Main_Figure_2

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2023-06-13

\#Load required R packages

```r
#Load packages
if(!require("pacman")) install.packages("pacman")
pacman::p_load(pacman, ggplot2, tidyverse, tidyr, lubridate, data.table, tsibble, wesanderson, reticula
```

\#Load data

```r
##Master data cross-sectional####
Master <-read.csv("../Data/R_input_files//Clinical_AMR_Microbiome_R2.csv") %>%
  as_tibble()
Master$FEVfactor<-cut(Master$FEV1, breaks=c(0, 30, 50, 70, Inf))

###wrangle AMR data ####
AMRFam <- Master %>% #clinical variables + amr families
  as_tibble() %>%
  select(-29:-42,-64:-356)
AMRFam$FEVfactor<-cut(AMRFam$FEV1,  breaks=c(0, 30, 50, 70, Inf))
#set levels
AMRFam$ExacerbatorState <- factor(AMRFam$ExacerbatorState, levels=c("NonEx", "Exacerbator", "FreqEx"))
AMRFam$Country <- factor(AMRFam$Country, levels=c("SG", "KL", "DD", "MI"))
AMRFam$Aetiology_short <- factor(AMRFam$Aetiology_short, levels=c("idiopathic", "postInfect", "postTB",
AMRFam$SampleID <- factor(AMRFam$SampleID, levels = AMRFam$SampleID[order(AMRFam$SC_AMR_alt)])
AMRFam$FEVfactor<-fct_rev(AMRFam$FEVfactor)
AMRFam <- AMRFam %>%
  gather(Resistome, RPKM, starts_with(c("Acridine.dye", "Aminocoumarin.antibiotic", "Aminoglycoside", "A

AMRFam$CTRL<-ifelse(is.na(AMRFam$BSI), "CTRL", "PATIENT")
```

\#Figure 2

```r
##1. Colour scheme AMR.
AMR.col.scheme=c("#026EB8","#06A955","#5D2E83","#2A2A73","#fc8403","#EBA5F3","#fc5017","#5CA5DB","#db690

##1. Exacerbation_Resistome###
Exacerbation_Resistome<-ggplot(data=AMRFam[which(is.na(AMRFam$Severity) == FALSE),],aes(x=ExacerbatorSta
  geom_bar(aes(), stat="identity", position = "fill") +
  scale_fill_manual(values = AMR.col.scheme)+
  scale_y_continuous(labels = scales::percent)+
  scale_x_discrete(labels = c('N.Ex (0)','Ex (1-2)', 'F.Ex (3+)'))+
  theme(legend.position="right",
        #axis.text=element_blank(),
        axis.title=element_blank(),
        #axis.title=element_text(size=14),
        #axis.text.x = element_blank(),
```

```r
      #axis.text.x = element_text(angle = 90),
      panel.background = element_rect(fill = NA),
      axis.line = element_line(size = 0.5, colour = "black"),
      legend.title = element_blank(),
      legend.text = element_text(face = "italic"))+
  guides(fill=guide_legend(nrow=2), size = .1)+
  xlab("")+
  ylab("Relative abundance (%)")+
  #facet_wrap(~BphageFamMT$Continent, scales="free_x")+
  theme(
    strip.background = element_rect(
      color="white", fill="white", size=1, linetype="solid"),
    strip.text.x = element_text(size = 12)
  )


##1. FEv1###
AMRFamMTfev<-subset(AMRFam, FEVfactor != "NA")
FEV1amr<-ggplot(data=AMRFamMTfev,aes(x=FEVfactor, y=RPKM, fill=Resistome))+
  geom_bar(aes(), stat="identity", position = "fill") +
  scale_fill_manual(values = AMR.col.scheme)+
  scale_y_continuous(labels = scales::percent)+
  scale_x_discrete(labels = c('>70%','50-70%','30-50%','<30%'))+
  theme(legend.position="right",
        #axis.text=element_blank(),
        axis.title=element_blank(),
        #axis.title=element_text(size=14),
        #axis.text.x = element_blank(),
        #axis.text.x = element_text(angle = 90),
        panel.background = element_rect(fill = NA),
        axis.line = element_line(size = 0.5, colour = "black"),
        legend.title = element_blank(),
        legend.text = element_text(face = "italic"))+
  guides(fill=guide_legend(nrow=2), size = .1)+
  xlab("")+
  ylab("Relative abundance (%)")+
  #facet_wrap()+
  theme(
    strip.background = element_rect(
      color="white", fill="white", size=1, linetype="solid"),
    strip.text.x = element_text(size = 12)
  )


##4. Severity_Resistome####
Severity_Resistome<-ggplot(data=AMRFam[which(is.na(AMRFam$Severity) == FALSE),],aes(x=Severity, y=RPKM,
  geom_bar(aes(), stat="identity", position = "fill") +
  scale_fill_manual(values = AMR.col.scheme)+
  scale_y_continuous(labels = scales::percent)+
  theme(legend.position="right",
        #axis.text=element_blank(),
        axis.title=element_blank(),
        #axis.title=element_text(size=14),
        #axis.text.x = element_blank(),
        #axis.text.x = element_text(angle = 90),
```
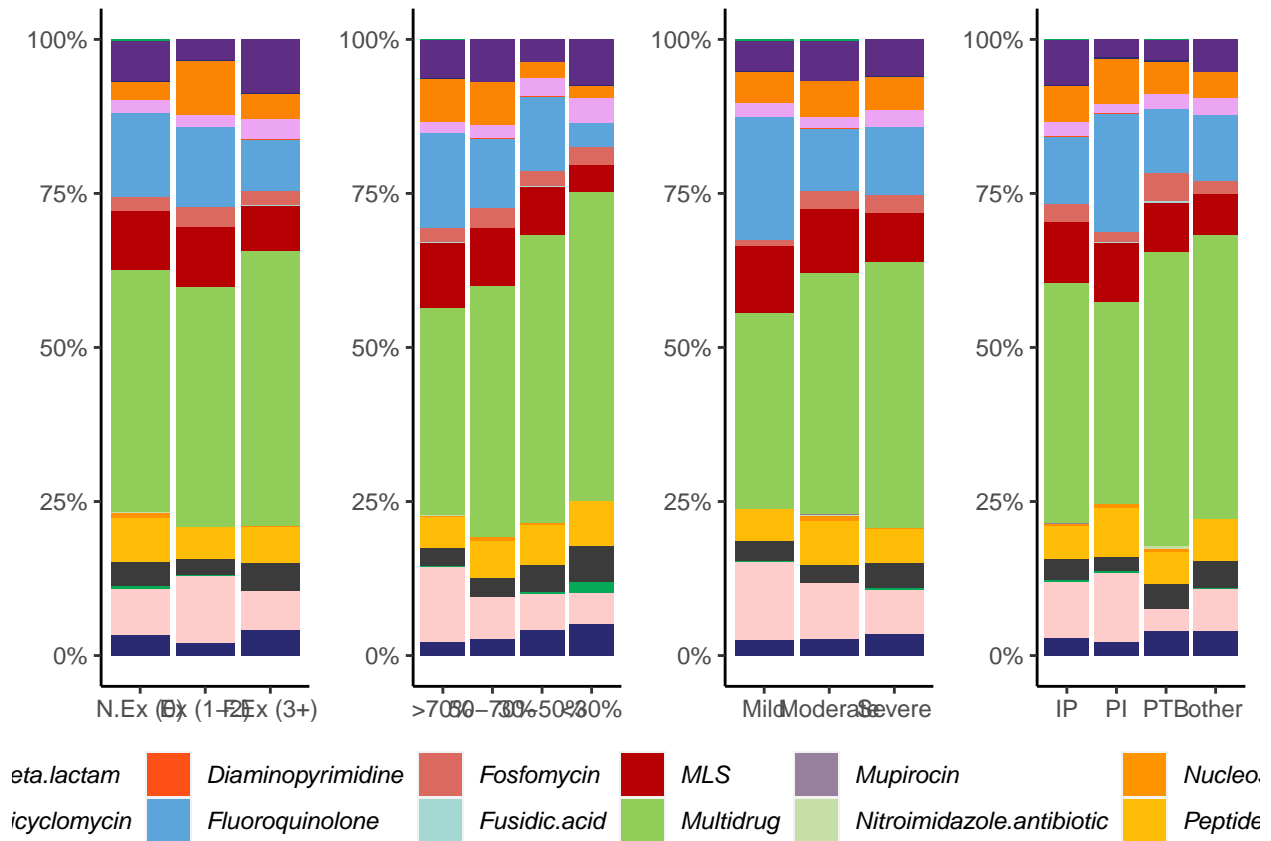
```r
      panel.background = element_rect(fill = NA),
      axis.line = element_line(size = 0.5, colour = "black"),
      legend.title = element_blank(),
      legend.text = element_text(face = "italic"))+
  guides(fill=guide_legend(nrow=2), size = .1)+
  xlab("")+
  ylab("Relative abundance (%)")+
  #facet_wrap(~BphageFamMT$Continent, scales="free_x")+
  theme(
    strip.background = element_rect(
      color="white", fill="white", size=1, linetype="solid"),
    strip.text.x = element_text(size = 12)
  )


##4. Aetiology_Resistome ####
Aetiology_Resistome_Fig3<-ggplot(data=AMRFam[which(is.na(AMRFam$Severity) == FALSE),],aes(x=Aetiology_s
  geom_bar(aes(), stat="identity", position = "fill") +
  scale_fill_manual(values = AMR.col.scheme)+
  scale_y_continuous(labels = scales::percent)+
  scale_x_discrete(labels = c('IP','PI', 'PTB', "other"))+
  theme(legend.position="none",
        #axis.text=element_blank(),
        axis.title=element_blank(),
        #axis.title=element_text(size=14),
        #axis.text.x = element_blank(),
        #axis.text.x = element_text(angle = 90),
        panel.background = element_rect(fill = NA),
        axis.line = element_line(size = 0.5, colour = "black"),
        legend.title = element_blank(),
        legend.text = element_text(face = "italic"))+
  guides(fill=guide_legend(nrow=2), size = .1)+
  xlab("")+
  ylab("Relative abundance (%)")+
  #facet_grid(~AMRFam$Country, scales="free_x")+
  theme(
    strip.background = element_rect(
      color="white", fill="white", size=1, linetype="solid"),
    strip.text.x = element_text(size = 12)
  )
Figure_2<-ggarrange(#Geography_Resistome,
  Exacerbation_Resistome,
  FEV1amr,
  Severity_Resistome,
  Aetiology_Resistome_Fig3,
  #Microbiology_Resistome,
  #Antibiotics_Resistome,
  font.label = list(size = 5),
  common.legend = TRUE, legend = "bottom", nrow = 1 )
Figure_2
```

Legend:
- ...eta.lactam
- Diaminopyrimidine
- Fosfomycin
- MLS
- Mupirocin
- Nucleo...
- ...icyclomycin
- Fluoroquinolone
- Fusidic.acid
- Multidrug
- Nitroimidazole.antibiotic
- Peptide...

```r
#Kruskal-Wallis analysis with FDR correction.
#unmatched n=251
cohort<-subset(Master, is.na(Master$SC_AMR_alt) == FALSE & Matching == "Matched")
cohort<-Master
cohort$Disease_state<-ifelse(cohort$Country == "CTRL", "CTRL", "BE")

#FEVfactor
df2 <- cohort %>%
  select(5,43:63,357) %>%
  gather(key, value, -Disease_state) %>%
  group_by(key) %>%
  do(tidy(kruskal.test(x= .$value, g = .$Disease_state)))
df_Disease_state <- df2[df2$key %in% colnames(cohort[43:63]), ]
df_Disease_state$FDR<-p.adjust(df_Disease_state$p.value, n=21, method = "fdr")

#FEVfactor
df2 <- cohort %>%
  select(1:28,43:63,356) %>%
  gather(key, value, -FEVfactor) %>%
  group_by(key) %>%
  do(tidy(kruskal.test(x= .$value, g = .$FEVfactor)))
df_FEV1 <- df2[df2$key %in% colnames(cohort[43:63]), ]
df_FEV1$FDR<-p.adjust(df_FEV1$p.value, n=21, method = "fdr")

#exacerbations
df2 <- cohort %>%
```

```r
  select(1:28,43:63,356) %>%
  gather(key, value, -ExacerbatorState) %>%
  group_by(key) %>%
  do(tidy(kruskal.test(x= .$value, g = .$ExacerbatorState)))
df_ExacerbatorState <- df2[df2$key %in% colnames(cohort[43:63]), ]
df_ExacerbatorState$FDR<-p.adjust(df_ExacerbatorState$p.value, n=21, method = "fdr")

#severity
df2 <- cohort %>%
  select(1:28,43:63,356) %>%
  gather(key, value, -Severity) %>%
  group_by(key) %>%
  do(tidy(kruskal.test(x= .$value, g = .$Severity)))
df_Severity <- df2[df2$key %in% colnames(cohort[43:63]), ]
df_Severity$FDR<-p.adjust(df_Severity$p.value, n=21, method = "fdr")

#Aetiology
df2 <- cohort %>%
  select(1:28,43:63,356) %>%
  gather(key, value, -Aetiology_short) %>%
  group_by(key) %>%
  do(tidy(kruskal.test(x= .$value, g = .$Aetiology_short)))
df_Aetiology_short <- df2[df2$key %in% colnames(cohort[43:63]), ]
df_Aetiology_short$FDR<-p.adjust(df_Aetiology_short$p.value, n=21, method = "fdr")

#Country
df2 <- cohort %>%
  select(1:28,43:63,356) %>%
  gather(key, value, -Country) %>%
  group_by(key) %>%
  do(tidy(kruskal.test(x= .$value, g = .$Country)))
df_Country <- df2[df2$key %in% colnames(cohort[43:63]), ]
df_Country$FDR<-p.adjust(df_Country$p.value, n=21, method = "fdr")
```