Global surveillance of antimicrobial resistance through global sewage

Pimlapas Leekitcharoenphon (Shinny)
Research Group for Genomic Epidemiology
DTU Food
Technical University of Denmark (DTU)

pile@food.dtu.dk
@ShinnyPimlapas

Global surveillance of AMR

Can human sewage be used to detect and combined with modelling explain global emergence and trends in AMR ?

Sample collection - 2016

79 samples from 60 countries have been collected and analysed

https://cge.cbs.dtu.dk/services/ResFinder/

Center for Genomic Epidemiology

Username
Password
New Reset Login

Home | Services | Instructions | Output | Overview of genes | Article abstract

ResFinder 3.0

ResFinder identifies acquired antimicrobial resistance genes and/or find chromosomal mutations in total or partial sequenced isolates of bacteria.

View the version history of this server.

The database is curated by:
Valeria Bortolaia
(click to contact)

Chromosomal point mutations ☐

Acquired antimicrobial resistance genes ☐

Select type of your reads

Assembled Genome/Contigs*

If you get an "Access forbidden. Error 403": Make sure the start of the web adress is https and not just http. Fix it by clicking here.

Isolate File

Name	Size	Progress	Status

Upload Remove

MGmapper: Reference based mapping and taxonomy annotation of metagenomics sequence reads

Thomas Nordahl Petersen[1]*, Oksana Lukjancenko[2], Martin Christen Frølund Thomsen[1], Maria Maddalena Sperotto[1], Ole Lund[1], Frank Møller Aarestrup[2], Thomas Sicheritz-Pontén[1]*

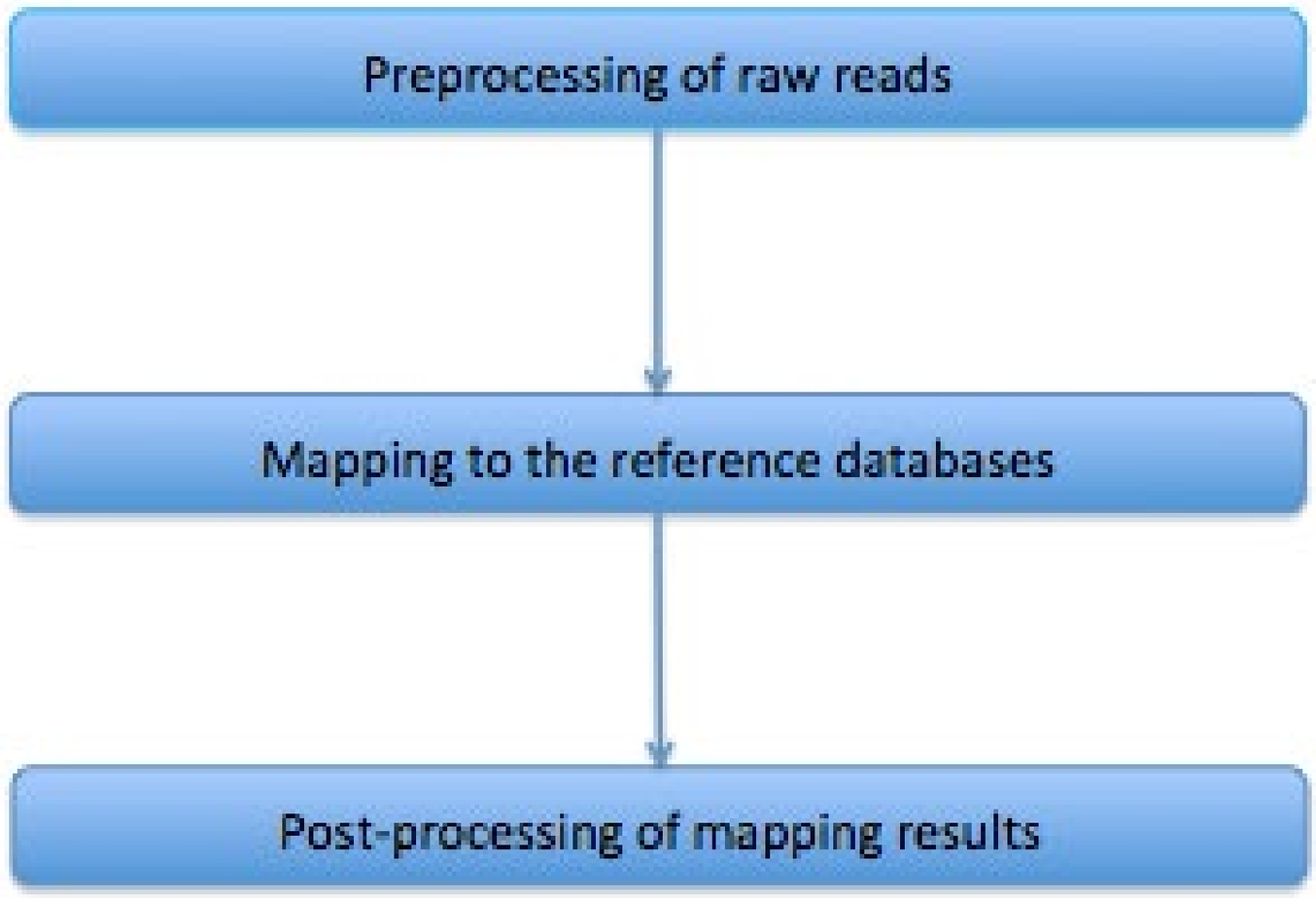
1 Department of Bio and Health Informatics, Technical University of Denmark, Kongens Lyngby, Denmark,
2 National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark

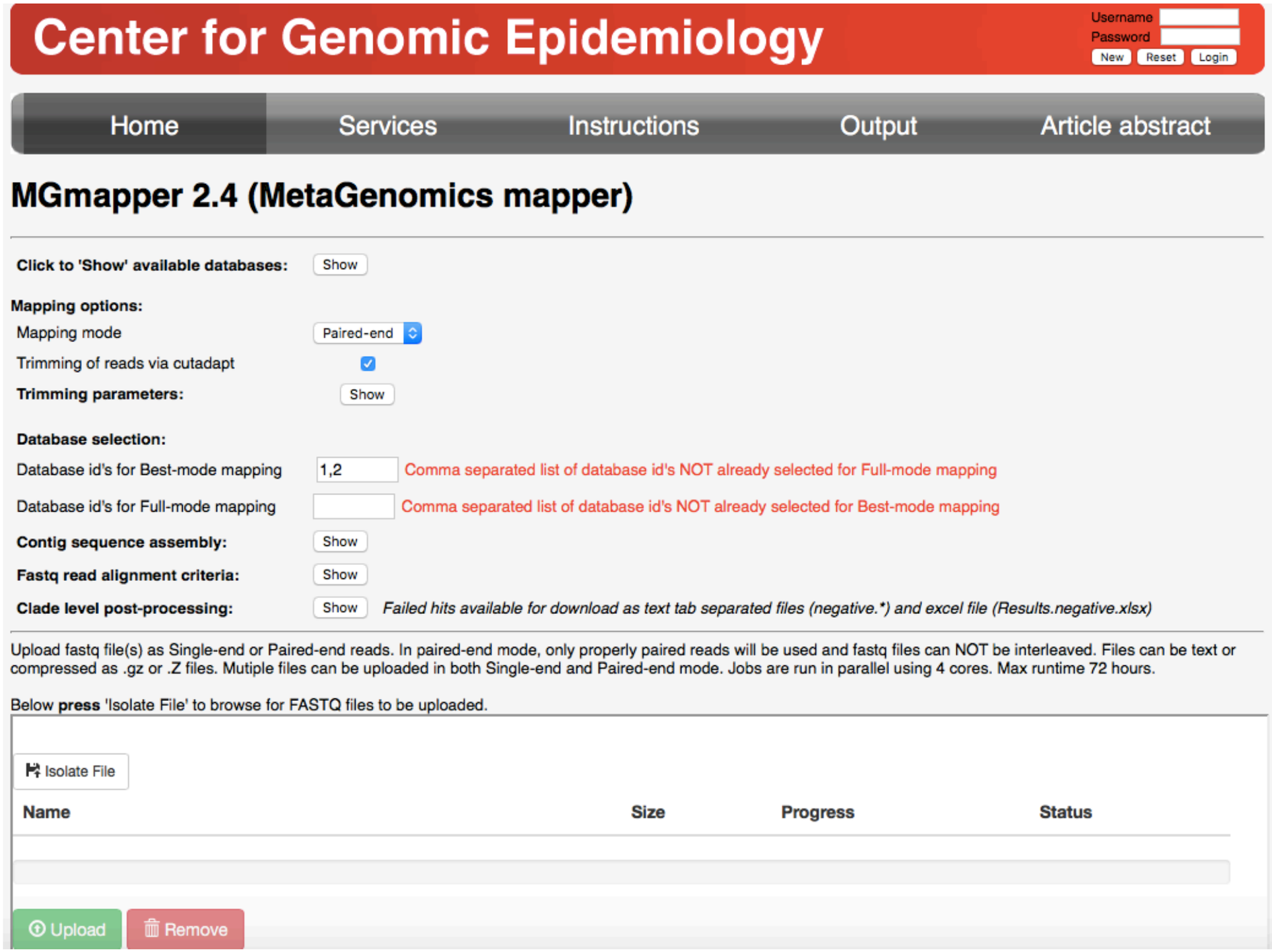
* tnp@cbs.dtu.dk (TNP); thomas@cbs.dtu.dk (TSP)

Abstract

An increasing amount of species and gene identification studies rely on the use of next generation sequence analysis of either single isolate or metagenomics samples. Several methods are available to perform taxonomic annotations and a previous metagenomics benchmark study has shown that a vast number of false positive species annotations are a problem unless thresholds or post-processing are applied to differentiate between correct and false annotations. MGmapper is a package to process raw next generation sequence data and perform reference based sequence assignment, followed by a post-processing analysis to produce reliable taxonomy annotation at species and strain level resolution. An in-vitro bacterial mock community sample comprised of 8 genuses, 11 species and 12 strains was previously used to benchmark metagenomics classification methods. After applying a post-processing filter, we obtained 100% correct taxonomy assignments at species and genus level. A sensitivity and precision at 75% was obtained for strain level annotations. A comparison between MGmapper and Kraken at species level, shows MGmapper assigns taxonomy at species level using 84.8% of the sequence reads, compared to 70.5% for Kraken and both methods identified all species with no false positives. Extensive read count statistics are provided in plain text and excel sheets for both rejected and accepted taxonomy annotations. The use of custom databases is possible for the command-line version of MGmapper, and the complete pipeline is freely available as a bitbucked package (https://bitbucket.org/genomicepidemiology/mgmapper). A web-version (https://cge.cbs.dtu.dk/services/MGmapper) provides the basic functionality for analysis of small fastq datasets.

MGmapper


Preprocessing of raw reads
Mapping to the reference databases
Post-processing of mapping results



https://cge.cbs.dtu.dk/services/MGmapper/

https://bitbucket.org/genomicepidemiology/mgmapper

https://www.coursera.org/learn/metagenomics

mapping AMR result

Gene	Description	GeneSize	ALB.17	AUS.18	AUS.18a	AUT.70
16S_rRNA_methyltransferase|	gentamicin	732	0	0	0	0
16S_rRNA_methyltransferase|	gentamicin	1263	0	0	0	0
16S_rRNA_methyltransferase|	gentamicin	765	0	0	0	0
16S_rRNA_methyltransferase|	gentamicin	765	0	0	0	0
16S_rRNA_methyltransferase|	gentamicin	804	0	0	0	0
16S_rRNA_methyltransferase|	gentamicin	804	0	0	0	0
16S_rRNA_methyltransferase|	gentamicin	765	0	0	0	0
AAC|GQ343136.1|	gentamicin	867	50	2	2	6
AAC|GQ343186.1|	sisomycin	429	0	0	0	0
AAC|KJ695106_1|	gentamicin	501	0	0	0	0
beta_lactamase|GQ342998.1|	amikacin	891	0	0	0	0
beta_lactamase|GQ342999.1|	amikacin	891	0	0	0	0
beta_lactamase|GQ343000.1|	amikacin	906	0	0	0	0
beta_lactamase|GQ343002.1|	amikacin	873	0	0	0	0
beta_lactamase|GQ343003.1|	amikacin	891	0	0	0	6
beta_lactamase|GQ343008.1|	amikacin	1263	2	0	0	0
beta_lactamase|GQ343010.1|	amikacin	1167	0	0	0	0
beta_lactamase|GQ343015.1|	amikacin	1263	0	0	0	6
beta_lactamase|GQ343019.1|	amikacin	891	0	0	0	0

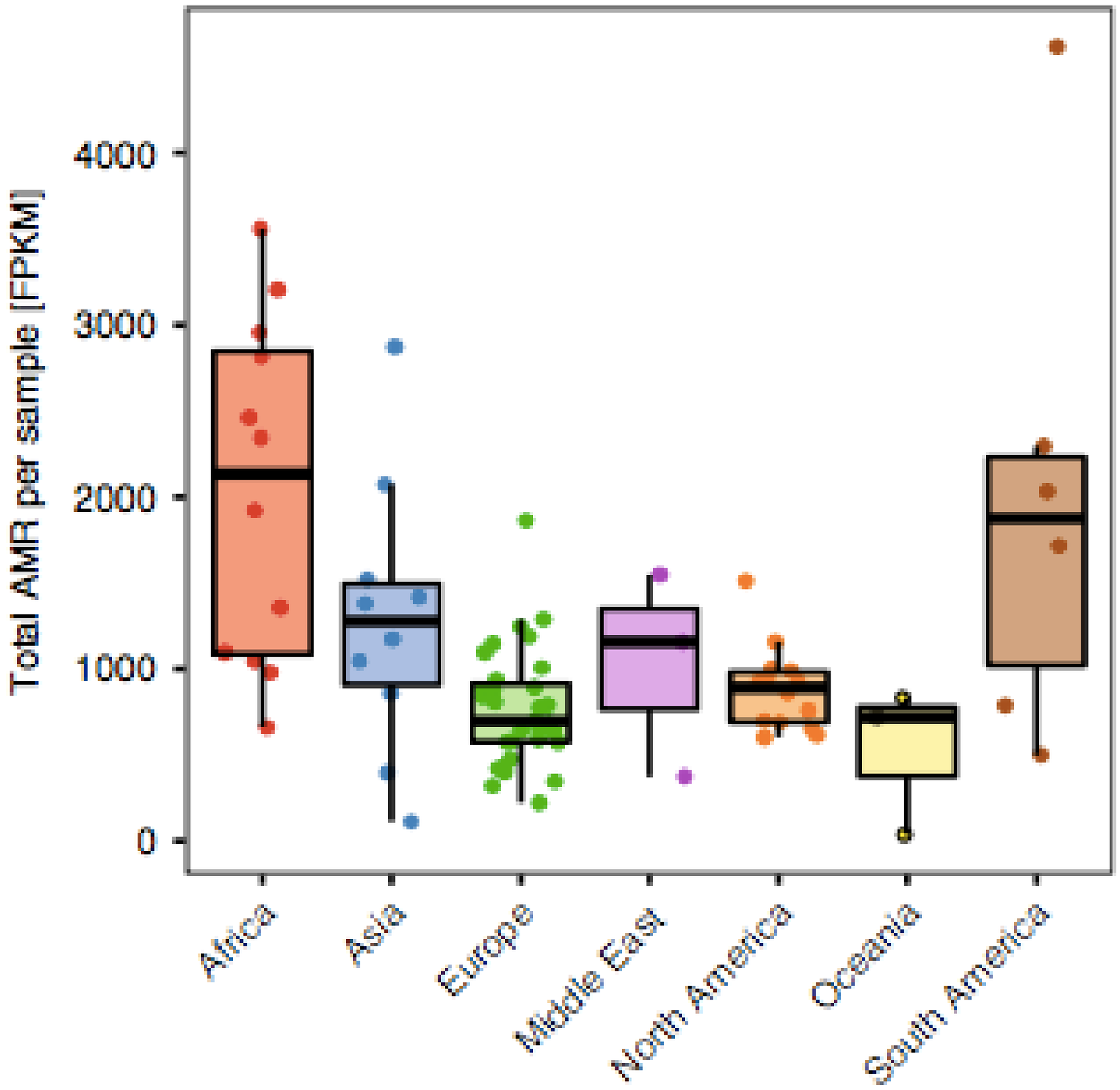
Sample	ReadCount_Bacteria
ALB.17	29715190
AUS.18	11724196
AUS.18a	3778910
AUT.70	15734026
BGR.66	19108694
BRA.53	17108324
BRA.53a	7733328
BWA.19	6599146
CAN.22	1423112
CAN.22a	868336
CAN.22b	11949640
CAN.22c	21282768

FPKM

Fragments Per Kilobase reference per Million bacterial fragments

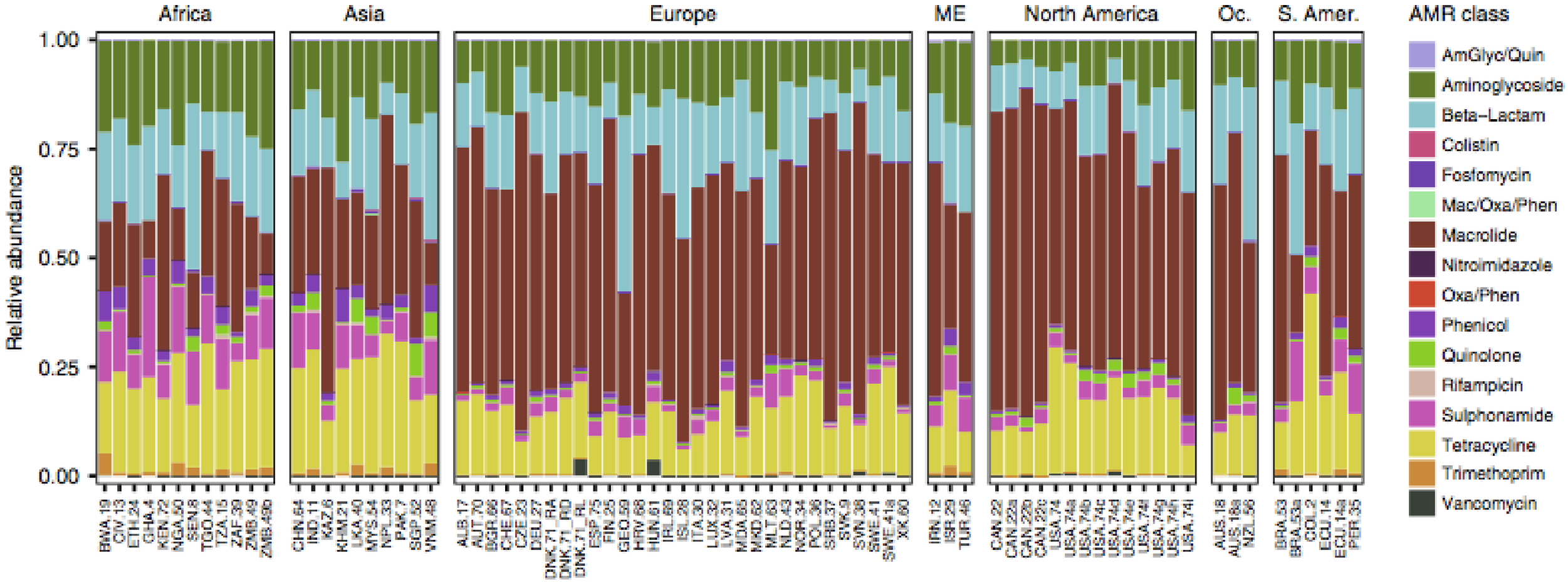
Total FPKM

Fragments Per Kilobase reference per Million bacterial fragments



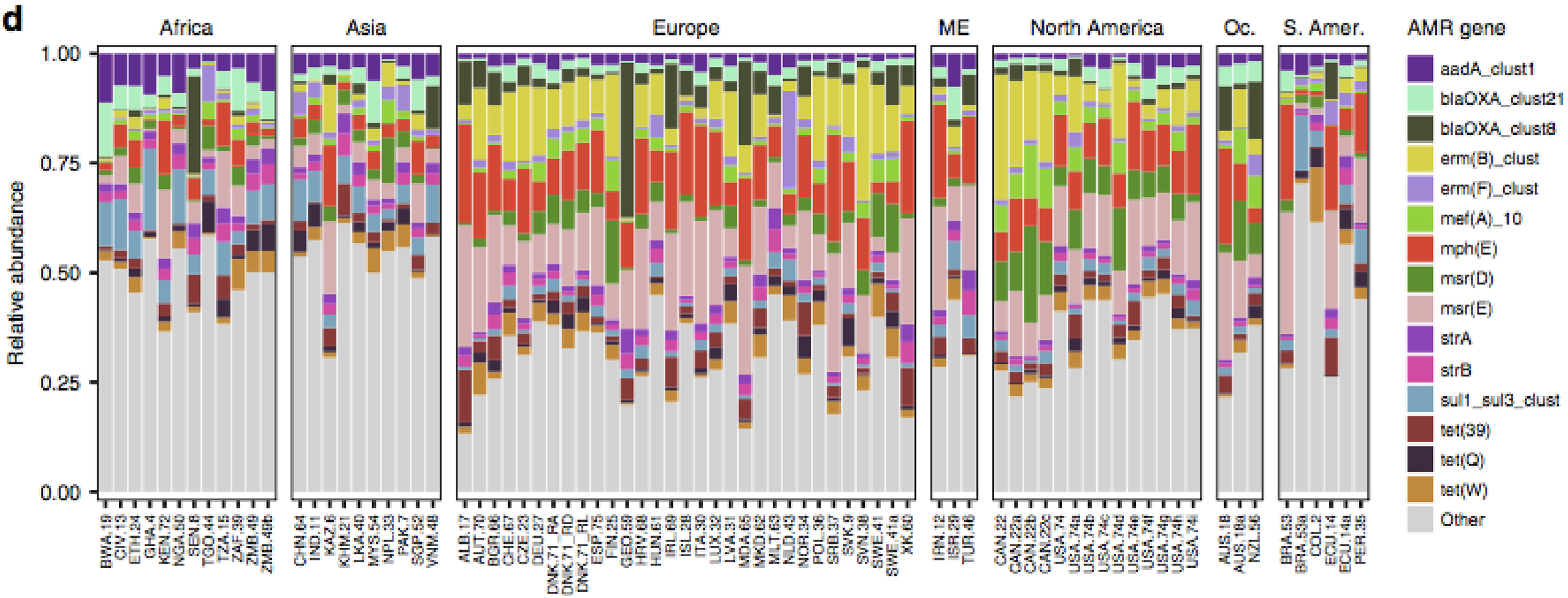
Hendriksen RS, et al. Nature Communications, 2019

AMR classes



Hendriksen RS, et al. Nature Communications, 2019

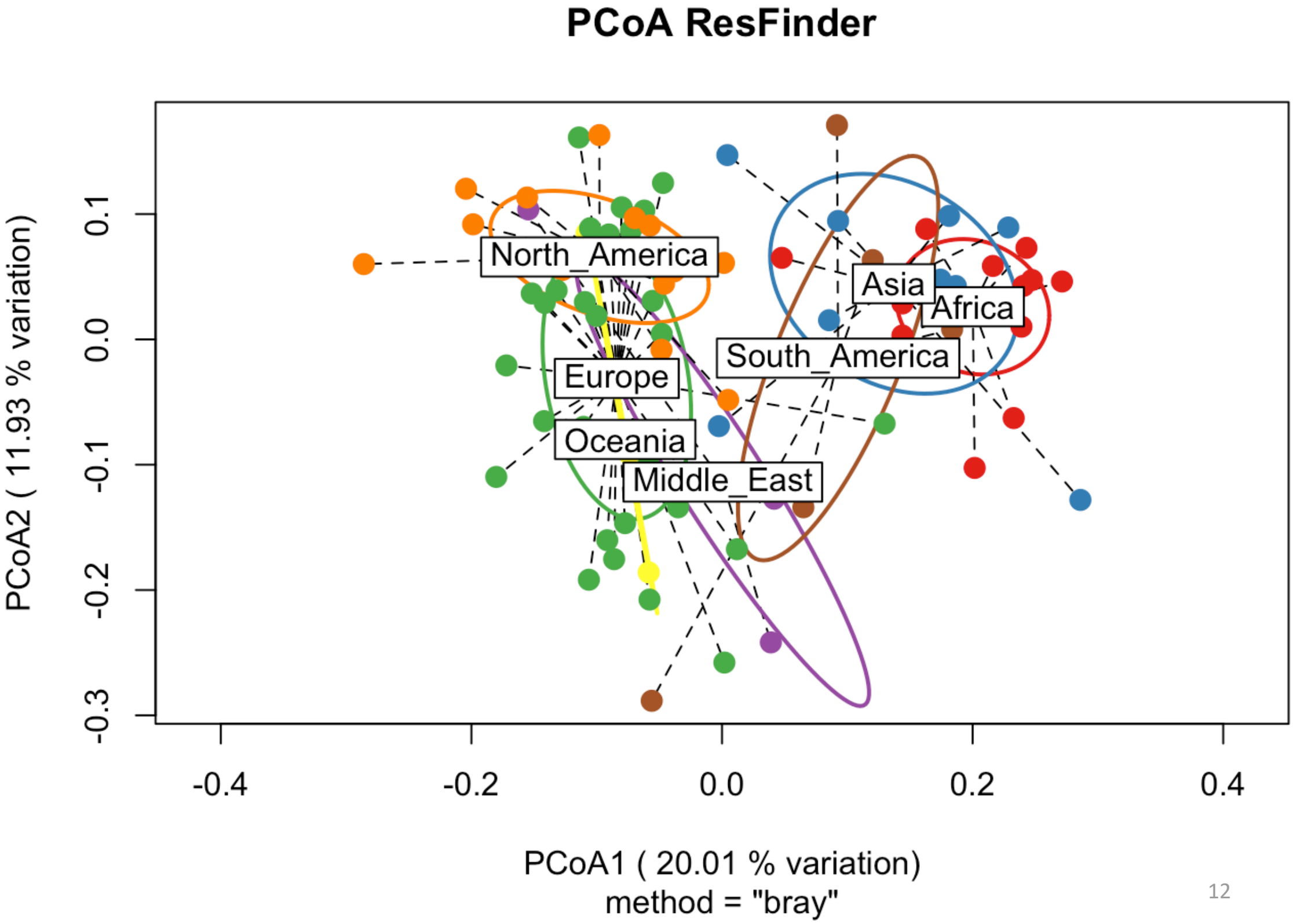
AMR genes



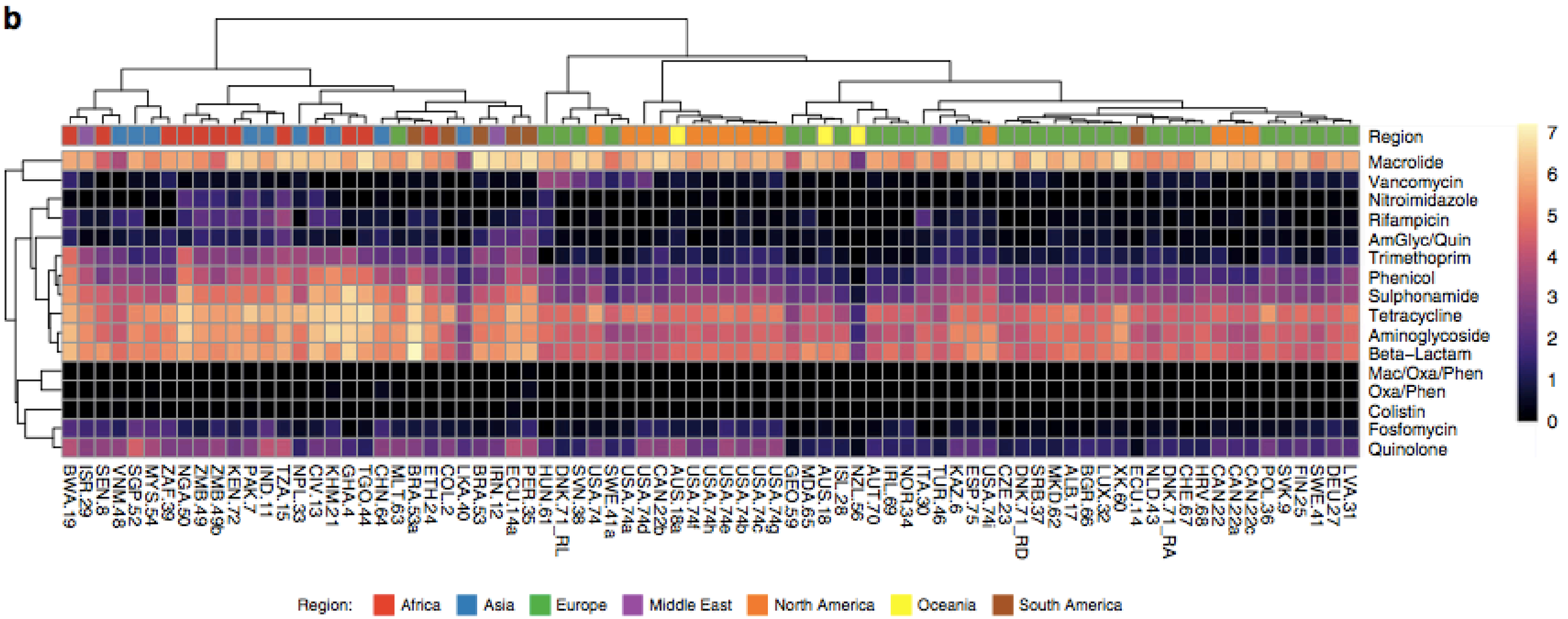
Hendriksen RS, et al. Nature Communications, 2019

Resistome clustering in sewage across regions

Hellinger-transformed (decostand function in vegan package) and Bray-Curtis dissimilarity



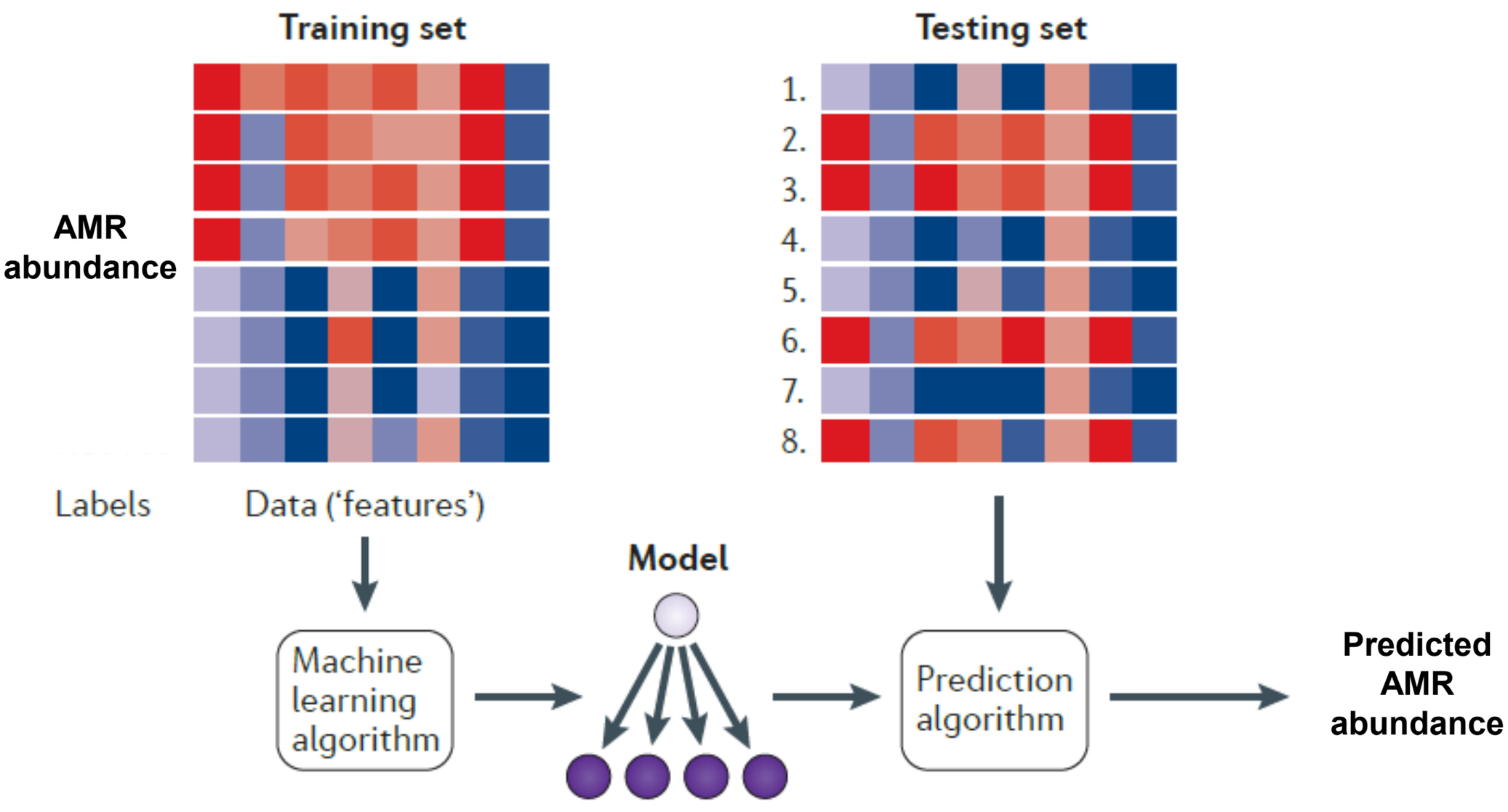
AMR class


b
Region
Macrolide
Vancomycin
Nitroimidazole
Rifampicin
AmGlyc/Quin
Trimethoprim
Phenicol
Sulphonamide
Tetracycline
Aminoglycoside
Beta−Lactam
Mac/Oxa/Phen
Oxa/Phen
Colistin
Fosfomycin
Quinolone
7
6
5
4
3
2
1
0
BWA.19
ISR.29
SEN.8
VNM.48
SGP.52
MYS.54
ZAF.39
NGA.50
ZMB.49b
ZMB.49
KEN.72
PAK.7
IND.11
TZA.15
NPL.33
CIV.13
KHM21
GHA.4
TGO.44
CHN.64
MLT.63
BRA.53a
ETH24
COL2
LKA.40
BRA.53
IRN.12
ECU.14a
PER.35
HUN.61
DNK.71_RL
SVN.38
USA.74
SWE.41a
USA.74d
USA.74a
CAN.22b
AUS.18a
USA.74f
USA.74h
USA.74e
USA.74b
USA.74c
USA.74g
GEO.59
MDA.65
AUS.18
ISL.28
NZL.56
AUT.70
IRL.69
NOR.34
ITA.30
TUR.46
KAZ.6
ESP.75
USA.74i
CZE.23
DNK.71_RD
SRB.37
MKD.62
ALB.17
BGR.66
LUX.32
XK.60
ECU.14
NLD.43
DNK.71_RA
CHE.67
HRV.68
CAN.22
CAN.22a
CAN.22c
POL.36
SVK.9
FIN.25
SWE.41
DEU.27
LVA.31
Region:
Africa
Asia
Europe
Middle East
North America
Oceania
South America

Drivers for AMR

Factor
Temperature
Flight connections
Antimicrobial use
Human development index

Predict AMR level using socio-economic data



Deeper look into socio-economic data from World bank

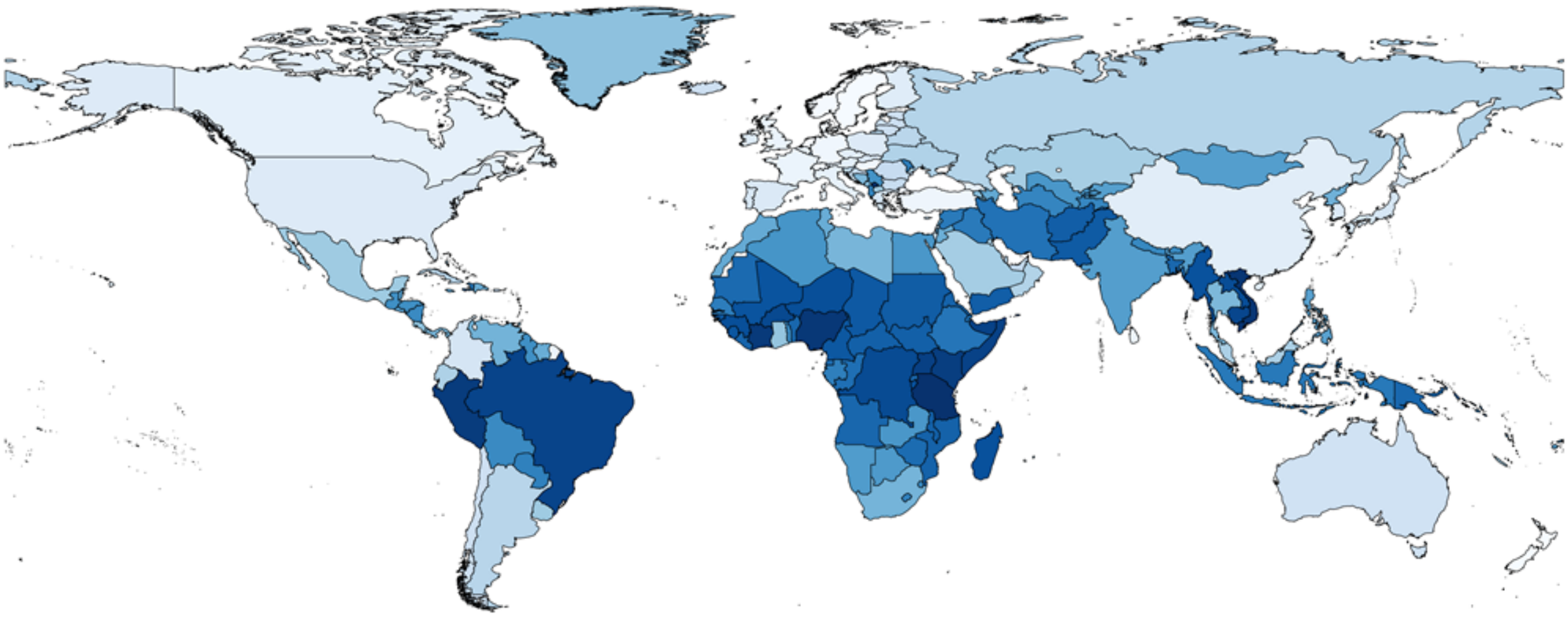
Predictors of higher AMR

- Mortality rate
- Death, by communicable diseases and maternal, prenatal and nutrition conditions
- Risk of maternal death
- Open defecation
- Diarrhoea prevalence in children
- Risk of impoverishing expenditure for surgical care
- Informal employment
- Time to import

Predictors of lower AMR

- Investment in water and sanitation
- Completeness of death reporting
- Educational attainment
- Number of surgical procedures
- Life expectancy at birth
- Number of Physicians
- Births attended by skilled health staff
- Grace period on external debt

Global resistance prediction



Hendriksen RS, et al. Nature Communications, 2019

Samples

Pilot (2016)

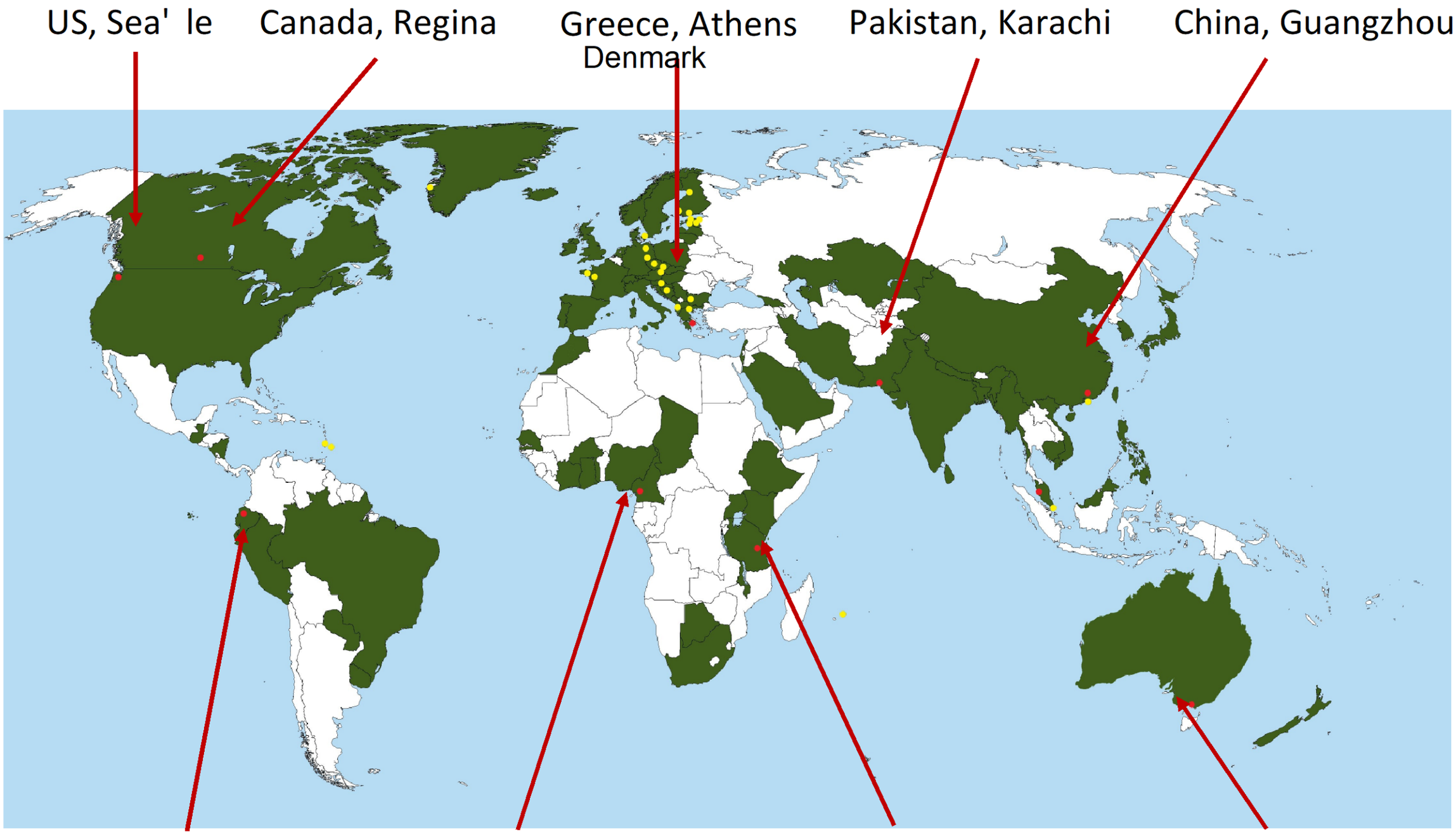
June 2017

November 2017

June 2018

November 2018

Sample collection – Longitudinal
Monthly samples in one year



X

Conclusion

- Sewage is a good source for an ethically acceptable and economically feasible global surveillance and prediction of AMR and infectious diseases
- Drug use explains only minor part of AMR
- There are other factors that drive the occurrence of AMR such as sanitation, health …

Acknowledgement

- Technical University of Denmark (DTU)
  - Frank Aarestrup
  - Rene Hendriksen
  - Thomas Nordahl Petersen
  - Patrick Munk
  - Sunje J. Pamp
  - Patrick Njage
  - Timo Roder
  - Jette Kjeldgaard
- University of Edinburgh
  - Bram Van Bunnik
  - Mark Woolhouse
- Erasmus Medical Center
  - Marion P. Koopmans
  - David Nieuwenhuijse
- Global sewage surveillance project consortium

ARTICLE

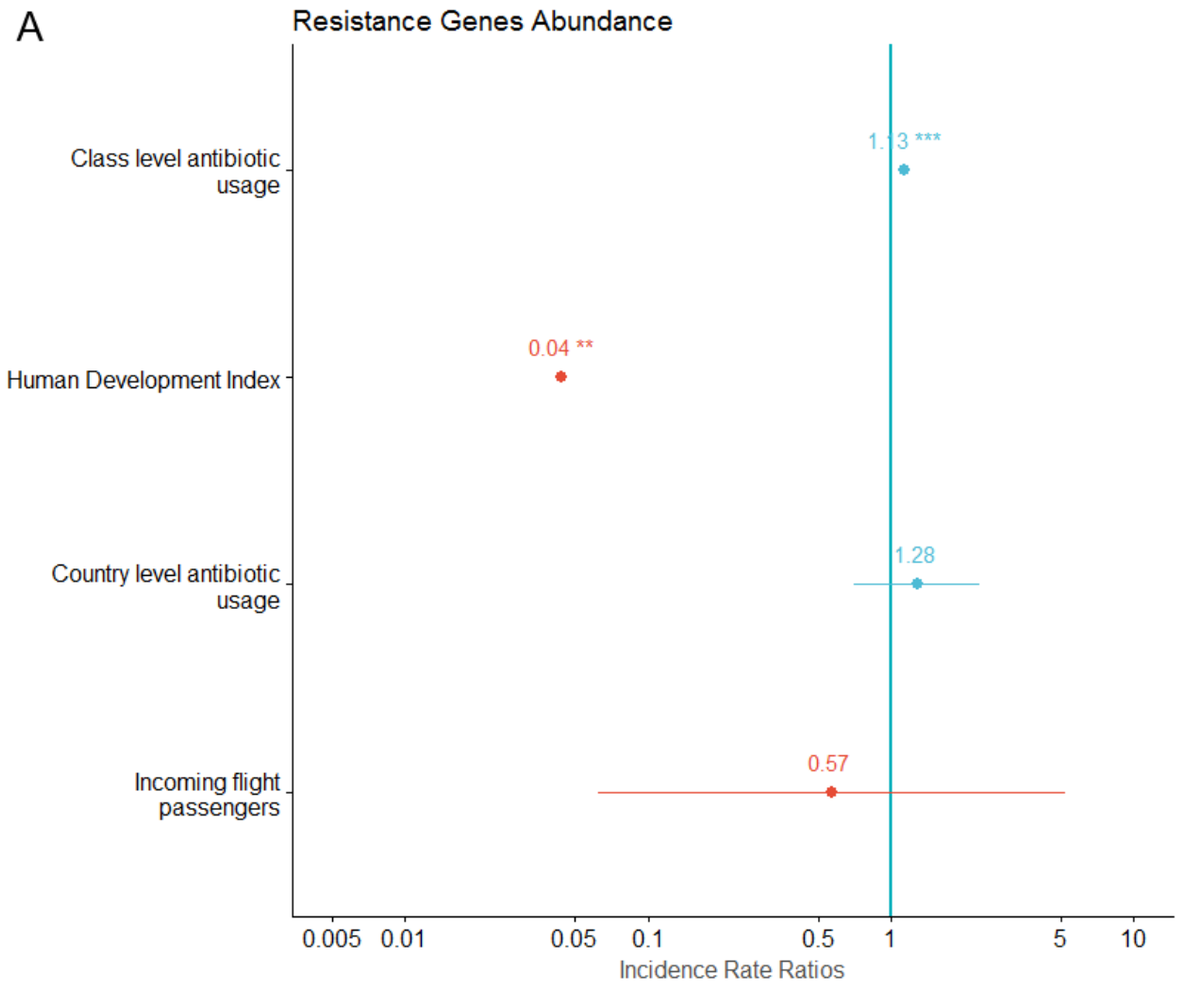
https://doi.org/10.1038/s41467-019-08853-3 OPEN

Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage

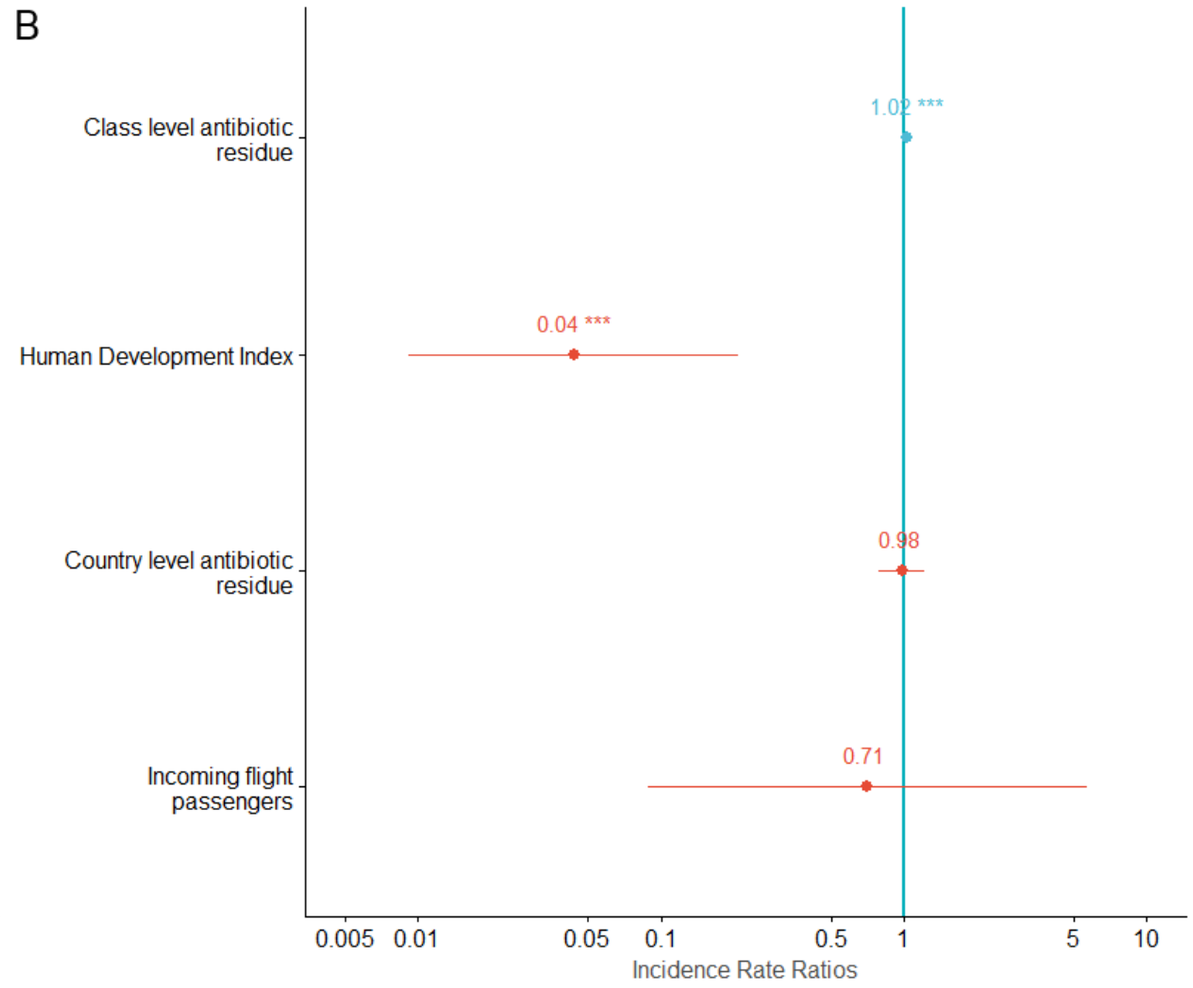
Rene S. Hendriksen[1], Patrick Munk[1], Patrick Njage[1], Bram van Bunnik[2], Luke McNally[3], Oksana Lukjancenko[1], Timo Röder[1], David Nieuwenhuijse[4], Susanne Karlsmose Pedersen[1], Jette Kjeldgaard[1], Rolf S. Kaas[1], Philip Thomas Lanken Conradsen Clausen[1], Josef Korbinian Vogt[1], Pimlapas Leekitcharoenphon[1], Milou G.M. van de Schans[5], Tina Zuidema[5], Ana Maria de Roda Husman[6], Simon Rasmussen[7], Bent Petersen[7], The Global Sewage Surveillance project consortium[#], Clara Amid[8], Guy Cochrane[8], Thomas Sicheritz-Ponten[9], Heike Schmitt[6], Jorge Raul Matheu Alvarez[10], Awa Aidara-Kane[10], Sünje J. Pamp[1], Ole Lund[7], Tine Hald[1], Mark Woolhouse[2], Marion P. Koopmans[4], Håkan Vigre[1], Thomas Nordahl Petersen[1] & Frank M. Aarestrup[1]

Thank you

A

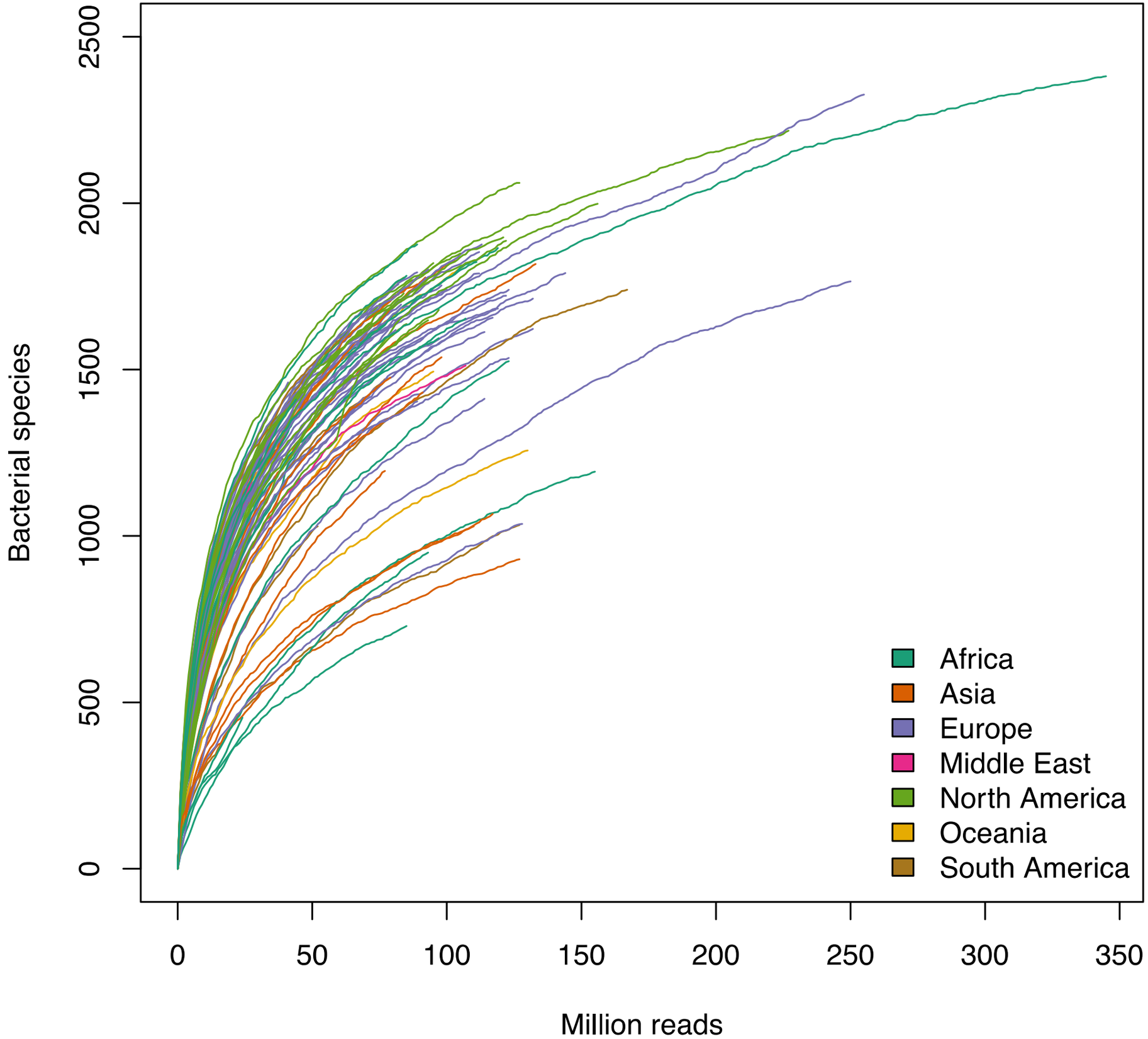

Resistance Genes Abundance
Class level antibiotic usage
Human Development Index
Country level antibiotic usage
Incoming flight passengers
1.13 ***
0.04 **
1.28
0.57
0.005
0.01
0.05
0.1
0.5
1
5
10
Incidence Rate Ratios

B


Class level antibiotic residue
Human Development Index
Country level antibiotic residue
Incoming flight passengers
1.02 ***
0.04 ***
0.98
0.71
0.005
0.01
0.05
0.1
0.5
1
5
10
Incidence Rate Ratios

Conclusion

- Sewage is a good source for an ethically acceptable and economically feasible global surveillance and prediction of AMR and infectious diseases
- AMR abundance is mainly influenced by local/national parameters
- Drug use explains only minor part of AMR
- There are other factors that drive the occurrence of AMR such as sanitation, health …

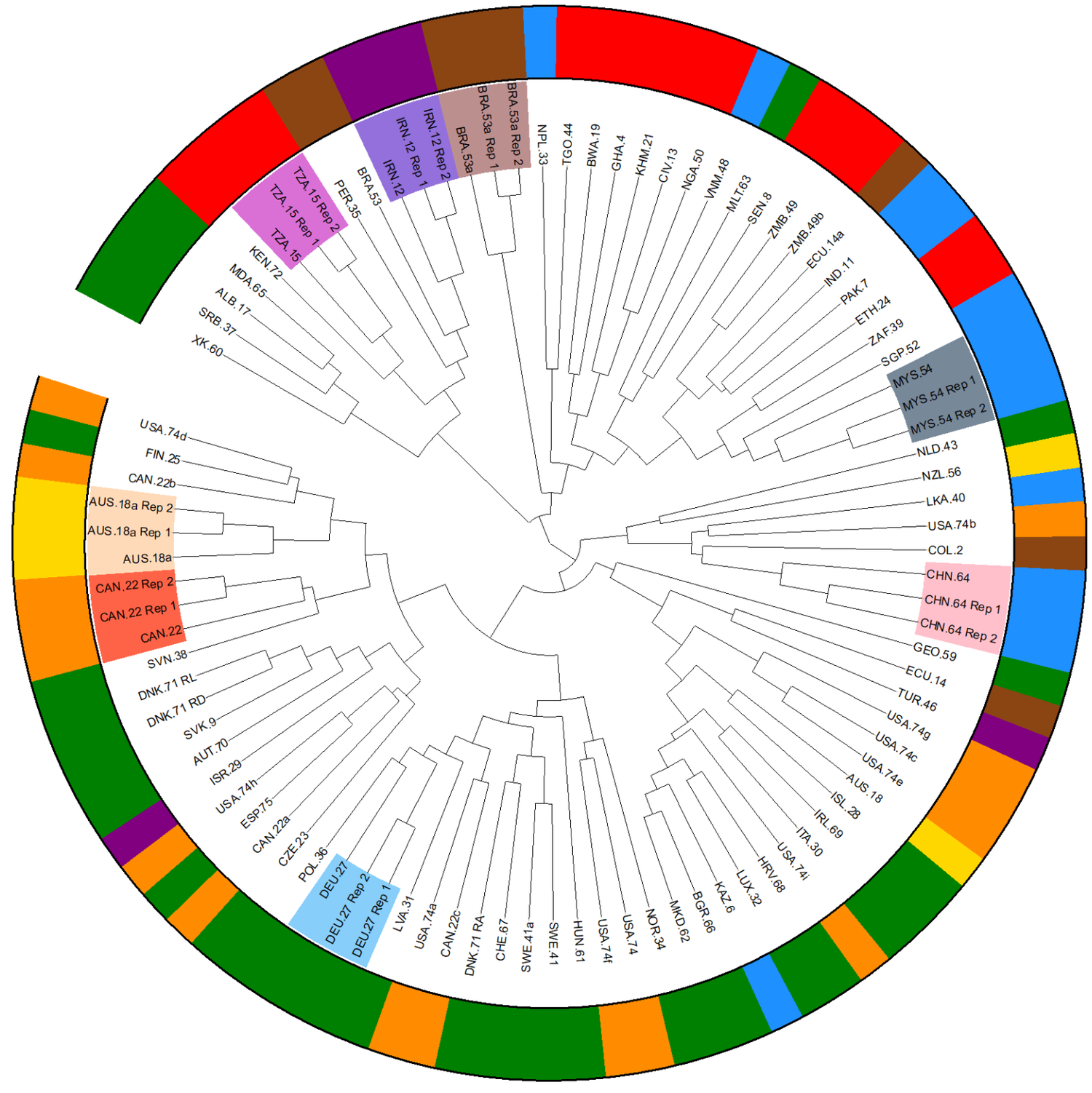

Bacterial species
0
500
1000
1500
2000
2500
0
50
100
150
200
250
300
350
Million reads
Africa
Asia
Europe
Middle East
North America
Oceania
South America

Continent

- Africa
- Asia
- Europe
- Middle_East
- North_America
- Oceania
- South_America

Triple-sampled sewage

- Germany
- Iran
- Tanzania
- Australia
- Canada
- Brazil
- Malaysia
- China



Sample collection protocol

Global Sewage Surveillance Project

– global surveillance of infectious diseases and antimicrobial resistance from sewage

---

Protocol for sewage collection, June 2017

1. EXECUTIVE SUMMARY

No single approach exists for adequately monitoring large populations for the emergence of novel pathogens. Recent developments in high-throughput sequencing offer the ability to rapidly identify nucleic acids from various organisms in clinical and environmental samples. Sewage systems transport human faecal material from a large number of individuals. A point-prevalence metagenomic analysis will be applied to sewage samples collected globally from the main sewage system of major cities prior to treatment plants inlet. The project will serve as proof-of-concept, which could initiate a global surveillance of human infectious diseases including antimicrobial resistance from sewage collected in major cities around the world to detect, control, prevent and predict human infectious diseases.

The Global Sewage Surveillance Project is a continuation of the 2016 Pilot Global Sewage Surveillance Project and will continue from 2017 to 2018 with sampling windows each year in June and November.

2. PROJECT DESCRIPTION

Background

Human and animal populations are increasingly confronted with novel, emerging or re-emerging infectious, zoonotic, and communicable diseases including those that are multi-drug resistant. Many of these events can be attributed to increased globalization, urbanization, climate change, population growth, and intensive farming. According to the WHO, more than 25% of the total 58 million worldwide annual deaths are the direct result of infectious diseases.

Surveillance of pathogens and antimicrobial resistance are essential in disease control and prevention strategies. Human disease surveillance is often hampered due to ethical problems with sensitivity of data collected from clinical samples or healthy individuals. Exposure to human waste is a well-established risk factor, why sewage has been suggested as an alternative to clinical or individual human samples for population based surveillance. Since such samples are anonymized it would also avoid many ethical concerns.

The rapid developments in high-throughput sequencing and metagenomic analysis offers the potential to simultaneously determine the presence and prevalence of a large number of DNA

Packing instruction

11 (packing example)

With the palm of your hand, press gently on the tape allowing it to fix well to the plastic bag

12 (packing example)

Place the closed bag containing the polystyrene box inside the cardboard box

Make sure the polystyrene box does not move inside the cardboard box when 'shaking' the package

Make sure to tape all way around the parcel three times, i.e. once in the middle fixing the flaps, and twice all the way around the sides of the parcel as indicated on the photo

Make sure the ends of the tape fix each other (see photo)

13 (packing example)

Make sure the tape correctly fixes the flaps to the cardboard box

14 (packing example)

Make sure that all corners and ends of the fortified tape is fixed to the cardboard box

Sample details

Global Sewage Surveillance JUNE 2017 - SAMPLE DETAILS

Introduction

THE PURPOSE OF THIS SURVEY is to capture an overview of the collected samples.

If you have any questions or feedback for the submission of information via this survey, please contact Rene Hendriksen (rshe@food.dtu.dk), at the Technical University of Denmark.

Note: An asterisk (*) indicates a question that requires an answer.

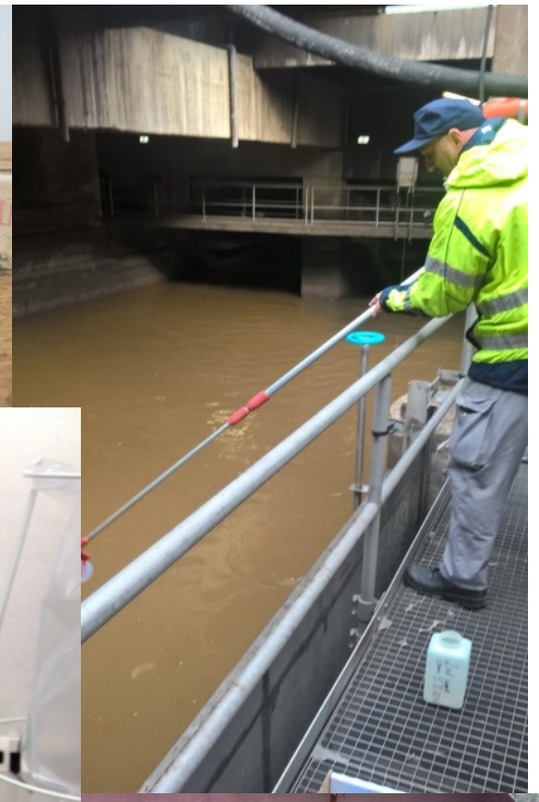
--- --- ---

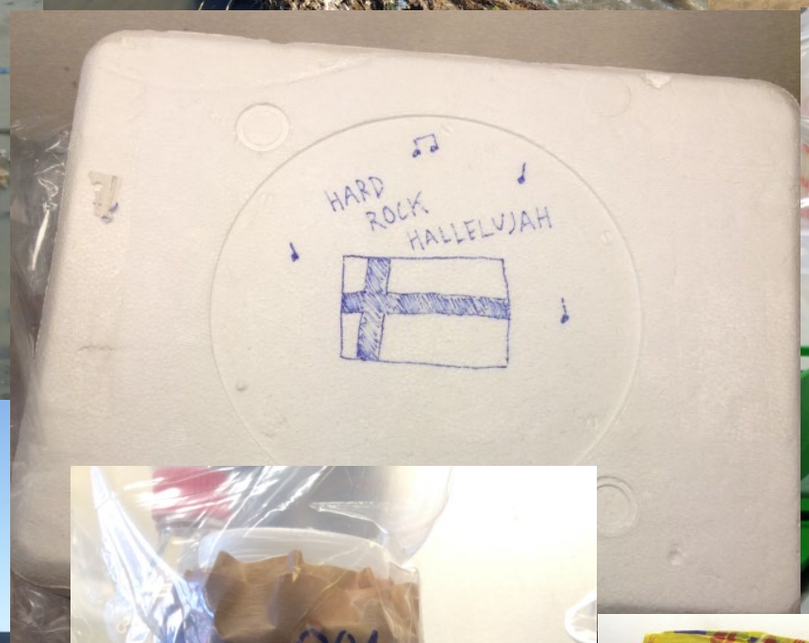
Background
No single approach exists for adequately monitoring large populations and their environments for the emergence of novel pathogens. Recent developments in high-throughput sequencing offer the ability to rapidly identify nucleic acids from various organisms in clinical and environmental samples. Sewage systems are recognized as an important source of human pathogens, especially in crowded settings with poor infrastructure.

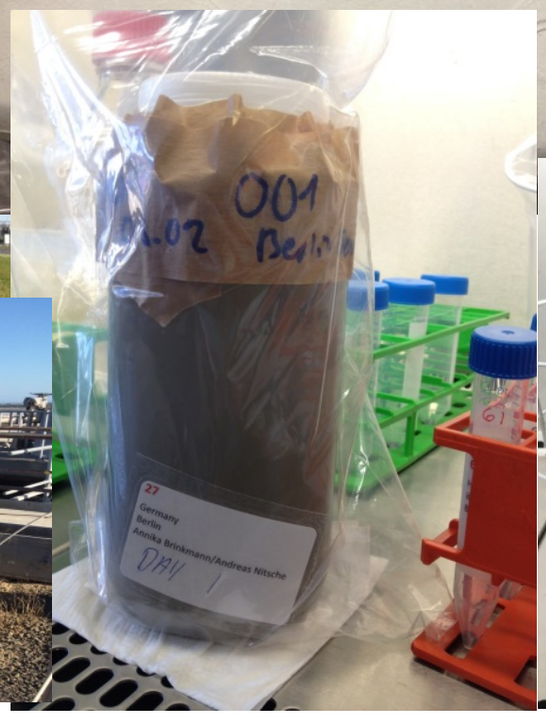
Purpose
A point-prevalence metagenomic analysis will be applied to sewage samples collected globally from the main sewage system of major cities prior to treatment plants inlet. The project will serve as proof-of-concept for applying metagenomic approaches, which could initiate a global surveillance of human infectious diseases including antimicrobial resistance from sewage collected in major cities around the world to detect, control, prevent and predict human infectious diseases.

Procedure
From each location, one representative sewage samples of 2L is collected from the main sewage flow from the city's main sewage pipelines prior to waste water treatment plant inlets or from the main outlet to rivers or similar. Samples can be obtained following the first filtering step, but it is important that there has been no processing of the sewage.
It is preferred to collect concentrated flow proportion sampling over 24 hours, however, should this not be possible due to lack of equipment, three crude point samples should be collected in a short time interval, i.e. at least 5 minutes between each individual sample, to ensure as much randomness as possible. Store the containers at -80°C (preferred) and prepare shipping the samples to the DTU Food in Denmark (see Appendix 2 in the protocol).
Specific description is found in the protocol sent by email from the organizer prior to sampling (for questions, please contact Rene Hendriksen, rshe@food.dtu.dk)

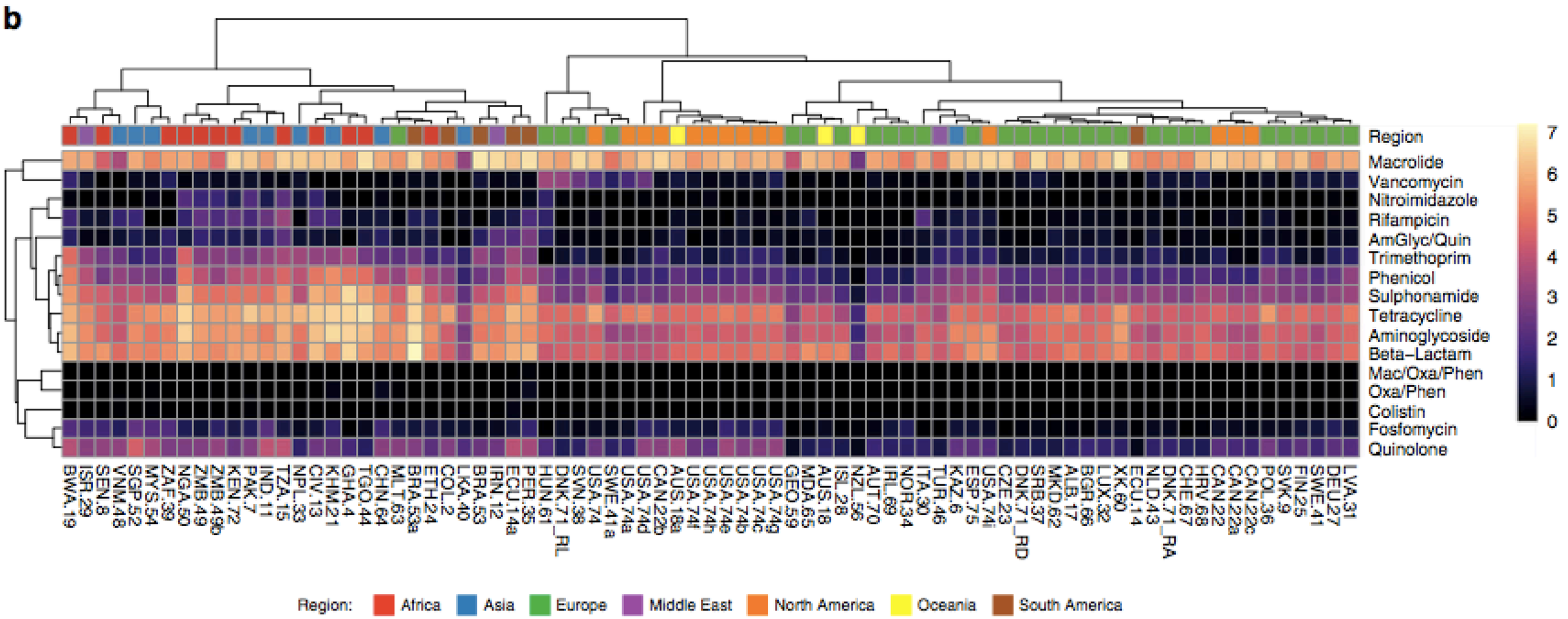
Sample collection – 2016 (pilot)



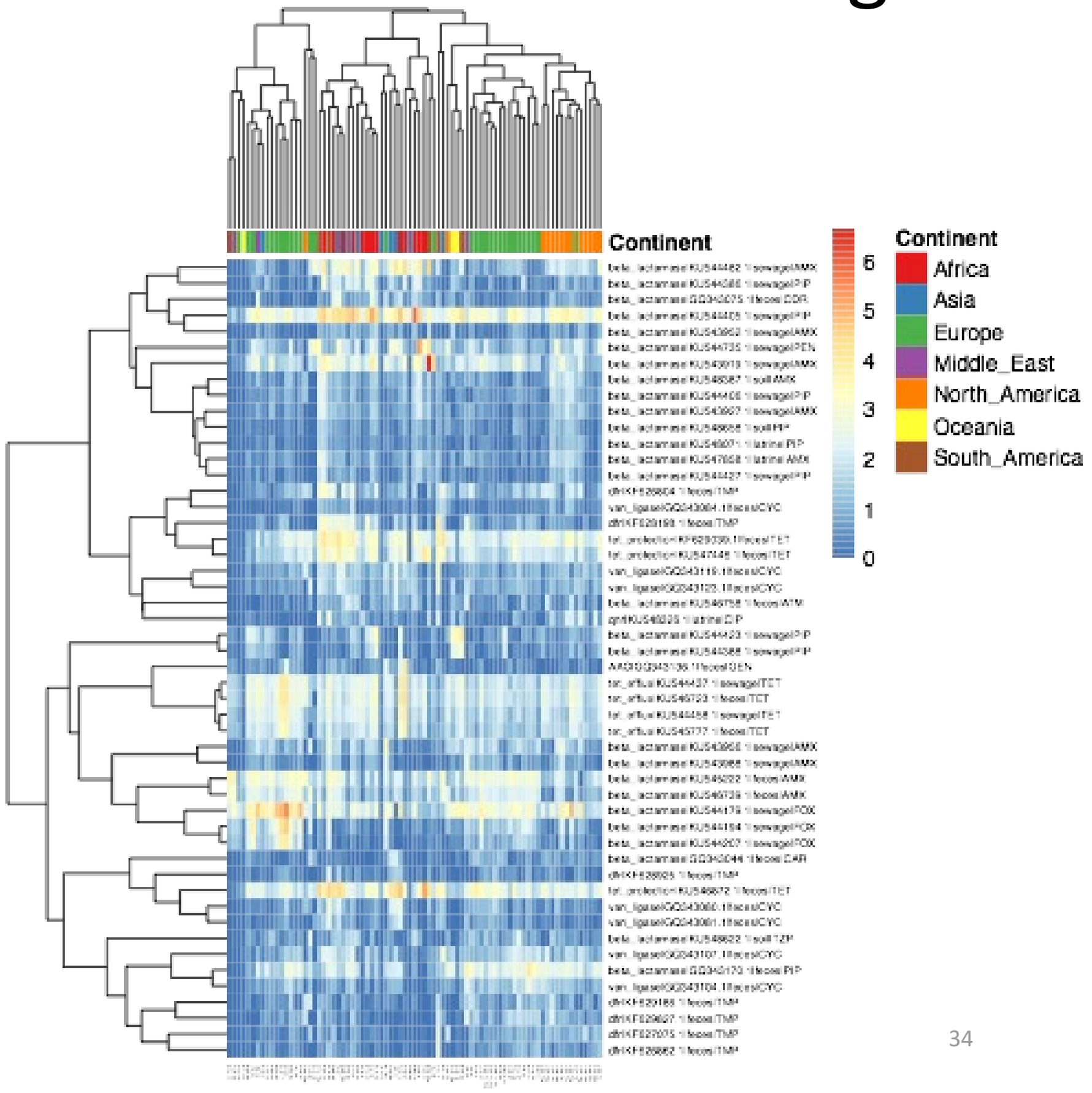


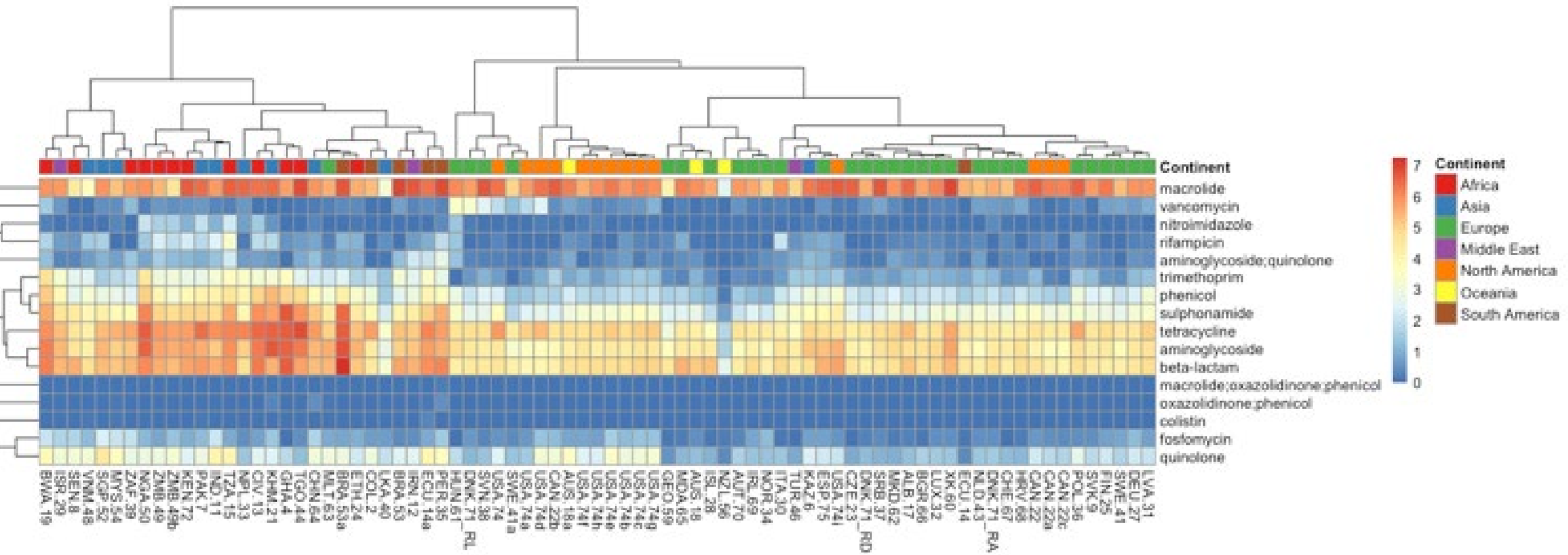


AMR class


b
Region
Macrolide
Vancomycin
Nitroimidazole
Rifampicin
AmGlyc/Quin
Trimethoprim
Phenicol
Sulphonamide
Tetracycline
Aminoglycoside
Beta−Lactam
Mac/Oxa/Phen
Oxa/Phen
Colistin
Fosfomycin
Quinolone
7
6
5
4
3
2
1
0
BWA.19
ISR.29
SEN.8
VNM.48
SGP.52
MYS.54
ZAF.39
NGA.50
ZMB.49b
ZMB.49
KEN.72
PAK.7
IND.11
TZA.15
NPL.33
CIV.13
KHM21
GHA.4
TGO.44
CHN.64
MLT.63
BRA.53a
ETH24
COL2
LKA.40
BRA.53
IRN.12
ECU.14a
PER.35
HUN.61
DNK.71_RL
SVN.38
USA.74
SWE.41a
USA.74d
USA.74a
CAN.22b
AUS.18a
USA.74f
USA.74h
USA.74e
USA.74b
USA.74c
USA.74g
GEO.59
MDA.65
AUS.18
ISL.28
NZL.56
AUT.70
IRL.69
NOR.34
ITA.30
TUR.46
KAZ.6
ESP.75
USA.74i
CZE.23
DNK.71_RD
SRB.37
MKD.62
ALB.17
BGR.66
LUX.32
XK.60
ECU.14
NLD.43
DNK.71_RA
CHE.67
HRV.68
CAN.22
CAN.22a
CAN.22c
POL.36
SVK.9
FIN.25
SWE.41
DEU.27
LVA.31
Region:
Africa
Asia
Europe
Middle East
North America
Oceania
South America

Top 50 functional resistance genes



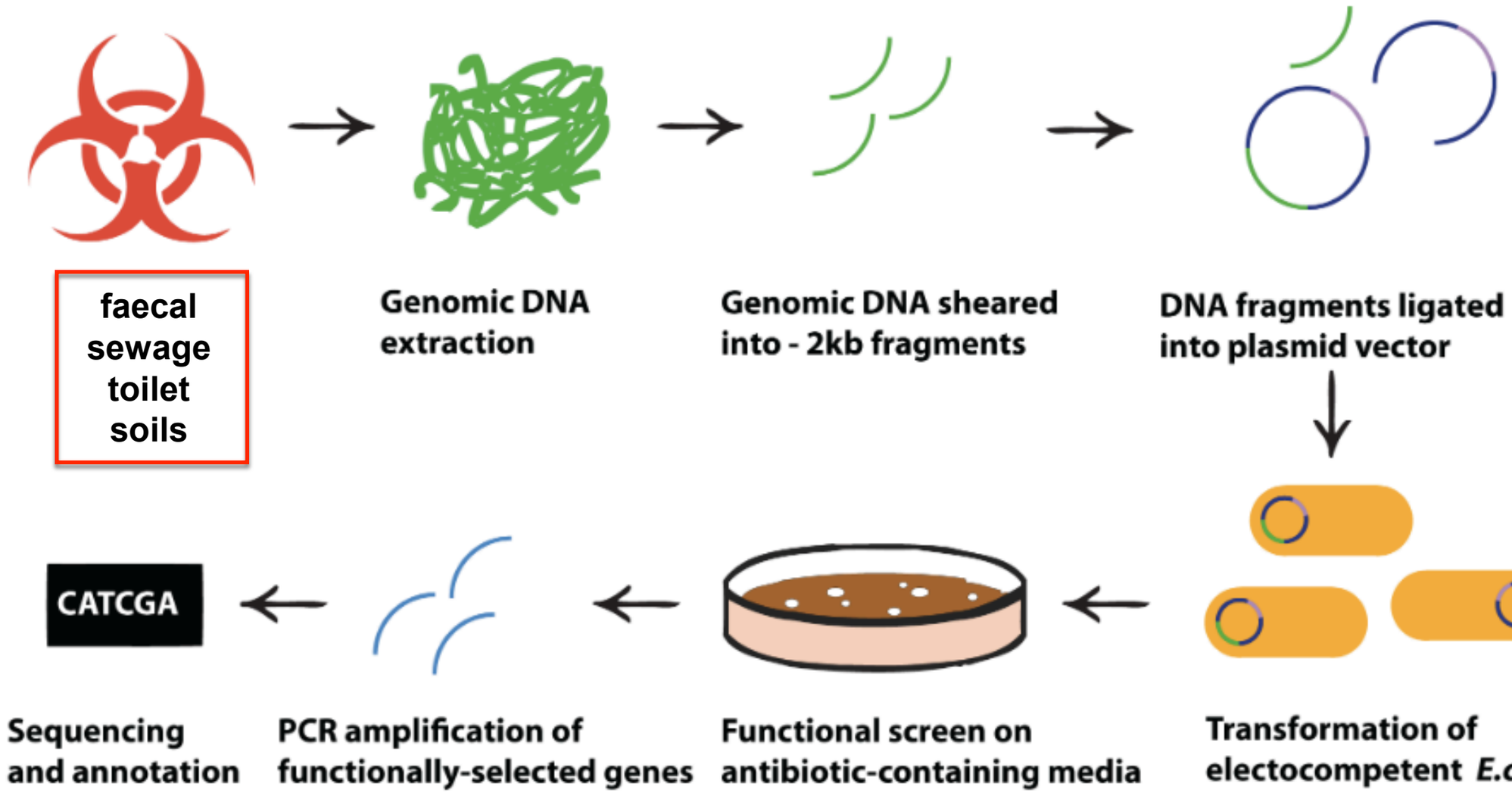

Continent
macrolide
vancomycin
nitroimidazole
rifampicin
aminoglycoside;quinolone
trimethoprim
phenicol
sulphonamide
tetracycline
aminoglycoside
beta-lactam
macrolide;oxazolidinone;phenicol
oxazolidinone;phenicol
colistin
fosfomycin
quinolone
7
6
5
4
3
2
1
0
Continent
Africa
Asia
Europe
Middle East
North America
Oceania
South America
LVA.31
DEU.27
SWE.41
FIN.25
SVK.9
POL.36
CAN.22c
CAN.22a
CAN.22
HRV.68
CHE.67
DNK.71_RA
NLD.43
ECU.14
XK.60
LUX.32
BGR.66
ALB.17
MKD.62
SRB.37
DNK.71_RD
CZE.23
USA.74i
ESP.75
KAZ.6
TUR.46
ITA.30
MOR.34
IRL.69
AUT.70
NZL.56
ISL.28
AUS.18
MDA.65
GEO.59
USA.74g
USA.74e
USA.74b
USA.74c
USA.74h
USA.74f
AUS.18a
CAN.22b
USA.74d
USA.74a
SWE.41a
USA.74
SVN.38
DNK.71_RL
HUN.61
PER.35
ECU.14a
IRN.12
BRA.53
LKA.40
COL.2
ETH.24
BRA.53a
MLT.53
CHN.64
TGO.44
GHA.4
KHM.21
CIV.13
NPL.33
TZA.15
IND.11
PAK.7
KEN.72
ZMB.49b
ZMB.49
MOA.50
ZAF.39
MYS.54
SGP.52
SEN.8
VNM.48
ISR.29
BWA.19

Functional resistance

- Resistance phenotype based on a functional metagenomic antibiotic resistance determinants
- Four functional metagenomic studies were considered, that analysed human faecal samples, sewage, latrines and soils
- Only ARDs selected on antibiotics towards which they are assumed to confer resistance have been selected
- Homology reduction with ResFinder using cd-hit

1. Pehrsson, E. C. et al. Interconnected microbiomes and resistomes in low-income human habitats. Nature 533, 212Ð216 (2016).
2. Moore, A. M. et al. Pediatric fecal microbiota harbor diverse and novel antibiotic resistance genes. PloS One 8, e78822 (2013).
3. Sommer, M. O. A., Dantas, G. & Church, G. M. Functional characterization of the antibiotic resistance reservoir in the human microflora. Science 325, 1128Ð1131 (2009).
4. Forsberg, K. J. et al. Bacterial phylogeny structures soil resistomes across habitats. Nature 509, 612Ð616 (2014).
5. Seemann, T. Prokka: rapid prokaryotic genome annotation. Bioinforma. Oxf. Engl. 30, 2068Ð2069 (2014).
6. Fu, L., Niu, B., Zhu, Z., Wu, S. & Li, W. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinforma. Oxf. Engl. 28, 3150Ð3152 (2012).

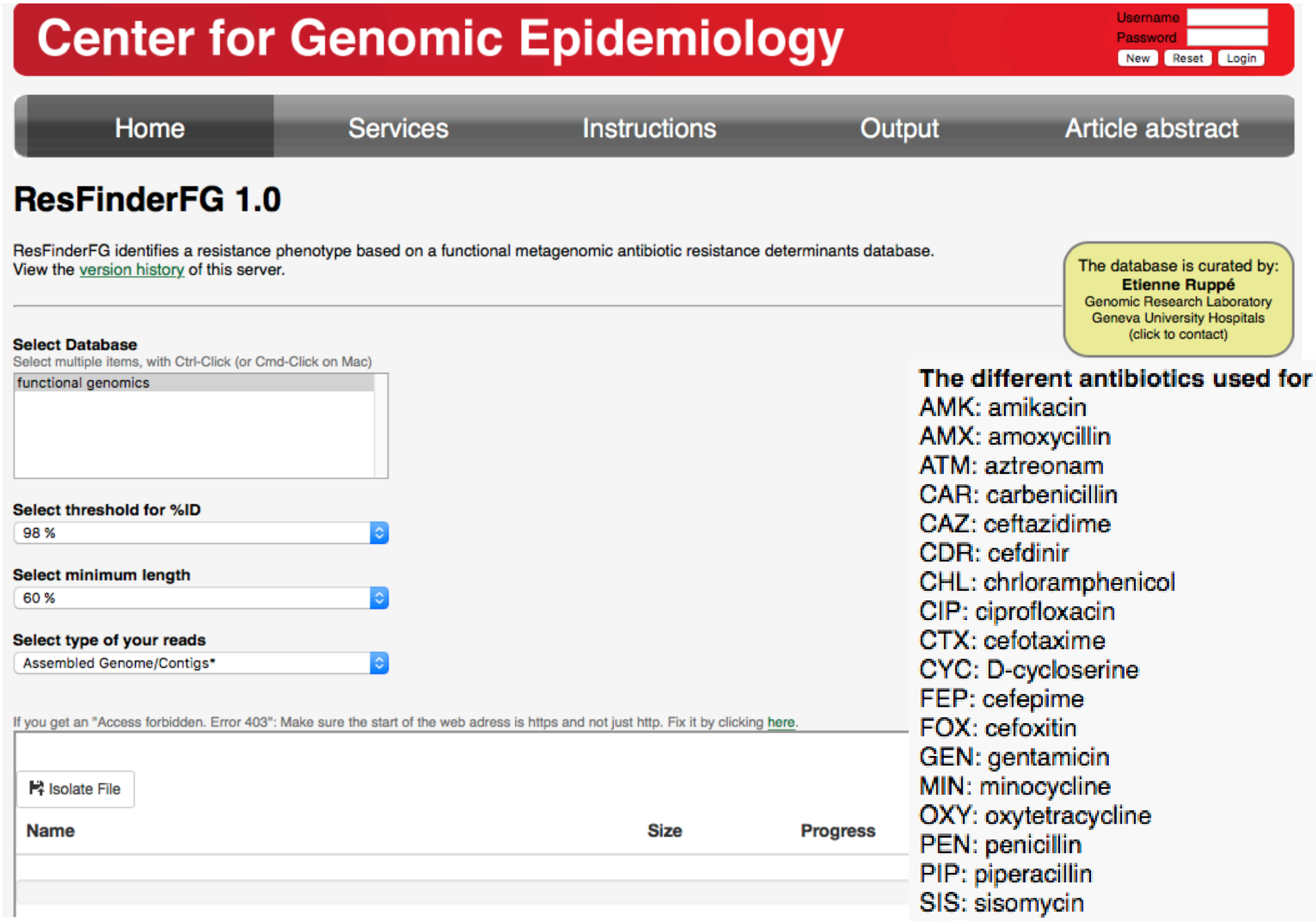
Functional metagenomics



Moore AM1, Munck C, Sommer MO, Dantas G. Front Microbiol. 2011 Oct 17;2:188

1. Pehrsson, E. C. et al. Interconnected microbiomes and resistomes in low-income human habitats. Nature 533, 212Ð216 (2016).
2. Moore, A. M. et al. Pediatric fecal microbiota harbor diverse and novel antibiotic resistance genes. PloS One 8, e78822 (2013).
3. Sommer, M. O. A., Dantas, G. & Church, G. M. Functional characterization of the antibiotic resistance reservoir in the human microflora. Science 325, 1128Ð1131 (2009).
4. Forsberg, K. J. et al. Bacterial phylogeny structures soil resistomes across habitats. Nature 509, 612Ð616 (2014).
5. Seemann, T. Prokka: rapid prokaryotic genome annotation. Bioinforma. Oxf. Engl. 30, 2068Ð2069 (2014).
6. Fu, L., Niu, B., Zhu, Z., Wu, S. & Li, W. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinforma. Oxf. Engl. 28, 3150Ð3152 (2012).

https://cge.cbs.dtu.dk/services/ResFinderFG/



The different antibiotics used for selection are:

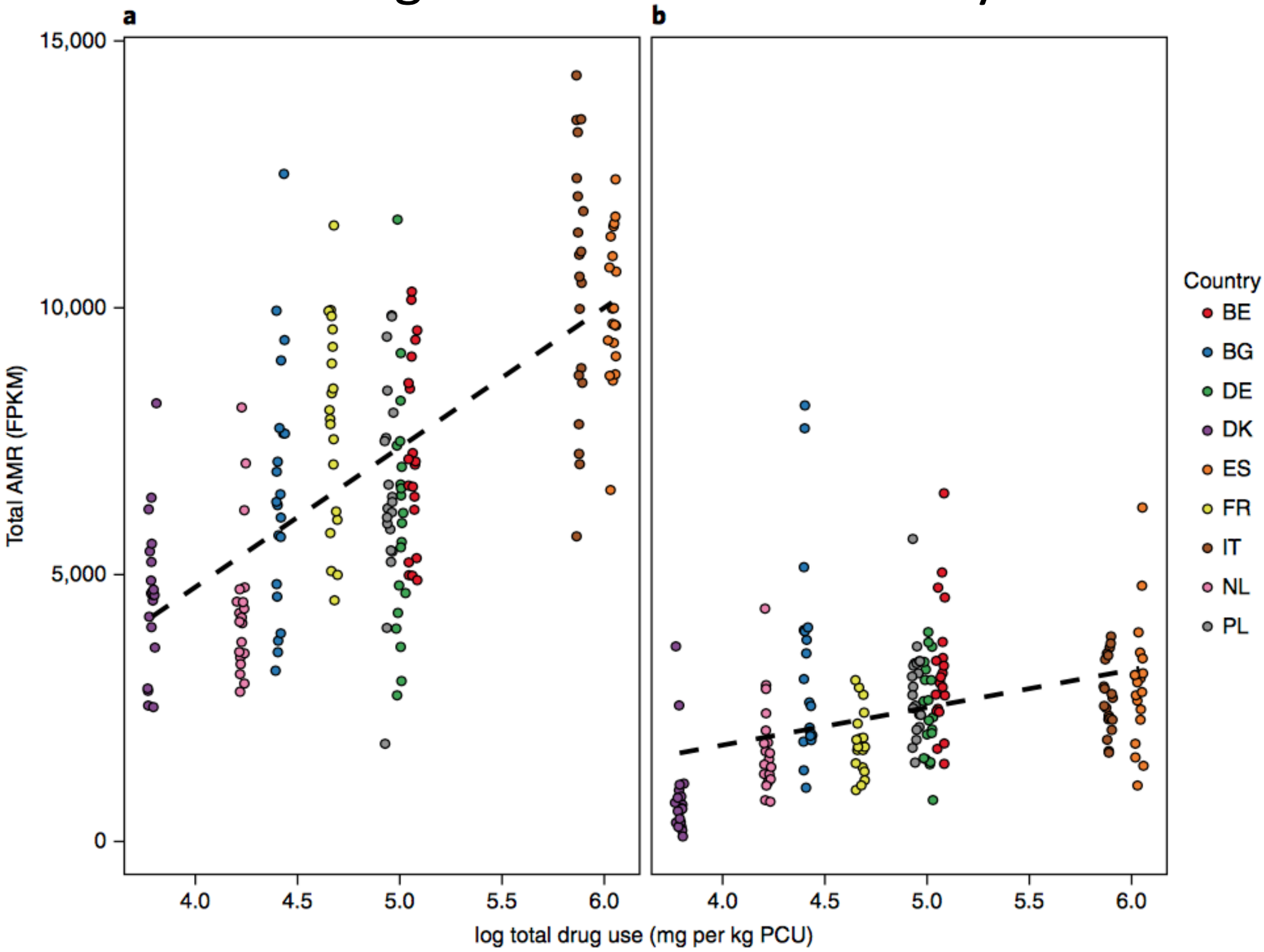
AMK: amikacin
AMX: amoxycillin
ATM: aztreonam
CAR: carbenicillin
CAZ: ceftazidime
CDR: cefdinir
CHL: chrloramphenicol
CIP: ciprofloxacin
CTX: cefotaxime
CYC: D-cycloserine
FEP: cefepime
FOX: cefoxitin
GEN: gentamicin
MIN: minocycline
OXY: oxytetracycline
PEN: penicillin
PIP: piperacillin
SIS: sisomycin
SXT: cotrimoxazole
TET: tetracycline
TGC: tigecycline
TMP: trimethoprim
TZP: piperacillin-tazobactam

ResFinder -> 3026 genes

Functional resistance -> 2514 genes

Pig

Poultry



Munk P. et al. Nature Microbiology. 898–908 (2018)

ResFinder

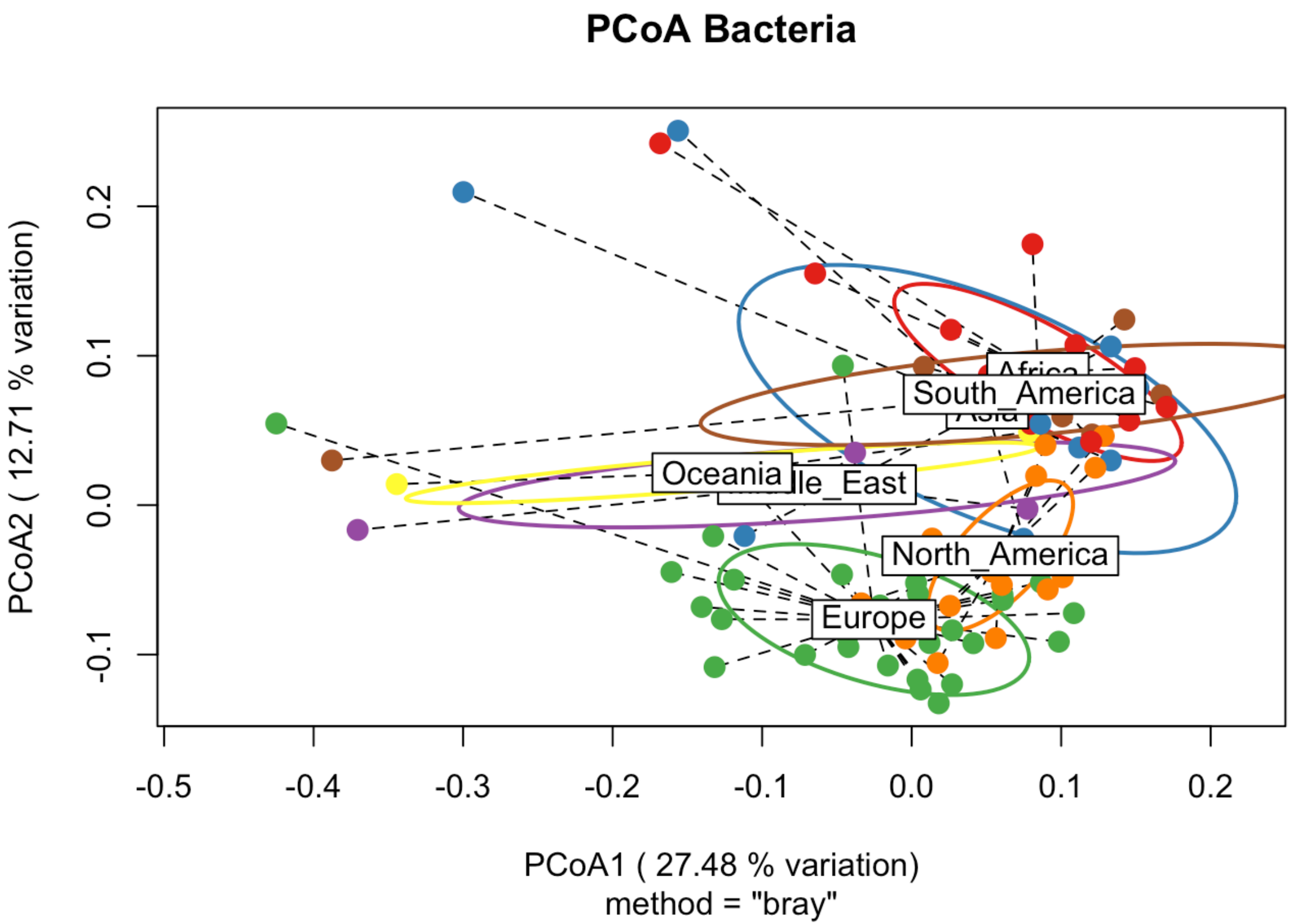
- aminoglycoside
- aminoglycoside;quinolone
- beta-lactam
- colistin
- fosfomycin
- glycopeptide
- macrolide
- macrolide;oxazolidinone;phenicol
- nitroimidazole
- oxazolidinone
- oxazolidinone;phenicol
- phenicol
- quinolone
- rifampicin
- sulphonamide
- tetracycline
- trimethoprim

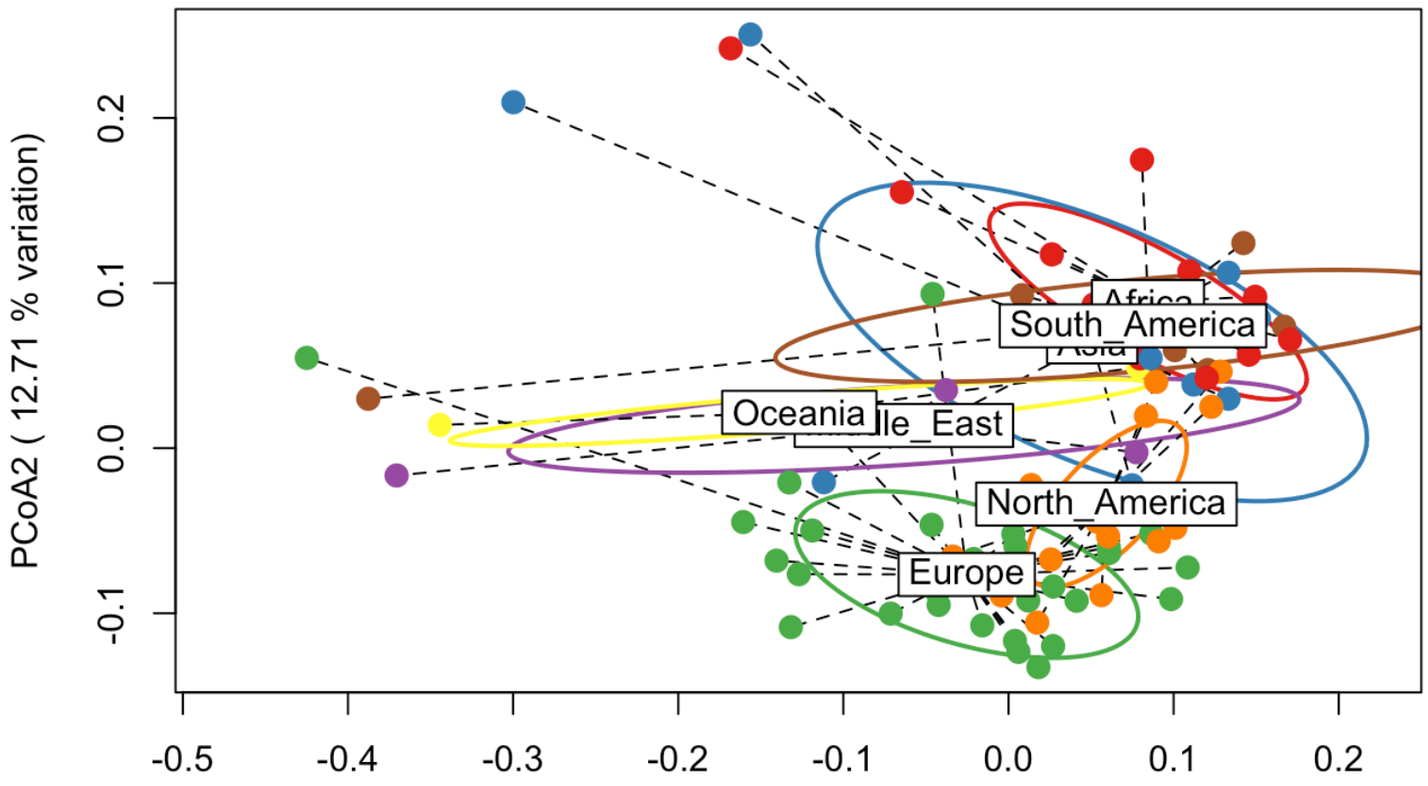
Functional resistance

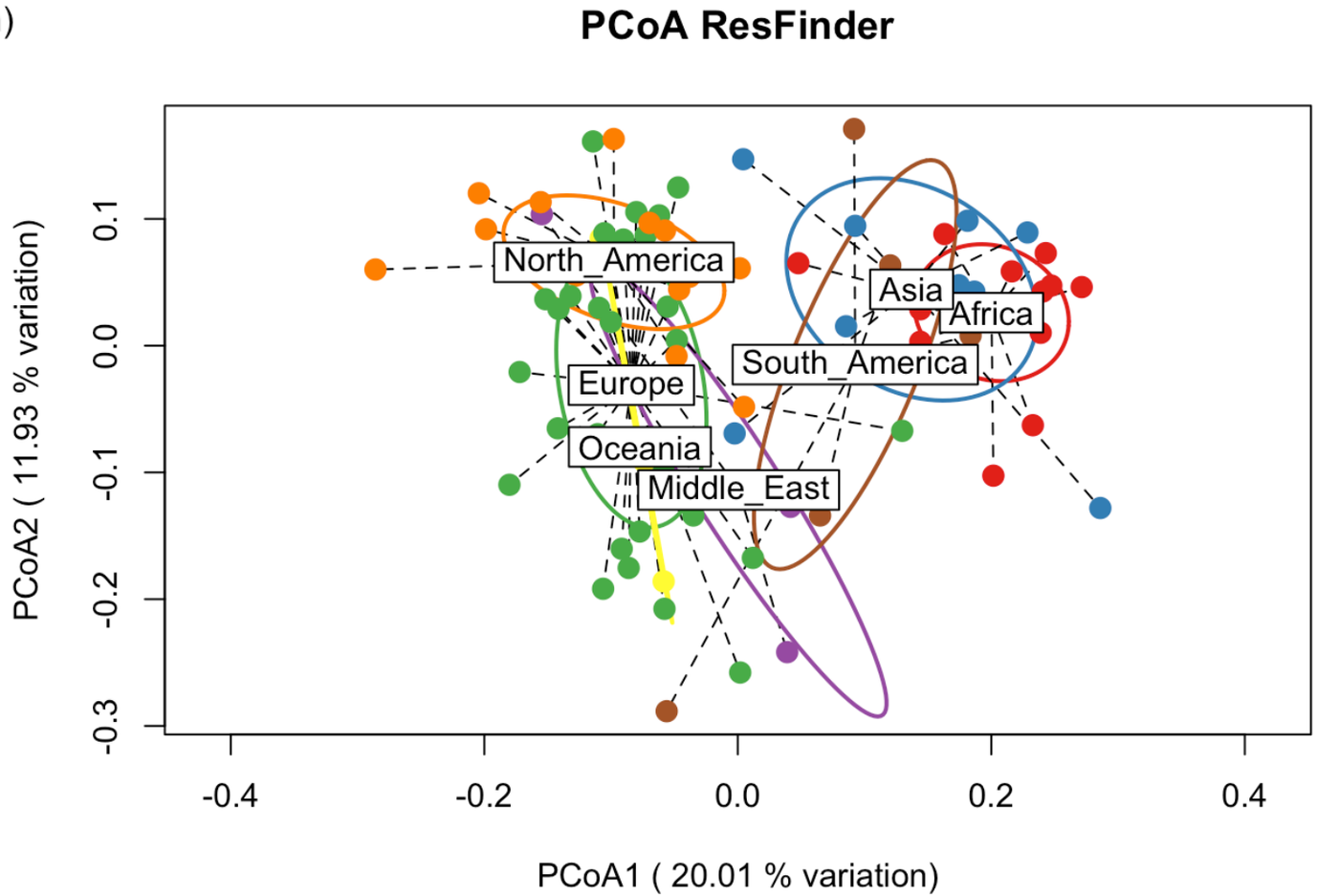
- aminoglycoside
- beta_lactam
- cotrimoxazole
- D_cycloserine
- phenicol
- quinolone
- tetracycline
- trimethoprim

- Critically important (Highest priority)
- Critically important (High priority)
- Highly important
- Important

What are the other drivers of the occurrence of AMR ?


PCoA Bacteria
South_America
Oceania
North_America
Europe
PCoA2 (12.71 % variation)
PCoA1 (27.48 % variation)
method = "bray"
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
-0.1
0.0
0.1
0.2

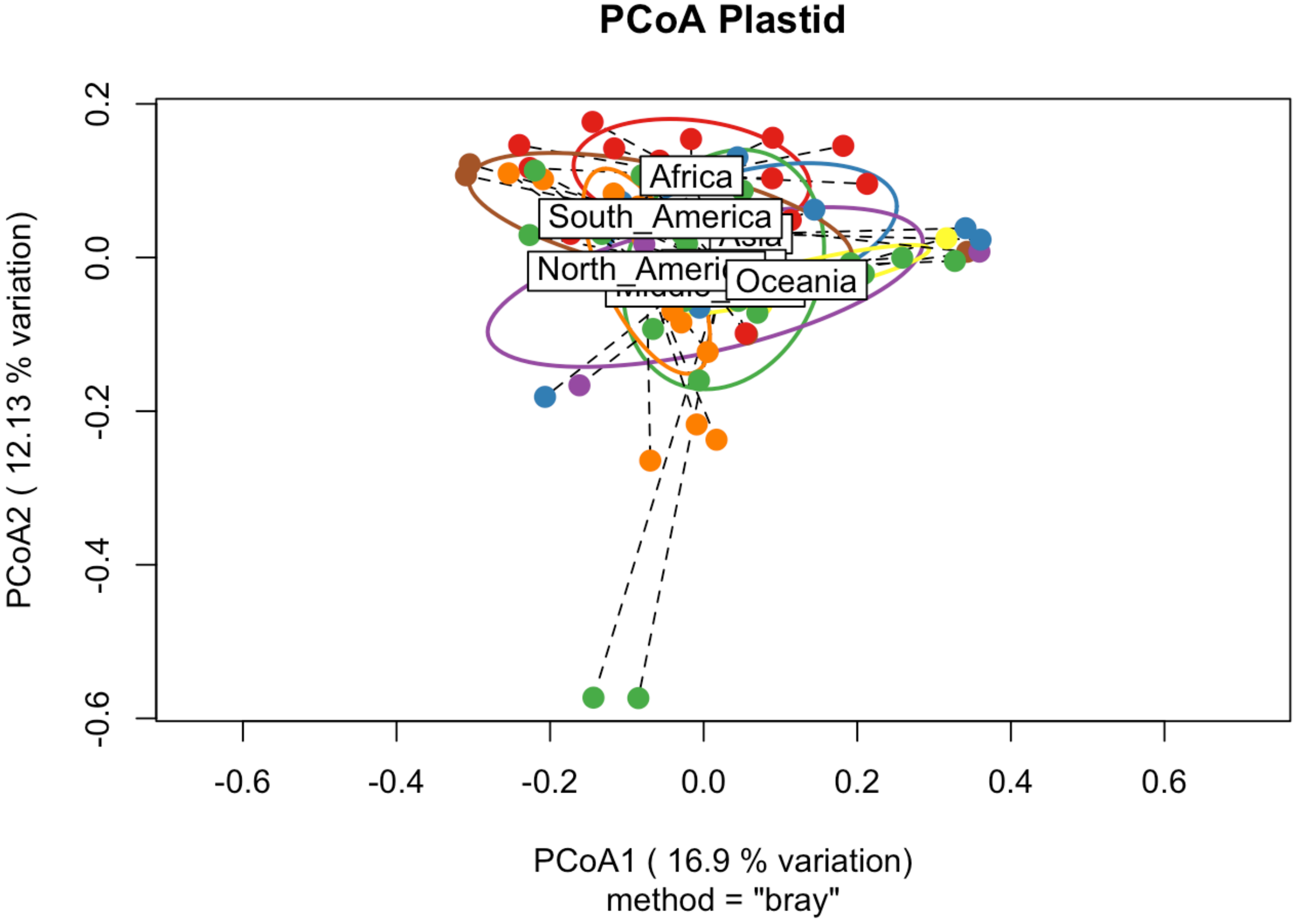

PCoA Bacteria
PCoA2 (12.71 % variation)
PCoA1 (27.48 % variation)
method = "bray"
South_America
Oceania
North_America
Europe
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2

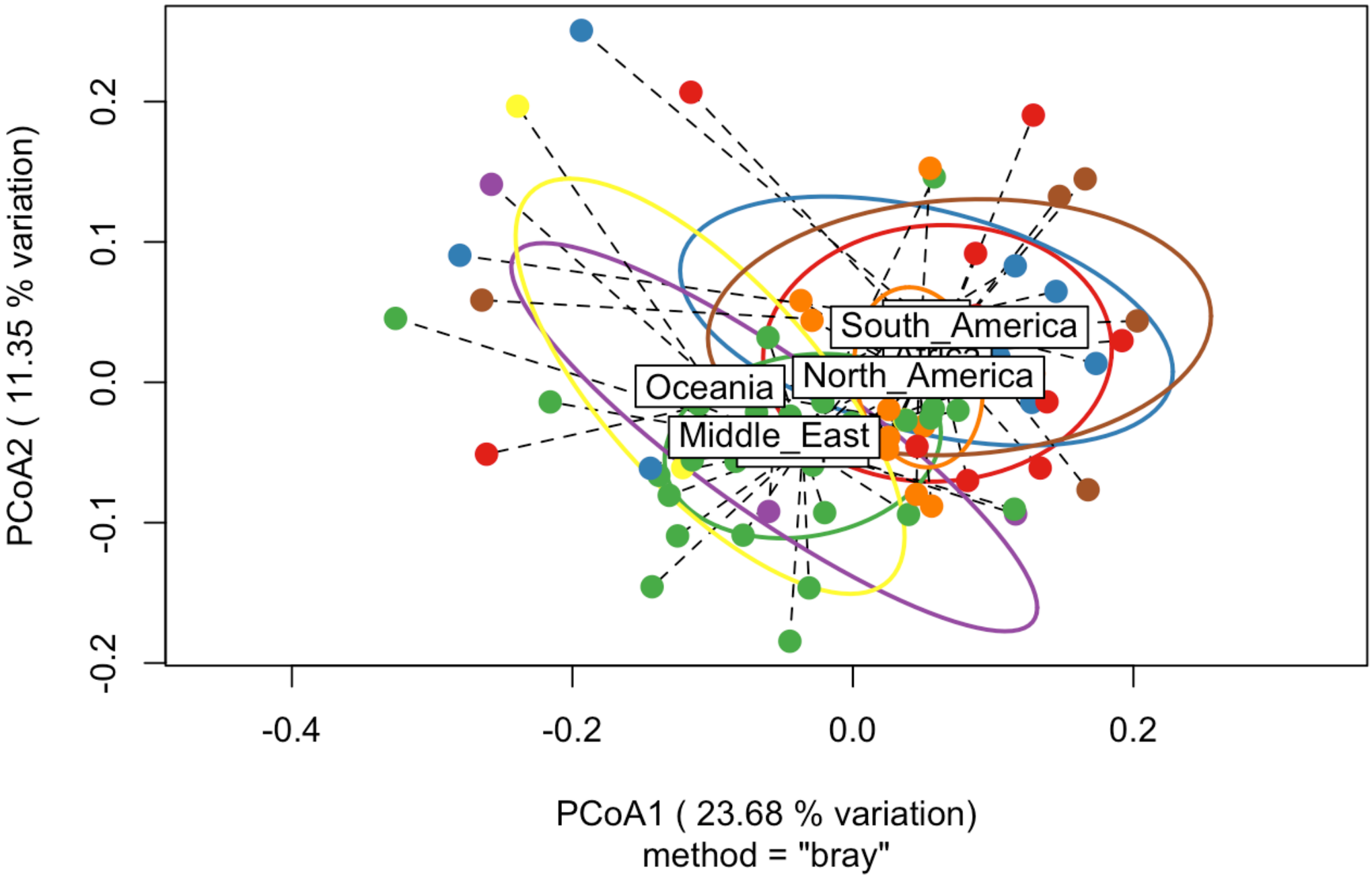

PCoA ResFinder
PCoA2 (11.93 % variation)
PCoA1 (20.01 % variation)
North_America
Asia
Africa
South_America
Europe
Oceania
Middle_East
-0.3
-0.2
-0.1
0.0
0.1
-0.4
-0.2
0.0
0.2
0.4

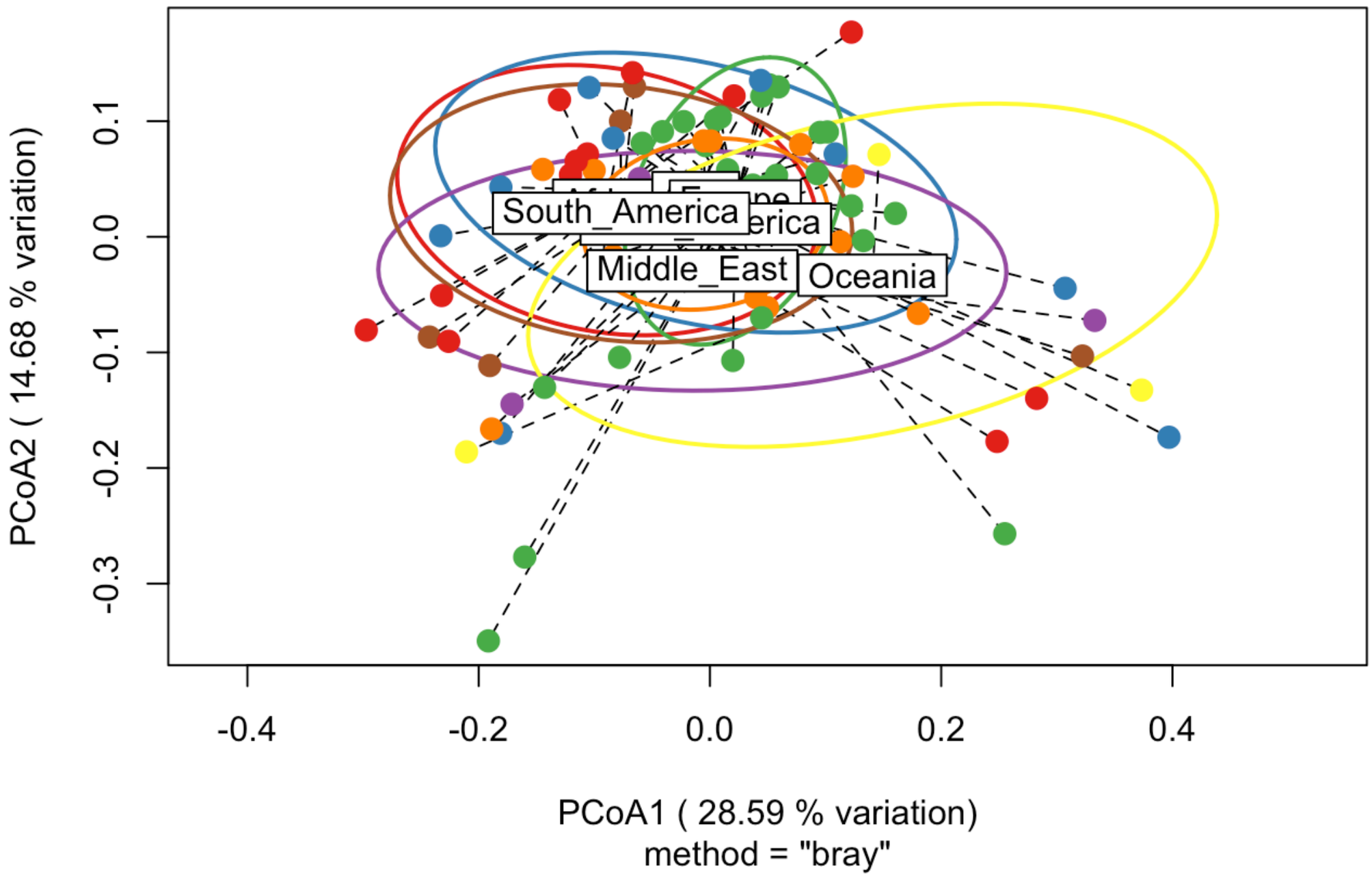
Future perspective

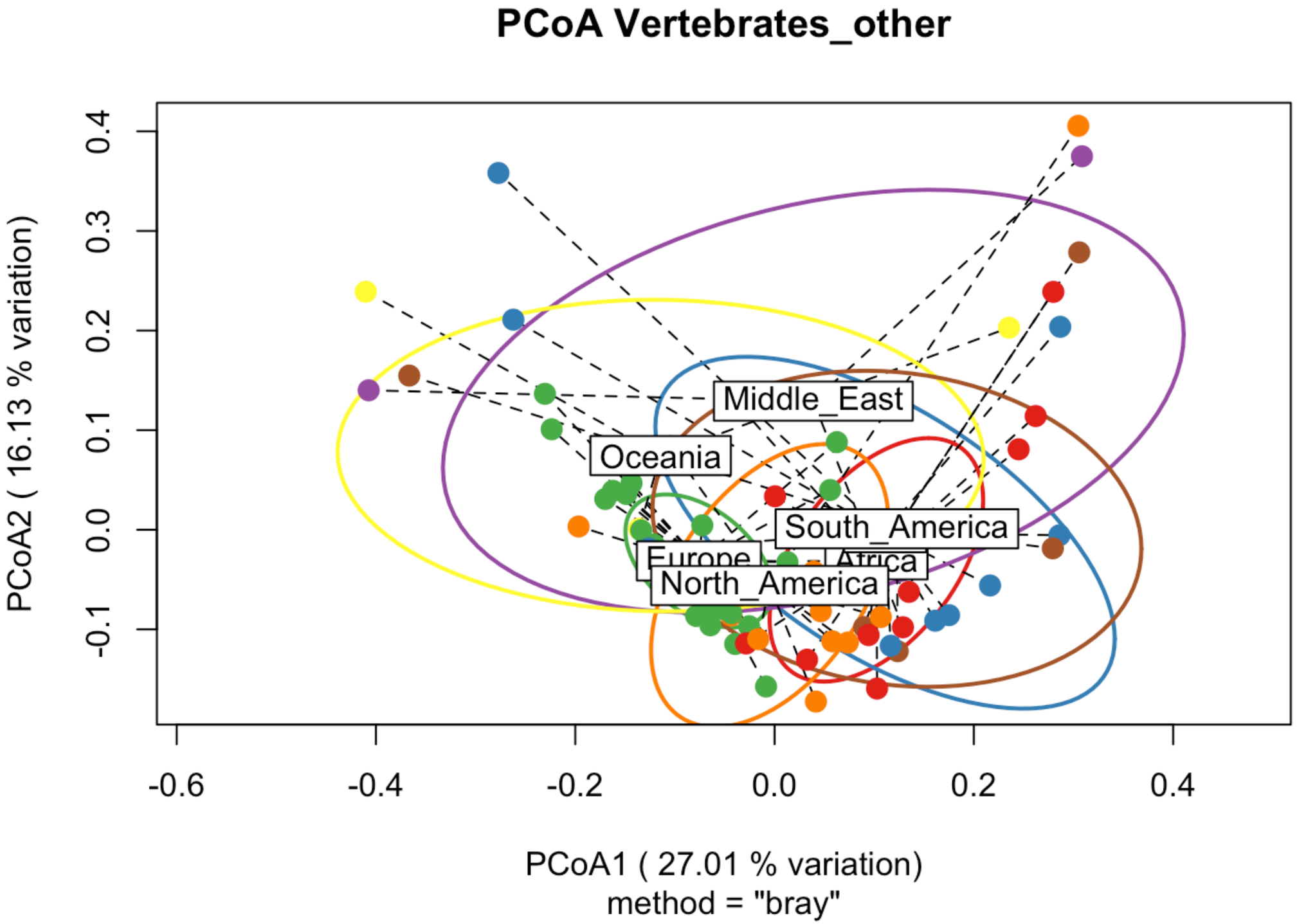
- Diversity and Abundance
  - AMR in gene level
  - AMR organisms
- Other resistance such as metal resistance and chromosomal point mutation

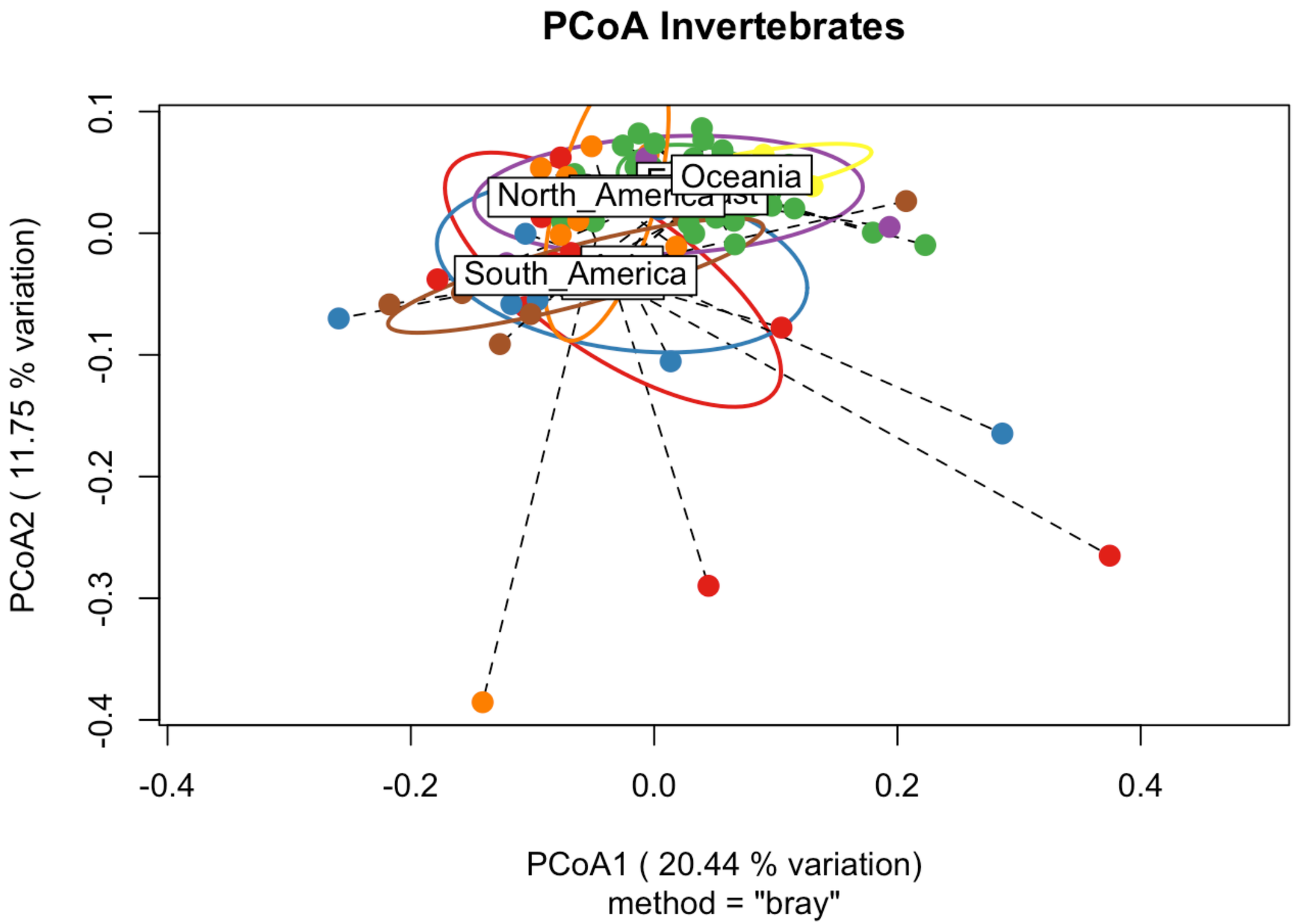
What are the other drivers of the occurrence of AMR ?

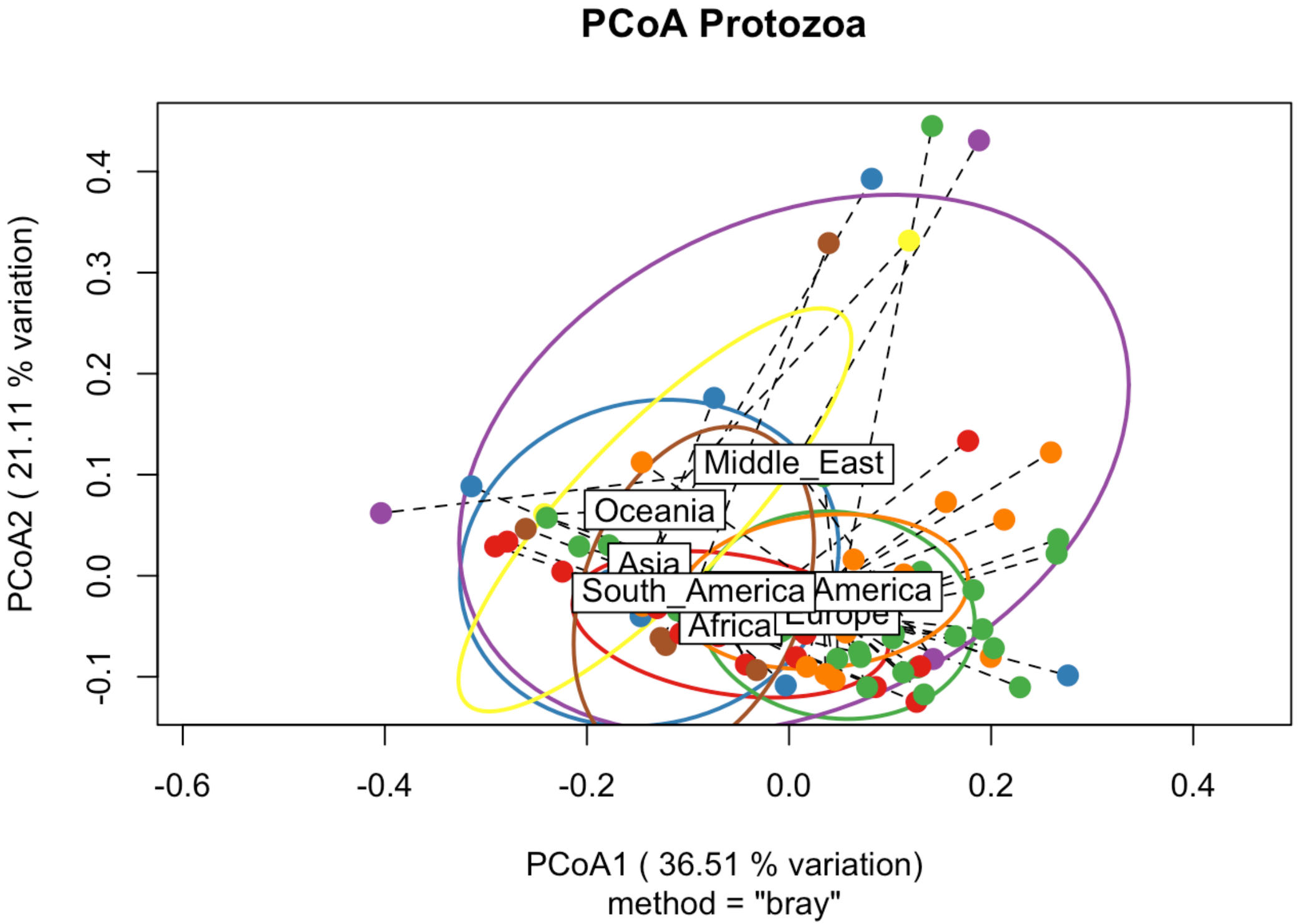

PCoA Plastid
Africa
South_America
Oceania
0.2
0.0
-0.2
-0.4
-0.6
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
PCoA2 (12.13 % variation)
PCoA1 (16.9 % variation)
method = "bray"

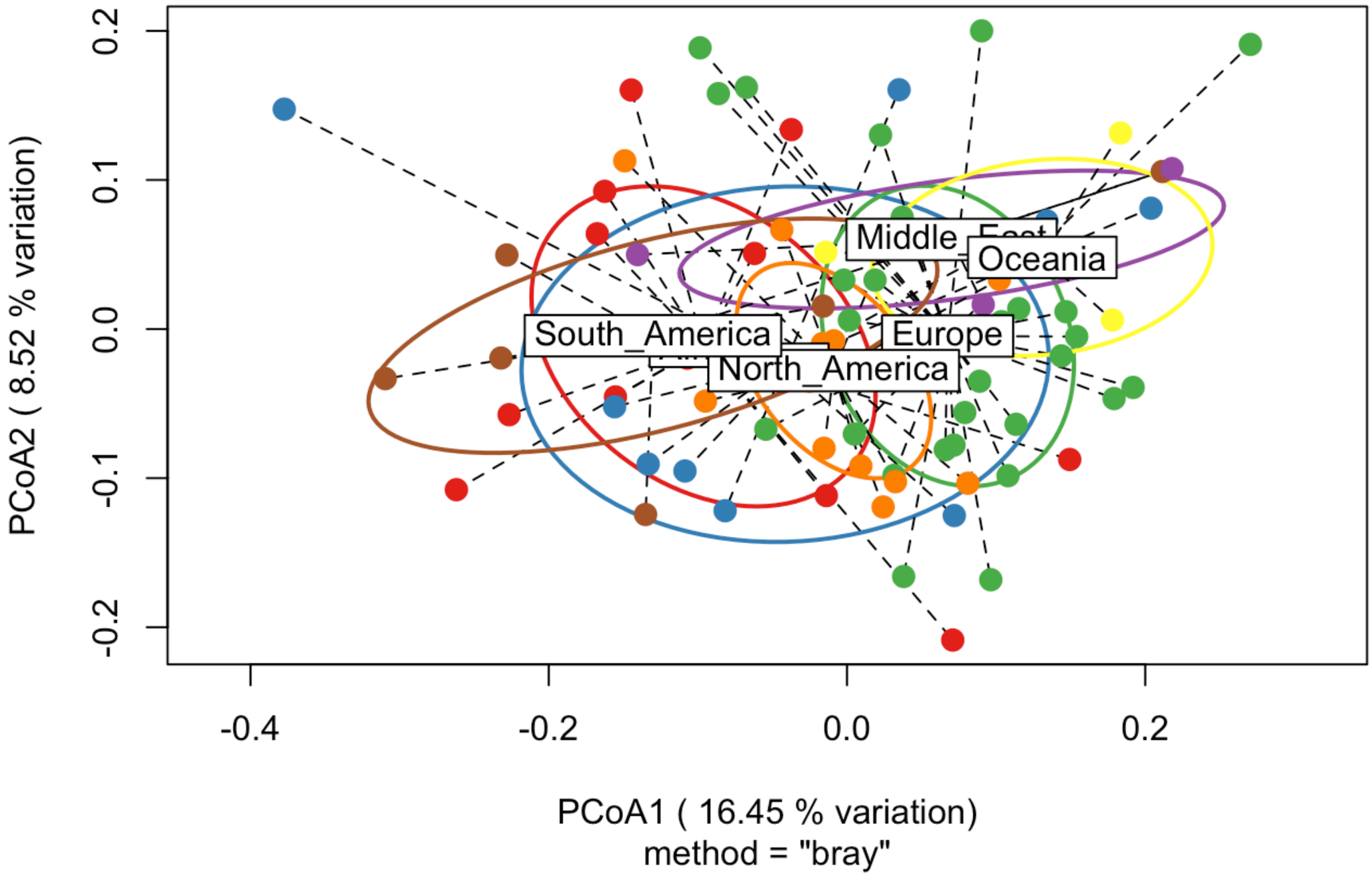

PCoA Plant
South_America
North_America
Oceania
Middle_East
PCoA2 (11.35 % variation)
PCoA1 (23.68 % variation)
method = "bray"
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2

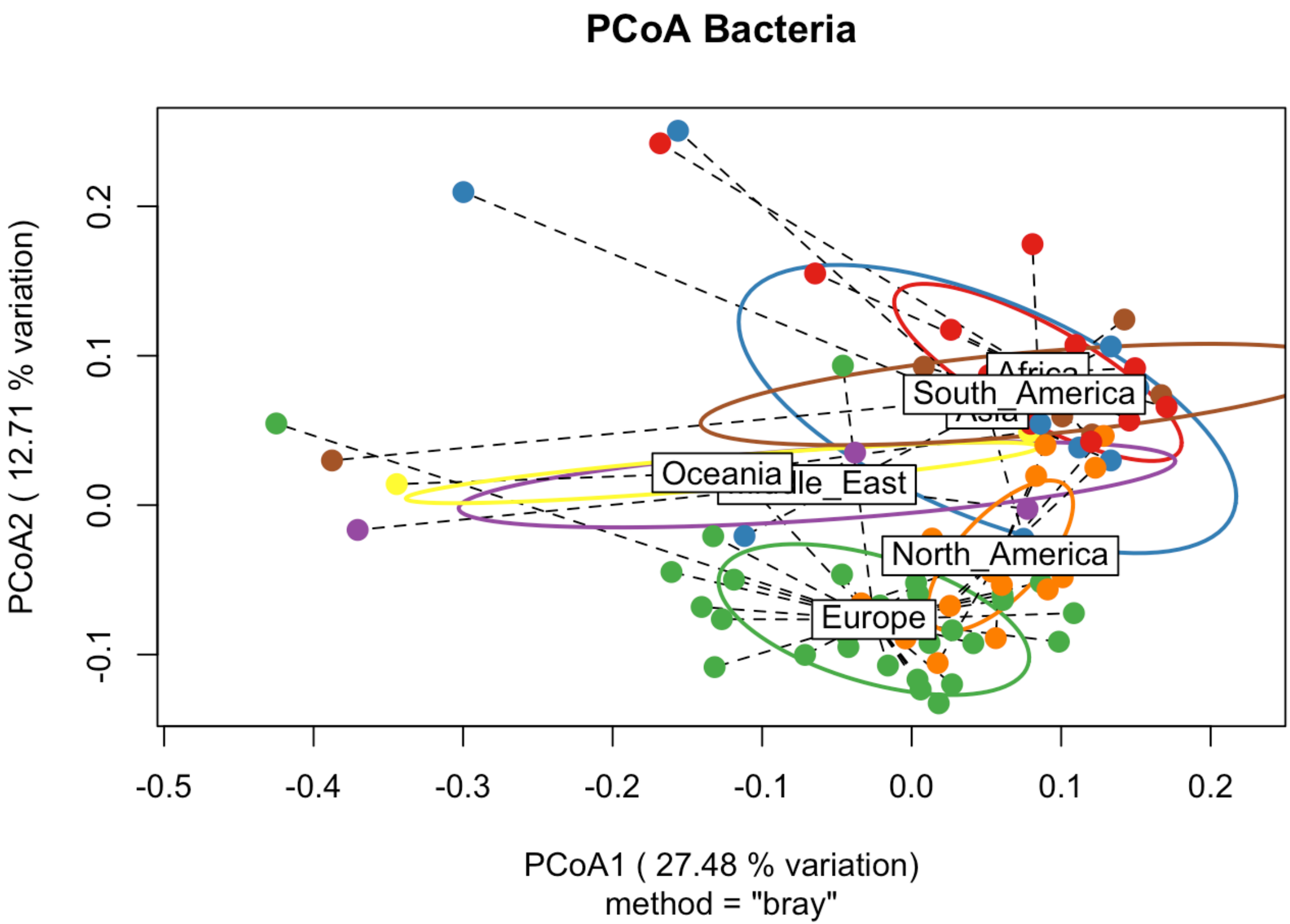

PCoA Vertebrate_mammals
South_America
Middle_East
Oceania
PCoA2 (14.68 % variation)
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.2
0.0
0.2
0.4
PCoA1 (28.59 % variation)
method = "bray"


PCoA Vertebrates_other
Middle_East
Oceania
South_America
Europe
Africa
North_America
PCoA2 (16.13 % variation)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.6
-0.4
-0.2
0.0
0.2
0.4
PCoA1 (27.01 % variation)
method = "bray"

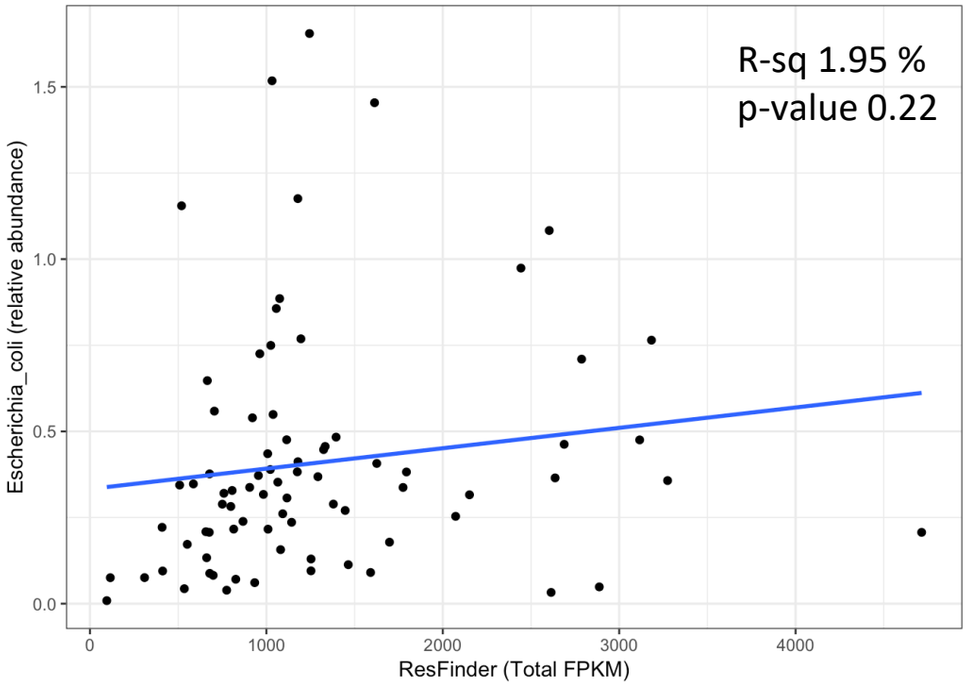

PCoA Invertebrates
Oceania
North_America
South_America
PCoA2 (11.75 % variation)
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.4
-0.2
0.0
0.2
0.4
PCoA1 (20.44 % variation)
method = "bray"


PCoA Protozoa
PCoA2 (21.11 % variation)
0.4
0.3
0.2
0.1
0.0
-0.1
Middle_East
Oceania
Asia
South_America
America
Africa
Europe
-0.6
-0.4
-0.2
0.0
0.2
0.4
PCoA1 (36.51 % variation)
method = "bray"

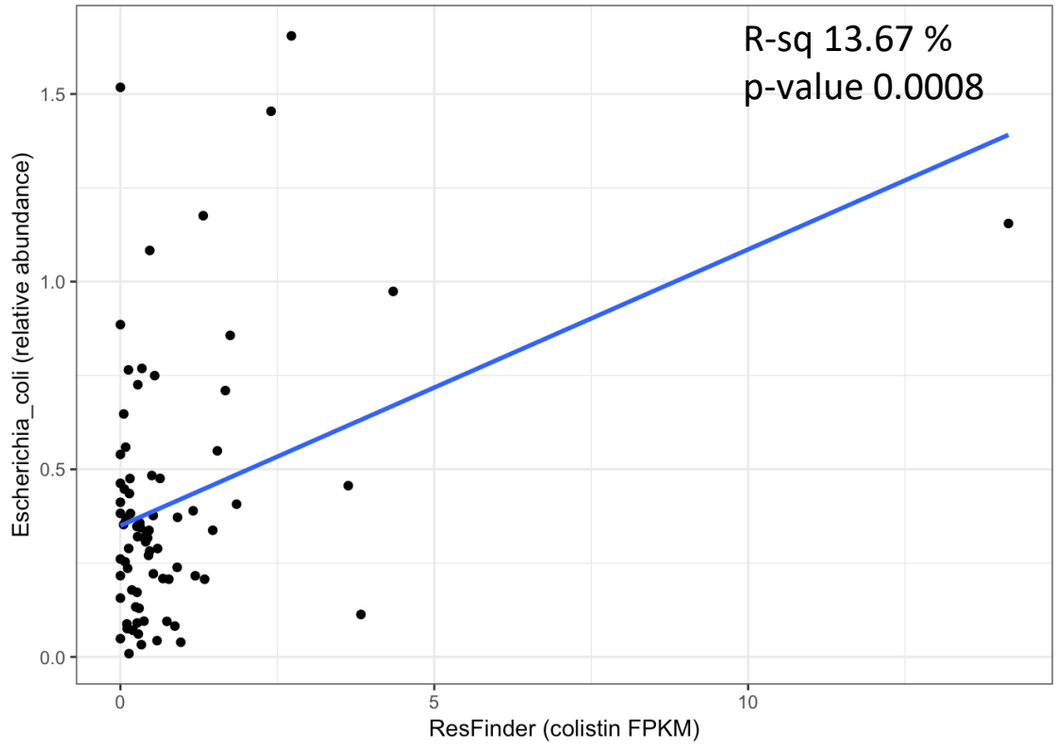

PCoA Fungi
Middle_East
Oceania
South_America
Europe
North_America
PCoA2 (8.52 % variation)
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2
PCoA1 (16.45 % variation)
method = "bray"


PCoA Bacteria
South_America
Oceania
North_America
Europe
PCoA2 (12.71 % variation)
PCoA1 (27.48 % variation)
method = "bray"
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
-0.1
0.0
0.1
0.2

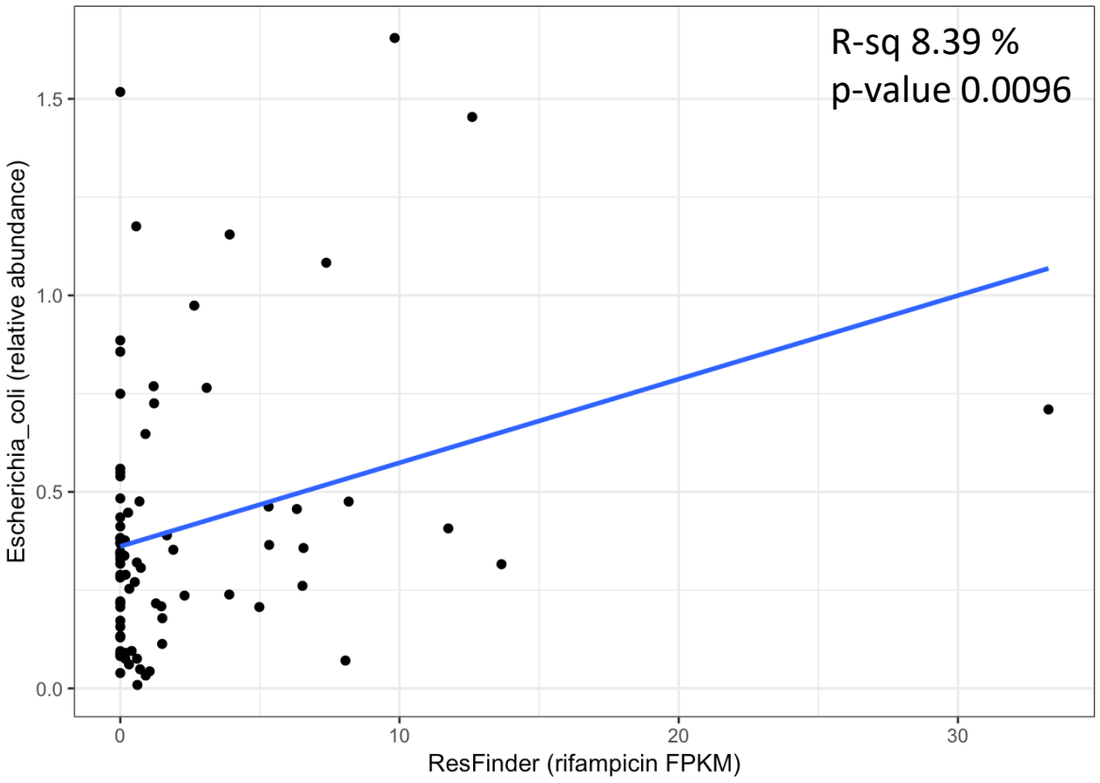
AMR genes and E.coli association



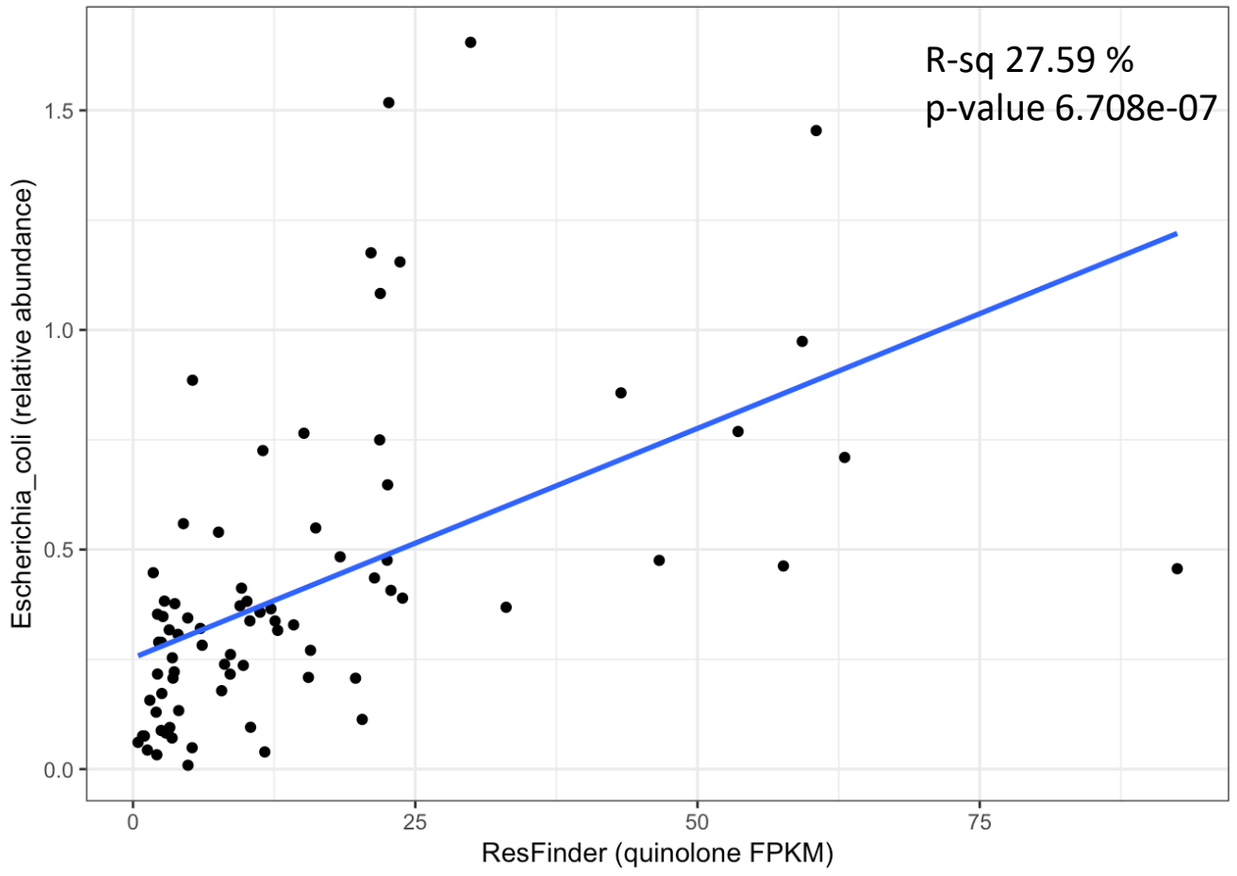
ResFinder (total FPKM)



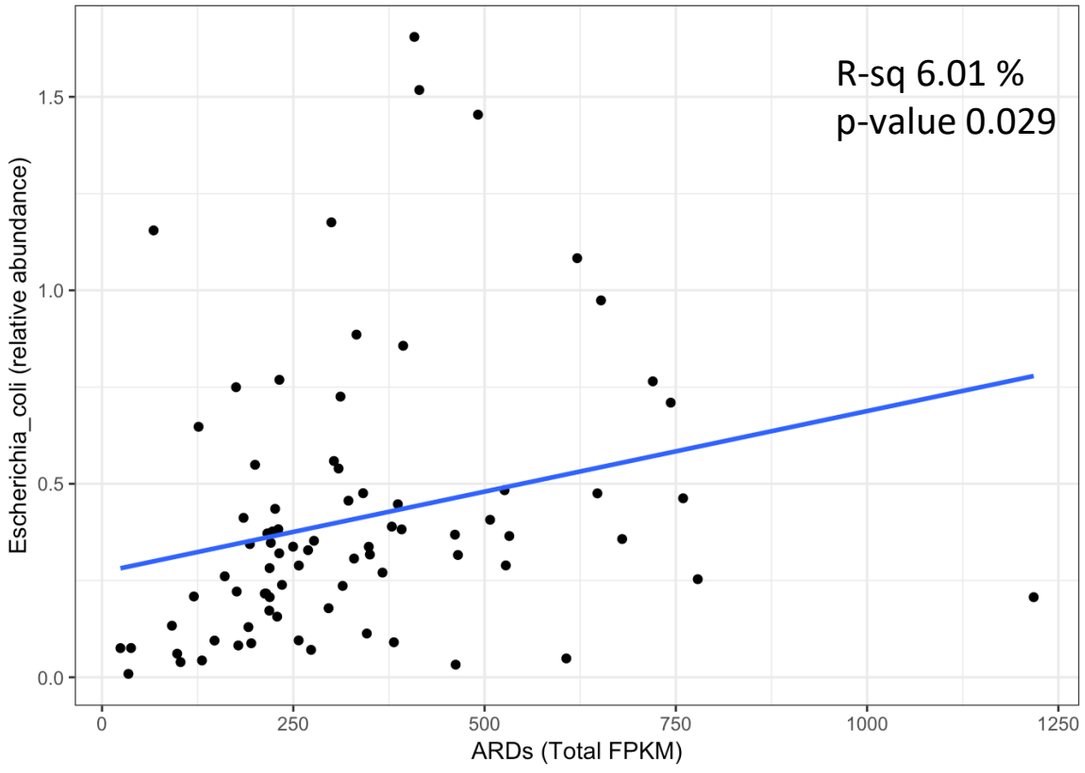
colistin



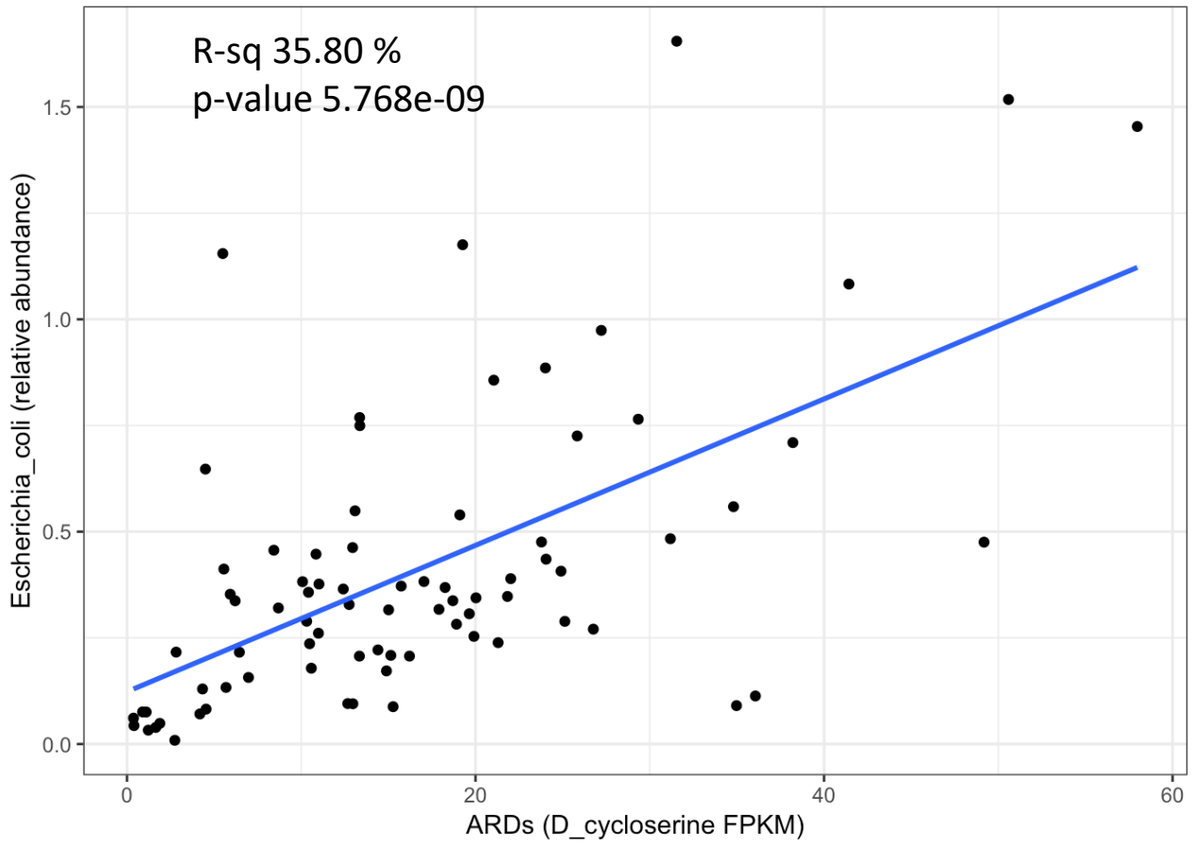
rifampicin

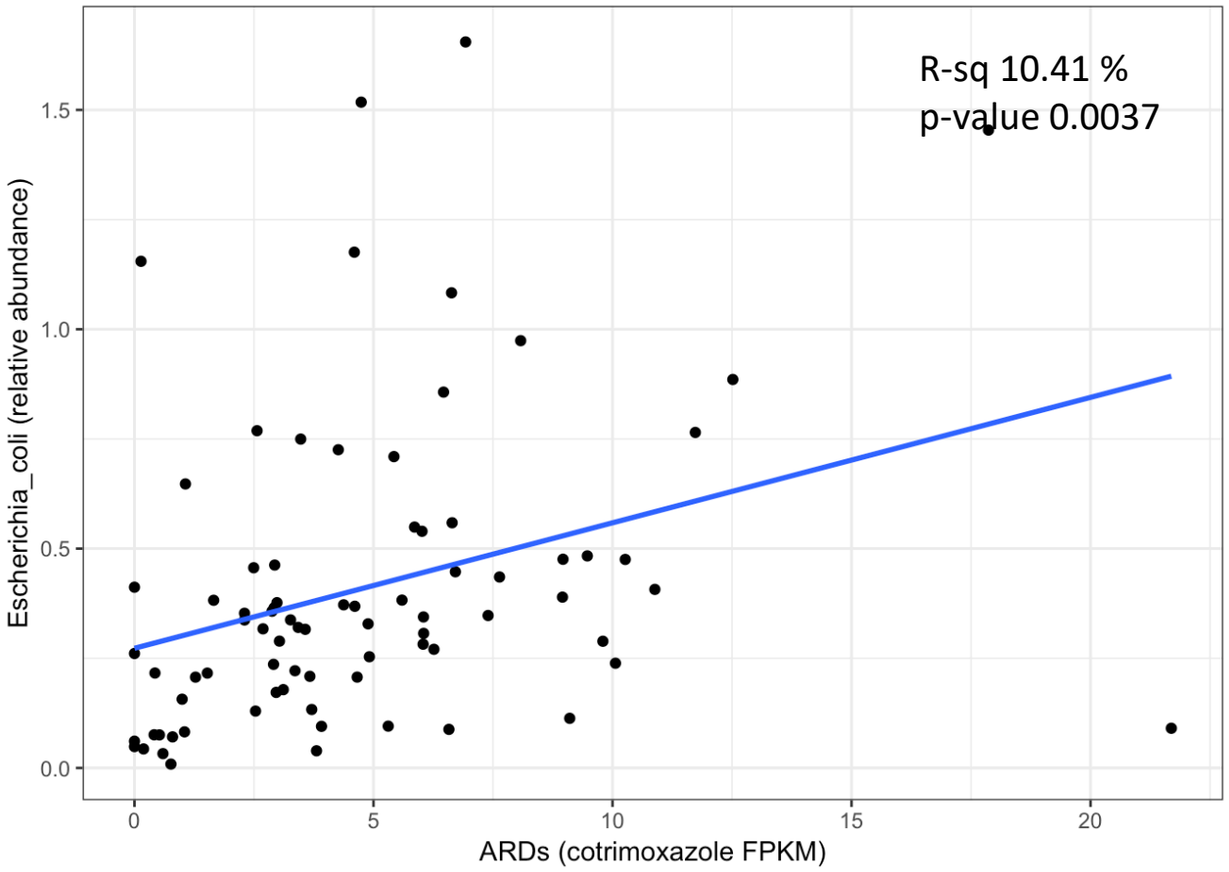


quinolone



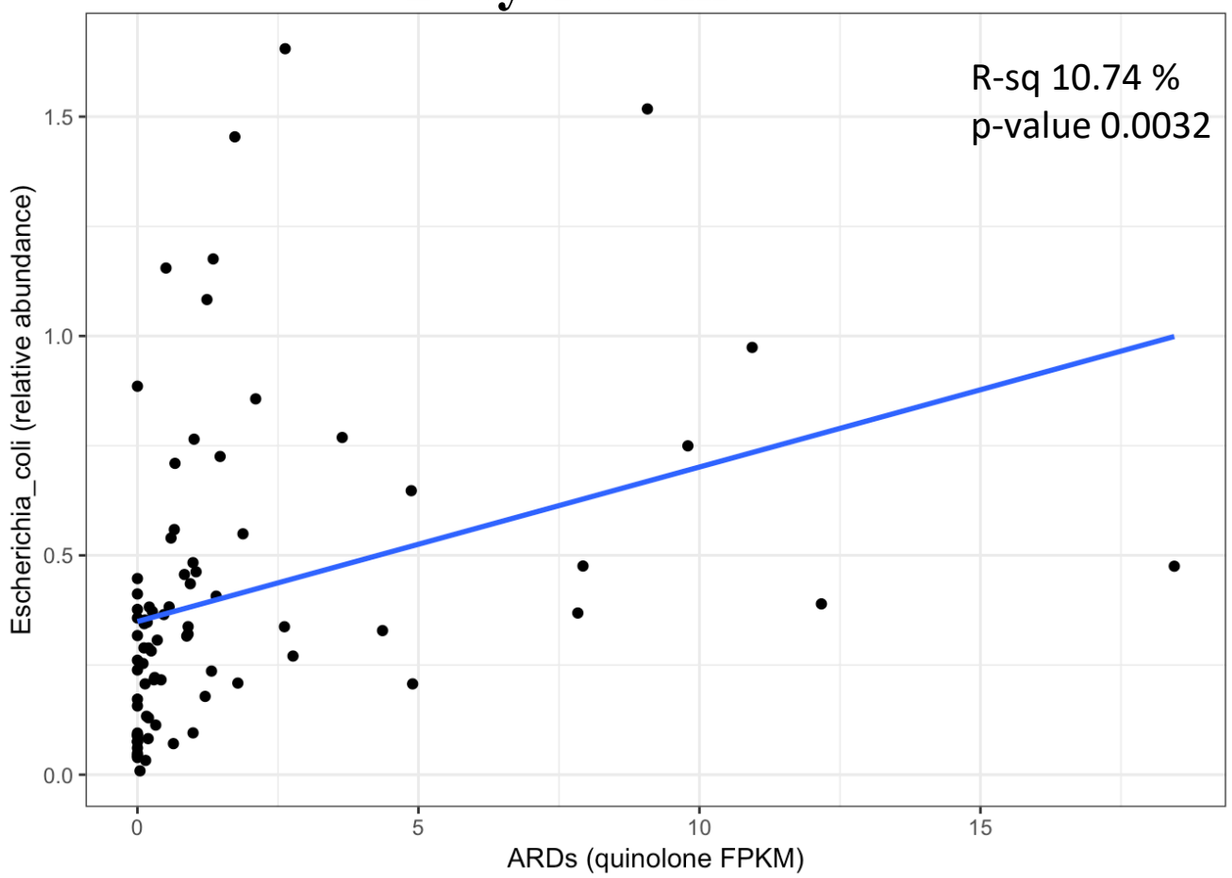
Functional resistance (total FPKM)

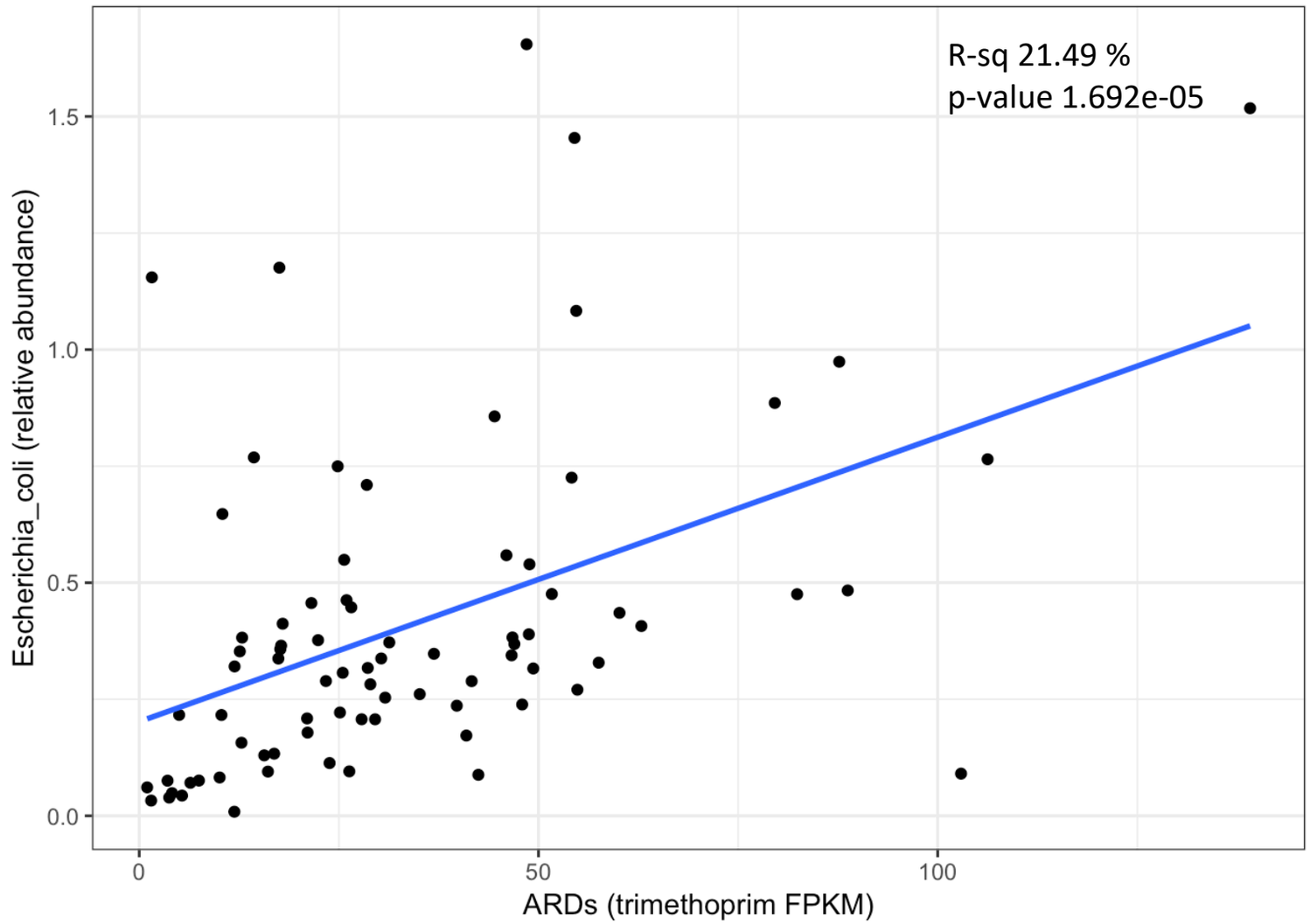




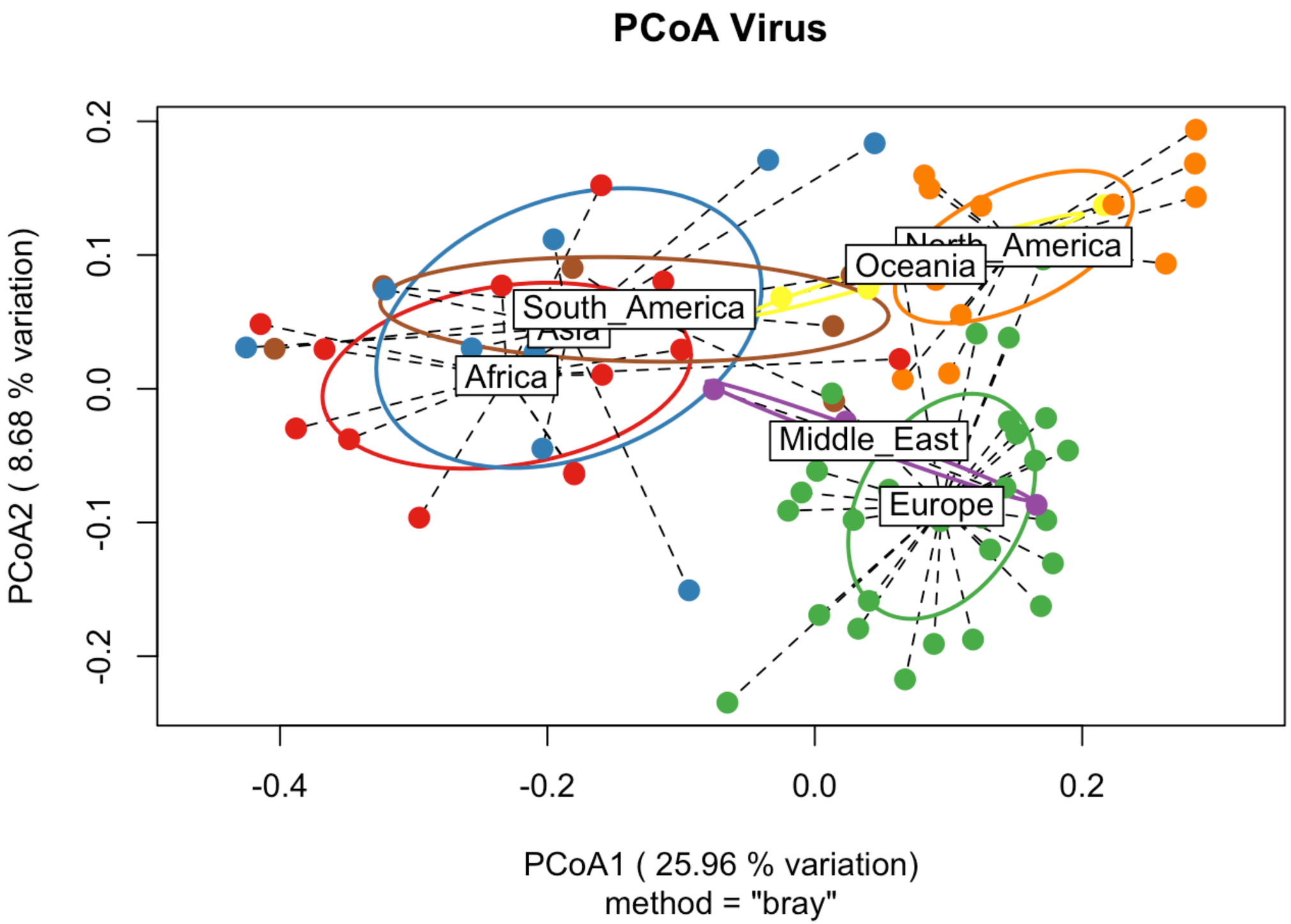
cotrimoxazole

D-cycloserine





trimethoprim


PCoA Virus
PCoA2 (8.68 % variation)
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2
PCoA1 (25.96 % variation)
method = "bray"
South_America
Africa
Oceania
North_America
Middle_East
Europe