Forschungskolleg IKARUS »Infektionen im Kardiovaskulären System – Pathophysiologie, Therapie und Diagnostik«

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Das klinische Forschungskolleg IKARUS (Infektionen im Kardiovaskulären System) am Universitätsklinikum Jena zielt darauf ab, neue diagnostische und therapeutische Ansätze zur Bekämpfung bakterieller Infektionen im Herz-Kreislaufsystem zu entwickeln. Solche Infektionen treten häufig an natürlichen und künstlichen Herzklappen oder Herzschrittmachern auf und nehmen aufgrund der alternden Bevölkerung und des medizinischen Fortschritts stetig zu. Besonders herausfordernd ist die Behandlung dieser Infektionen, da die Bakterien in der Lage sind, sich auf Oberflächen von Implantaten anzusiedeln und Biofilme zu bilden, die sie vor Antibiotika und dem Immunsystem schützen.

Das durch die Else-Kröner-Fresenius-Stiftung geförderte IKARUS-Forschungskolleg bündelt interdisziplinäre Expertise in den Bereichen Infektionsmedizin, Herzchirurgie, Kardiologie und Medizinische Mikrobiologie. Ziel ist es, innovative Strategien zur Diagnostik und Therapie solcher Infektionen zu entwickeln. Dazu gehört neben der Untersuchung der Pathophysiologie bakterieller Infektionen im kardiovaskulären System auch die Erforschung neuer therapeutischer Interventionen und Diagnostikverfahren.

Das Kolleg fördert den wissenschaftlichen Nachwuchs im Rahmen des *Clinician Scientist*-Programms und bietet sieben jungen Ärztinnen und Ärzten die Möglichkeit, ihre klinische Tätigkeit mit wissenschaftlicher Forschung zu verbinden. Hierbei steht die Förderung der Forschungskompetenzen im Vordergrund, um langfristig neue Behandlungsansätze zu entwickeln und die Versorgungsqualität für betroffene Patienten zu verbessern. Das Universitätsklinikum Jena bietet hierfür aufgrund seiner langjährigen Erfahrungen im Bereich Infektionsmedizin und Sepsis exzellente Voraussetzungen.

Extrahepatic replication and genomic signatures of the hepatitis E virus in the kidney

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Introduction:

The hepatitis E virus (HEV; species Paslahepevirus balayani) is a common human pathogenic and zoonotic virus that can cause both acute fulminant and chronic hepatitis. Despite its reputation as a hepatotropic virus, HEV infection is also associated with a number of extrahepatic diseases, including kidney disorders. However, the extent to which HEV replicates in kidney cells remains unclear. The present study aims to investigate the capacity of HEV to propagate in kidney cells *in vitro* and to assess whether HEV displays mutational signatures that correlate with compartmentalisation *in vivo*.

Methods:

We use HEV cell culture models to study the replication cycle and the effect of antivirals in human kidney cell lines and primary cells. In addition, we identified patients with chronic HEV infection from which we then sequenced viral RNA of urine origine, stools and plasma to analyse the viral sequence composition and to gain insight into intra-host diversity and compartmentalisation.

Results:

A wide range of human kidney cell lines and primary human supports viral entry, replication and propagation of HEV *in vitro*. Interestingly, the broad-spectrum antiviral ribavirin was less effective in inhibiting HEV replication in some kidney cells. Sequencing of HEV RNA-directed RNA polymerase coding region from plasma, stool and urine and subsequent phylogenetic analysis revealed diversification of HEV into tissue-specific viral subpopulations. In particular, the viruses derived from urine were found to be distinct from those derived from plasma and stool.

Conclusions:

In conclusion, kidney cells support the propagation of HEV *in vitro* and exhibit reduced sensitivity to antiviral treatment. Furthermore, HEV patient-derived sequences demonstrated compartmentalisation into distinct clusters that correlated with sample source. Collectively, these data indicate the potential for extrahepatic replication of HEV, which may result in clinically significant disease or serve as a reservoir for patient relapse.

RSV Treatment Beyond Direct Antivirals: Exploring the Host Cells for Potential Targets for Therapeutic Intervention

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Respiratory Syncytial Virus (RSV) is a leading cause of death in infants under the age of one year, the elderly, and immunocompromised patients. Despite recent vaccine approvals, challenges remain in achieving widespread public acceptance and providing effective protection for immune-compromised patients. Existing antiviral drugs, targeting viral proteins drawback from the potential development of resistance by mutagenesis. An alternative is to compromise proteins in the host cell which are essential for the virus but not for the host. Our approach is to identify host factors, and particularly transcription factors, that have been hijacked by the virus to reprogram gene expression induced by RSV.
We used multiple previously published gene expression and host-factor screening data. Followed by gene set enrichment analyses, we select the most prominent pathways to employ gene regulatory network models to identify regulators being responsible for the changed gene expression.
We found immune response and morphogenesis/development to be enriched in upregulated genes due to infection, while cell cycle and metabolism being enriched in downregulated genes. Applying gene regulatory models led to the prediction of HNF4A, IRF4, NRF1 and STAT2 as regulators of RSV induced changes.
While experimental validation is needed, these findings may lead to a better understanding of reprogrammed infected host cells by the virus paving the way for new therapeutic options.

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Left ventricular assist device (LVAD) - assoziierte driveline-Infektionen als eine spezifische Form von komplizierten Weichgewebeinfektionen - Probleme und mögliche therapeutische Optionen

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Einleitung:

Left ventricular assist device (LVAD) - assoziierte driveline-Infektionen stellen eine potenzielle lebensbedrohliche Situation dar, bei der eine Entfernung des Implantats nicht in Frage kommt. Die Infektion stellt sich als Lokalbefund um die Eintrittsstelle der Driveline dar, wird jedoch in gängigen Definitionen nicht als Weichgewebeinfektion bzw. als akute bakterielle Infektion der Haut- und Weichgewebe (acute bacterial skin and skin structure infection, abSSSI) anerkannt. Dies schränkt den Gebrauch vieler Antibiotika als “off-label use” ein.

Methode:

Es erfolgte zunächst eine selektive Literaturrecherche über Pub Med zu den Themen “LVAD driveline-Infektionen” sowie “komplizierte Haut- und Weichgebeinfektionen” unter Berücksichtigung nationaler und internationaler Leitlinien. Anschliessend wurde ein interdisziplinärer Expertenkonsens zur Einordnung von LVAD-driveline-Infektionen in das Gesamtkonzept komplizierter Weichgewebeinfektionen bzw. akuter bakterieller Infektionen der Haut- und Weichgewebe erarbeitet und therapeutische Optionen dargestellt.

Ergebnisse:

873 Artikel wurden aus der primäre Literaturrecherche ermittelt, von denen n=67 in die Analyse einbezogen wurden. Es konnte gezeigt werden, dass LVAD-Infektionen die anatomischen, klinischen und mikrobiologischen Kriterien von komplizierten Haut- und Weichgewebeinfektionen (HWGI) bzw. akuten bakteriellen Infektionen der Haut- und Weichgewebe erfüllen (Tab. 1) und daher auch als solche definiert sind. Ausgehend von dieser konsentierten Einordnung wurde eine Therapieschema entwickelt, dass auch neuere Optionen wie z.B. Glykopeptide mit verlängerter Halbwertzeit als “On-label-use” einschliesst (Abb. 1).

Infektionstyp	Oberflächliche/tiefe SSI Kardiochirurgie	LVAD driveline infection
Anatomische Lokalisation	Haut- und Weichgewebe	Haut- und Weichgewebe
Klinische Symptome	Rötung Überwärmung Eitrige Sekretion Schmerzen Funktionseinschränkung	Rötung Überwärmung Eitrige Sekretion Schmerzen
Erregersprektrum	S. aureus (30-50%) KNS (15%) Enterokokken (10%)	S. aureus (50%) KNS (10%) Enterokokken (10%) Pseudomonas spp. (8%)

Tab. 1: Merkmale komplizierter HWGI bzw. abSSSI und LVAD-Infektionen im Vergleich

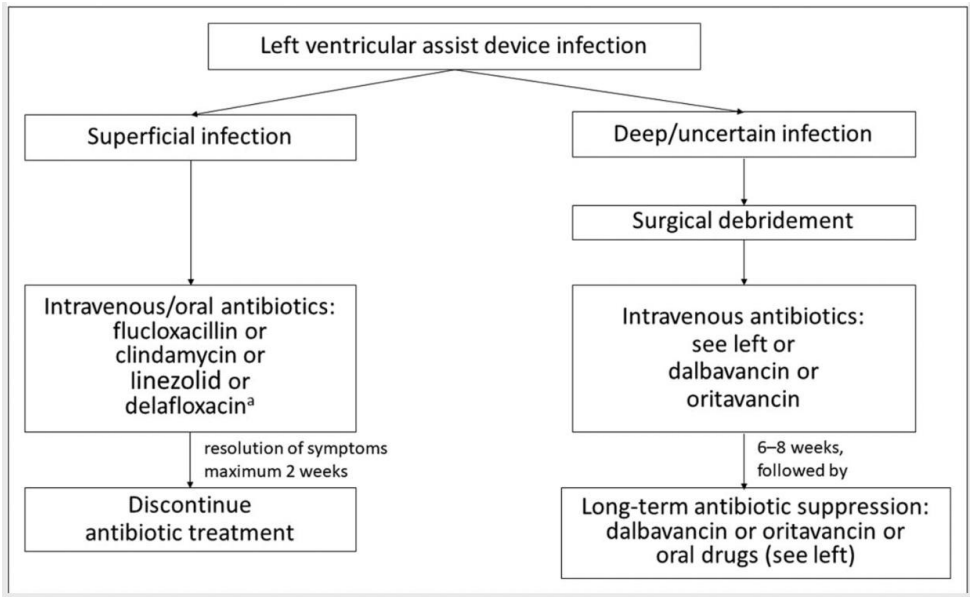

Abb. 1: Therapieoptionen bei verschiedenen Ausprägungen von LVAD-Infektionen

Schlussfolgerungen:

LVAD driveline-Infektionen erfüllen anatomisch, klinisch und mikrobiologisch die Kriterien einer komplizierten Weichgewebeinfektion bzw. einer akuten bakteriellen Infektion der Haut- und Weichgewebe. Diesem Ansatz folgend könnte das Armamentarium an “on label”- Therapieoptionen für diese schweren Infektionen erweitert und damit auch die Lebensqualität der Patienten verbessert werden.

The Role of Drug Repurposing in Containment of Emerging Viral Disease Caused by SARS-CoV-2

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The presented work focuses on the role of drug repurposing in containment of an emerging disease, taking SARS-CoV-2 as a case study. It emphasizes the potential benefits of repurposing existing drugs as a strategy to quickly identify new antiviral therapies. The core of presented work is centered on identifying inhibitors targeting key proteins of the SARS-CoV-2 replication cycle and the development of a validation pipeline of assays to support putative future needs in rapid development of inhibitors for emerging viruses. It shows both target-based biochemical assays and cell-based phenotypic assays to screen for potential inhibitors of SARS-CoV-2 proteases (3Clpro and PLpro), the RNA-based-RNA polymerase, the helicase, and virus entry mechanisms. The poster highlights the main results of performed drug repurposing screens and hit validation, as well as highlights the complexity of transitioning from biochemical assays to cell-based assays, emphasizing that also repurposed compounds showing efficacy in biochemical tests often fail to replicate these results in cellular environments.

Multidrug resistance to oral antibiotics among *Escherichia coli* urine isolates from patients at outpatient departments in Germany and in vitro activity of nitroxoline, Germany, 2010-2023

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Study Group 'Antimicrobial Resistance' of the Paul-Ehrlich-Society for Infection Therapy

Background:
Escherichia coli is the main cause of urinary tract infections (UTI). Treatment of UTI can be difficult due to the spread of multidrug-resistant isolates conferring resistance to standard oral antibiotics. Nitroxoline is an antimicrobial agent recommended in the treatment of acute or recurrent UTI. The objectives of this study were to i) evaluate the frequency of multidrug-resistant strains among uropathogenic *E. coli* from outpatients in Germany and ii) determine their susceptibility to nitroxoline over the period 2010-2023.

Methods:
Isolates were collected prospectively at 20 laboratories during five surveillance studies conducted by the Paul-Ehrlich-Society for Infection Therapy. Species identification and antimicrobial susceptibility testing (broth microdilution) were performed at a reference laboratory. Antimicrobial susceptibilities were investigated using industrially manufactured plates (Bruker-Merlin, Germany) including the following antibiotics: amoxicillin, amoxicillin-clavulanic acid, cefuroxime, cefpodoxime, ciprofloxacin, trimethoprim, fosfomycin, nitrofurantoin and nitroxoline. Presence of ESBL genes was confirmed in isolates with ESBL-phenotype by PCR/sequencing.

Results:
In total, 1.984 isolates were collected between 2010 and 2023. Almost 50% of isolates were resistant to at least one drug tested (Table). Combined resistance to amoxicillin, cefuroxime, ciprofloxacin and trimethoprim was detected in 5.4%, 2.0%, 2.8%, 3.3% and 4.1% of isolates collected in 2010/11, 2013/14, 2016/17, 2019/20, and 2022/23, respectively, while rates of ESBL-producing isolates were 8.3%, 3.3%, 7.3%, 10.8% and 6.6%, respectively. Nitroxoline was tested against 1.073 isolates, including all isolates in 2010/11 (n=399) and subsets of isolates in 2013/14 (n=212), 2019/20 (n=240) and 2022/23 (n=222). Resistance to nitroxoline was not observed.

Conclusion:
Resistances of oral antibiotics used to treat infections with uropathogenic *E. coli* from German outpatients in 2022/2023 followed trends of years 2010 to 2020. Lowest resistance rates were observed for Fosfomycin, nitrofurantoin and nitroxoline, with no nitroxoline-resistant isolates detected over the entire period 2010 to 2023.

Table: Resistance rates (%) of *E. coli* isolates from 2010 to 2023

Antibacterial agent	Breakpoint (mg/L) [1]	Study interval					Trend [2]
		2010/11 (n=399)	2013/14 (n=395)	2016/17 (n=400)	2019/20 (n=400)	2022/23 (n=390)	
Resistance to at least one drug	Not applicable	58.9	46.1	49.5	50.0	52.8	0.0122
Amoxicillin	> 8	43.5	41.5	41.5	45.5	40.5	0.6247
Amoxicillin-clavulanic acid	> 8	32.5	34.7	14.8	19.0	16.7	< 0.0001
Cefuroxime	> 8	10.0	5.1	12.4	12.3	10.8	0.0034
Cefpodoxime	> 1	8.5	4.3	9.0	12.0	9.0	0.0039
Ciprofloxacin	> 0,5	20.1	14.2	16.8	10.8	13.1	0.0030
Trimethoprim	> 4	34.1	26.1	28.0	28.0	29.5	0.1337
Fosfomycin	> 8	5.0	3.0	6.3	7.8 [3]	7.2 [3]	0.0320
Nitrofurantoin	> 64	0.8	1.0	1.0	1.5	0.3	0.4621
Nitroxoline [4]	> 16	0	0	Not tested	0	0	Not calculated

[1] EUCAST breakpoints for all orally administered antibiotics. [2] Chi-squared-test for linear trend. [3] Resistance was confirmed by agar dilution. [4] Isolates tested: 2010/11, n=399; 2013/14, n=212; 2019/20, n=240; 2020/23, n=222

Resistance to antimicrobial agents in *Escherichia coli* and *Klebsiella pneumoniae* from hospitalized patients: results of the PEG surveillance study, 1984-2022/23

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Introduction:

The Study Group 'Antimicrobial Resistance' of the Paul-Ehrlich-Society for Infection Therapy (PEG) regularly monitors the frequency of antimicrobial resistance among clinical isolates of frequently encountered bacterial pathogens. Project H (Hospital) of the PEG surveillance study comprises 20-30 medical laboratories (mostly affiliated with tertiary care medical centers) located throughout Germany (80-90%) and in Austria and Switzerland. Here, we describe the temporal changes of resistance in *Escherichia coli* and *Klebsiella pneumoniae* to clinically relevant antibiotics.

Materials and Methods:

Isolates were collected in regular intervals from 1984 to 2022/23, with more than 8.600 *E. coli* isolates and more than 3.600 *K. pneumoniae* isolates investigated (Figures 1 and 2). Only isolates considered by the respective investigators to have caused infections were included. Susceptibility testing was performed using commercially manufactured microtiter plates from Bruker, Bornheim, Germany. Species identification was confirmed by biotyping and MALDI-TOF mass-spectrometry.

Results:

The observed period of 38 years shows a considerable increase in resistance for a number of antibiotics. Following the introduction of the first fluoroquinolones (FQ) into clinical use between 1984 and 1986, resistance to the FQ (ciprofloxacin) increased in both *K. pneumoniae* and *E. coli*. The highest ciprofloxacin resistance rates were seen between 2001 and 2016 (>12%) in *K. pneumoniae* and between 2004 and 2016 (>20%) in *E. coli*. Since the beginning of the study, there was also a considerable increase in resistance of *E. coli* to "old" antibiotics such as ampicillin, cotrimoxazole and gentamicin. Since 2010, resistance rates against these antibiotics have shown an unchanged or decreasing trend, but resistance rates for ampicillin and cotrimoxazole remain high (30-50%).

Cefotaxime was introduced in Germany in 1980. At this time, no cefotaxime-resistant *E. coli* were detected. Low resistance rates (<3 %) were observed until 2001. In *K. pneumoniae,* cefotaxime resistance remained low (<5%) until 1998. After that, resistance in both species rose to around 17% by 2010 and remained roughly unchanged thereafter. The first carbapenem (imipenem) was introduced in Germany in 1984, followed by meropenem in 1995. Carbapenem (meropenem) resistance remained low in *E. coli* (<1%) and *K. pneumoniae* (<3%) up until today.

Conclusion:

Our study gives a broad overview about changes in antimicrobial resistance rates in *E. coli* and *K. pneumoniae* over a 38-year period. Despite resistance rates for carbapenems still remain at a low level, our comprehensive data demonstrate that an increase of resistance rates can suddenly occur at some point in time. This highlights the importance of the regularly performed PEG surveillance study for a continuous, systematic collection and analysis of antimicrobial resistance data.