IdiPAZ Instituto de Investigación Hospital Universitario La Paz
Hospital Universitario La Paz
Paseo de La Castellana, 261
Edificio Norte, 4ªPlanta
28046 - Madrid

qMAR

Quantification of the intestinal load of a targeted set of resistance genes to Monitor Antibiotic Resistance in paediatric transplant patients

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The aims

The gut microbiome is involved in numerous processes that include protection against colonization and infection by harmful microorganisms. The extensive use of antibiotics could result in an imbalance between the helpful and harmful bacteria, leading to detrimental effects on the health of an individual and increasing the risk of having multidrug resistant organisms in the intestines. This is especially concerning for paediatric transplant patients that have special needs and are heavily reliant on antibiotics.

Previous studies showed that determining the load of resistance genes among the gut microbial organisms could be a predictive factor for the development of harmful infections. Along these lines, qMAR's main objective is to evaluate the use of qPCR, a sensitive and accurate test, for monitoring the relative loads of resistance genes over time. Ultimately, this approach could be used as a **tool to monitor biomarkers that could help in Personalized Medicine approaches among paediatric transplant patients in order to improve on their course of treatment**.

The following research goals have been set for this project:

- To collect samples from paediatric transplant patients using non-invasive procedures.
- To track and quantify the antibiotic resistance genes over time using qPCR.
- To determine associations between clinical interventions, the relative load of resistance genes, and clinical outcome.

In order to achieve our objective, faecal samples and medical records will be collected from each paediatric transplant patients over a period of 12 months. Antibiotic susceptibility testing will be performed for the isolated bacteria in order to determine the presence of resistance. qPCR will then be performed directly on the samples in order to determine the load of epidemiologically relevant resistant genes over time. Multi-locus sequence typing will also be performed for determining the clonality of the isolates encountered in this study, allowing us to trace the origin and dissemination of resistant bacteria. Finally, statistical analyses will be performed in order to determine associations between clinical interventions, the load of resistance genes, and clinical outcomes.

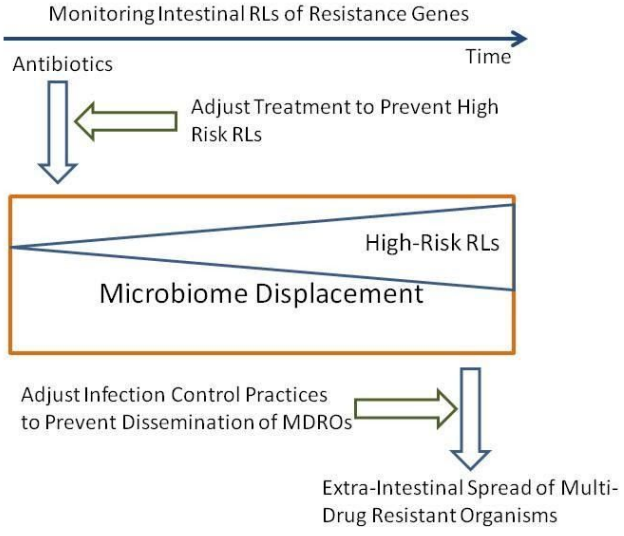
The main expected results are determining a threshold of resistance genes after which adverse clinical outcomes would be expected, and determining how clinical interventions are affecting the load of resistance genes and the microbiome.

What have done so far,

Throughout the project, we used rectal swabs that are routinely obtained from paediatric patients (434 swabs in total) in order to quantify the total amount of bacteria in the gut using qPCR directed towards the universal *16SrDNA* gene that is present in all bacteria. At the same time, qPCRs directed towards the genes of resistance endemic at the La Paz University Hospital were performed. These are the $bla_{\text{CTX-M-1-Family}}$ and $bla_{\text{OXA-1}}$ genes that are able to break down β-lactam antibiotics, and $bla_{\text{OXA-48}}$ and bla_{VIM} that are able to break down carbapenems which are used as last-line antibiotics in many cases. The intestinal Relative Loads (RLs) of these genes were normalized to the total bacterial load using the $\Delta\Delta C_t$ method.

The RLs determined for bla_{OXA-48} and bla_{VIM} were significantly higher in rectal swabs obtained from Transplant (Tx) patients as compared to those from Non-Transplant (non-Tx) patients ($P < 0.05$). The highest RLs were obtained from the Liver Tx ward, where large amounts of antibiotics are given to the patients, as per the transplantation protocols. The most common organism detected in the rectal swabs that harboured these resistance genes was *Klebsiella pneumoniae* (68.5%), followed by *Enterobacter cloacae* (8.3%), *Escherichia coli* (6.7%), *Klebsiella oxytoca* (5.9%), and several other organisms that were detected in less than 5% of the rectal swabs.

The hospital's records were screened in order to determine whether the same organism detected in the rectal swab has been isolated from a different clinically significant extra-intestinal site within 5 days of detecting the organism intra-intestinally. In total, 33.3% (n = 17) of the Tx patients and 21.4% (n = 15) of the non-Tx patients have had the same resistance genes and organism detected in the rectal swab and in an extra-intestinal clinically relevant sample. Of these extra-intestinal isolates, 68.42% were deemed to be infections while 31.58% were considered colonisations. Extra-intestinal *K. pneumoniae* isolates were recovered from the hospital's bacterial collection and clonality analysis was performed. This analysis showed that in 12 out of 13 cases, the same clone was detected intra- and extra-intestinally, demonstrating how the same organism was able to move outside of the intestinal environment.



The RLs of the intestinal resistance genes within 5 days of the detection of the extra-intestinal isolates ranged from 1.2% to 6.9% of the total intestinal bacterial population, depending on the specific gene. Since *Enterobacteriaceae* normally form less than 1% of the total intestinal bacterial population, this means that even a slight disequilibrium in this population can lead to the spread of multidrug resistant organisms (MDROs) to extra-intestinal sights. Receiver Operating Characteristic (ROC) analysis was used in order to divide the RLs into low-risk and high-risk groups for having extra-intestinal spread of resistant organisms. Antibiotic consumption data of paediatric Tx patients was also collected and it showed a significant correlation between consumption of non-carbapenem β-lactams in the previous 3 months and the high-risk groups for extra-intestinal spread of bacteria harbouring the $bla_{\text{CTX-M-1-Family}}$ and $bla_{\text{OXA-1}}$ genes ($p < 0.05$). Moreover, when plotting the antibiotic consumption in relation to the RLs detected for each patient, a clear relation was observed between the maintenance of high intestinal RLs and antibiotic consumption.

Taken together, our data demonstrates the usefulness of the tool developed through qMAR that allows for the tracking of the intestinal RLs of resistance genes over time. This, in turn, can be predictive of extra-intestinal spread of MDROs that can cause infections and/or play a role in transmission. Moreover, the fact that consuming certain antibiotics maintains the RLs in the high risk group allows for healthcare workers and infection control personnel to take pre-emptive measures to minimize the risk on the patient and the spread of MDROs in the hospital. Finally, tracking the RLs over time using an easy to implement, fast, and accurate tool allows for the personalization of treatment for each patient that will improve on patient outcome and prove beneficial to them, and their families.

How we have disseminated our results

Several activities have been performed where various parts of the qMAR project were progressively disseminated. These activities include:

- Five seminars given to researchers and healthcare professionals at the La Paz University Hospital
- Three public outreach events where the qMAR project was introduced
- Poster presented at the SEIMC National Conference
- qMAR presented at the REA Cluster Meeting and Monitoring Mission

- Abstract published in the ECCMID 2020 abstract book
- A project-special section in the IdiPAZ website was created
- A press release introducing the project and the researcher was made by IdiPAZ
- The hashtag #qMAR in conjunction with @EU_Commission @MSCActions, @IdipazScience, and @Micro_LaPaz has been used extensively on Twitter throughout qMAR's duration to announce the various seminars given and to promote the project
- The project has been posted on the researcher's Linked-In profile
- The qMAR project was featured on the Innovation Radar and marked as a useful Tool that stakeholders in the diagnostics field might wish to exploit

Science is coming

The following manuscripts have been prepared and are currently under review by the co-authors:

- Quantifying the Intestinal Load of Genes of Antibiotic Resistance among two Paediatric Patient Populations: Not all "Positives" are Equal
- The effect of Antibiotic Consumption on the Intestinal Loads of Resistance Genes and Extraintestinal Dissemination of Multidrug Resistant Organisms among Paediatric Liver Transplant Patients
- Relative Quantification of the Intestinal Loads of *Serratia* spp. among Neonates during an Outbreak

Finally, the results have already been exploited by several groups of the La Paz University Hospital where the data generated from qMAR was used in order to discuss infection control practices among the paediatric liver Tx patients. Further exploitation of these results is expected once the publications pass the peer-review process and are published in open-access journals.

The impact of qMAR

The main innovation of the qMAR project is using pre-existing technology in order to develop a tool for tracking the intestinal RLs of resistance genes over time. This tool is able to incorporate any number of resistance genes that are endemic in any hospital all over the world, is easy to implement, cheap, and provides information rapidly. Moreover, the link demonstrated between the effect of antibiotic consumption, the increase in RLs, and the dissemination of MDROs to extraintestinal sites adds to the innovative aspect of the project

where tracking the RLs over time can give valuable information for healthcare personnel to react in real-time in order to minimize adverse effects and the spread of MDROs. This innovative process has been demonstrated in the flexibility of its application to various settings where we were able to use its rationale in tracking an outbreak caused by *Serratia marcescens* in the hospital's neonatal unit, and apply it in order to normalize the C_t values obtained for the SARS-CoV-2 RT-PCR testing during the COVID-19 pandemic.

qMAR's tool can create a new market for diagnostic kits that are designed in such a way as to track the relative load of resistance genes instead of simply detecting their presence or absence. Moreover, it has an impact on policy makers where they could use it in order to better assess infection control measures and patient outcome. Also, since all the data will be published in open-source journals, this project could have a similar effect on policy-makers all over the world. In order to further the usefulness and the rapid implementation of the tool developed for qMAR, the process has been validated, extensively tested, and evaluated with healthcare professionals at the La Paz University Hospital in order to make it as close as possible to be readily available and market-ready. On a societal level, the tool developed in qMAR can be used in order to improve upon patient outcomes, and directly affect their own quality of life and that of their families.

How the Marie Skłowdowska-Curie Actions has helped the fellow to find his path

This project has also greatly impacted the researcher's career where he was able to receive hands-on training in cutting-edge technologies and widen his skill-set. Moreover, he was rewarded a 3-year contract through the Spanish Juan de La Cierva call at the same institution where qMAR was performed. He also was able to forge collaborations that resulted in him obtaining the JPI-AMR Network 2020 grant as a principal investigator and collaborate on two national Spanish research projects. Finally, the researcher himself was able to impact the hospital where this work was done through the various seminars and meetings that were performed, and the collaborations that he forged.

To stay tuned about qMAR news follow Elie Dahdouh in Twitter