet werden.

Material und Methoden: Für die vorliegende Arbeit wurde eine retrospektive Datenanalyse von 50 LeprapatientInnen (15 Frauen und 35 Männer) aus Salem, im Bundesstaat Tamil Nadu in Indien, durchgeführt. Dazu wurden 66 Wundabstriche von chronischen Wunden der Füße oder Unterschenkel der PatientInnen entnommen. Daraufhin wurden Kulturen zur Analyse des Keimspektrums und des Antibiogramms angelegt.

Ergebnisse: In 95,5% der Proben befanden sich zwei oder mehr unterschiedliche Bakterienstämme; der Median ergab vier Bakterienarten pro Abstrich. Der am häufigsten isolierte Mikroorganismus war *Pseudomonas aeruginosa* (26/66; 39,4%), knapp gefolgt von *Staphylococcus aureus* (24/66 mit 25 Isolaten; 36,4%) und β-hämolytischen *Streptokokken* der Gruppe G und C, abgekürzt GGS und GCS (20/66 und 5/66; 30,3% und 7,6%).

Enterobacter cloacae (13/66; 19,7%), *Klebsiella pneumoniae* (11/66; 16,7%), *Proteus mirabilis* (9/66; 13,6%), *Escherichia coli* (6/66 mit 8 Isolaten; 9,1%), *Acinetobacter baumannii* (8/66; 12,1%) und *Proteus vulgaris* (6/66; 9,1%) waren ebenfalls häufig detektierbar.
27 (40,9%) der 66 Proben beinhalteten zumindest einen multiresistenten Keim. Insgesamt konnten 33 multiresistente Bakterien nachgewiesen werden. Die Liste der multiresistenten Organismen wird von *MRSA, Methicillin-resistenten Staphylococcus aureus,* (23/33; 69,7%) angeführt, gefolgt von extended spectrum ß-lactamase (ESBL)-produzierenden *Proteus vulgaris* (4/33; 12,1%). Zudem gab es drei (9,1%) 3MRGN-*E. coli,* multi-resistente Gram-negative (ebenfalls ESBL-produzierend), einen (3,0%) ESBL-produzierenden *E. coli* sowie einen (3,0%) ESBL-produzierenden 3MRGN-*Proteus mirabilis* beziehungsweise einen (3,0%) ESBL-produzierenden *Enterobacter cloacae*. Kein Erreger wurde als 4MRGN-Stamm klassifiziert.

Fazit: Die relativ große Anzahl multiresistenter Bakterien unterstreicht die Bedeutung einer sorgfältig durchdachten Antibiotikatherapie.
Basierend auf der Auswertung der vorhandenen Daten aus Salem in Indien, sollten die hohen *MRSA*-Raten hinsichtlich einer oralen empirischen Therapie berücksichtigt werden: In diesen Fällen stehen dem behandelnden Arzt Fusidinsäure per os, Erythromycin und Clindamycin zur Verfügung. Für *Pseudomonas* and *Enterobacteriaceae* können Ciprofloxacin und in einigen Fällen Amoxicillin/Clavulansäure oder orale Cephalosporine verwendet werden.
Da ähnliche Studien divergierende Ergebnisse erbrachten, könnten weitere Studien zu lokalen Resistenzraten die Wahl geeigneter Antibiotika für die jeweilige medizinische Einrichtung erleichtern.

Abstract

Background: Leprosy, also called Morbus Hansen, is a chronic granulomatous infectious disease caused by *Mycobacterium leprae* or *Mycobacterium lepromatosis*.
It is nowadays classified as an orphan disease; even though, nearly 215 000 new cases per year are detected worldwide - the majority in India, Brazil and Indonesia.
The mycobacteria mainly attack the peripheral nerves and the skin, but the eyes, upper airway mucosa or the testicles may also be affected.
Due to the characteristic loss of sensibility, patients often suffer from trophic ulcers - in some cases this requires antibiotic treatment which has to be tailored to local resistance patterns.

Objective: The purpose of this diploma thesis was to investigate the bacteriological profile and the resistance patterns of chronic lesions of leprosy patients followed by an evaluation of the output in view of the influence on empiric antimicrobial treatment, where necessary.

Materials and Methods: For the present thesis a retrospective data analysis was carried out among 50 leprosy patients (15 women and 35 men) from Salem, district of Tamil Nadu, India. Thereto, 66 wound swabs from chronic lesions on the feet or lower legs of patients were collected. Subsequently, cultivations with the analysis of the bacteriological profile and resistance patterns were undertaken.

Results: 95.5% of the samples contained two or more different bacteria strains; the median was at four bacteria per swab. The most commonly isolated organism was *Pseudomonas aeruginosa* (26/66; 39.4%) closely followed by *Staphylococcus aureus* (24/66 with 25 isolates; 36.4%) and β-haemolytic *streptococci* of group G and C, abbreviated GGS and GCS (20/66 and 5/66; 30.3% and 7.6%).
Enterobacter cloacae (13/66; 19.7%), *Klebsiella pneumoniae* (11/66; 16.7%), *Proteus mirabilis* (9/66; 13.6%), *Escherichia coli* (6/66 with 8 isolates; 9.1%), *Acinetobacter baumannii* (8/66; 12.1%) and *Proteus vulgaris* (6/66; 9.1%) were also frequently detected.
27 (40.9%) of the 66 samples included at least one multi-resistant pathogen. Altogether 33 multi-resistant bacteria could be determined. The list of multidrug-resistant, MDR, organisms is headed by *MRSA, methicillin-resistant Staphylococcus aureus,* (23/33;

69.7%) and is continued by extended spectrum ß-lactamase (ESBL)-producing *Proteus vulgaris* (4/33; 12.1%). Additionally, there were three (9.1%) 3MRGN-*E. coli,* multi-resistant Gram-negatives (also ESBL-producing), one (3.0%) ESBL-producing *E. coli* as well as one (3.0%) ESBL-producing 3MRGN-*Proteus mirabilis* respectively one (3.0%) ESBL-producing *Enterobacter cloacae*.
None of the microorganisms could be assigned to a 4MRGN strain.

Conclusion: The quite high number of multidrug-resistant bacteria shows the importance of carefully considered antibiotic treatment. Based on the analysis of the present data from Salem, India, the high *MRSA*-rates have to be taken into account for oral empiric therapy: In this case oral fusidic acid, erythromycin or clindamycin are available.
For *Pseudomonas* and *Enterobacteriaceae* ciprofloxacin or in some cases amoxicillin/ clavulanic acid or oral cephalosporins might be used.
As similar studies revealed varying results, further research on local resistance rates may facilitate the choice of appropriate antibiotics for the respective medical institution.

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Glossary and abbreviations

A. baumannii	Acinetobacter baumannii
BB	borderline-borderline
BCG	Bacillus Calmette-Guérin
BI	bacterial index
BL	borderline-lepromatous
BLI	β-lactamase-inhibitors
BT	borderline-tuberculoid
CD4+	cluster of differentiation 4 positive (T-helper cells)
CD8+	cluster of differentiation 8 positive (cytotoxic T- cells)
CRE	carbapenem-resistant Enterobacteriaceae
DTMC	Doctor Typhagne Memorial Charitable
ECDC	European Centre for Disease Prevention and Control
E. cloacae	Enterobacter cloacae
E. coli	Escherichia coli
ENL	erythema nodosum leprosum
ESBL	extended-spectrum-β-lactamase
EUCAST	European Union Committee on Antimicrobial Susceptibility Testing
GCS	group C streptococci
GGS	group G streptococci
GHD	Department for Global Health and Development
ICRC	Indian Cancer Research Centre
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IND	indeterminate
K. pneumoniae	Klebsiella pneumoniae
LL	lepromatous
LPEP	leprosy post-exposure prophylaxis
LPh	Lucio's phenomenon
MB	multibacillary

MDR	multidrug-resistant/ multidrug-resistance
MDT	multidrug therapy
M. habana	Mycobacterium habana
MI	morphological index
MIP	Mycobacterium indicus pranii= Mycobacterium w
M. leprae	Mycobacterium leprae
M. lepromatosis	Mycobacterium lepromatosis
MRGN	multi-resistant Gram-negatives
MRSA	methicillin-resistant Staphylococcus aureus
M. tuberculosis	Mycobacterium tuberculosis
M. ulcerans	Mycobacterium ulcerans
M. vaccae	Mycobacterium vaccae
NLEP	National Leprosy Eradication Programme
P. aeruginosa	Pseudomonas aeruginosa
PB	paucibacillary
PCR	polymerase chain reaction
PGL-1	phenolglycolipid-1
P. mirabilis	Proteus mirabilis
P. vulgaris	Proteus vulgaris
ROM	rifampicin, ofloxacin, minocycline
RR	reversal reaction
RT-PCR	reverse transcriptase polymerase chain reaction
S. aureus	Staphylococcus aureus
sp./spp.	species
TT	tuberculoid
VRE	vancomycin-resistant enterococci
WHO	World Health Organisation

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1 Introduction

1.1 Leprosy

Leprosy, also called Morbus Hansen, has always been a feared and stigmatizing disease that is –contrary to the perception of the Western civilisation- still present in many parts of the world. When thinking of leprosy, mutilated extremities, exclusion and irremediableness come to mind. However, this is not necessarily correct, as medical progress could improve the situation of patients to a large extent. But despite the possibility of an antibiotic therapy and already conducted research, the disease still raises unsolved questions. Hereafter, the latest state of knowledge concerning leprosy shall be outlined.

1.2 Historical background of leprosy

Leprosy is considered to be the oldest human-specific contagious disease: Genome analyses of *Mycobacterium leprae* isolates namely retraced the infection for more than 100,000 years. Presumably the development of these bacteria in humans respectively in their ancestors even dates back millions of years.
Studies could identify the origin of the Hansen's bacilli in Africa. (1)

„In corpore hominis *ita dinoscitur,*
si variatim inter sanas cutis partes color diversus appareat,
aut si ita se ubique diffundat,
ut omnia unius coloris quamvis adulterini faciat.“(2)

Freely translated this means: "Leprosy is thus diagnosed on the human body: If a different colour appears between the healthy skin areas or if it [the skin alteration] spreads everywhere so that it covers all with a single but anomalous colour."
That quote originates from *Isidore of Seville* also called *Isidorus Hispalensis*, a medieval author who lived approximately from 560 anno domini to 636 anno domini. (3)

However, this more or less applicable description is not at all the first written evidence of the Hansen's disease: The earliest documents dealing with the genuine leprosy trace back to at least 600 before Christ, maybe even to 1400 before Christ in India when writings

were drafted about a disease called *Kushta*. (4) The Egyptian *Ebers Papyrus* (1550 before Christ), the Sanskrit *Atharva Veda* (1000 before Christ) and the Old and New Testament are not generally considered as reliable sources describing leprosy.
The oldest skeletal proof of the disease also originates from India in the second millennium before Christ. (5)
Researchers hypothesise that it was *Alexander the Great* and his army who contributed to the propagation of *Mycobacterium leprae* to Europe in 327-325 before Christ.
The reason, why leprosy disappeared in the European area, cannot be indicated satisfactorily nowadays. It could be due to the higher living standards, cross-reactive antibodies against tuberculosis and leprosy or the pestilence which decimated the number of susceptible people.
From Europe the disease was then disseminated in Western Africa and the Western hemisphere as a result of the journeys of discovery and the slave trade. (4)

Although leprosy had been known for thousands of years, only *Gerhard-Henrik Armauer Hansen*, a Norwegian doctor, discovered the *Mycobacterium leprae*, the cause of Morbus Hansen, in 1873. (6)
However, it took several decades till an effective treatment for leprosy was found: In the 1940s the doctors started to prescribe promin, a sulfone, which unfortunately led to many side effects such as anaemia and allergic dermatitis. (7)
For this reason they used dapsone: Since 1946, it was used as a monotherapy, but drug resistance soon developed. (8)
This evolution was finally decisive for the WHO's recommendation of the multidrug therapy (MDT) containing dapsone and rifampicin with or without clofazimine- depending on whether the patient suffers from pauci- or multibacillary leprosy. (9)

In 2008, *Xiang Han et al.* attracted attention by identifying another species causing leprosy: *Mycobacterium lepromatosis*. The bacterium was discovered in two Mexican men with diffuse lepromatous leprosy: Therefore, it was assumed that *Mycobacterium lepromatosis* was responsible for the more ominous cases- yet, this could not be confirmed. (10, 11)

Stigma related to leprosy is as old as the disease itself: Ignorance and superstition now and then have resulted in injustice. Since Hansen's disease has been thought to be the

punishment for sins respectively the outcome of a curse, it has been accepted and forced that leprosy patients were isolated in leprosy colonies or leprosaria (leprosy hospitals).
It is still a common situation that the diagnosis of Morbus Hansen walks along with repudiation and banishment. The main reason for this behaviour is the disfigured physical appearance of patients and maybe also the odour of infected lesions.
Furthermore, religious and cultural aspects play an important role: Christianity, Hinduism and Buddhism circulated the assumption that leprosy was a fair penalty for sins in the current or previous life. In China Hansen's disease was supposed to be sexually transmitted by prostitutes and accordingly the consequence of immoral conduct.
The more pragmatic root of stigmatisation is the fear of transmission of the disease- mistakenly believed incurable which is closely related to visible skin manifestation.
Likewise, racism kept stigma alive to some degree, for leprosy was considered as the disease of inferior and poor people. Even nowadays the illness is associated with poverty, unemployment and lack of education. (12)

1.3 Epidemiology of Leprosy

Collected data from 143 countries revealed that 214 783 new cases were reported in 2016, while 171 948 people were under multidrug therapy. At that time the global incidence was 2.9/ 100 000/ year; the highest regional incidence was 8.2/ 100 000/ year in South-East Asia.
Whereas in 1983 21.1 leprosy cases per 10 000 people were detected, the prevalence rate declined to 0.27/ 10 000 till 2016; this corresponds to a decrease of 99%.
During this period more than 16 million patients underwent a leprosy therapy. (13, 14)

The distribution of leprosy is quite different: *Figure 1* indicates that the Hansen's disease is mainly found in the emerging and developing nations. (15)

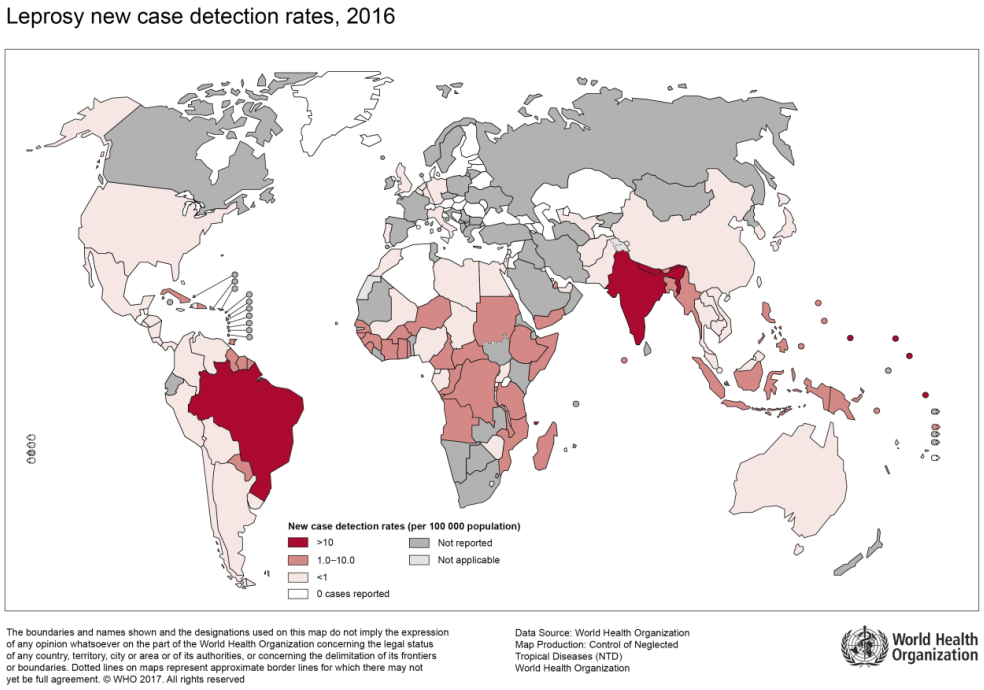

Figure 1: Leprosy new case detection rates (15)

About 95% of the newly diagnosed patients were registered in only 22 so-called "global priority countries" which mostly belong to Africa and South-East Asia. This circumstance has not gravely changed since 2007.

The leprosy hot spots are located in India, Brazil and Indonesia, where more than 10 000 cases are detected. India is the unchallenged leader with more than 135 000 leprosy cases. Nevertheless, it could eradicate it as a public health problem in 2005 -the World Health Organisation defined this goal as a prevalence <1 per 10 000. (14)

Country	**Number of new cases detected**			
	2007	**2010**	**2013**	**2016**
Brazil	39 125	34 894	31 044	25 218
India	137 685	126 800	126 913	135 485
Indonesia	17 723	17 012	16 856	16 826
Global total	258 133	228 474	215 656	214 783

Table 1: New leprosy case detection trends in three global priority countries, 2006–2017 [modified after (14)]

T*able 2* shows that nearly 60% of the new leprosy patients are male and that 8.5% of the patients are children.
Although leprosy is hence much more disseminated in adults, it is the occurrence in children and adolescents that indicates the continuous transmission. (16)

	No. of new cases detected (2016)	**No. of females among new cases**	**No. of children among new cases**	**No. of MB among new cases**
Global total	214 783	84 202 (39.2%)	18 230 (8.5%)	127 013 (59.1%)

Table 2: Global leprosy situation by WHO region and country, December 2016-March 2017 [modified after (14, 15)]

1.4 Aetiology of leprosy

Leprosy is defined as a chronic granulomatous infectious disease caused by *Mycobacterium leprae* or *Mycobacterium lepromatosis*. Also known as the Hansen's disease, it attacks the skin, the peripheral nerves, the upper respiratory tract's mucosa and the eyes. (10, 17)
Morbus Hansen is caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis*. They are "relatives" of *M. tuberculosis* and *M. ulcerans*, the pathogen of Buruli ulcer, with whom they share some characteristics: The genus *Mycobacterium* is classified by its acid resistance, the slow growth and its lipid-rich and waxy cell wall with mycolic acids. (18, 19)
However*, M. leprae* and *M. lepromatosis* vary concerning their genome: The difference of coding genes accounts 7%, but only 82% of the pseudogenes are identical. All in all, these mycobacteria are well conserved and provoke similar clinical signs. (20)

1.4.1 Mycobacterium leprae

Mycobacterium leprae is a slightly bent, acid-resistant rod-shaped bacterium with a width of 0.3µm and a length of 1-5µm.
The pathogenic agent is obligatory intracellular, but outside the body it remains infectious about nine days under tropical conditions. (18, 19)

These bacteria show a tropism for macrophages and Schwann cells and need a temperature between 27 and 30°C in order to survive and proliferate. This is likely the reason, why they are more frequent in cooler body parts such as skin, peripheral nerves, upper airway mucosa, testicles and the eyes. (21, 22)
Hansen's bacillus only divides every 10-14 days, which might be a causative factor for the long incubation period of many years.
So far, the bacterium could not be cultivated in-vitro; thus, the research on leprosy is hampered.
In 1960, it was possible for the first time to conduct an in-vivo-culture in mice, eleven years later researchers switched to leprosy-infected nine-banded armadillos.(23)
The main natural reservoir is situated in humans. However, mycobacteria can also be found in armadillos in the Southern States of the USA and South America as well as red squirrels in the British Isles, some captive primates, soil and water.(24, 25)

1.4.2 Mycobacterium lepromatosis

Mycobacterium lepromatosis was first discovered in Mexico where it is endemic (as well as in the Caribbean countries) and where it is even more common than *M. leprae*. Individual cases also appeared in India, Brazil, France, USA, Canada, Iran, Singapore et cetera. In the endemic regions *M. lepromatosis* and *M. leprae* may lead to mixed infections. (20, 26)
The more recently discovered *Mycobacterium lepromatosis* was also detected in squirrels in the United Kingdom. (24)
The bacillus is associated with Lucio's phenomenon, a special form of leprosy mainly found in Mexico. It is characterized by dolorous, necrotic-haemorrhagic lesions. (20)

1.5 Pathogenesis of leprosy

The following statements only refer to *M. leprae* since the pathogenesis of *Mycobacterium lepromatosis* hitherto lacks solid research results.

1.5.1 Transmission of leprosy

Despite the fact, that leprosy is known for millennia, the process of transmission is still not conclusively proven.

It is assumed that nasal secretion or minute pathogen-containing particles, also known as Flügge-droplets, spread the microbes via inhalation. This requires a close and frequent contact with a leprosy patient.
In an Indian study, 60% of untreated multibacillary leprosy cases correlated with a detectable *M. leprae*-DNA in swabs from the nasal mucosa. Though, it is also supposed that even persons, in whom leprosy has not emerged, can act as a carrier, for they exhibit transient nasal contamination with mycobacteria. The study of *Job et al.* indicated that this affected 4% of leprosy patients' contacts before multidrug therapy and 6% one month after multidrug therapy. (21, 25, 27)

Skin erosions constitute another possible pathway of transmission: Several cases of leprosy lesions evolving over tattoos or vaccination scars and after injuries have been published. Moreover, there exists some evidence that the exfoliation of stratum corneum cells contributes to the environmental dissemination. The bacilli are picked up by basal cells from the papillary dermis and can go to the superficial layer inside the keratinocytes or accompanying them.
At some point the epithelium is shed and the leprosy mycobacteria, whose extracorporeal viability amounts several days, are able to invade other people. 1g of lepromatous tissue contains about seven billion mycobacteria, in other forms of the disease 1g comprises only one million bacilli.
This process, where dermal components are engulfed by the epidermis in order to get rid of them, is called transepidermal elimination. (22, 28-31)
In the study of *Job et al.*, the histological examination determined that 60% of the patients had acid-fast bacteria in their keratin layer; while, *Mycobacterium leprae*-DNA could be found in 80% of the cases by polymerase chain reaction (PCR) of skin washings.
17% respectively 1% of the contact persons showed a PCR-positivity for *M. leprae*-DNA in the skin during the pre-treatment respectively treatment period. After two months of multidrug therapy (MDT), none of the infected persons still had a positive result. (27)

The environment should not be neglected as a potential transmission route: Soil and water may play an important role in this context. (32)
The zoonotic reservoir of leprosy in nine-banded armadillos is well documented and at least in the South-Eastern United States credible links between infected armadillos and humans could be uncovered. (33)

Insects like flies, mosquitoes and bedbugs were also discussed as possible carriers of leprosy, but no evidence could be found for this hypothesis. (25)
Additional paths of infection as via breast milk, vertical transmission and blood have still not been excluded. (34-37)

1.5.2 Aetiopathology of leprosy

Just a few weeks as in infants or up to 30 years in adults elapse between the infection and the first clinical symptoms of leprosy; the average is around three to ten years. This long incubation period can be explained by the slow reproduction of *M. leprae*, the low antigenicity or its metabolic elimination. (21, 38)
During this period, the mycobacteria are not idle, though: They have to conquer numerous obstacles, such as the intact epithelium, secretions with antimicrobial substances and IgA, as well as cells of the innate and acquired immune system.
If the bacilli are capable to overcome these barriers, they start to invade Schwann cells, macrophages and dendritic cells. (39)

1.5.3 Classification of leprosy

The *Ridley-Jopling classification* applies clinical, histological and immunological criteria in order to grade leprosy: TT (tuberculoid), BT (borderline tuberculoid), BB (borderline borderline), BL (borderline lepromatous) and LL (lepromatous). (40)
At both ends of the spectrum the disease is clinically stable, whereas the borderline subtypes tend to acute episodes of inflammation, called leprosy reactions.
Tuberculoid leprosy is related to an adequate immune response; therefore, mycobacteria are reduced. Conversely, the lepromatous form shows an antigen-specific unresponsiveness leading to an uncontrolled proliferation of mycobacteria.
The WHO defines another categorisation into paucibacillary (PB), low bacterial load, and multibacillary (MB), high bacterial load. This is also influenced by immunology: The tuberculoid type is paucibacillary (≤ five lesions); lepromatous leprosy is multibacillary (> five lesions). (38, 39, 41)
Since 2002, the *Madrid classification* is in use and subdivides leprosy into the indeterminate, tuberculoid, borderline and lepromatous form. The indeterminate type (IND) is supposed to prelude the disease. This initial phase lacks an assignable

immunological pattern and can either progress to a stable pole or be self-limiting. (21, 22, 42)

1.5.4 Leprosy reactions

Although Morbus Hansen is a chronic disease, there occur acute phases of inflammation, known as leprosy reactions. These reactional states are the manifestation of an immune response alteration mainly caused by medical treatment, pregnancy, concomitant infections or stress. There exist two different types of leprosy reactions: reversal reactions (RR, type I) and erythema nodosum leprosum (ENL, type II). (38)

Type I reversal reactions (RR) appear in borderline-tuberculoid, borderline-borderline and borderline-lepromatous leprosy. It constitutes a delayed-type (=type IV) hypersensitivity mediated by CD4+ cells and is directed towards the tuberculoid pole. (38, 43)
Under therapy the bacterial load is decreased, so reversal reactions tend to the tuberculoid pole. Conversely, bacilli can multiply easily within untreated cases and provoke subpolar lepromatous leprosy. (21)

Erythema nodosum leprosum is due to type III hypersensitivity, the accumulation of immune complexes. Erythema nodosum leprosum (ENL, type II) is predominant in borderline-lepromatous and lepromatous leprosy. 60% of lepromatous cases suffer from type II reactions, partly even several times. Such reactions exhibit an acute exacerbation under therapy- mainly in lepromatous leprosy patients, scarcely in borderline-lepromatous cases. (21, 22)
There exists an erythema nodosum leprosum variant called Lucio's phenomenon (LPh) that is primarily observed in Mexico but that can also be found in other Central and South American countries. Lucio's phenomenon is classified as a necrotic panvasculitis with endothelial proliferation, thrombosis and vascular occlusion. It is found in diffuse lepromatous leprosy and is often associated with *M. lepromatosis*.
It is thought that Lucio's phenomenon and erythema nodosum leprosum in general can be traced to immune complexes: IgG and C3 inside dermal vessel walls and circulating immune complexes are detectable. This immune response may be triggered by stress, strain, bacterial endotoxins, medication etc. (21, 44, 45)

1.6 Symptoms of leprosy

Hansen's disease may affect the skin, peripheral nerves, the eyes, upper respiratory tract mucosa, testicles or other organs.

Leprosy mycobacteria attack cutaneous peripheral nerves, first and foremost the posterior tibial, the cubital, the medial and lateral peroneal nerve. Perineural fibrosis sparks off the thickening of nerves, whereby the nerves get palpable. Additionally, pain, sensory and motor damage appear.

Numbness, anhidrosis and impaired thermoreception result from the involvement of small cutaneous nerve fibres. (17, 22)

The immune response is the major factor impacting the clinical manifestation: Tuberculoid leprosy is one end of the spectrum, lepromatous leprosy the other. (21)

An overview of the clinical differences is depicted in chapter diagnosis, *table 3*.

1.6.1 Clinical signs of indeterminate leprosy

Indeterminate (IND) leprosy patients show single hypopigmented macular lesions sometimes with poorly delimitable edges. In fair skinned people, lesions seem erythematous. There is no pain sensitivity loss, but at the end of this stage derated thermosensitivity and hypohidrosis signalize the transition to a more proceeded phase. This period may take up to five years. (23, 46)

1.6.2 Clinical signs of tuberculoid leprosy

Tuberculoid (TT) leprosy is characterized by single or few asymmetrical erythematous plaques often with an edge bead and a hypochromic atrophic centre. The anaesthesia inside the small well-defined lesions leaves out the face because of the copious sensory nerves there. In early stages the loss of sensitivity is often missing.

As an expression of motor impairment, tuberculoid leprosy patients experience muscular weakness that precedes paralysis. Ulnar claw, fall hand, benediction hand or facial nerve paresis with mask face (facies antonina) may develop.

Due to denervation of skin appendages, some patients suffer from alopecia and anhidrosis within the lesions. Besides, myelin sheath thickening, hyperkeratosis and ulceration may appear.

If the optic nerves are affected, corneal opacity, irreversible eye damage or even blindness are the consequences. (21-23)

1.6.3 Clinical signs of lepromatous leprosy

The clinical presentation of lepromatous (LL) leprosy is shaped by multiple symmetrical and bilateral papules and nodules. The confluent lesions are hypopigmented, erythematous or coppery with indistinct borders. Sensation is frequently not harmed; nevertheless, dryness of skin and alopecia can be determined. As long as it is the lepromatous and no borderline form, several peripheral nerves are compromised- mainly in the cooler body parts. The advanced state is characterized by infiltrations causing lepromas (nodules and plaques). Furthermore, patients complain of peripheral oedema in their lower limbs and hypaesthesia.

Finally, lepromas form the leonine facies with diffuse facial infiltration and madarosis (bilateral loss of the eyebrows and eyelashes). The resorption of the alveolar process of maxilla and the anterior nasal spine often affects the loss of the superior incisors which gives the patient the typical facies leprosa.

Since mycobacteria disseminate systemically, Hansen's disease may manifest in various organs, such as the eyes, kidneys, testicles, bones, joints, blood vessels, lymphatic nodes and mucosa of the upper airway.

The rare multibacillary histoid subtype shows asymmetrical (sub-)cutaneous nodules that demarcate from the normal environing skin. The papules and plaques are shiny and skin-coloured, mainly covering the back, face and buttocks. (21-23, 38, 47)

1.6.4 Clinical signs of borderline leprosy

The different immune responses of borderline cases are mirrored by various clinical signs. The resemblances of borderline-tuberculoid and tuberculoid lesions are evident, but borderline-tuberculoid (BT) lesions are more numerous and smaller. Sensory loss also walks along with nerve thickening, yet in a larger number, irregular and less pronounced.

Borderline-borderline (BB) leprosy evinces symptoms of tuberculoid and lepromatous leprosy. The asymmetrical erythematous plaques have a hypochromic oval centre (foveal spot), distinct inner and indistinct outer edges.

In contrast to lepromatous leprosy, borderline-lepromatous (BL) skin lesions are less symmetrical and partly lack sensation. (21)

1.6.5 Clinical signs of leprosy reactions

The chronic course of Hansen's disease may be interrupted by acute phases of inflammation, also known as leprosy reactions. These reactional states are characterized by an immune response alteration and are caused by medical treatment, pregnancy, infections or stress.

Because of the provoked nerve damage, these acute reactions may augment morbidity- if untreated, during or even after treatment.
Reversal reactions, type I, exhibit oedematous, erythematous and scaling lesions combined with hyperaesthesia and possible ulcerations, vesicles and necrosis.
Besides, neuritis, which should be treated immediately, and peripheral oedema are reported. Depending on the closeness to tuberculoid or lepromatous pole, a systemic manifestation is less or more likely. (21, 22)

Erythema nodosum leprosum manifests as a sudden clinical deterioration with general symptoms: fever, myalgia, arthralgia, malaise, lymph node swelling and oedema.
The symmetrical subcutaneous nodules are located on the lower extremities or more seldom on the trunk, are inflamed and painful. Neuritis, renal or hepatic damage are possible additional findings.
Necrotic erythema nodosum leprosum, as a subspecies of type II reactions, is caused by vascular obliteration, which is in turn owed to immune complex deposition and the subsequent leukocytoclastic vasculitis. (21, 22)
The other erythema nodosum leprosum form, Lucio's phenomenon, presents dolorous red or purple macules with irregular borders. The necrotic-haemorrhagic lesions, induced by vascular obliteration, first appear on the lower limbs and then move on to the torso and the upper extremity.
At the same time, general symptoms as in classical erythema nodosum leprosum may occur. The diffuse macules convert to blisters, necrotic scab and eventually to atrophic scars. Advanced Lucio's phenomenon is reflected by thromboses, skin infarction or haemorrhages. Recurrent lesions can destroy superficial body parts as the nose or the auricle; Lucio's phenomenon might even result in death. (22, 45, 48)

1.7 Complications of leprosy

The complications of Morbus Hansen are manifold and affect the health of the patients as well as their living environment. Neurological, skeletal and soft tissue, systemic and social changes may be in store for the patients.

1.7.1 Neurological changes in leprosy

Not only do mycobacteria attack dermal free nerve endings -originating sensitivity impairment, but they also penetrate peripheral nerve trunks provoking neuritis. This process takes place slowly and may deteriorate during reactional phases. Functional changes may be accompanied by pain. When the pain associated to neuritis chronifies, this is termed a neuropathic pain.

Peripheral leprosy neuropathy comprises a motor, a sensory and an autonomic part: Myasthenia, paresis, paralysis, amyotrophy, tendon retraction and ankylosis (motor); hypaesthesia or anaesthesia (sensory); vasomotor dysregulation, anhidrosis and reduced sebaceous gland function (autonomic).

M. leprae has a predilection of the facial and the trigeminal nerve in the face, of the ulnar, radial and median nerve in the upper limbs and of the posterior tibial and common fibular nerve in the lower extremity.

Nerve lesions of the face: Infestation of the ophthalmic ramus of the trigeminal nerve diminishes the nasal and corneal perception, so that patients are prone to infections, ulcers and blindness. Damage of the zygomatic branch of the 7^{th} cranial nerve is responsible for lagophthalmos, orbicular paralysis and possible ectropion.

In case of malfunction of autonomic nerve fibres, the decreased mucous secretion and perfusion provoke atrophic rhinitis.

Nerve lesions of the upper extremity: Sensory and autonomic impairment of the ulnar nerve affect its innervation area; namely the medial edge of the hand, the 4^{th} and 5^{th} finger. As motor function is concerned, the metacarpophalangeal joints remain in hyperextension and the interphalangeal articulations in flexion which leads to the so-called claw hand. Additionally, thenar, hypothenar and interosseous spaces may atrophy.

Among other things, benediction hand, ulnar deviation and sensitivity impairment of the palmar side of the thumb, index, middle finger and the radial part of finger IV are evolved by median nerve attack.

The seldom radial nerve invasion is always accompanied by ulnar and median nerve lesions (triple paralysis) and elicits drop wrist, forearm atrophy and sensitivity loss of the dorsal part of finger I-III and the radial half of the ring finger.
Nerve lesions of the lower extremity: Because of the common fibular nerve damage, foot drop and atrophy of the anterior and lateral leg can be found.
If the posterior tibial nerve is affected, claw toes, plantar anaesthesia and muscle atrophy as well as paralysis of the intrinsic muscles are caused. (21)

1.7.2 Skeletal and soft tissue changes in leprosy

95% of leprosy patients suffer from nonspecific musculoskeletal complaints; ulcerations, fractures, deformities and polyarthritis may occur.
Ulcers rank among the most frequent consequences of leprosy. Usually, they evolve in anaesthetic compression areas -mainly in the sole (malum perforans), scarcer in the palm or the cornea. Trophic ulcers are indolent and often superinfected with an impeded healing process and return repeatedly, so that physical disability appears normal.
Most of the musculoskeletal and soft tissue damages can be traced back to nerve impairment: Neurotrophic-triggered atrophy, recurrent trauma due to anaesthesia and analgesia or bone resorption following unequal load distribution because of paralysis and contractures.
Osteoporosis, the second most common unspecific sign in Morbus Hansen, is provoked by hypogonadism which is a result of testicular atrophy. Osseous bacterial invasion inducing periostitis or osteomyelitis presents another mechanism. (21-23, 49, 50)

1.7.3 Systemic changes in leprosy

Multibacillary cases, mainly of lepromatous leprosy, develop systemic changes on the basis of bacteraemia or reactional states. Comorbidities or adverse drug reactions may also trigger systemic alteration.
Respiratory system: For leprosy is supposed to be transmitted by airborne droplets, it is not surprising that changes especially in the upper airways are described- particularly within erythema nodosum leprosum. In comparison, the lower respiratory tract is seldom affected. Possible symptoms are chronic rhinitis with nasal congestion, rhinorrhoea and epistaxis. (21, 51)
Eyes: Eye damage can be elicited by direct infiltration and optic nerve disturbance. Madarosis, lagophthalmos, corneal opacity, iridocyclitis and cataracts are probable eye

lesions. At the time of diagnosis, 11% of multibacillary patients present with vision loss. (22, 23, 52)

Kidneys: Leprosy nephropathy is especially found in multibacillary forms. Reactional processes, mainly erythema nodosum leprosum with immune complexes, and secondary amyloidosis are the major responsible factors. Glomerulonephritis (30% of the leprosy cases), interstitial nephritis and pyelonephritis are reported in leprosy. Renal failure is the most common cause of death of lepromatous leprosy patients. (21, 53)

Testicles: Testicular involvement arises remarkably often- mainly in type II reactions. Probably this can be explained by the propensity of bacilli to temperatures between 30 and 33°C, since in the testicles there are 35°C. The affection presents as testicular pain, atrophy and orchitis leading to hypogonadism with infertility, sexual impotence, loss of libido, osteoporosis and gynaecomastia. (21, 49)

Haematological and lymphatic system: 90% of lepromatous leprosy types have a bacteraemia; Lucio's phenomenon may even take life-threatening dimensions because of sepsis and secondary infections.

High bacilli load is seen in reticuloendothelial cells of the spleen, liver and bone marrow. The bone marrow infiltration may engender myelosuppression with pancytopenia. (21, 48)

Cardiovascular system: Cardiac involvement, like ventricular hypertrophy or arrhythmias, emerges more frequent in multibacillary than in paucibacillary leprosy. Infiltration of sympathetic or parasympathetic nerves provokes autonomic dysfunction. Moreover, the infestation of vessels expresses as coronary heart disease or ischaemic ulcers. (21)

1.7.4 Stigma caused by leprosy

Stigmatization is a serious and widespread problem of Hansen's disease. Since ancient times, leprosy patients have to endure rejection and expulsion, so that the outcasts and sometimes also their families are obliged to live in leprosy communities. There exist hundreds of "leprosy-colonies" in India; in Brazil 33 communities are registered. Moreover, patients are also prone to self-stigmatize themselves and withdraw from public life.

Stigma or the fear of it influences peoples' reaction: Some of them try to ignore and hide their symptoms; other patients rather seek help before the illness aggravates.

Thereby, the socio-cultural component is pivotal: The social position of women, religion and traditions have a great impact on stigmatization -possibly leading to sexual inactivity or even divorce. (12, 54, 55)

1.8 Risk factors for leprosy

Several studies have identified the following risk factors for getting leprosy .

1.8.1 Genetic factors influencing leprosy

The majority of exposed people do not develop a disease, as about 95% of the world's population is naturally resistant to leprosy mycobacteria. (4)
It is therefore the genome which determines a person's susceptibility to Morbus Hansen- both according the infection by itself and the clinical features.

The first references to these facts were furnished by twin studies, which could indicate a correlation between the similarity of genes and the disease outbreak respectively the markedness of leprosy. The most significant study carried out by *Chakravartti* and *Vogel* yielded that nearly 60% of the examined monozygotic twins are both affected by Hansen's disease; this contributes to a concordance rate three times higher than in binovular twins. Among the infected twin pairs, 86.5% (identical twins) respectively 75% (fraternal twins) were afflicted by the same leprosy subtype. (41)

Segregation analyses help to ascertain, whether there is evidence that one or multiple major genes underlie the infection per se and the different varieties of a disease. From several studies resulted that the mode of inheritance for leprosy in general or non-lepromatous Morbus Hansen is recessive or co-dominant. (56)

1.8.2 Household

The greatest risk arises multibacillary cases: Household respectively dwelling contacts of those patients were five respectively eight times more often affected by Morbus Hansen than non-contacts. Household and dwelling contacts of paucibacillary patients had a double risk of contracting leprosy. This can be traced back to the physical proximity but also to the frequent genetic similarity. (57, 58) In this context dwelling is defined as the place where people sleep. (59)
Exposure to a household with more than seven members is accompanied by a three times higher risk of disease compared to households of one to four residents. (60)
75% of the household members fall ill during the first year after the diagnosis of the index case. (61)

1.8.3 Age

Research results about the impact of age are ambiguous. However, there exist at least some meaningful studies that attribute a bimodal distribution to Hansen's disease.

As shown in *figure 2*, 15- till 19-year-old and ≥ 40-year-old persons seem to be the most susceptible groups. (57)

The first peak apparently shifted to older individuals, since in former studies the highest prevalence was found in children between ten and fourteen years. (59, 62)

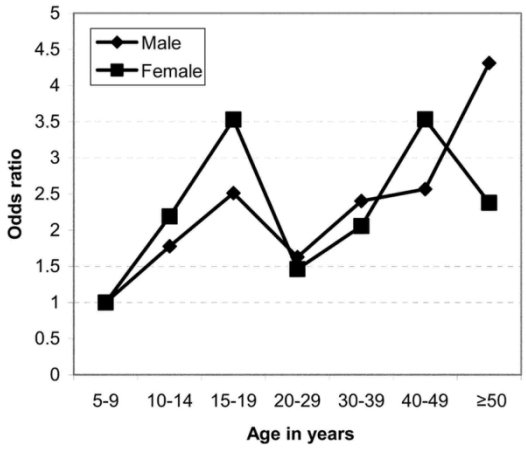

Figure 2: *Odds ratios for leprosy in contacts, by age and sex* (57)

1.8.4 Gender

With respect to a potential correlation of a person's sex and leprosy, it arises that different study conclusions are quite contradictory. The male/female-ratio shows a strong deviation depending on the geographic area: In Asia men seem to be more prevalent, whilst in Africa the situation is reversed.

This discrepancy is supposed to be induced by socio-cultural factors and not by biological differences. The fear of stigma combined with the low status of women and their restricted mobility, superstition and lack of knowledge about Hansen's disease are possible explanations of the female underrepresentation in leprosy reports of some regions.

Notwithstanding the male/female-ratio, the registered multibacillary cases were rather imputable to men. (55)

1.8.5 Poverty

Leprosy is popularly known as a disease of poor people – and indeed, clear evidence could be found that poverty -mainly in connection with insufficient nutritional supply- is a main risk factor for getting Morbus Hansen. (63)

1.9 Diagnosis of leprosy

Leprosy diagnosis follows a typical workflow: History taking, physical exam consisting of inspection, nerve palpation and neurological examination and finally histopathological, immunological and/or molecular-biological tests.
As per WHO-guidelines, one of the following cardinal signs suffices to diagnose Morbus Hansen:

1. one or more skin lesions matching leprosy manifestation with unequivocal sensory loss with or without thickened nerves
2. positive skin smear

According to how many lesions can be found, two subgroups are classified: Paucibacillary (PB) and multibacillary (MB). Paucibacillary cases with a maximum of five lesions normally have negative smear results; positivity of skin smears rates as an indicator of multibacillary leprosy regardless of the number of skin manifestations. Clinically multibacillary is defined as leprosy with more than five lesions. As shown in *table 2* nearly 60% of the newly diagnosed cases are multibacillary. (17, 38)

1.9.1 Smear test in leprosy

Apart from clinical examination smear tests are the crucial instrument of leprosy diagnosis. This requires tissue fluid of the ear lobe, nasal mucosa or suspect skin lesions. The smears are applied to a microscope slide and stained with Ziehl-Neelsen; then the microbiologist looks for rod-shaped mycobacteria in hundred visual fields. The frequency of detected bacteria is indicated with Ridley's logarithmic scale, also called bacterial index (BI).
1+ means a low bacterial load, 6+ is the highest index; in paucibacillary leprosy this test is scarcely positive. The severity level of infection and the therapeutic response can be estimated by the bacterial index.

Additionally, the therapeutic effect is illustrated by the morphological index (MI), the ratio between regularly stained, intact and therefore vital mycobacteria and the total number of counted bacteria. (22, 46)

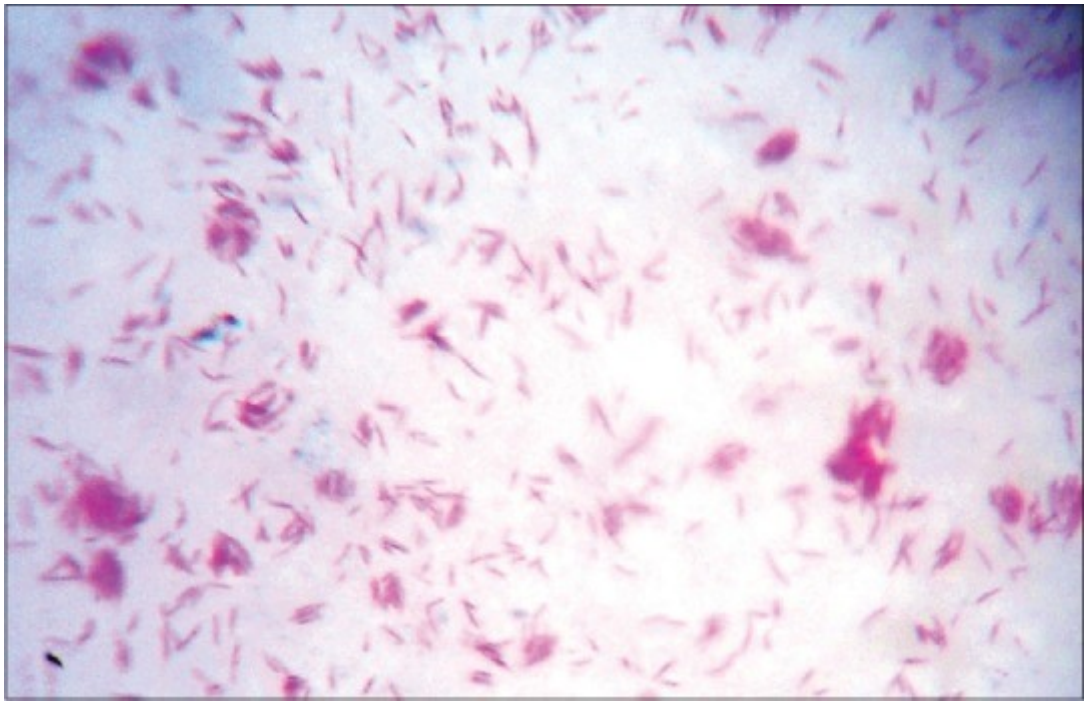

Figure 3: Ziehl-Neelsen stain showing acid-fast bacilli with a bacterial index of 6+ (64)

1.9.2 Skin biopsy in leprosy

For tissue sampling the doctor chooses the edge of the suspect lesion including subcutaneous layers. Then staining is implemented- either with the Fite-Faraco stain, Ziehl-Neelsen stain or fluorescent stain, which is the most sensitive one.
Skin biopsies serve the evidence of histological leprosy alterations and the classification of leprosy forms. As classification is concerned, the histopathological finding always has to suit to clinical manifestations.
Indeterminate (IND) leprosy: Initially, only few mycobacteria are seen perineural, subepidermal and inside arrector pili muscles; however, lymphocytes inside the nerves are frequent. There are no granulomas but slight perineural and perivascular inflammation.
Tuberculoid (TT) leprosy: Epithelioid granuloma formation is the most important histological criterion of tuberculoid leprosy. The granulomas are composed of multinucleated giant cells (Langhans cells), macrophages, epithelioid cells and lymphocytes. Observations have been able to show that CD4+ cells attached to macrophages lie in the partly necrotic centre, whereas CD8+ lymphocytes form the wall.

The granulomas are located perineural, perivascular or subepidermal where they may ulcerate. This walks along with intraneural lymphocytic infiltration and low bacterial load.
Borderline-tuberculoid (BT) leprosy: While granulomas leave out subepidermal areas, they are prominent around the nerves with lymphocyte infiltrates and may girdle arrector pili muscles and sweat glands. Langhans cells and lymphocytes are diminished in comparison to tuberculoid cases.
Borderline-borderline (BB) leprosy: From immunological and clinical view, borderline-borderline leprosy is located in the middle, this is also true for histopathology: There are epithelioid granulomas less demarcated than in tuberculoid leprosy and without Langhans cells. At the same time granular macrophages and potentially numerous bacteria are found.
Borderline-lepromatous (BL) leprosy: High bacterial load is accompanied by granular and sometimes foamy macrophages and a tenuous -primarily perineural- lymphocytic infiltrate.
Lepromatous (LL) leprosy: This multibacillary subtype is characterized by foamy macrophages (Virchow cells) in diffuse or nodular order, scanty lymphocytes and onion-like nerves. Immunohistochemistry indicates the predominance of CD8+ cells -in contrast to tuberculoid leprosy with CD4+ preponderance.
Histoid leprosy is named after the pronounced spindle-shaped histiocytes which are arranged in storiform (whorled) patterns.
Leprosy reactions: Reversal reactions reveal a CD4+ dominant infiltrate, numerous macrophages and a thickened epidermis.
Erythema nodosum leprosum is the only type with a neutrophilic infiltrate. Lucio's phenomenon can be diagnosed by granulomatous necrotizing panvasculitis with endothelial proliferation. The wall of –mainly medium-sized- arteries is infiltrated by macrophages and mycobacteria. Smaller vessels are affected by leukocytic destruction (leukocytoclastic vasculitis). (21, 38, 45-47, 65)

1.9.3 Polymerase chain reaction in leprosy

The polymerase chain reaction, PCR, allows the amplification of leprosy-specific genes with a specificity of almost 100% and a sensitivity of 34-80% within paucibacillary leprosy respectively 90% amongst multibacillary patients. RT-PCR, reverse transcriptase polymerase chain reaction, and sequencing are helpful in order to detect antibiotic resistance genes. The differentiation between *Mycobacterium lepromatosis* and M. *leprae* can also be achieved by PCR.(20, 23)

1.9.4 Serology in leprosy

IgM antibodies against phenolglycolipid-1 (PGL-1), a cell surface constituent of *M. leprae*, can be used for the serological investigation of lepromatous leprosy (80-100% seropositive).

Because of poor seropositivity this is not adapted for paucibacillary leprosy, though. (46, 66)

1.9.5 Lepromin test

The lepromin test is not the first choice for diagnosis, but it can be a clue for the prediction of the individual immune response.

In short, 0.1ml of heat-killed leprosy bacilli are intradermally injected into the forearm. Lest violet erythematous papules larger than 5mm appear within 24-48h, this expression of hypersensitivity is called *Fernández reaction*. The same process observed after 3-4 weeks is termed *Mitsuda reaction*.

Unlike in borderline or LL patients, the cell-mediated immune system works as in tuberculoid leprosy and is thus responsible for these reactions. (22, 46)

Table 3 displays a synopsis for the distinction of leprosy forms by means of inspection, sensitivity, lepromin, smear tests and skin biopsies.

Forms and characteristics of leprosy					
characteristics	**tuberculoid**	**borderline-tuberculoid**	**borderline-borderline**	**borderline-lepromatous**	**lepromatous**
number of lesions	single (≤ 3)	few (≤ 10)	several (10-30)	numerous (>30), asymmetrical	innumerable, symmetrical
size	variable (mainly large)	variable (partly large)	variable	variable (mainly small)	small
surface of lesions	hypopigmented, very dry, partly scaly	dry, scaly, bright	dull or slightly shiny	shiny	shiny
sensations in lesions (apart from face)	absent	markedly or moderately diminished	moderately or slightly diminished	slightly diminished	minimally diminished or not affected
hair growth in lesions	absent	markedly diminished	moderately diminished	slightly diminished	not affected initially
bacilli in skin	nil	nil or 1+	1-3+	3-5+	>5+, with globi
bacilli in nose	nil	nil	nil	usually nil	plenty, with globi
lepromin test	+++	++ or +	-	-	-

Table 3: Characteristics of tuberculoid, borderline-tuberculoid, borderline-borderline, borderline-lepromatous and lepromatous leprosy [modified after (38, 67)]

1.10 Treatment of leprosy

1.10.1 Pharmacological therapy of leprosy

First line medication for leprosy

As recommended by the WHO, multidrug therapy (MDT) includes rifampicin and dapsone for paucibacillary leprosy and additively clofazimine for multibacillary cases.

Dapsone: Formerly used as a monotherapy, the bacteriostatic dapsone is now combined in order to combat resistance. There may occur dose-dependent haemolysis and methaemoglobinaemia which are aggravated by glucose-6-phosphate dehydrogenase deficiency. Cephalea, gastrointestinal symptoms or fatigue are among the side effects. This antibiotic, which inhibits folic acid synthesis, can be taken unhesitatingly during pregnancy.

Rifampicin*:* The strong bactericidal activity of rifampicin is based on the suppression of RNA-polymerase. It is fully effective by periprandial intake and should always be combined with the other antibiotics. Rifampicin may cause hepatotoxicity and intrahepatic cholestasis (especially in combination with alcohol abuse). Besides, gastrointestinal complaints, erythematous macules two to three hours after intake and reddish-orange discolouration of body fluids are provoked. Enzyme induction with an expedited metabolism of for example oral contraceptives must be minded.

Clofazimine*:* This antibiotic is rather antiphlogistic than bactericidal and is used for multibacillary and erythema nodosum leprosum cases. Three-quarters of the patients develop red-brown skin discolouration after a few weeks of therapy, which persists even months and years after the end of the therapy. Especially young women often decide to terminate clofazimine medication because of the hyperpigmentation. Further side effects are discolouration of body fluids and conjunctivae, xerosis and gastrointestinal symptoms. (46)

Table 4 summarizes the dose regimen of paucibacillary respectively multibacillary leprosy for adults and children. First line drug therapy of paucibacillary leprosy lasts six months, for multibacillary leprosy it takes twelve months.

It should be noted that dosage adjustment for children younger than ten years has to be carried out: Rifampicin 10mg/kg, dapsone 2mg/kg, clofazimine 1mg/kg daily and 6mg/kg monthly.

During pregnancy there may be a deterioration of leprosy with potential irreversible sequelae; therefore, treatment of pregnant women is essential to such an extent. The advised multidrug therapy is considered to be safe for mothers and their offspring. The three substances are excreted into human milk to different degrees: Rifampicin to 5%, dapsone to 15% and clofazimine to 22%. Rifampicin is said to be reconcilable with breastfeeding; while the opinions and recommendations of dapsone during nursing diverge, as there have been case reports of haemolytic anaemia. Clofazimine seems to be harmless : The only observed side effect during breastfeeding was skin discolouration of the child. (68, 69)

Dose regimen in leprosy

	paucibacillary		**multibacillary**	
adults	daily:	dapsone 100mg	daily:	dapsone 100mg clofazimine 50mg
	monthly:	rifampicin 600mg dapsone 100mg	monthly:	rifampicin 600mg dapsone 100mg clofazimine 300mg
children (< ten years dosage adjustment according to body weight)	daily:	dapsone 50mg	daily:	dapsone 50mg clofazimine 50mg (every other day)
	monthly:	rifampicin 450mg dapsone 50mg	monthly:	rifampicin 450mg dapsone 50mg clofazimine 150mg
	↓ six months		↓ twelve months	

Table 4: *Dose regimen for leprosy patients recommended by the WHO* [modified after (68)]

Second line medication for leprosy

Provided first line drugs forfeit their efficacy due to antibiotic resistance, there are some alternatives: Ofloxacin (400mg per day), minocycline (100mg per day) and clarithromycin (500mg per day). Depending on the found resistances, they are used in different combinations but never as a monotherapy. (46)

ROM-therapy of leprosy

Single skin lesions of indeterminate or tuberculoid cases without nerve impairment are treated by the so-called ROM-therapy. The term ROM stands for **r**ifampicin (600mg daily), **o**floxacin (400mg daily) and **m**inocycline (100mg daily). Children older than five years get half the dose. (46)

Treatment of leprosy reactions

Reversal reactions are medicated with prednisolone 40-60mg daily for two weeks, then prednisolone has to be tapered (5mg every 14 days) while keeping the disease progression in view.

For erythema nodosum leprosum thalidomide 100-400mg daily is prescribed and may be given for months; every 20-30 days it then is lowered by 100mg. Its teratogenicity prohibits the intake during pregnancy; thus, contraceptive measures are necessary. Sometimes the prescription of prednisolone (40mg for five days with subsequent gradual reduction) is advisable. Lucio's phenomenon is treated by systemic corticosteroids. Although the therapeutic approach is different in the respective leprosy reactions, in any case the multidrug therapy has to be continued. (46)

1.10.2 Management of leprosy-associated deformities

Even though or all the more because nerve damage and following deformities are permanent, patients need to obtain adequate further measures except medication.

They should undergo active and passive physical treatment of paretic or contracted limbs and appropriate skin care. Preventive measures against accumulating damages such as adjusted orthopaedic shoes are pivotal constituents of treatment.

Reconstructive surgeons shall be integrated in the care of leprosy patients in order to alleviate functional and aesthetic consequences. Therefore, rhinoplasty, correction of lid closure, claw hand and foot drop are part of the therapy.

Leprosy is not only a medical problem but also demands political responsibility: Socio-economic actions have to be undertaken, such as financial start-ups for career start or occupational training programs.

Above all, comprehensible and comprehensive clarifications are indispensable: Information about transmission, preventability of complications and antibiotic resistance as well as encouragement and a strong stance against stigmatization have to be provided. (46)

1.11 Prevention of leprosy

1.11.1 Immunoprophylactic vaccination of leprosy

Hereinafter, only the role of *Bacillus Calmette-Guérin* and *Mycobacterium indicus pranii* will be elucidated; although there are some more -less common used- vaccines like *M. vaccae*, *ICRC* and *M. habana.* (70)

Bacillus Calmette-Guérin vaccine of leprosy patients

Bacillus Calmette-Guérin, abbreviated *BCG,* is a vaccine that is primarily applied in infancy in order to fight tuberculosis but that also shields from Hansen's disease. It has sufficiently been corroborated that *BCG* is beneficial as a protective factor especially for multibacillary cases. (58)

The positive effect of the intradermal *BCG*-injection ranges from 20 to 90% according to various studies: It is particularly high in people maintaining close contact to leprosy patients and in combination with chemoprophylaxis with rifampicin.

Moreover, there might be regional and time-dependent differences. The *BCG* protection declines over time but can be in part determined even 30 years later. (71)

Vaccinated people, who nonetheless contract Morbus Hansen, mainly suffer from the tuberculoid form; so that *BCG* is seen as a safeguard against multibacillary leprosy.

This phenomenon could be elicited by the boosting of the cell-mediated immunity due to the similarity of *M. leprae* and *Bacillus Calmette-Guérin* antigens. Another explanatory approach is the so-called trained immunity, where an epigenetic adaption of innate cells is initiated. This innate immune memory causes a cytokine release in presence of particular pathogens during the first three months after vaccination.

Positivity in the Mitsuda test may result; therefore, this examination should be accomplished before a vaccination.(58, 72, 73)

***Mycobacterium indicus pranii* vaccine of leprosy patients**

In May 2017, India's National Leprosy Eradication Programme, NLEP, launched a vaccination pilot project for leprosy contacts in selected districts. The deployed vaccine is called *Mycobacterium indicus pranii* (*MIP*) and can be used as an inactivated vaccine for leprosy as well as for tuberculosis.

M. indicus pranii (*MIP*), also known as *Mycobacterium w*, is a cultivable saprophyte and was named after *Gursharan Pran Talwar,* who had already developed the vaccine in the 1990s.
The measured protective effect is rather divergent: The results vary from about 26% to 68%. (70, 74, 75)

1.11.2 Reporting of leprosy cases

There exists an international reporting obligation to the World Health Organisation for the purpose of determining the incidence and prevalence of leprosy patients.
India complies with this demand by means of a web-based reporting system called "Nikusth", which has been established by the National Leprosy Eradication Program. (76)
For Austria, there also exists a reporting obligation for leprosy: Since 1990, there were ten reports of leprosy without an event of death- all cases were imported cases, the last leprosy case was recorded in 2010 in Styria. (77)

1.11.3 Contact management of leprosy

It has been observed that contact persons who receive a single dose of rifampicin are up to 50-60% less often affected by Morbus Hansen in the first two years. There have been made efforts to introduce leprosy post-exposure prophylaxis (LPEP) into national leprosy programs. So far, India, Indonesia, Myanmar, Nepal, Sri Lanka and Tanzania as well as -at least in an altered way- Brazil and Cambodia established the leprosy post-exposure prophylaxis.(76, 78)
Furthermore, contacts –in particular household members- should be screened for skin lesions. Awareness campaigns and mobile dermatological mass screenings help to diagnose new cases earlier.
For this reason, India's National Leprosy Eradication Programme has initiated the Sparsh leprosy awareness campaign that is intended to counteract stigmatisation and discrimination. It is expected that these measures can also increase the number of persons who seek medical help.
Furthermore, the NLEP has also originated leprosy case detection campaigns in highly endemic regions.(76)

1.12 Prognosis of leprosy

Prognosis of leprosy depends on early diagnosis, treatment and the subtype: Indeterminate leprosy is mostly self-limiting and immune competence of tuberculoid leprosy often leads to spontaneous healing too, whilst lepromatous cases are progressive and can be fatal. Causes of death are secondary infections, renal failure and amyloidosis. (22)

1.13 Differential diagnoses of leprosy

The great variety of clinical manifestation complicates diagnosis in relation to differential diagnoses. False positive results may be the onset of social stigma with all its consequences, whilst false negative diagnoses may wreak delayed therapy with evolving deformities.

Indeterminate leprosy can be mistaken for vitiligo, tinea versicolor or alba, hypochromic nevus or post-inflammatory hypopigmentation. Though, tuberculoid and borderline cases resemble granuloma anulare and multiforme, tinea rosea, lupus erythematodes, psoriasis, sarcoidosis etc. Lepromatous patients should not be mistaken with cases of mycosis fungoides, scleroderma and ichtiosys. Other multibacillary lesions exhibit similarities with lymphomas, xanthomas, neurofibromatosis, leishmaniasis or syphilis. Neuropathy is not at all limited to Morbus Hansen, but is also caused by diabetes, alcohol abuse, trauma, inflammation and others. Nerve thickening can be seen in amyloidosis and Charcot-Marie-Tooth disease too. The systemic changes like in multibacillary Hansen's disease must be examined regarding other causal factors.

The list of differential diagnosis could still be continued and shows once again the importance of valid clinical criteria and test methods. (21-23)

1.14 Growing problem of multi-resistant bacteria

In general, the occurrence of multidrug-resistant bacteria gains ground worldwide.
This especially arises from the inadequate use of antibiotics -on the one hand due to an overuse in humans, on the other hand because of the application of antibiotics in animal husbandry. The dose, therapy duration, compliance of patients, prescription requirement, the availability of different substances etc. also play an important role in this context. (79)

Regarding the above-mentioned problem of multi-resistance in bacteria, India suffers from one of the highest resistance rates in the world leading to a threatening health burden and thereby skyrocketing costs.
At the same time, malnutrition, poor hygiene standards and poor public health infrastructures further infectious diseases in India, whose mortality rate amounts about 417 per 100 000 persons. In India the purchase of antibiotics has been facilitated due to the improving economic situation and higher salaries, while investments into public health and sanitation have been largely neglected. Compared to other countries the immunisation rate in vaccine-preventable diseases with 72% is relatively low and only 36% of the Indian population have access to improved sanitary facilities -mainly sewage systems are in need of improvement.
As medical care cannot be granted for a large number of people, over-the-counter remedies are popular; however, this favours in turn the emergence of multidrug-resistant microbes. (80, 81)

According to the recent publications of the WHO concerning the problem of multi-resistant bacteria, *Acinetobacter*, *Pseudomonas* and *Enterobacteriaceae*, like *Klebsiella*, *Proteus* and *Escherichia coli*, are the most dangerous bacteria as regards multidrug-resistance worldwide. (82)
This is also reflected in data of the ECDC (European Centre for Diseases Prevention and Control) of 2016: 55.4% of the *Acinetobacter* isolates were resistant to at least one surveyed antibiotic substance (aminoglycosides, carbapenems, fluoroquinolones) and still about 43% of the *Acinetobacter* strains had a combined resistance to these antibiotics. About one-third (33.9%) of *Pseudomonas aeruginosa* was resistant to at least one of the five examined antibiotic groups: 16.3% of *Pseudomonas aeruginosa* isolates were resistant to piperacillin/tazobactam, 15% to fluoroquinolones and carbapenems, 13% to ceftazidime,

and 10% to aminoglycosides. In 4.4% of the cases the bacterium even showed resistance to all five groups.
Data is quite similar for *Klebsiella pneumoniae*: A resistance rate of 34.5% to at least one investigated antibiotic and resistances of 4.4% to all four substance groups were reported. It was determined that *Klebsiella* was resistant to third-generation cephalosporins in 25.7%, to fluoroquinolones in 24.6%, aminoglycosides in 19% and carbapenems in 6.1% of the isolates.
In even 58.6%, *Escherichia coli* was resistant to at least one examined antibiotic: The list of resistance rates is headed by aminopenicillins with 57.4%, followed by fluoroquinolones (21%), third-generation cephalosporins (12.4%) and aminoglycosides (9.8%). Just a little amount (<0.1%) of *E. coli* isolates was resistant to the combination of these antimicrobial groups. (83)
As published by the Center for Disease Dynamics, Economics & Policy, the most frequent multi-resistant pathogens are the same in India, but resistance rates are even higher. 50% of *Pseudomonas aeruginosa* and 70% of *Acinetobacter baumannii*, *E. coli* and *Klebsiella pneumoniae* were resistant to third-generation cephalosporins and fluoroquinolones. The carbapenem resistance ranges from 10% in *E. coli* to 70% in *Acinetobacter baumannii*. (84)

1.15 Bacterial flora inside chronic wounds

Chronic wounds are defined as lesions that persist without healing tendency for more than 12 weeks. There are aetiological differences -venous, arterial, neuropathic or mechanic causes- that are not reflected in the bacterial colonisation of wounds, though. The most commonly found bacteria -irrespective of the underlying genesis- are *Staphylococcus aureus* (53%), followed by *Pseudomonas aeruginosa* (25%) and different *Enterobacteriaceae* (49%). (85-87)

Chronic lesions like trophic ulcers are the main reason why leprosy ranks among the most impairing diseases; sufficient treatment is therefore inevitable.
However, in case of the need of an antibiotic therapy, treatment can be limited by the occurrence of multi-resistant bacteria, what constitutes a major problem in developing countries- especially in India. Although multi-resistance in bacteria is a general problem in

India, the microbiological spectra and resistance patterns are regionally different, so that the necessity arises to explore the local resistance patterns. The knowledge of local resistance rates has important implications for the empiric choice of antibiotics and thus for the patients' outcome. (80)

Taking these circumstances into account, the question arises whether multidrug-resistant pathogens also play a crucial role in chronic wounds of leprosy patients and whether this fact may have an impact on the antibiotic therapy.
In order to get to the bottom of this issue, smears of lesions of leprosy patients from Salem, South India, have been obtained and analysed concerning the microbiological spectrum and resistance patterns.
The interpretation of this data is intended to better understand the local resistance patterns of leprosy-associated wounds and consequently to facilitate the choice of antimicrobial treatment when microbiological investigations are not available.

1.16 Resistance and multidrug-resistance in the microbiologic laboratory

Drug-resistances are differentiated between primary and secondary resistance.
Primary or intrinsic resistant bacteria have a natural gap of effectiveness for certain antibiotics; that means that every organism within a species is resistant to one or a number of drugs. Secondary resistances, on the other hand, are acquired- either by mutations or by gene transfer.
Bacteria have developed three possibilities to exchange their genes: conjugation, transformation and transduction. Conjugation describes the process of gene transfer from one bacterium to the other in the presence of ring-shaped extrachromosomal DNA-molecules, also known as plasmids. The absorption of free DNA is called transformation; whereas, bacteriophages act as mediators in case of transduction.
The evolution of resistances per se is based on different mechanisms: One possibility is the mutation of target proteins, like the DNA-topoisomerase that is normally susceptible to fluoroquinolones. Due to structural alterations, though, the targets get resistant to the antibiotics.

Such resistance genes may be transferred horizontally which happens in case of resistant *Streptococcus pneumoniae*, *Neisseria meningitidis* and *MRSA* (m*ethicillin-resistant Staphylococcus aureus*).

Drugs can also be rendered harmless by enzymes: For example, β-lactams -such as penicillins, cephalosporins, monobactams and carbapenems- are hydrolysed by β-lactamases. The aggravated version for third- and fourth-generation cephalosporins is called ESBL, extended spectrum β-lactamases, and occurs mainly within *Enterobacteriaceae*, like *Escherichia coli*, *Proteus spp*. and *Klebsiella pneumoniae*. Aminoglycosides like gentamicin, amikacin and kanamycin are destroyed by aminoglycoside phosphoryl-/ adenyl- or acetyltransferases.

Moreover, special proteins may bind to ribosomes or DNA-topoisomerases so that tetracyclines respectively fluoroquinolones are ineffective. Drug-specific or multidrug efflux pumps can emerge as well.

Finally, some bacteria such as *E. coli*, *Pseudomonas aeruginosa* or *Klebsiella pneumoniae* may get less permeable because of porin mutations. This mechanism presents though a quite rare condition, as this also causes a reduced influx of nutrients. (88, 89)

The more alterations cumulate, the more likely multidrug-resistance (MDR) develops. There exist various definitions for multidrug-resistance depending on the respective microbe. However, all of them have the acquired non-susceptibility to specific antimicrobial classes in common- accordingly, intrinsic resistances are not taken into account. (90)

In connection with multidrug resistant pathogens, Gram-negative *Enterobacteriaceae* might produce ESBL. In addition, 3MRGN (multi-resistant Gram-negatives) and 4MRGN are noteworthy subclasses that are resistant to three of four or all four groups of antibiotics- namely: Acylureidopenicillins, third-generation cephalosporins, carbapenems and fluoroquinolones. The Robert-Koch institute has published the following table (*table* 5) in order to classify 3MRGN and 4MRGN. (91)

Classification of 3 and 4 MRGN					
		Enterobacteriaceae* or *Acinetobacter baumannii		***Pseudomonas aeruginosa***	
Antibiotic class	**Substances**	**3MRGN**	**4MRGN**	**3MRGN**	**4MRGN**
Acylureido-penicillins	Piperacillin	R	R	only one of the four antibiotic classes is effective (susceptible)	R
third-generation cephalosporins	Ceftazidime Cefotaxime	R	R		R
Carbapenems	Imipenem Meropenem	S	R		R
Fluoroquinolones	Ciprofloxacin	R	R		R

Table 5: Antibiotics for the identification of 3MRGN and 4MRGN; R= resistant or intermediate, S= susceptible [modified after (91)]

Within *Staphylococcus aureus* the resistant version is referred to as *MRSA – methicillin resistant Staphylococcus aureus*. After acquiring the so called MecA gene, *MRSA* are resistant to all penicillins, cephalosporins and carbapenems. Due to this fact, *MRSA* are also considered as a multidrug-resistant, MDR, pathogen.

MDR *Pseudomonas aeruginosa* and *Acinetobacter,* vancomycin-resistant e*nterococci* (VRE), carbapenem-resistant *Enterobacteriaceae* (CRE), drug-resistant *Mycobacterium tuberculosis, Neisseria gonorrhoeae* or *Streptococcus pneumoniae* constitute other menacing pathogens. (92)

Hereinafter, a description of the most common and later in this study found potentially multidrug-resistant pathogens is given.

1.17 Staphylococcus aureus and MRSA

The colonisation of humans with *Staphylococcus aureus* amounts from 20-50% - mainly inside the nasal antrum and on the perineum but also in the colon, rectum and vagina. The Gram-positive bacterium usually settles during the neonate phase, whereby the majority is

only an asymptomatic carrier. However, *S. aureus* is one of the most common infectious agents: It induces 70-80% of wound infections, 50-60% of osteomyelitis, 15-40% of prosthesis infections, 30% of sepsis and endocarditis and 10% of pneumonias. The list of virulence factors is long and some strains additionally produce enterotoxins or toxic-shock-syndrome-toxins, which lead to diarrhoea with vomiting and toxic shock syndrome. Furthermore, *Staphylococcus aureus* is one of the most resilient human pathogens. As a bacterium thriving in dry milieus such as dust, it survives temperatures of 60°C for about half an hour; besides, it can leave the gastrointestinal tract unscathed.
S. aureus exploits its high tenacity and spreads rapidly via smear infection. It mostly infects the skin and mucosa and triggers purulent lesions.
Especially persons with granulocytopenia, pre-damaged skin and foreign bodies -like catheters or implants- are in danger of getting infected.
Surgical treatment and supplementary antibiotics are the procedure of choice for local infections. Initially, *Staphylococcus aureus* was susceptible to β-lactams (penicillins, cephalosporins apart from ceftazidime), macrolides, clindamycin, fosfomycin, glycopeptides (vancomycin, teicoplanin), rifampicin, linezolid and fusidic acid.
Based on selection though, *staphylococci* have soon developed penicillinases which ruin the efficacy of penicillin- nowadays this affects nearly 90% of *S. aureus* in hospital settings. The creation of so called BLI, β-lactamase-inhibitors like sulbactam, clavulanic acid or tazobactam, provided remedy just for a little while, as a modified penicillin binding protein emerged shortly thereafter. *MRSA*, *methicillin-resistant Staphylococcus aureus*, developed, which is resistant to methicillin, the first penicillinase-stable penicillin. *MRSA* are resistant to all penicillins, cephalosporins and monobactam antibiotics, including all carbapenems. They very often show additional resistances to macrolides, aminoglycosides, tetracycline, lincosamides and chloramphenicol. The number of effective antibiotics is therefore quite limited: Vancomycin, teicoplanin, tigecycline, daptomycin and linezolid can be used to fight *MRSA.* In order to control the dissemination of *MRSA* in the clinical setting; in some cases, especially in intensive care units, patients should be isolated. In case of nasal colonisation mupirocin can eliminate *staphylococci* and disinfecting soaps may reduce cutaneous bacteria load. (19, 88, 93)

1.18 Streptococci of group G and C

The Gram-positive *streptococci* are categorised by means of their haemolysis: α-s*treptococci* haemolyse blood agar only incompletely, whereas haemolysis is complete for ß-haemolytic and absent in the presence of γ-haemolytic *streptococci*.
ß-haemolytic s*treptococci* are further divided into Lancefield-serogroups (A-H, K-V) which is based on different C-polysaccharides on the surface of the *streptococci*. Even though group A and B s*treptococci* are said to be clinically more relevant, in the following the focus will be on group C (GCS) and G (GGS) *streptococci*, because in the present study GCS and especially GGS were found in large numbers.
The disease burden of group C and group G *streptococci* resembles one another and seems to be underestimated. It mainly includes suppuration, wound and skin infections like necrotizing fasciitis, cellulitis and impetigo. Moreover, sepsis, pharyngitis with possible subsequent rheumatic fever and endocarditis or puerperal sepsis can be triggered.
Streptococci spp. have got an intrinsic resistance to fusidic acid, ceftazidime and low-level aminoglycosides. There is no definition for multidrug-resistance in *streptococci* isolates. (19, 94, 95)

1.19 Pseudomonas aeruginosa

The tendency for environmental and antibiotic-resistance is characteristic for *Pseudomonas aeruginosa*, which can only survive under aerobe conditions. The Gram-negative bacterium can be ubiquitously found in soil, wetland and sanitary facilities and is even capable of multiplying inside some disinfectants. It may contaminate food so that it is sometimes identified on faeces. Occasionally, *Pseudomonas* also colonises the throat and nose of healthy people; therefore, endogenous infections are possible.
Loads of bacterial virulence factors allow the opportunistic pathogen to cause infections that may end up lethal. The bacterium is known to be a frequent and dreaded nosocomial microbe of pneumonia, wound and urinary infections as well as of dermatitis (whirl pool dermatitis), keratitis, otitis externa and sepsis. Very often *Pseudomonas aeruginosa* colonizes chronic wounds and ulcers, where the odour as well as a light green colour of the surface is characteristic. Immunodeficiency is a predisposing factor regarding *Pseudomonas* infections- especially neutropenia and the loss of cutaneous and mucosal integrity, like within cystic fibrosis, is unfavourable in this context.

The high intrinsic resistance of *Pseudomonas aeruginosa* involves aminopenicillins with ß-lactamase inhibitors, as well as some cephalosporins, like cefalexin, cefuroxime or ceftriaxone and others, such as ertapenem or trimethoprim/sulfamethoxazole.
Pseudomonas aeruginosa rapidly develops further resistances when treated.
In connection with this retrospective study, the following naturally resistant antibiotics shall be mentioned: Amoxicillin/clavulanic acid, cefuroxime, cefotaxime, tetracycline, trimethoprim/sulfamethoxazole and moxifloxacin.
Usually, the bacterium is susceptible to piperacillin/tazobactam, aminoglycosides, ceftazidime and cefepime (antipseudomonal cephalosporins), ciprofloxacin and carbapenems. In case of the necessity of an antibiotic therapy, combination therapies are often recommended in order to prevent the development of resistances in *Pseudomonas aeruginosa*. (19, 90, 91, 94, 96)

1.20 Enterobacteriaceae

Enterobacteriaceae present a family of Gram-negative bacteria that are able to survive and multiply both under aerobe and anaerobe conditions and that degrade glucose either with or without the presence of oxygen.
Escherichia coli, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Proteus vulgaris* respectively P. *mirabilis* –that were all found in the taken samples- are constituents of the normal human microbiome, mainly the gastrointestinal tract. Nevertheless, they may engender infections when occurring in other parts of the body. *Salmonella*, *Shigella* and *Yersinia*, on the other hand, are obligatory pathogenic.
Hereafter, the description will merely concentrate on the facultative pathogens.

1.20.1 Enterobacter cloacae

The flagellated *Enterobacter cloacae* can be found in water, soil, sewage and on food and is a commensal in human and animal guts.
Moreover, it is an important nosocomial pathogen for respiratory and urinary tract infections as well as for bacteraemia -mainly in immunocompromised people.
Enterobacter cloacae has an intrinsic resistance to amoxicillin/clavulanic acid, ampicillin, some first generation cephalosporins and cefoxitin (2nd generation cephalosporin). High rates of secondary resistances lead to a major problem, since MDR, multidrug resistant,

bacteria also gain ground due to cephalosporinases, carbapenemases and ESBL, extended spectrum β-lactamases. Carbapenems, especially imipenem, are the most active agents for *Enterobacter cloacae* infections. (19, 97)

1.20.2 Klebsiella pneumoniae

Soil, water and plants are the common habitats of *Klebsiella spp.*; besides, 30% of healthy people are colonised with this bacterium in their intestine or upper airways. *K. pneumoniae* causes a special type of pneumonia (Friedländer-pneumonia) and aggravated chronic bronchitis; other infections are mainly urinary tract infection, sepsis and soft tissue infections. The production of ESBL and/or carbapenemases is problematic for the treatment of *Klebsiella pneumoniae*. (19)

1.20.3 Proteus mirabilis and vulgaris

Proteus spp. are particularly moveable because of their flagellation. The putrefactive agents live in sewage, soil, cadavers and overripe cheese and are part of the intestinal flora. Some strains are able to produce urease. Urease splits urea into ammoniac and CO_2 whereby the pH and therefore the tendency for magnesium-ammonium-phosphate kidney stones raises. *P. mirabilis* provokes urinary tract infections, while *P. vulgaris* triggers mainly wound infections or sepsis. (19, 94)

1.20.4 Escherichia coli

E. coli can be optionally or mandatorily pathogenic as well as apathogenic and is an indicator of faecal contamination. Perfect conditions prevail at about 37°C, but these bacteria can also exist at 4 to 46°C.

Escherichia coli is the most common cause of urinary infections (nearly 80%) that can devolve into a pyelonephritis. 30% of sepsis stem from *E. coli* –during neonate period it is even the most frequent pathogen in the event of sepsis or meningitis. It also elicits appendicitis, cholangitis, cholecystitis and peritonitis or nosocomial pneumonia and wound infections.

An *E. coli* infection indicates the use of second or third-generation cephalosporins, fluoroquinolones, carbapenems or cotrimoxazole, while especially ampicillin and sometimes also piperacillin do not work. (19)

1.21 Acinetobacter baumannii

Acinetobacter spp. are environmental, mucous and cutaneous bacteria that often rank among the multi-resistant pathogens. These bacteria survive in dry and moist areas and are isolated from soil, water, aliment and animal excrements. Besides, they can also colonise the pharynx, gastrointestinal tract, urethra and vagina.

The spectrum of diseases covers nosocomial and community-acquired pneumonia, tracheobronchitis, urinary infections, bacteraemia, wound infections and cellulitis.

Because of *Acinetobacter's* high intrinsic resistance, empiric treatment is difficult and should be adapted after resistance testing.

Primary resistances of *Acinetobacter* include ampicillin, amoxicillin/clavulanic acid, tetracycline, trimethoprim/sulfamethoxazole and most of the cephalosporins except antipseudomonal types such as cefepime and ceftazidime.

2 Material and Methods

2.1 Study design

The following study represents a retrospective data analysis of the microbiological spectrum and resistance patterns of swabs taken from chronic wounds of leprosy patients from Salem, in the state of Tamil Nadu, India from November 2016 (see *figure 4*).
In this study, the bacterial load, the localisation of sampling, the most commonly found microbes, the frequencies of multi-resistant pathogens and the resistance rates were assessed. For this purpose, calculations, tables and diagrams were created. For the analysis all data of the patients were anonymised.

Figure 4: Map of India with highlighting of the district of Tamil Nadu

2.2 Sample collection

The data of the study originates from the project "Leprosy on the road" which is based on a long-standing partnership between the St. Mary's Hospital (DTMC-Trust = Doctor Typhagne Memorial Charitable) in Salem, India and the Medical university of Graz, Austria. Salem is a city located in the eponymous district of the South-Eastern state Tamil Nadu.

As part of the mentioned initiative, members of the Department of Global Health and Development, briefly GHD, set off from Graz to Salem in November 2016.

The GHD-team, which consisted of doctors, nurses and students, aimed at promoting surgical care, public health and hygienic measures for leprosy patients in this area.

During routine examinations as part of the DTMC-Trust's life-long care program, wound swabs of chronic leprosy-associated lesions were collected with the consent of patients. Thus, their names, their gender and the localisation of the sampling were recorded.

All patients had already been diagnosed with leprosy and had finished their leprosy treatment.

2.3 Microbiological spectrum analyses and resistance testing

Subsequent to the sampling, swabs were transported in a tube with 1mL of liquid Amies-medium (eSwab™, Becton Dickinson) and stored for further processing in the microbiological laboratory. As a first step, a Gram-staining was performed, afterwards a microbiological spectrum analysis was carried out using the routinely used laboratory methods with cultivation of the material on different agar plates and incubation under aerobic and anaerobic conditions at 37°C for 24- 48 hours. The identification of the bacteria was conducted using the MALDI-TOF instrument (bioMérieux). The resistance testing and interpretation of the results was accomplished according to the actual EUCAST-guidelines (European Union Committee on Antimicrobial Susceptibility Testing, www.eucast.org). The resistance testing was only performed on facultative pathogens; commensals were listed without resistance patterns.

2.4 Data evaluation

The collected data consists of the patients' names, gender, age and localisation of the sampling. Patient names were compared to exclude doubles and were later anonymised. Analyses concerning the gender and the localisation of the investigated samples were performed. The results of the microbiological laboratory tests were then analysed regarding Gram-staining, the bacterial spectrum and the found resistance patterns. For a vivid depiction of the data, diagrams of frequencies and percentages were created.

2.5 Limitations

The presented data constitutes a local snapshot rather than a long-term evaluation of the bacterial flora of the chronic wounds of the included patients. During the study period, samples of wounds were taken, when patients were at home and agreed to the examination. The sampling was performed without describing the local situation of inflammation of the wound, but none of the patients was taking antibiotics at this time.
Contamination of chronic wounds with bacteria from soil or surface water was also possible, as the patients often do not use shoes and ulcers are rarely treated with dressings. Moreover, the number of patients tested might be too small in order to provide significant results for the whole region of Tamil Nadu. The collected data originates only from one ethnic group; thus, the results might not be transferrable to other ethnicities.
Nevertheless, this study may foster further improvement in term of empiric antibiotic treatment of chronic leprosy-associated ulcers in the district of Salem.

3 Results

3.1 Demographic data of leprosy patients from Salem

The following analysis is based on the data of 66 swabs taken from 50 leprosy patients with chronic lesions.

35 (70%) out of 50 persons were male and 15 (30%) female - this gender distribution is again visualised in *figure 5*. The age of the patients ranged between 23 and 85 years, with a median of 61.9 years.

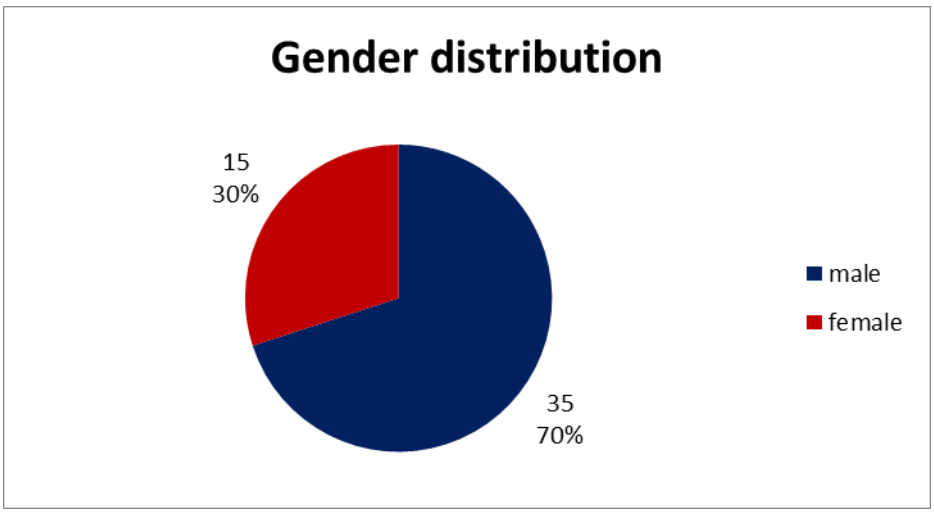

Figure 5: Gender distribution of samples of chronic lesions in Indian leprosy patients

3.2 Localisation of wound samples

With reference to the localisation of sampling, the following picture emerges: 35 (53.0%), out of 66 samples were taken from the sole, nine each (13.6%) from the tibia respectively the toes, five (7.6%) from the ankle, three (4.5%) from the heel, two each (3.0%) from the dorsum of the feet respectively the tibia/ankle and one (1.5%) from the ankle/dorsum. These results are depicted in *figure 6*.

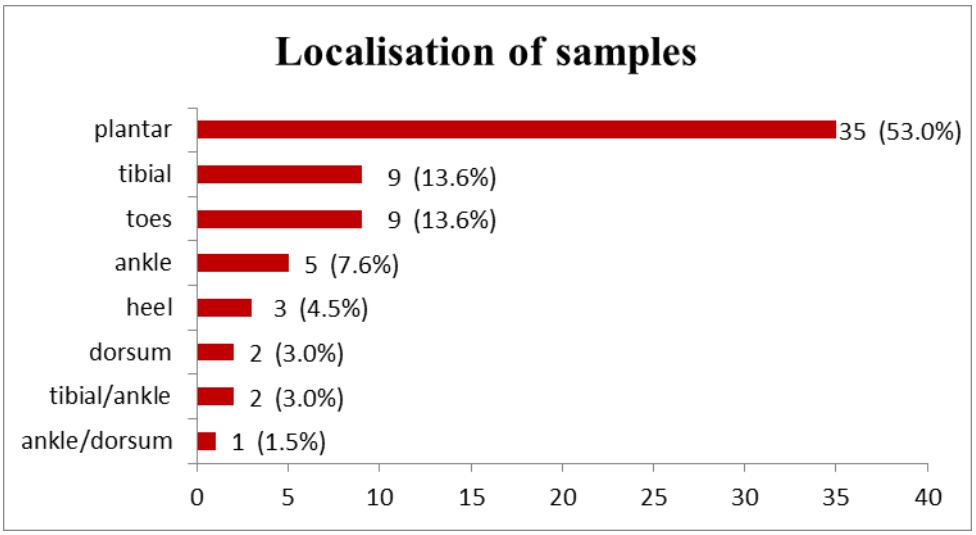

Figure 6: Number and localisation of samples of chronic lesions in Indian leprosy patients (n=66)

3.3 Bacterial flora of chronic lesions in Indian leprosy patients

In the 66 swabs a total of 289 microorganisms were found, assigned to at least 63 different bacteria species and fungi.

They can roughly be divided into the subsequent groups: 70 microorganisms (24.2%) were non-fermenting bacteria, 59 (20.4%) *Enterobacteriaceae*, 43 (14.9%) *streptococci*, 27 (9.3%) *staphylococci*, 13 (4.5%) *anaerobes*, nine (3.1%) aerobic *Bacillaceae*, three (1.0%) *Candida* spp. and 65 (22.5%) miscellaneous bacteria.

The 70 non-fermenting bacteria consisted of: 26 *Pseudomonas aeruginosa*, eight *Acinetobacter baumannii,* eight *Stenotrophomonas maltophilia*, seven *Shewanella algae,* five *Pseudomonas putida*, four *Chryseobacterium indologenes,* two *Pseudomonas stutzeri*, two *Acinetobacter haemolyticus*, two *Acinetobacter lwoffii*, one *Pseudomonas mendocina*, one *Pseudomonas fluorescens,* one *Chryseobacterium sp,* one *Sphingobacterium multivorum,* one *Achromobacter sp*. and one *Comamonas testosteroni.*

The group of *Enterobacteriaceae* contained 13 *Enterobacter cloacae*, eleven *Klebsiella pneumoniae*, nine *Proteus mirabilis*, eight *Escherichia coli*, seven *Morganella morganii*, six *Proteus vulgaris*, one *Citrobacter* sedlakii, one *Citrobacter sp*., one *Serratia marcescens*, one *Leclercia adecarboxylata* and one *Pandoraea sp*..

The *streptococci* were divided into 20 s*treptococci* of group G, five s*treptococci* of group C, five *Enterococcus faecalis*, five *Enterococcus spp*., four *Viridans group streptococci*, two *Enterococcus faecium*, one *Enterococcus avium* and one *Enterococcus casseliflavus*.

The genus of s*taphylococci* was represented by 25 *Staphylococcus aureus* and two *Staphylococcus sciuri*.

Among the *anaerobes* three *Bacteroides ovatus*, three *Bacteroides fragilis*, two *Bacteroides uniformis*, one *Bacteroides caccae*, one *Bacteroides sp*., one *Fusobacterium necrophorum*, one *Fusobacterium varium* and one *Prevotella melaninogenica* were found.

The group of miscellaneous bacteria comprised 25 *Corynebacterium spp*., ten *Corynebacterium striatum*, seven *Aeromonas hydrophila/ caviae*, five *Neisseria mucosa*, four *Alcaligenes faecalis*, three *Alcaligenes spp*., two *Myroides* spp., two *Arcanobacterium haemolyticum*, one *Corynebacterium tuberculostearicum*, one *Pasteurella multicoda*, one *Neisseria subflava*, one *Rhodococcus equi,* one *Neisseria sp*., one *Bifidobacterium sp*. and one *Kocuria sp.*.

Each of the samples contained at least one potential pathogenic bacterial species, while a maximum of eight detected bacteria was reached.
In three (4.5%) of 66 samples only one microorganism was found, four (6.1%) swabs included two bacteria, 14 (21.2%) samples contained three microbes, in 16 smears (24.2%) four microorganisms were found, twelve cases (18.2%) yielded five, eight (12.1%) swabs held six bacteria, in seven (10.6%) smears there were seven and in two (3.0%) cases even eight microbes. None of the swabs was sterile. The graphical presentation of the microbiological load is portrayed in *figure 7*.

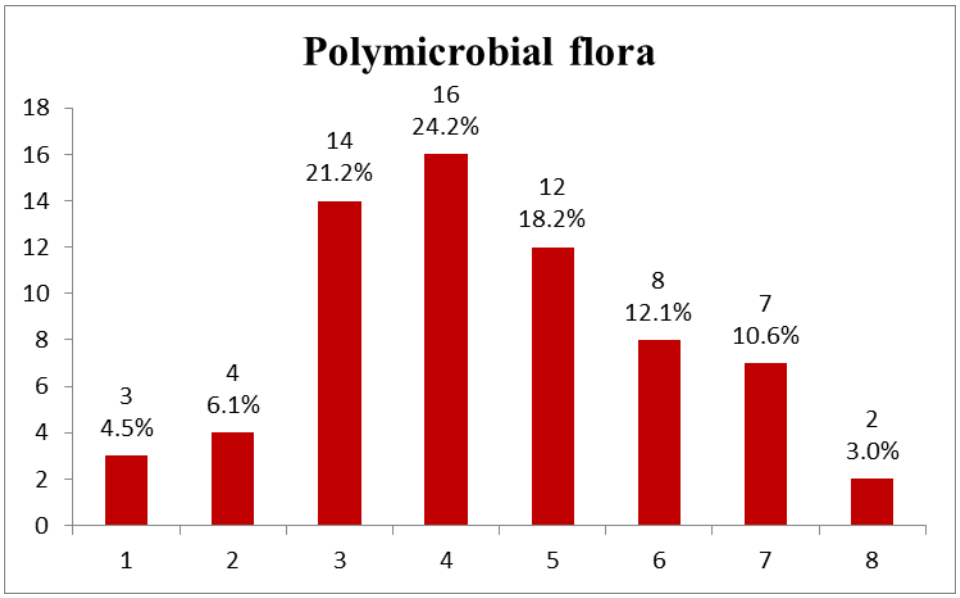

Figure 7: *Polymicrobial flora in samples of 66 chronic lesions in Indian leprosy patients: y-axis = number of samples; x-axis= number of detected microorganisms*

3.4 Gram stain and assignment to bacterial identification

166 out of the 289 detected microorganisms were Gram-negative bacteria which is 57.4%; 120 were Gram-positive bacteria presenting 41.5% and three (1.0%) were fungi (see *figure 8*).

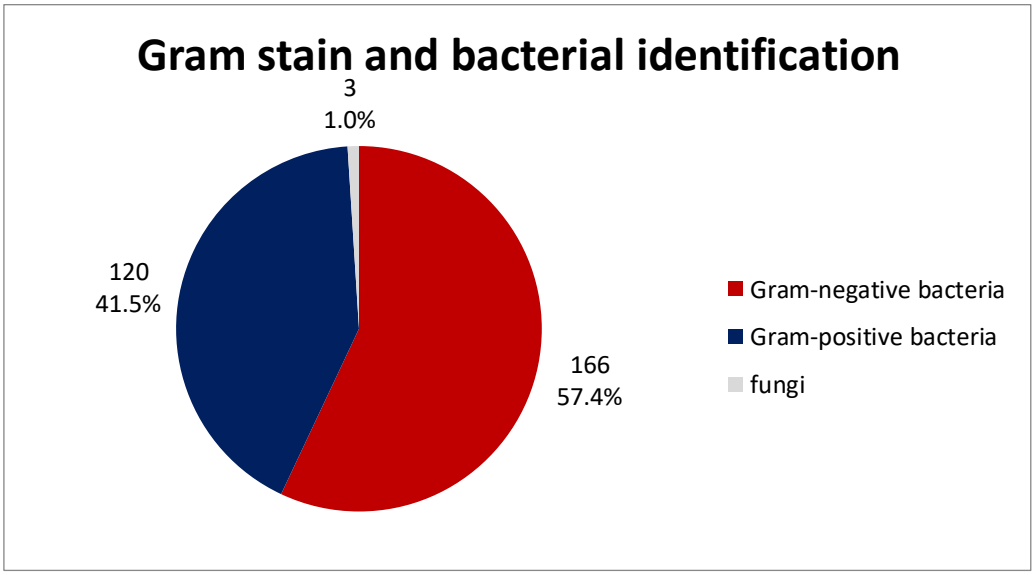

Figure 8: *Gram stain of detected microbes in samples of chronic lesions in Indian leprosy patients*

3.5 Resistance patterns

An antibiotic resistance pattern analysis was performed for at least 198 (68.5%) of 289 detected microorganisms. Amongst the 198 examined microbes were 70 non-fermenting bacteria, 59 *Enterobacteriaceae*, 26 s*treptococci,* 25 s*taphylococci* and 18 miscellaneous bacteria.

The results of the resistance patterns of the most frequent microbes, found in the swabs, are presented in detail hereinafter.

Resistance patterns including the respective percentage of resistances are depicted in *table 6* and *7*.

The interpretation concerning multi-drug resistance was carried out by means of the definition of the Robert-Koch institute (see above in *table 5*).

3.5.1 Staphylococcus aureus

In 24 (36.4%) of 66 swabs *Staphylococcus aureus* was found; one sample contained two different strains; therefore, a total of 25 *S. aureus* were detected.

Concerning the resistance pattern 23 of 25 (92.0%) were *MRSA* (*methicillin-resistant Staphylococcus aureus*)*;* whereby, one patient was colonized with both *MRSA* strains. According to that, 22 (33.3%) of the 66 swabs contained *methicillin-resistant Staphylococcus aureus*. Only two *S. aureus* isolates were susceptible to methicillin.

Per definition, *MRSA* are resistant to all ß-lactam antibiotics (including penicillins, carbapenems and cephalosporins); however, further resistances to other tested antibiotics were found as follows: Ciprofloxacin 96% (24/25), gentamicin 72% (18/25), trimethoprim/sulfamethoxazole 56% (14/25), tetracycline 52% (13/25), erythromycin 36% (9/25) and clindamycin 36% (9/25).

None of the *S. aureus/MRSA* isolates was resistant to linezolid, rifampicin, mupirocin or fusidic acid; while, phenoxymethylpenicillin and amoxicillin were resistant in all cases.

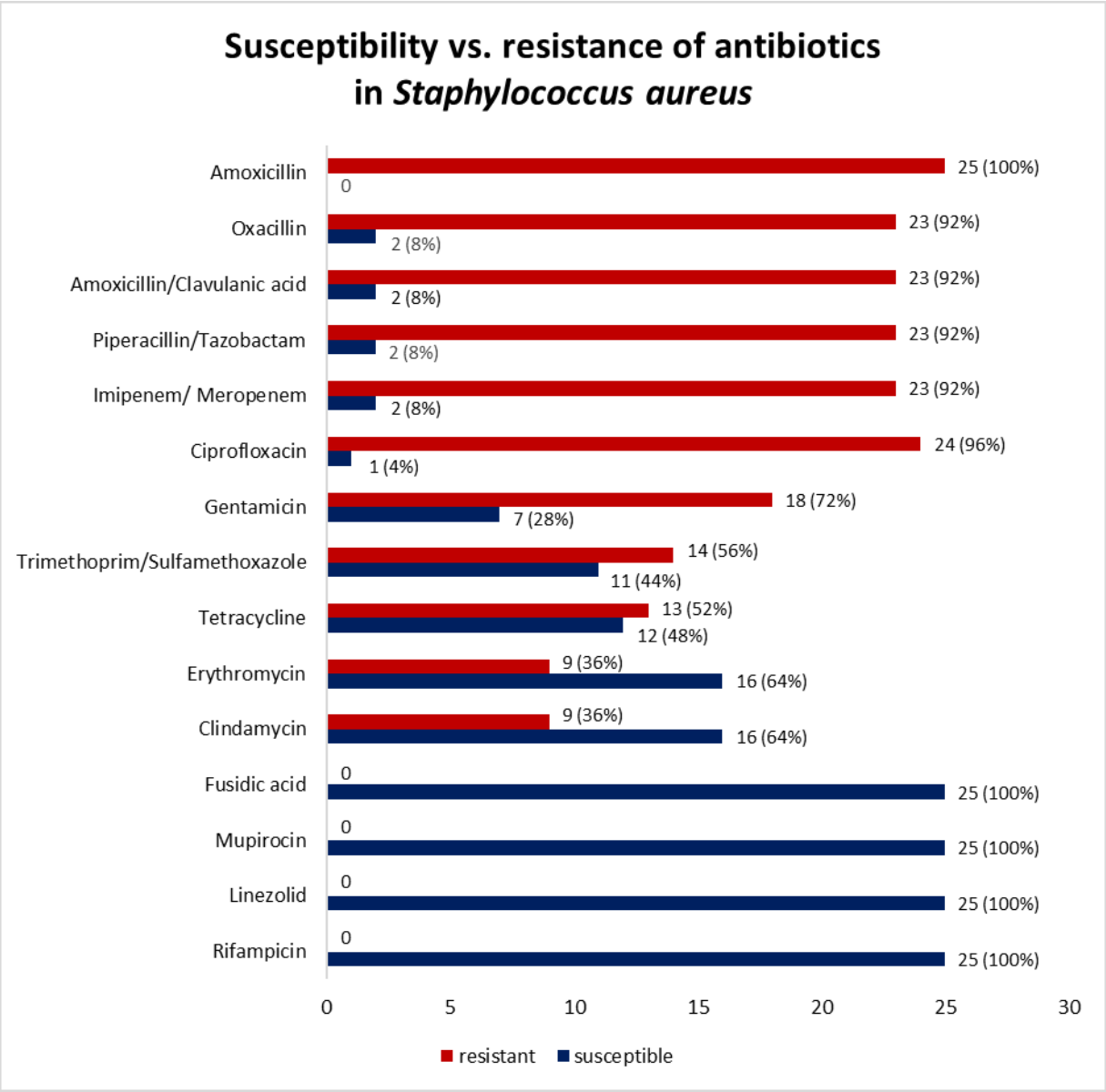

Figure 9: *Distribution of susceptibility and resistance of Staphylococcus aureus to tested antibiotics detected in samples of chronic lesions in Indian leprosy patients*

3.5.2 Streptococci of group G and C

20 (30.3%) out of 66 swabs contained *streptococci* of group G (GGS); in five (7.6%) samples *streptococci* group C (GGC) were found.

All group G and C *streptococci* were declared resistant to aminoglycosides, ceftazidime, ciprofloxacin and tetracycline.

For the other tested antibiotics following resistance rates were found: Levofloxacin and moxifloxacin were determined as resistant in 88% (in total 22/25; in GGS: 90%= 18/20; in

GCS: 80%= 4/5), erythromycin in 56% (in total 14/25; in GGS: 50%= 10/20; in GCS: 80%= 4/5) and clindamycin in 48% (in total 12/25; in GGS: 45%= 9/20; in GCS: 60%= 3/5).

All isolates were susceptible to phenoxymethylpenicillin, amoxicillin with and without clavulanic acid, piperacillin/tazobactam, carbapenems and the tested cephalosporins except ceftazidime.

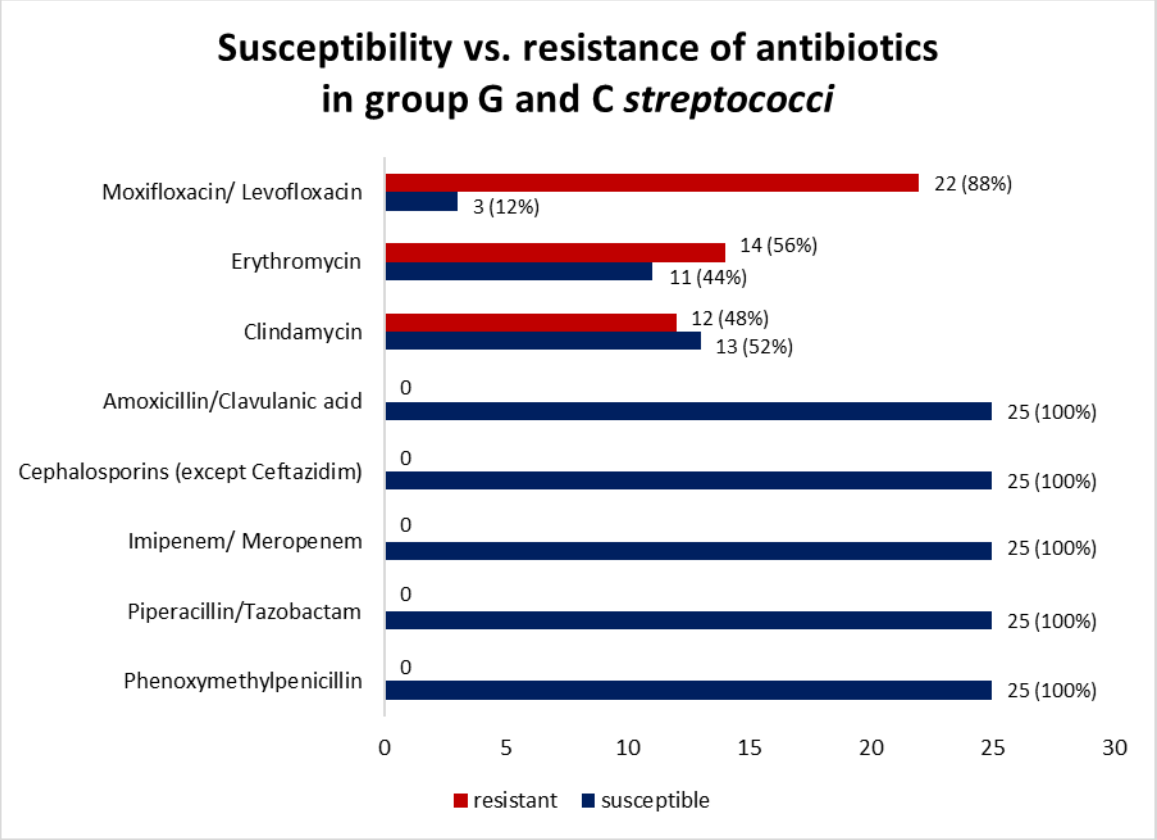

Figure 10: *Distribution of susceptibility and resistance of group G streptococci and group C streptococci to tested antibiotics detected in samples of chronic lesions in Indian leprosy patients*

3.5.3 Pseudomonas aeruginosa

26 (9.0%) out of 289 microorganisms were *Pseudomonas aeruginosa*, which were detected in 39.4% of the 66 samples.

All were - consistent with their intrinsic resistance - reported resistant to amoxicillin with and without clavulanic acid, to cefuroxime, cefotaxime, tetracycline, moxifloxacin and trimethoprim/sulfamethoxazole.

For the other tested antibiotics, the following resistance rates were determined: Ciprofloxacin 15.4% (4/26), gentamicin 11.5% (3/26), cefepime 3.8% (1/26) and ceftazidime 3.8% (1/26). All *Pseudomonas aeruginosa* were susceptible to piperacillin/tazobactam, carbapenems and amikacin.
None of the *Pseudomonas aeruginosa* was assigned to a 3 or 4 MRGN-strain.
Figure 11 shows the analysis of the resistance patterns.

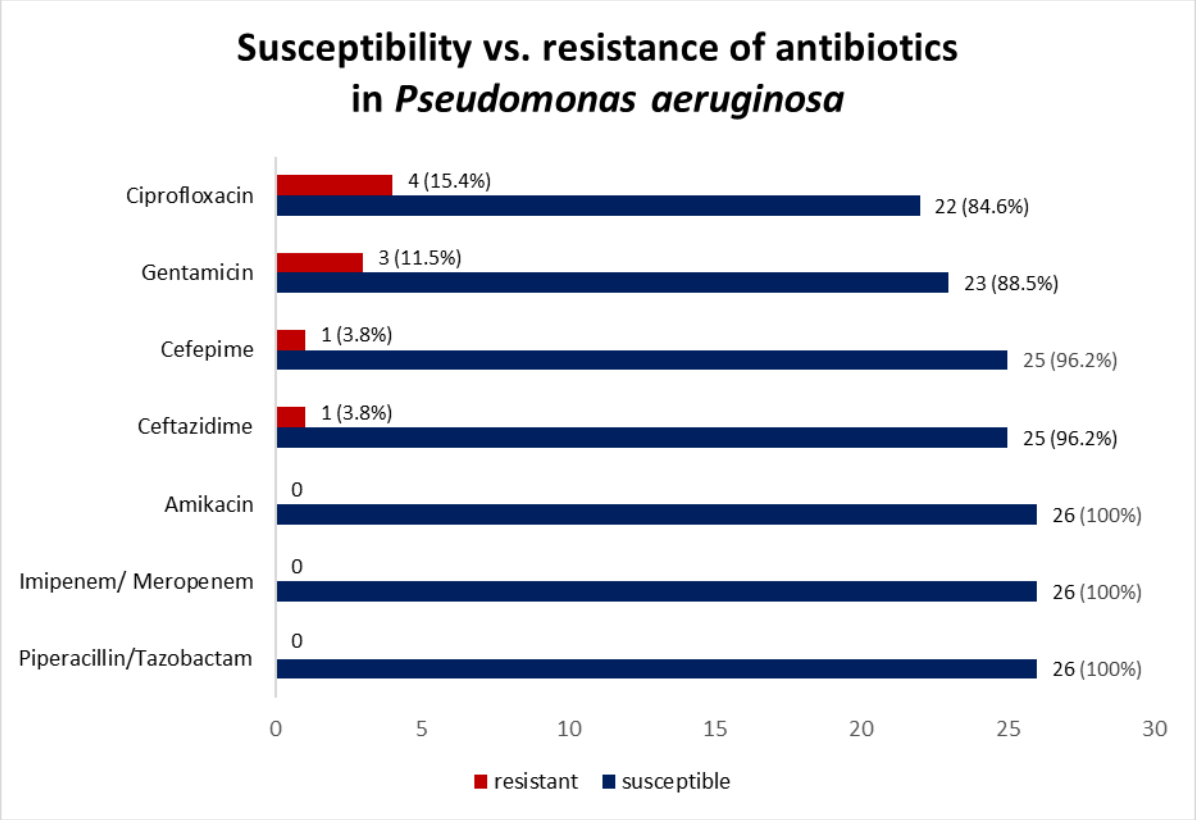

Figure 11: Distribution of susceptibility and resistance of Pseudomonas aeruginosa to tested antibiotics detected in samples of chronic lesions in Indian leprosy patients

3.5.4 Enterobacter cloacae

In the 66 swabs 13 (19.7%) *Enterobacter cloacae* were identified in total - including one isolate with extended spectrum β-lactamase (ESBL)- production.
Beyond the natural resistance of *Enterobacter cloacae,* resistances were detected as follows: Cefotaxime was tested resistant in 15.4% (2/13); resistance was also found in 7.7% (1/13) for piperacillin/tazobactam, aminoglycosides (gentamicin and amikacin), trimethoprim/sulfamethoxazole and ceftazidime.

All *Enterobacter cloacae* were susceptible to carbapenems, cefepime and ciprofloxacin. None of the *Enterobacter cloacae* was assigned to a 3 or 4MRGN, multi-resistant Gram-negative.

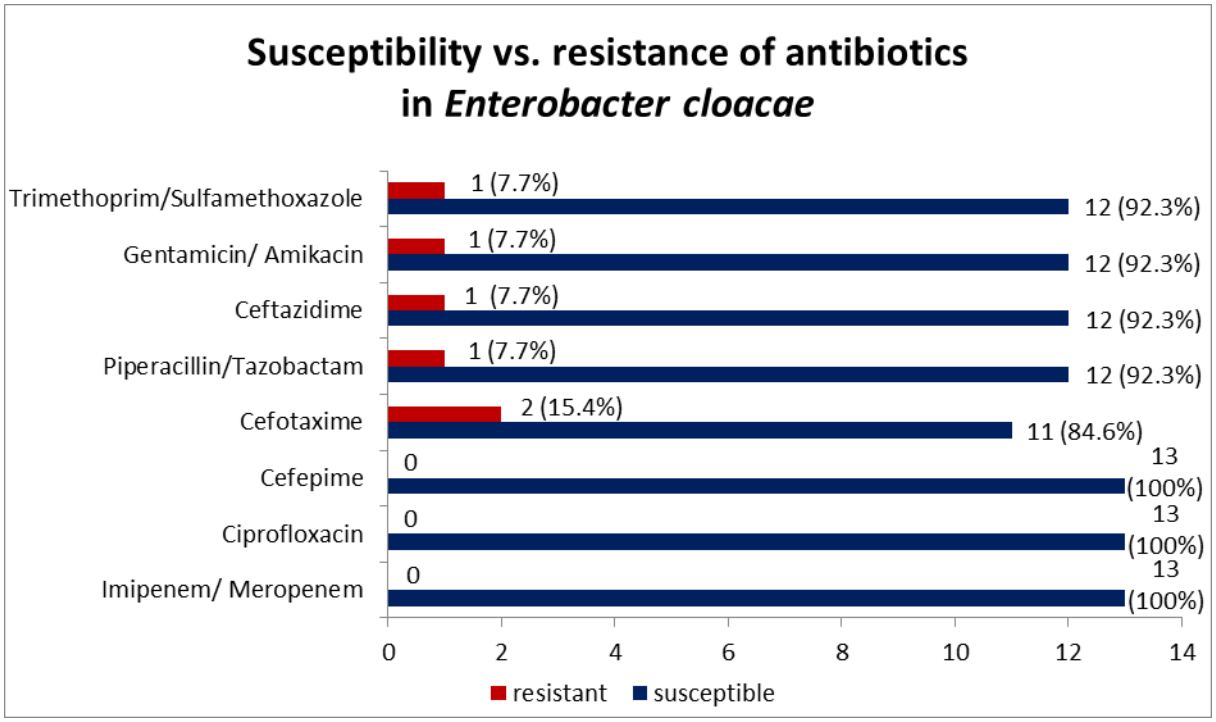

Figure 12: *Distribution of susceptibility and resistance of Enterobacter cloacae to tested antibiotics detected in samples of chronic lesions in Indian leprosy patients*

3.5.5 Klebsiella pneumoniae

Klebsiella pneumoniae was the second most common member of *Enterobacteriaceae* after *Enterobacter cloacae* and was detectable in eleven of the 66 swabs (16.7%).

All *Klebsiella pneumoniae* were only resistant to amoxicillin, hence neither a multidrug-resistant nor an ESBL-producing strain could be identified.

All *Klebsiella pneumoniae* strains were susceptible to amoxicillin/clavulanic acid, the tested cephalosporins, carbapenems, aminoglycosides, trimethoprim/sulfamethoxazole, ciprofloxacin and moxifloxacin.

3.5.6 Proteus mirabilis and vulgaris

In total nine (13.6%) of 66 swabs comprised *Proteus mirabilis* and six (9.1%) *Proteus vulgaris,* including one ESBL-producing *P. mirabilis* and four ESBL- producing *P. vulgaris.*

For *Proteus mirabilis* the following picture emerged: Ciprofloxacin was assigned as resistant in 55.6% (5/9), amoxicillin and trimethoprim/sulfamethoxazole in 44.4% (4/9), aminoglycosides in 33.3% (3/9), cefepime and ceftazidime in 11.1% (1/9). Amoxicillin/clavulanic acid, piperacillin/tazobactam and carbapenems were tested susceptible for *P. mirabilis*.

One *Proteus mirabilis* could be identified as 3MRGN, this strain was ESBL-producing at the same time.

All *Proteus vulgaris* strains were resistant to amoxicillin and cefuroxime (intrinsic resistance). Further resistances for *Proteus vulgaris* were also found: The strains were resistant to trimethoprim/sulfamethoxazole, gentamicin and ciprofloxacin in five (83.3%) of six cases, to ceftazidime and cefepime in four cases (66.7%) and to amoxicillin/clavulanic acid in two cases (33.3%). All *P. vulgaris* isolates were susceptible to piperacillin/tazobactam, carbapenems and amikacin; no 3 or 4MRGN *Proteus* vulgaris was detected.

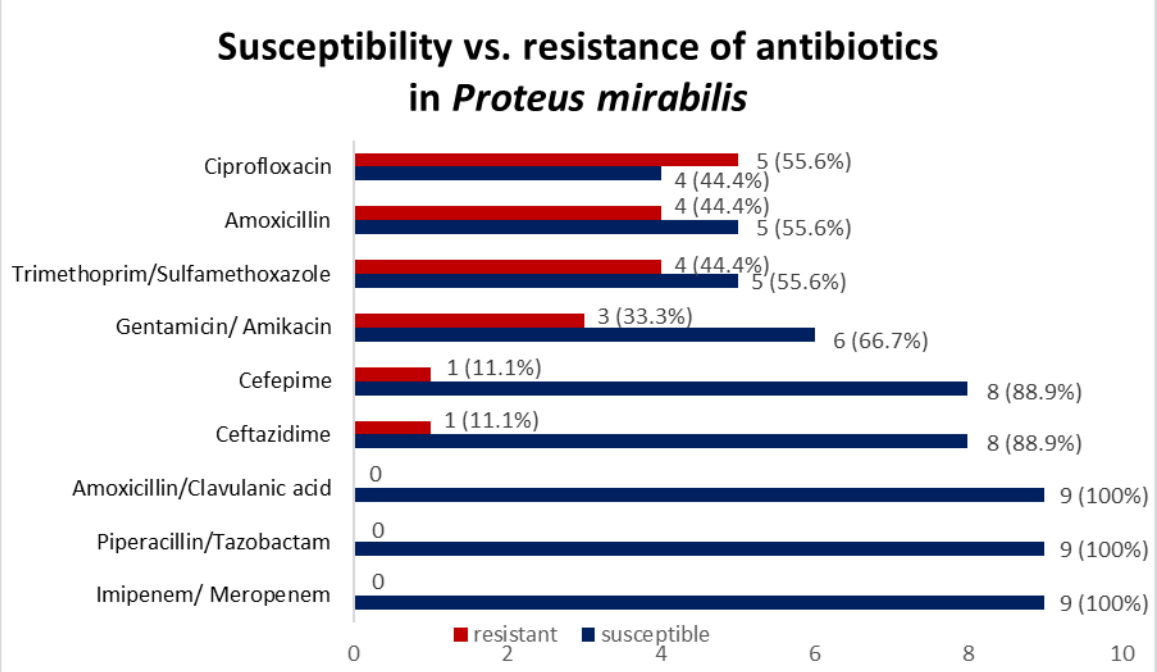

Figure 13: *Distribution of susceptibility and resistance of Proteus mirabilis to tested antibiotics detected in samples of chronic lesions in Indian leprosy patients*

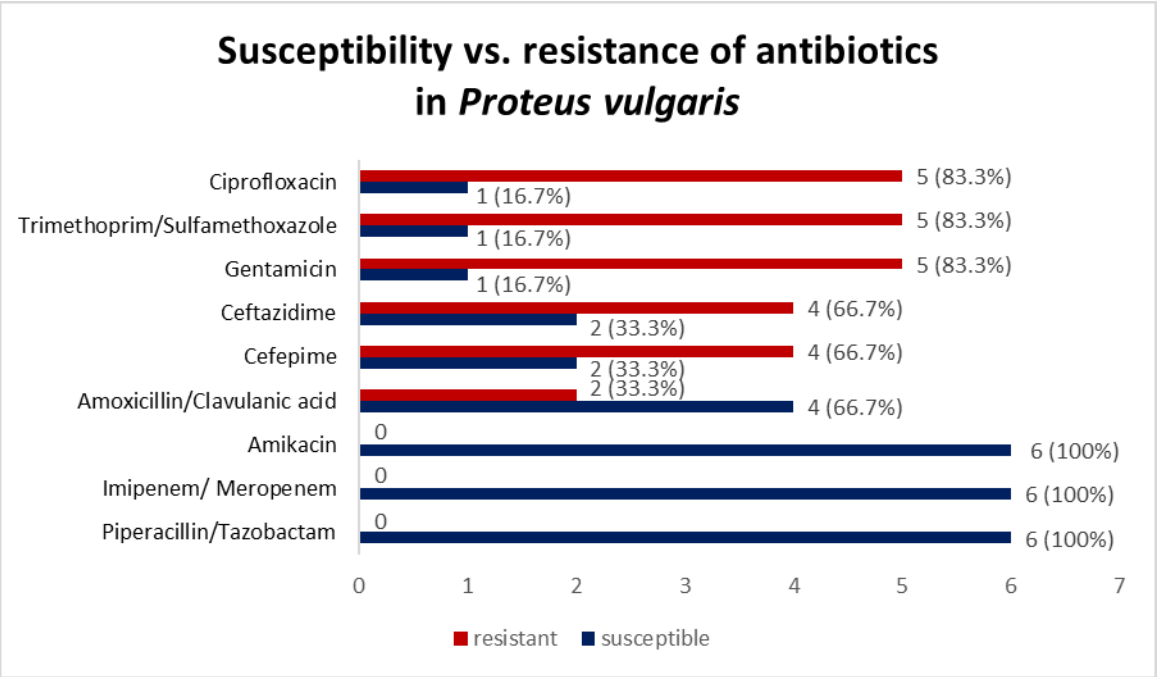

Figure 14: *Distribution of susceptibility and resistance of Proteus vulgaris to tested antibiotics detected in samples of chronic lesions in Indian leprosy patients*

3.5.7 Escherichia coli

Six (9.1%) out of 66 swabs held *E. coli*; two samples contained two different strains; thus, in total eight *E. coli* were detectable.

Resistance patterns were as follows: Amoxicillin was resistant in 87.5% (7/8), ceftazidime and ciprofloxacin in 50% (4/8), cefepime in 37.5% (3/8), amoxicillin/clavulanic acid, piperacillin/tazobactam and trimethoprim/sulfamethoxazole in 25% (2/8) and gentamicin were assigned resistant in 12.5% (1/8).

All strains were susceptible to the tested carbapenems and amikacin.

Three (37.5%) of eight *E. coli* were identified as 3MRGN and four (50%) were ESBL-producer.

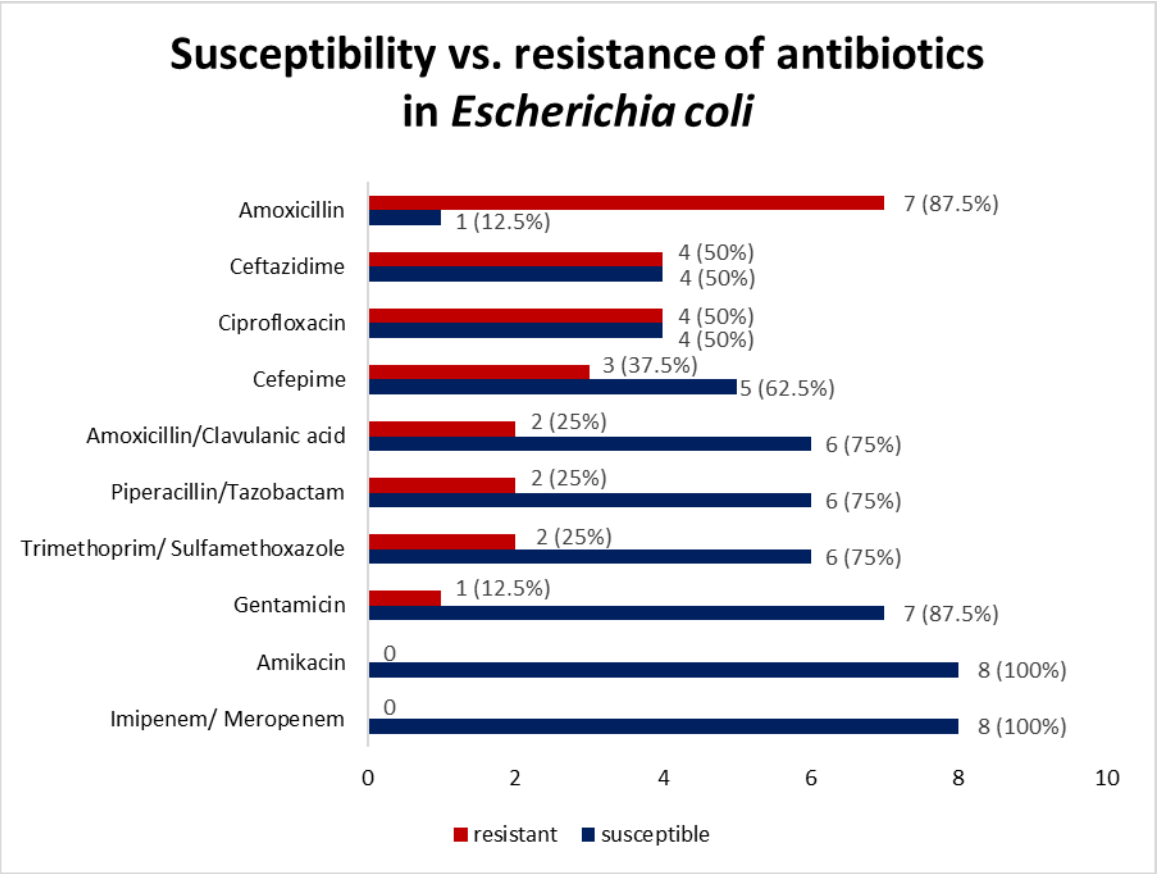

Figure 15: *Distribution of susceptibility and resistance of Escherichia coli to tested antibiotics detected in samples of chronic lesions in Indian leprosy patients*

3.5.8 Acinetobacter baumannii

In 12.1% (8/66) of the swabs *Acinetobacter baumannii* could be identified. According EUCAST-guidelines, *A. baumannii* were reported intrinsically resistant to amoxicillin, amoxicillin/clavulanic acid, cephalosporins, moxifloxacin and tetracycline. All other antibiotics - carbapenems, aminoglycosides, trimethoprim/sulfamethoxazole and ciprofloxacin - were tested susceptible in *Acinetobacter baumannii*.

A synopsis of the susceptibility and resistance rates is presented in *table 6* and *7*.

Susceptibility and resistance rates										
Antibiotics	***Staphylococcus aureus***		***Streptococci* of group G & C**		***Pseudomonas aeruginosa***		***Enterobacter cloacae***		***Klebsiella pneumoniae***	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Phenoxymethyl-penicillin	0 (0%)	25 (100%)	25 (100%)	0 (0%)	-	-	-	-	-	-
Oxacillin	2 (8%)	23 (92%)	-	-	-	-	-	-	-	-
Amoxicillin	0 (0%)	25 (100%)	25 (100%)	0 (0%)	0 (0%)	26 (100%)	0 (0%)	13 (100%)	0 (0%)	11 (100%)
Amoxicillin/ Clavulanic acid	2 (8%)	23 (92%)	25 (100%)	0 (0%)	0 (0%)	26 (100%)	0 (0%)	13 (100%)	11 (100%)	0 (0%)
Piperacillin/ Tazobactam	2 (8%)	23 (92%)	25 (100%)	0 (0%)	26 (100%)	0 (0%)	12 (92.3%)	1 (7.7%)	11 (100%)	0 (0%)
Cefuroxime iv	2 (8%)	23 (92%)	25 (100%)	0 (0%)	0 (0%)	26 (100%)	-	-	11 (100%)	0 (0%)
Cefotaxime	2 (8%)	23 (92%)	25 (100%)	0 (0%)	0 (0%)	26 (100%)	11 (84.6%)	2 (15.4%)	11 (100%)	0 (0%)
Ceftazidime	0 (0%)	25 (100%)	0 (0%)	25 (100%)	25 (96.2%)	1 (3.8%)	12 (92.3%)	1 (7.7%)	11 (100%)	0 (0%)
Cefepime	2 (8%)	23 (92%)	25 (100%)	0 (0%)	25 (96.2%)	1 (3.8%)	13 (100%)	0 (0%)	11 (100%)	0 (0%)

Antibiotics	*Staphylococcus aureus*		*Streptococci* of group G & C		*Pseudomonas aeruginosa*		*Enterobacter cloacae*		*Klebsiella pneumoniae*	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Imipenem	2 (8%)	23 (92%)	25 (100%)	0 (0%)	26 (100%)	0 (0%)	13 (100%)	0 (0%)	11 (100%)	0 (0%)
Meropenem	2 (8%)	23 (92%)	25 (100%)	0 (0%)	26 (100%)	0 (0%)	13 (100%)	0 (0%)	11 (100%)	0 (0%)
Gentamicin	7 (28%)	18 (72%)	0 (0%)	25 (100%)	23 (88.5%)	3 (11.5%)	12 (92.3%)	1 (7.7%)	11 (100%)	0 (0%)
Amikacin	-	-	0 (0%)	25 (100%)	26 (100%)	0 (0%)	12 (92.3%)	1 (7.7%)	11 (100%)	0 (0%)
Tetracycline	12 (48%)	13 (52%)	0 (0%)	25 (100%)	0 (0%)	26 (100%)	-	-	-	-
Trimethoprim/ Sulfamethoxazole	11 (44%)	14 (56%)	-	-	0 (0%)	26 (100%)	12 (92.3%)	1 (7.7%)	11 (100%)	0 (0%)
Ciprofloxacin	1 (4%)	24 (96%)	0 (0%)	25 (100%)	22 (84.6%)	4 (15.4%)	13 (100%)	0 (0%)	11 (100%)	0 (0%)
Moxifloxacin	3 (12%)	22 (88%)	3 (12%)	22 (88%)	0 (0%)	26 (100%)	12 (92.3%)	1 (7.7%)	11 (100%)	0 (0%)
Levofloxacin	2 (8%)	23 (92%)	3 (12%)	22 (88%)	-	-	-	-	-	-

Antibiotics	*Staphylococcus aureus*		*Streptococci* of group G & C		*Pseudomonas aeruginosa*		*Enterobacter cloacae*		*Klebsiella pneumoniae*	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Erythromycin	16 (64%)	9 (36%)	11 (44%)	14 (56%)	-	-	-	-	-	-
Clindamycin	16 (64%)	9 (36%)	13 (52%)	12 (48%)	-	-	-	-	-	-
Fusidic acid	25 (100%)	0 (0%)	-	-	-	-	-	-	-	-
Rifampicin	25 (100%)	0 (0%)	-	-	-	-	-	-	-	-
Linezolid	25 (100%)	0 (0%)	-	-	-	-	-	-	-	-
Mupirocin	25 (100%)	0 (0%)	-	-	-	-	-	-	-	-

Table 6: Susceptibility and resistance rates of Staphylococcus aureus, streptococci of group G and C, Pseudomonas aeruginosa, Enterobacter cloacae and Klebsiella pneumoniae: S= susceptible, R= resistant (intermediate strains are summarised within the resistant column), -: Antibiotics not tested for this respective group

Susceptibility and resistance rates								
Antibiotics	**Proteus mirabilis**		**Proteus vulgaris**		**Escherichia coli**		**Acinetobacter baumannii**	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Amoxicillin	5 (55.6%)	4 (44.4%)	0 (0%)	6 (100%)	1 (12.5%)	7 (87.5%)	0 (0%)	8 (100%)
Amoxicillin/ Clavulanic acid	9 (100%)	0 (0%)	4 (66.7%)	2 (33.3%)	6 (75%)	2 (25%)	0 (0%)	8 (100%)
Piperacillin/ Tazobactam	9 (100%)	0 (0%)	6 (100%)	0 (0%)	6 (75%)	2 (25%)	-	-
Cefuroxime iv	8 (88.9%)	1 (11.1%)	0 (0%)	6 (100%)	4 (50%)	4 (50%)	0 (0%)	8 (100%)
Cefotaxime	8 (88.9%)	1 (11.1%)	2 (33.3%)	4 (66.7%)	4 (50%)	4 (50%)	0 (0%)	8 (100%)
Ceftazidime	8 (88.9%)	1 (11.1%)	2 (33.3%)	4 (66.7%)	4 (50%)	4 (50%)	0 (0%)	8 (100%)
Cefepime	8 (88.9%)	1 (11.1%)	2 (33.3%)	4 (66.7%)	5 (62.5%)	3 (37.5%)	0 (0%)	8 (100%)
Imipenem	9 (100%)	0 (0%)	6 (100%)	0 (0%)	8 (100%)	0 (0%)	8 (100%)	0 (0%)
Meropenem	9 (100%)	0 (0%)	6 (100%)	0 (0%)	8 (100%)	0 (0%)	8 (100%)	0 (0%)

Antibiotics	Proteus mirabilis		Proteus vulgaris		Escherichia coli		Acinetobacter baumannii	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Gentamicin	6 (66.7%)	3 (33.3%)	1 (16.7%)	5 (83.3%)	7 (87.5%)	1 (12.5%)	8 (100%)	0 (0%)
Amikacin	6 (66.7%)	3 (33.3%)	6 (100%)	0 (0%)	8 (100%)	0 (0%)	8 (100%)	0 (0%)
Trimethoprim/ Sulfamethoxazole	5 (55.6%)	4 (44.4%)	1 (16.7%)	5 (83.3%)	6 (75%)	2 (25%)	8 (100%)	0 (0%)
Ciprofloxacin	4 (44.4%)	5 (55.6%)	1 (16.7%)	5 (83.3%)	4 (50%)	4 (50%)	8 (100%)	0 (0%)
Moxifloxacin	4 (44.4%)	5 (55.6%)	1 (16.7%)	5 (83.3%)	4 (50%)	4 (50%)	0 (0%)	8 (100%)

Table 7: Susceptibility and resistance rates of Proteus mirabilis, Proteus vulgaris, Escherichia coli and Acinetobacter baumannii: S= susceptible, R= resistant (intermediate strains are summarised within the resistant column), -: Antibiotics not tested for this respective group

3.6 Multi-resistance of pathogens in chronic leprosy-lesions

Amongst the 198 examined microbes 33 (16.7%) were multi-resistant pathogens namely *MRSA*, 3MRGN and/or ESBL-producing *Enterobacteriaceae*.

Within the 66 samples, 22 swabs included one multi-resistant pathogen, four swabs contained two and one swab carried three multi-resistant bacteria. To summarise, in 27 (40.9%) swabs at least one multi-resistant microorganism was detected.

Within the 33 multi-resistant bacteria, *MRSA* was the most common one, with 23/33 (69.7%); followed by ESBL-producing *Proteus vulgaris* with 4/33 (12.1%). Furthermore, there were three (9.1%) 3MRGN-*E. coli* (also ESBL-producing) and one (3.0%) ESBL-producing *E. coli* as well as one (3.0%) ESBL-producing 3MRGN-*Proteus mirabilis* respectively one (3.0%) ESBL-producing *Enterobacter cloacae*.

None of the microorganisms could be assigned to a 4MRGN strain.

Figure 18 and *19* depict the results graphically.

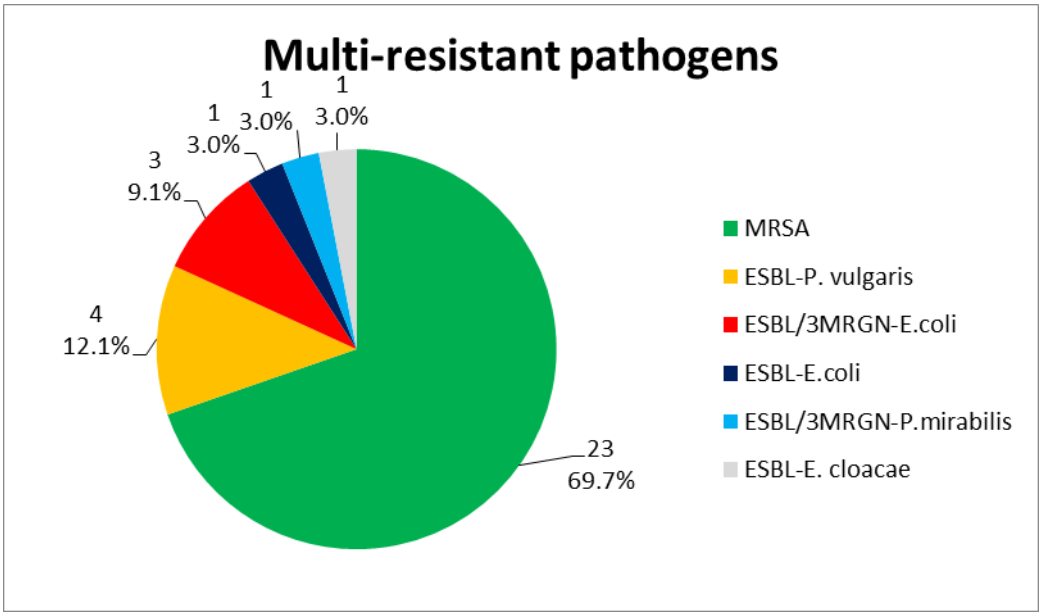

Figure 16: Absolute numbers and percentages of MDR bacteria in samples of chronic lesions in Indian leprosy patients

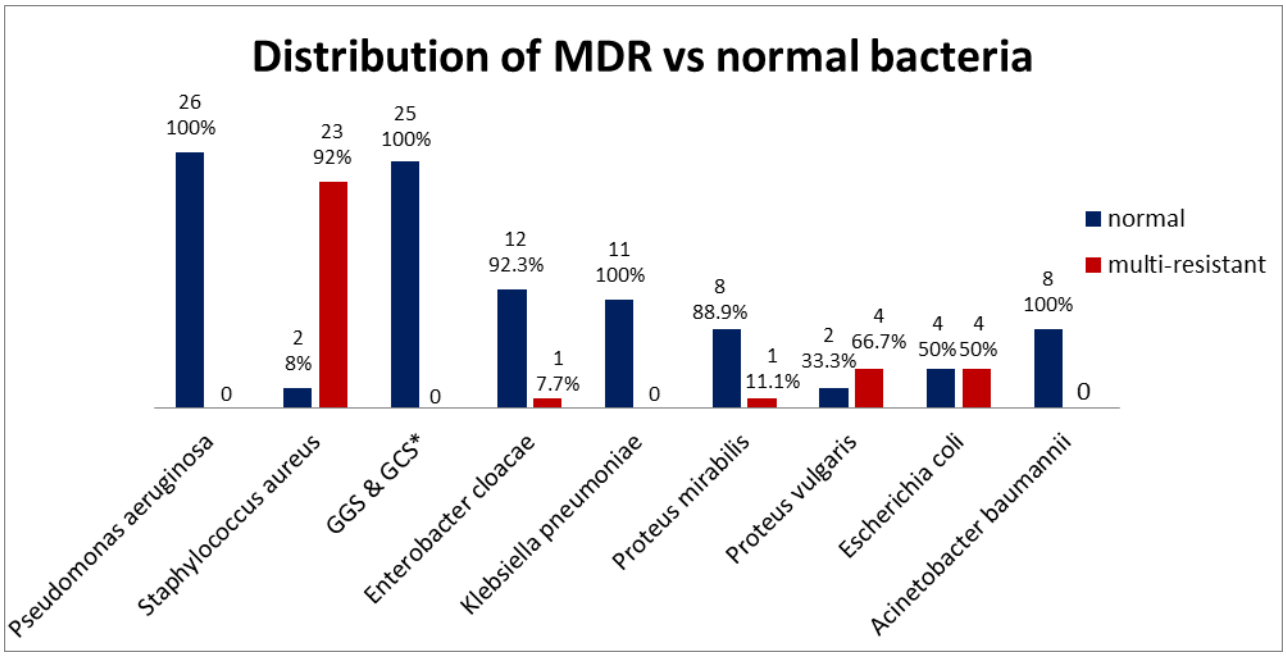

*Figure 17: Distribution of MDR bacteria versus normal bacteria detected in samples of chronic lesions in Indian leprosy patients; *: A definition for MDR streptococci group G and C does not exist.*

4 Discussion

Even though leprosy is classified as an orphan disease still 215 000 new cases occur worldwide each year. In absolute numbers this means, that India is by far the most effected country with about 135 000 newly diagnosed persons per year. Although the figures show a decline of new cases, Hansen's disease remains a health burden that should not be underestimated.

Many challenges are associated with Morbus Hansen: From nerve impairment with subsequent morbidity and social stigmatisation, systemic involvement or leprosy reactions to lethal courses.
Deformities and neuropathic chronic ulcers may even remain after successful treatment and are therefore important factors related to the quality of life and rehabilitation. Malnutrition, poor hygiene standards and poor health care structures as well as known high antibiotic resistance rates in India are some of the aspects retarding or impeding the recovery process.

The aim of this thesis was the description of the microbiological spectrum and resistance patterns within chronic leprosy-associated wounds, followed by an interpretation of the results with regard to their impact on empiric antibiotic treatment of such lesions, if necessary.

4.1 Demographic data and localisation of wound samples

The present study included 35 (70%) men and 15 (30%) women, which roughly mirrors the gender distribution of leprosy worldwide. According to the WHO report from December 2016 to March 2017, men constitute 60% of the leprosy patients. (14, 15)
The majority of swabs were taken from the sole - this is logically explicable because the greatest mechanical stress is transmitted to the feet. Additionally, the lack of appropriate footwear corresponds to a higher risk of getting injured on the sole, where microbes from the soil can easily penetrate the wound.

4.2 Bacterial flora of leprosy-associated chronic lesions

In literature several studies concerning leprosy wounds and their resistance patterns could be found. These studies were not only carried out in India but also in Brazil, Ethiopia and Mali. (50, 98-103)

One needs to emphasise that this study was performed in a completely different geographical region. The Indian study of Majumdar and colleagues investigated Calcutta in the state of West Bengal (East India), the study of Shravani et al. took place in Hyderabad in the state of Telangana (South-Central India). (101, 102)

Our study was undertaken in Salem in the state of Tamil Nadu in South-Eastern India. The lesions of leprosy patients from Salem were commonly infected multiply (95.5%) with a median of four microbes per sample; only 4.5% comprised solely one microorganism. In other studies, multiple organisms (two or more microbes) were much rarer: 5%, 19.6%, 22.7% respectively 36%. (50, 99, 101, 103)

In the present study, 57.4% of Gram-negative bacteria and 41.5% of Gram-positive bacteria as well as 1% of fungi were identified. This strongly correlates to the data of Lema et al. with 56% of Gram-negative and 44% of Gram-positive bacteria. (99)

The analysis of the data from Salem revealed *Pseudomonas aeruginosa* as the most common microorganism inside chronic ulcerated wounds of leprosy patients, closely followed by *MRSA* and group G *streptococci*. *Pseudomonas aeruginos*a appeared in 39.4%, *MRSA* in 34.8% respectively *Staphylococcus aureus* generally in 37.9% and group G *streptococci* in 30.3% of the swabs. Furthermore, group C streptococci, *Enterobacteriaceae* (*Proteus spp*., *Enterobacter cloacae*, *Klebsiella pneumoniae*, *E. coli*) and *Acinetobacter baumannii* were frequently found. Compared to the other studies, the microbiological spectrum was very similar; however, the percentages of the found bacteria differed. (50, 98-100, 102, 103)

4.3 Resistance patterns of leprosy-associated lesions

The resistance patterns were also investigated and discussed in the studies mentioned above and demonstrate the importance of local investigations.
Resistance rates of *Staphylococcus aureus* were considerably higher in patients from Salem: 92% of *Staphylococcus aureus* were *MRSA* compared to 8.7% in the study of Shravani et al. Ciprofloxacin resistance rates were also higher: 96% of the isolates of the present study were resistant, whilst in the other studies Ciprofloxacin resistance ranged between 4.8% and 30.9%.
Conversely, erythromycin in *Staphylococcus aureus* performed better with a resistance rate of 36% - Ramos et al. ascertained a resistance rate of 26.7%, Shravani et al. of 56.1%, Lema et al. of 75% and Ferreira et al. of 90.5%. (50, 99, 100, 102)

While we found a high number of *streptococci*, unfortunately, no comparable study for group G and C *streptococci* could be found. Nevertheless, it can be recorded that all *streptococci* of group G and C were susceptible to penicillin, amoxicillin/clavulanic acid or meropenem while in contrast all had to be reported as resistant to ciprofloxacin, amikacin and gentamicin.

For *Pseudomonas aeruginosa*, the results were more gratifying since resistance rates were much lower than in the preceding studies: All isolates collected from the leprosy patients from Salem were susceptible to the carbapenems in comparison with a resistance rate of 33.3% in the study of Shravani et al. The ciprofloxacin resistance rate was 15.4%; whereas, it ranked between 22.2% and 37.5% in the other studies. (50, 100, 102)

Albeit no comparable study could be found reporting about ciprofloxacin resistance in *Enterobacter cloacae*, it can be noted that fortunately in our study all *Enterobacter* isolates were susceptible to ciprofloxacin as well as to the carbapenems. In the study of Lema et al., *Enterobacter* was resistant to trimethoprim/sulfamethoxazole in 12.5% and susceptible to gentamicin in all cases, whereas in our study resistance rates of 7.7% for gentamicin and trimethoprim/sulfamethoxazole were detected. (99)

For *Klebsiella pneumoniae* there is one study of Shravani et al. that reports a resistance rate for piperacillin/tazobactam of 32.2%, for imipenem and meropenem of 16.1% and for

ciprofloxacin of 35.4%, while all *Klebsiella* isolates were susceptible to the mentioned antibiotics in the patients from Salem. (102)

For *Proteus mirabilis*, carbapenems would have worked in all cases. In comparison, Shravani et al. found a resistance rate of 37.5%. By contrast, the results for ciprofloxacin were significantly worse: In the present study, *Proteus mirabilis* was resistant in 55.6%; while other studies published rates between 12.8% and 32%. The same discrepancy between the studies applies for *Proteus vulgaris.* (50, 99, 100, 102)

For *E. coli* percentages of resistance rates were highly divergent in the discussed studies. In the study of Ferreira et al., all *E. coli* isolates were susceptible to ciprofloxacin, while in the study of Ramos et al., about 30% were resistant. In this study, half of the *E. coli* showed resistance to ciprofloxacin. (50, 100)

Finally, the resistance rates of *Acinetobacter baumannii* shall be discussed: The study of Lema et al. published a resistance rate to ciprofloxacin of 20% which does not correspond to the data of the present study, where all *Acinetobacter* strains were susceptible to ciprofloxacin. (99)

It can thus be concluded that the resistance rates of bacteria found in chronic lesions of leprosy patients differ from region to region. Further local investigations with a representative number of samples may be helpful to support the respective medical institution.

4.4 Resistance rates of pathogens in leprosy-associated lesions

According to their resistance pattern, bacteria can be assigned to the different groups of multi-resistant bacteria -like MRSA, ESBL or 3 or 4 MRGN. Awareness of the local frequency of the occurrence of multi-resistant bacteria is necessary. This is based on the fact, that 50 percent of bacterial infections in Indian hospitals are resistant to commonly used antibiotics. (81)

In clinical isolates high rates of methicillin-resistant *Staphylococcus aureus* (MRSA) have been reported from all over India, ranging from 32% to 80%. This corresponds with our data, where we even found a rate of 92%. (81)

While in our study none of the *Pseudomonas aeruginosa* isolates was multidrug-resistant, Gill. et al. investigated a total of 1915 patient samples in a tertiary hospital in India and found that 52.3% of *P. aeruginosa* were multidrug-resistant (MDR), what has led to significant morbidity and mortality of the patients.(104)

Dramatically increasing numbers of ESBL-producing, respectively 3 or 4 MRGN *Enterobacteriaceae* are reported worldwide. (105, 106) For India, reports show that *Klebsiella pneumoniae* exhibits high MDR- and ESBL-rates; up to 74.4% (Rath et al.). (107) Fortunately, none of the samples from the leprosy patients from Salem contained 3 or 4 MRGN respectively ESBL-producing *Klebsiella,*

There was no 3 MRGN *Enterobacter cloacae isolate,* either; a single isolate was ESBL-positive- this represents 7.7%. Nevertheless, a Pakistan study yielded an ESBL-rate of 14.9%. in *Enterobacter cloacae* isolates. (108)

In patients from Salem, 37.5% of *Escherichia coli* were 3MRGN and 50% were ESBL producer. This complies with the numbers of the study of Rath et al., which assessed the ESBL-rate of *E. coli* with 40 to 60% in an Indian teaching hospital and in the adjoining community. (109)

For *Acinetobacter baumannii* there was a strong discrepancy between the present and a North Indian study: While *Acinetobacter* was neither multidrug nor carbapenem-resistant in the samples from Salem, MDR-rates of 87% and a carbapenem resistance (more precisely meropenem resistance) of 20% was published. (110)

4.5 Empiric antibiotic therapy of lesions in Indian leprosy patients

Approaches to an expedient antibiotic therapy are complex, since a wide range of antibiotics is on the market. The situation might be complicated - as demonstrated above - due to the occurrence of MDR bacteria.

For the antibiotic therapy of soft tissue infections which should be carried out orally, a variety of agents is available, namely penicillins, amoxicillin +/- clavulanic acid, first- or second-generation oral cephalosporins, fluoroquinolones, macrolides or clindamycin. (111)

As regards the patients from Salem, it has to be considered that 92 % of the *Staphylococcus aureus* isolates were *MRSA*. Therefore, the selection of antibiotics in this case is limited to oral fusidic acid, erythromycin or clindamycin - both with a resistance rate up to nearly 40%. Alternatives include rifoldin, linezolid, which is very expensive, or daptomycin, which is only available as an intravenous therapy.
Based on the fact, that nearly all samples showed a polymicrobial flora, *Pseudomonas* and *Enterobacteriaceae* must also be taken into consideration of an empiric antibiotic therapy. Amoxicillin/clavulanic acid, oral cephalosporins or ciprofloxacin may be the antibiotics of choice, depending on the local epidemiology.
For intravenous therapy piperacillin/tazobactam as well as carbapenems might be used. As no 4 MRGN was found in this study, carbapenems are active against nearly all bacteria except *MRSA*, which are - due to the underlying resistance mechanism - resistant to carbapenems.

The chronic, non-healing wounds in leprosy patients often persist for many years. Due to the resulting breakdown of the physiological conditions of the surrounding tissue, peroral antibiotics often cannot reach the wound. Thus, local and topical preparations, which were perfectly performed in all the patients we visited during the study period in Salem, are the best fundament in order to avoid severe and complicated soft tissue infections. (112)
The knowledge of the local bacterial spectrum and the local resistance patterns is only one piece of the puzzle in the fight against multi-resistant bacteria and in the fight against leprosy. Further local research on this topic will support all the admirable people of the DTMC trust to reach that aim.

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