



Microbiology

Multi-Drug-Resistant bacteria / MDR: laboratory diagnostics and prevention

June 2017 ● MeshHp

(VS)

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Antimicrobial resistance / MDR:

an increasing threat to health systems worldwide ... and often leads to:

- treatment failure
- high treatment costs
- increasing mortality

● Gram-positive MDR-Bacteria:

Staphylococcus aureus (MRSA)
Enterococcus faecium (VRE)

● Gram-negative MDR-Bacteria:

Enterobacteriaceae (ESBL)
Enterobacteriaceae (Carbapenemase)
Pseudomonas aeruginosa
Acinetobacter baumannii



Gram-positive: MRSA (Methicillin-resistant *Staphylococcus aureus*)

	Healthcare-associated MRSA (HA-MRSA)	Community acquired MRSA (CA-MRSA)	Livestock associated MRSA (LA-MRSA)
Appearance	public health system: hospitals etc.	common population: „community“	associated with handling animals: cows, pigs, sheep etc.
	older immunodeficient patients	even younger patients (not immunodeficient!)	even younger patients (not immunodeficient!)
Molecular-biological	mecA-Gene	mecA-Gene PVL-Gene (Cytokine)	mecA-Gene ST 398-Gene
Resistance	all β -Laktames, Fluorchinolones, Makrolides	all β -Laktames, Fusidineacid	all β -Laktames, Tetracycline



Gram-positive: MRSA - Diagnostics (Methicillin-resistant *Staphylococcus aureus*)

MRSA-Screening: chromogenic culture media

- ▶ specimen: smear-test (nose, pharynx, groin etc.)
- ▶ method:
 - culture directly from swab on selective culture media
 - 24 h incubation
 - interpretation (coloured colonies)



- ▶ result (according to manufacturer-information):

„... identification within 8-24 Std. possible“
„... no substitution for classic susceptibility-testing“

- ▶ target: specific MRSA-detection like MRSA-Screening (e.g. inpatients, depending on risk-factors)

MRSA-Infection – culture and susceptibility-test

▶ specimen: **smear-test (infected wounds etc.)**

▶ method: - culture directly from swab
on blood media (streaking)
- 24 h incubation



▶ result identification / susceptibility-testing:

- suspicious colonies: *VITEK XL/ Maldi-TOF*
and *VITEK XL/ Agar-Diffusion*
- first detection of MRSA within same patient
always confirmed by PCR at same day!
(*mecA-Gen (HA-MRSA)*, gene for *PVL (CA-MRSA)*
and sequence-type *ST398 (LA-MRSA)!*)



▶ target: **MRSA-detection for treatment of infections,
... maybe follow-up-controls**

Gram-positive: MRSA - Prevention (*Methicillin-resistant Staphylococcus aureus*)

triggered by: **Gyrase-inhibitors (e.g. Cipro- / Levofloxacin)**

Infection: **effective antibiotic treatment**
(susceptibility-testing!)

Colonisation: **Screening: smear-test nose**
(... and skin, axilla, groin, rectum)

Eradication: - nose: **Mupirocin ointment**
- skin: **washing with disinfectant**

Hygiene: - separation / isolation of patient
- environment: **disinfection !**
- **handhygiene !**



Gram-positive: VRE

(*Vancomycin-/Glycopeptid-resistent Enterococcus sp.*)

- *Enterococcus sp.* are usually resistant against Penicillin, Cephalosporines, Clindamycin, Co-Trimoxazol, Tetrazycine
- but usually sensitive against Glykopeptides (*Vancomycin/Teicoplanin*) and Linezolid !



Gram-positive: VRE - Diagnostics

(*Vancomycin-/Glycopeptid-resistent Enterococcus sp.*)

- susceptibility-testing: *Vancomycin*

„... if you are searching, you will find ...“



Gram-positive: VRE - Prevention

(*Vancomycin-resistent Enterococcus sp. (E.faecium)*)

triggered by: *Vancomycin* (oral / systemic)

Infection: **effective antibiotic treatment**
(susceptibility-testing!)

Colonisation: **Screening: smear-test rectum**
(... deep ! ... maybe also stool)

Eradication: - **NO ! ... would not be successful ...**

Hygiene: (risc-areas: separation / isolation of patient)
- **environment: disinfection !**
- **handhygiene !**



Gram-negative: ESBL and AmpC (Beta-Lactamases)

ESBL = Extended-Spectrum-Beta-Lactamases:

- resistance against Penicillines und Cephalosporines
- Gene-transfer (Plasmid): possible
- frequent variants are CTX-M-ESBL, TEM- and SHV-ESBL

AmpC-Beta-Lactamases

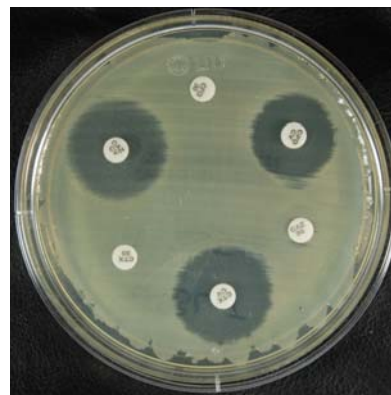
- resistance against Penicillines und Cephalosporines
- Gene-transfer (Plasmid): partly (!) possible
- frequent Plasmid-coded variants: CMY, DHA, ACC



Gram-negative: ESBL - Diagnostics (Extended Spectrum Beta Lactamases)

selective-culture-media
 help to find suspicious isolates.

resistance against:
3. Gen. Cephalosporines
 (group a/b: Cefotaxim,
 Ceftriaxone etc.)



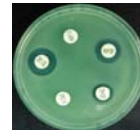
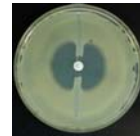
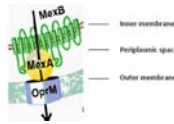


Gram-negative: Carbapenem Resistance (Efflux-Pump, Loss of Porines, Carbapenemases)

Efflux-pump (active transport):

- antibiotics: inside ► out !
(... frequently *Pseudom. aeruginosa*: > 80 %)

(Source: Dr. Yvonne Pfeifer, RKI Wernigerode, Germany)

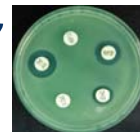
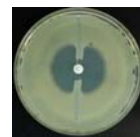


Loss of Porines:

- Outer Membrane Proteins / OMP
- mutations lead to loss of Porines / loss of Permeability !
- additionally ESBL/AmpC ► Carbapenem-Resistance ! (type ETP, MEM)
(... frequently *Enterobacter aerogenes*: > 90%, *K.pneumoniae* etc.)

Carbapenemases

- enzymatic hydrolysis of Carbapenems
- resistance: Penicillines, Cephalosporines, Carbapenems
- Gene-transfer between Gram-Negative Bacteria possible
- frequent variants are KPC, OXA-48, VIM, NDM
- identification via PCR
(Germany: Nat. Reference Center, Bochum)



**ESBL- and Carbapenemase-producing Bacteria
in many cases are additionally resistant against
other classes of antibiotics !
(Fluorchinolones, Aminoglykosides, Sulfonamides etc.)**



Gram-negative: Why „MRGN“ in Germany? (MRGN = Multi Resistant Gram Negative – Bacteria)

Problems with Gram-negative MDR-bacteria:

- increasing antimicrobial resistance within Gram-negative bacteria
- ESBL to be found in different bacteria genera / species
- always new genes / types of resistance-mechanisms found
- missing therapy-options and high mortality during Gram-negative MDR-infections

Target:

- **NO classification depending on resistance-mechanisms !**
- classification depending on resistance against groups of antibiotics (leading antibiotics), not considering virulence
- classification depending on loss of more than 2 of 4 important antibiotic groups for effective antimicrobial treatment

RKI / KRINKO: central institution for health protection
(supports the Federal Ministry of Health as a central scientific institution in the field of biomedicine) 10/2012

defined the MRGN-criteria of MDR with the main target:

► hygiene-relevant issues in hospitals !!

- 2MRGN:** resistance against Penicillines (Piperacillin) and 3rd Gen. Cephalosporines (Cefotaxim/Ceftazidim)
(Neo-Päd)
- 3MRGN:** resistance against Penicillines, 3. Gen. Cephalosporines + Fluorchinolones (Ciprofloxacin)
- 4MRGN:** resistance against Penicillines, 3. Gen. Cephalosporines, Fluorchinolones + Carbapenems (Imipenem/Meropenem) or detection of Carbapenemases

New classification (RKI / KRINKO): 3/4MRGN

Robert Koch-Institut | Epidemiologisches Bulletin Nr. 36

12. September 2011

Antibiotikagruppe	Leitsubstanz	Enterobacteriaceae		Pseudomonas aeruginosa		Acinetobacter spp.	
		3MRGN ¹	4MRGN ²	3MRGN ¹	4MRGN ²	3MRGN ¹	4MRGN ²
Acylureidopenicilline	Piperacillin/ Tazobactam	R	R	Nur eine der vier Antibiotika- gruppen wirksam (sensibel)	R	R	R
		R	R		R	R	R
		S	R		R	S	R
		R	R		R	R	R
Cephalosporine der 3./4. Generation	Cefotaxim und/ oder Ceftazidim	R	R		R	R	R
Carbapeneme	Imipenem und/ oder Meropenem	S	R		R	S	R
Fluorchinolone	Ciprofloxacin	R	R		R	R	R

Tab. 1: Klassifizierung multiresistenter gramnegativer Stäbchen auf Basis ihrer phänotypischen Resistenzeigenschaften

(R = resistent oder intermediär sensibel, S = sensibel)

¹ 3MRGN (Multiresistente gramnegative Stäbchen mit Resistenz gegen 3 der 4 Antibiotikagruppen)

² 4MRGN (Multiresistente gramnegative Stäbchen mit Resistenz gegen 4 der 4 Antibiotikagruppen)

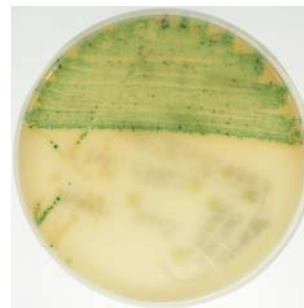


Gram-negative: MRGN - Diagnostics (MRGN = Multi Resistant Gram Negative – Bacteria)

... more complex than ESBL-diagnostics:
More combined resistances !

- ▶ pre-testing (screening) with selective culture media and application of additional antibiotics:

- incubation up to 48 hours
- dividing relevant bacteria from physiological flora



- ▶ identification/susceptibility-testing of suspicious colonies:

- additional 24 hours

► **extended susceptibility testing:**

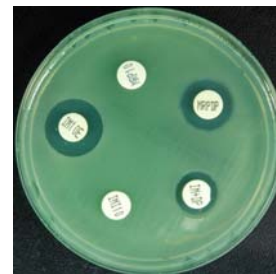
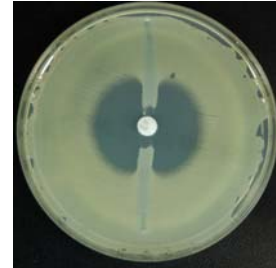
- additional antibiotics:
 e.g. **Tigecyclin, Colistin**

► **phenotypic confirmation-tests for detection of resistance-mechanisms:**

- Carbapenemases

► **confirmation of the resistance-mechanisms: genotype**

- especially during cases of outbreaks !



RUHR-UNIVERSITÄT BOCHUM

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DEPARTMENT OF MEDICAL MICROBIOLOGY

NATIONAL REFERENCE LABORATORY FOR MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA

- 1 week additional until final result

**typical result:
 3MRGN**

UNTERSUCHUNGSMATERIAL: Urin-Tauchmedium	ANTIBIOGRAMME	1.
ANGEFORDERTE UNTERSUCHUNGEN: Allgemeine pathogene Keime/Erreger (ggf. Resistenzbestimmung)	Ampi-/Amoxicillin	R
R E S U L T A T E :	Mezlocillin	R
KEIMZAHL: Keimzahl: > 100 000 KbE/ml	Piperacillin	R
NACHGEWIESENE KEIME: 1. <i>Klebsiella pneumoniae</i> CAVE! Multiresistenz! (3MRGN)	Amoxicillin+Clavulans.	R
BEMERKUNG: Multiresistenz 3MRGN bzw. 4MRGN: Resistenz gegenüber 3 bzw. 4 der Antibiotikagruppen Acylureidopenicilline, Cephalosporine der 3./4. Generation, Carbapeneme und Fluorchinolone.	Ampicil.+Sulbactam	R
HINWEIS zum Infektionsschutzgesetz: Folgende hier nachgewiesenen Keime sind aufgrund ihrer Resistenz in der Liste der gemäß §23 IfSG zu erfassenden Erreger aufgeführt: * <i>Klebsiella</i> spp.	Piperacil.+Tazobactam	R
	Cefuroxim	R
	Cefotaxim/Ceftriaxon	R
	Ceftazidim	R
	Imipenem	S
	Meropenem	S
	Tobramycin	I
	Ciprofloxacin	R
	Levofloxacin	R
	Cotrimoxazol	R
	Tetracyclin	R
	(S=sens. I=interm. R=resistent)	



Gram-negative: MRGN - Prevention (MRGN = Multi Resistant Gram Negative – Bacteria)

- triggered by:** Cephalosporines, Gyrase-inhibitors
- Infection:** **effective antibiotic treatment**
(susceptibility-testing!)
- Colonisation:** **Screening: smear-test rectum**
(... deep ! ... maybe also stool)
- Eradication:** - **NO ! ... would not be successful ...**
- Hygiene:** - separation / isolation of patient
- environment: disinfection !
- handhygiene !

Measures to prevent the spreading of MRGN

	active Screening and Isolation until result	prevention of transmission		Eradication
		ward	Risc-areas (ICU etc.)	
3MRGN E.coli	NO	basic hygiene	isolation	not recommended
4MRGN E.coli	risc-population (rectal, wounds, urine)	isolation	isolation	not recommended
3MRGN Klebsiella spp.	NO	basic hygiene	isolation	not recommended
4MRGN Klebsiella spp.	risc-population (rectal, wounds, urine)	isolation	isolation	not recommended
3MRGN Enterobacter spp.	NO	basic hygiene	basic hygiene	not recommended
4MRGN Enterobacter spp.	risc-population (rectal)	isolation	isolation	not recommended
other 3MRGN Enterobacteriaceae	NO	basic hygiene	basic hygiene	not recommended
other 4MRGN Enterobacteriaceae	risc-population (rectal)	isolation	isolation	not recommended
3MRGN Pseudom. aeruginosa	NO	basic hygiene	isolation	not recommended
4MRGN Pseudom. aeruginosa	risc-population (rectal, pharynx)	isolation	isolation	not recommended
3MRGN Acinetob. baumannii	NO	basic hygiene	isolation	not yet cleared
4MRGN Acinetob. baumannii	risc-population (nasopharynx, skin)	isolation	isolation	nor yet cleared



Gram-negative MDR bacteria: in Mongolia? (Study Ulaanbaatar July – September 2014)

**Gram-negative MDR are increasingly reported throughout Asia:
ESBL, AmpC- β -lactamases, carbapenemases !**

RESEARCH ARTICLE

Faecal Carriage of Gram-Negative Multidrug-Resistant Bacteria among Patients Hospitalized in Two Centres in Ulaanbaatar, Mongolia

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Results:

- first surveillance study (rectal swabs) that estimates faecal carriage of Gram-negative MDR bacteria in Mongolia: 1050 patients !
- rate of bacterial colonisation from 2 hospitals in UB:
 1. National Traumatology and Orthopaedics Research Centre / NTORC
 2. Burn Treatment Centre / BTC
- most MDR-carriers were colonized with E.coli (CTX-M) followed by CTX-M-producing Klebs. pneumoniae and Enterob. cloacae
- high colonisation-rate of β -lactamases-producing MDR in the study population.
- Carbapenemases were detected in both centres
Acinetobacter baumannii (OXA48) and Pseudomonas aeruginosa (VIM-2)
- Carbapenemase-producing bacteria only rarely detected

Conclusion of the study:

alarming!

... many critically ill patients in Mongolia are treated initially with 3rd-generation Cephalosporines !

unfortunately!

... Carbapenems - drugs of choice for severe ESBL-driven infections - are not widely available in Mongolia !

... uncontrolled use and misuse of antimicrobial drugs is widespread in Mongolia: commonly purchased without perscription !

important!

... routine surveillance, appropriate infection control practice and antibiotic prescribing policies

(to prevent further spread of Gram-negative MDR bacteria - especially of carbapenemases)

... only a combination of
hygiene
+
rational antibiotic treatment
can slower the progress /
expansion of MDR !



Thank you for your attention !