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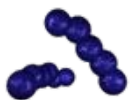
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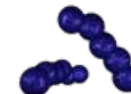
Deciphering the role of vaginal and sperm microbiota in the success of artificial insemination in sheep

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WHY AI?

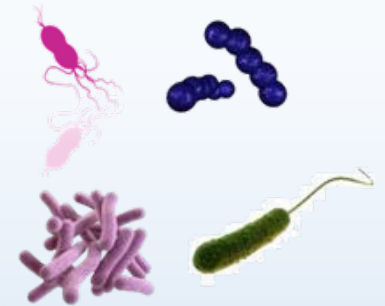
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In dairy sheep breeding programs, AI is an essential tool to:

- ▶ *Rams progeny test*
- ▶ *Connect herds for comparable EBVs across herds*
- ▶ *Dissemination of genetic improvement through genetic elite rams*



LOW SUCCESS AI RATES

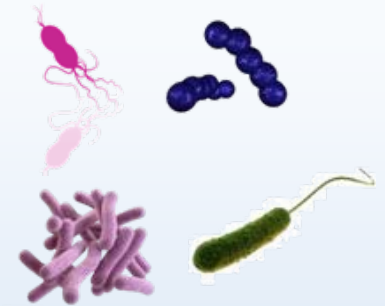
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Depending on breeds and production systems, AI in sheep has in general low success rates, among many other reasons, because:

- ▶ *Ewes winding and long reproductive tract*
- ▶ *Variable skills of inseminators*
- ▶ *Use of fresh semen*
- ▶ *Unknown step of the oestrus cycle*



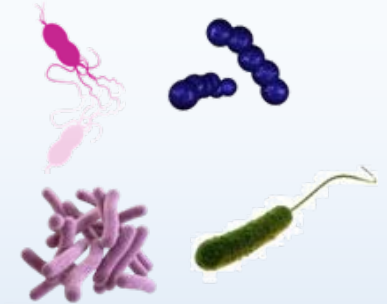
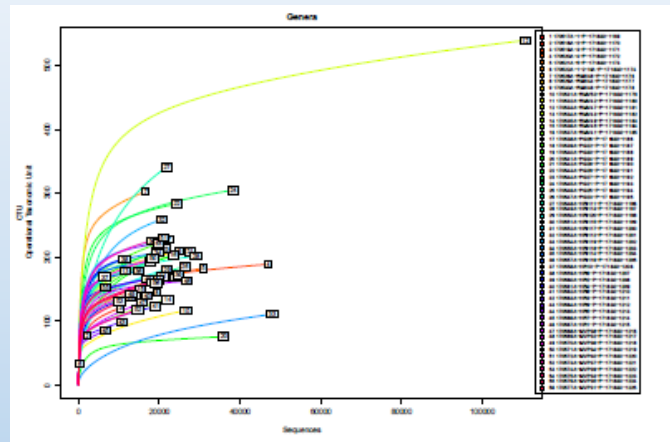
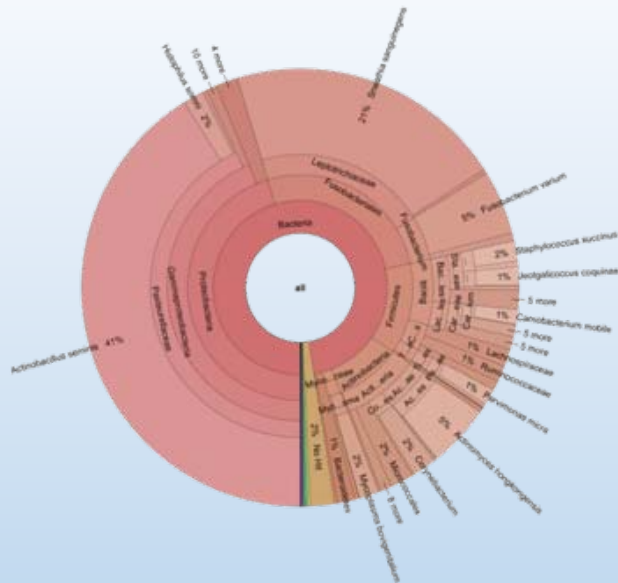
OBJECTIVE

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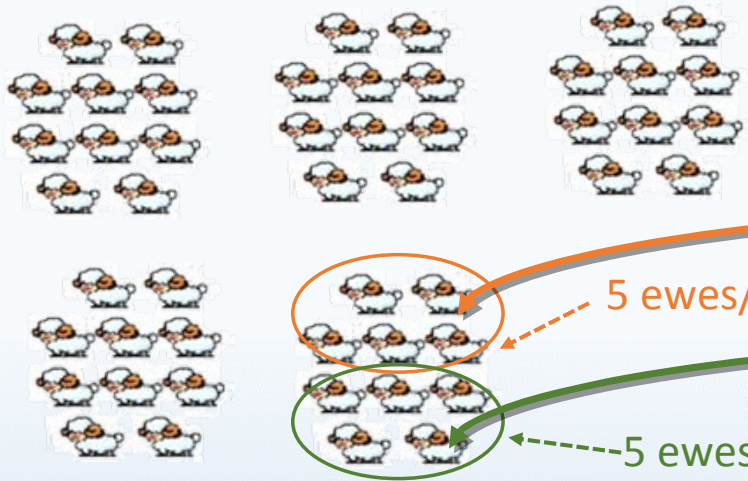
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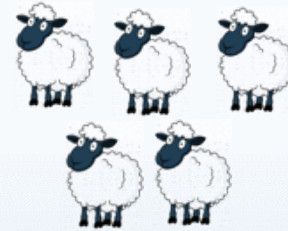
Characterize Vaginal and sperm Microbiota in the ovine specie and determine its role in the AI success using NGS techniques



EXPERIMENTAL DESIGN



- 5 AI rams
- Age 4 to 7 years
- Each ram AI 2 ewes/herd



- 5 herds, 10 ewes/herd
- Age 1.5 to 6 years
- At least one lambing



- AIs performed by a single veterinarian in the same day



METHODS

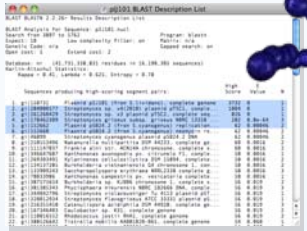
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BLAST *rRNA RefSeq*
NCBI database

6



5

Remove *Ovis aries* seq
GCA_000298735.2 Oar_v4.0



1 **DNA extraction**
Qiagen Kits



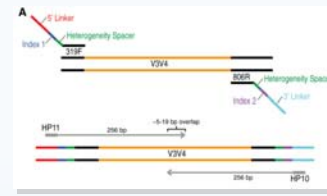
4

Sequences management
Pair merged PEAR
Remove adapters, low quality and short reads CUTADAPT >200nt
Remove chimera UCHIME



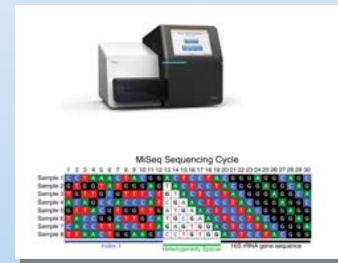
2

Illumina Libraries
V3-V4 16s rRNA gene



3

MiSeq Illumina
300nt PE



BIOINFORMATICS

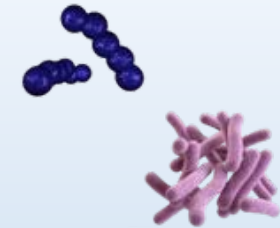
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▶ Differences in **microorganism abundance** at the **phylum, family, genera and species levels** were test for:

- *PRIDs treatment (NAB vs AB)*
- *rectal temperatures (in ranks of 0.5°C)*
- *herds*
- *AI rams*
- *ewes pregnancy status (pregnant P, non-pregnant NP)*

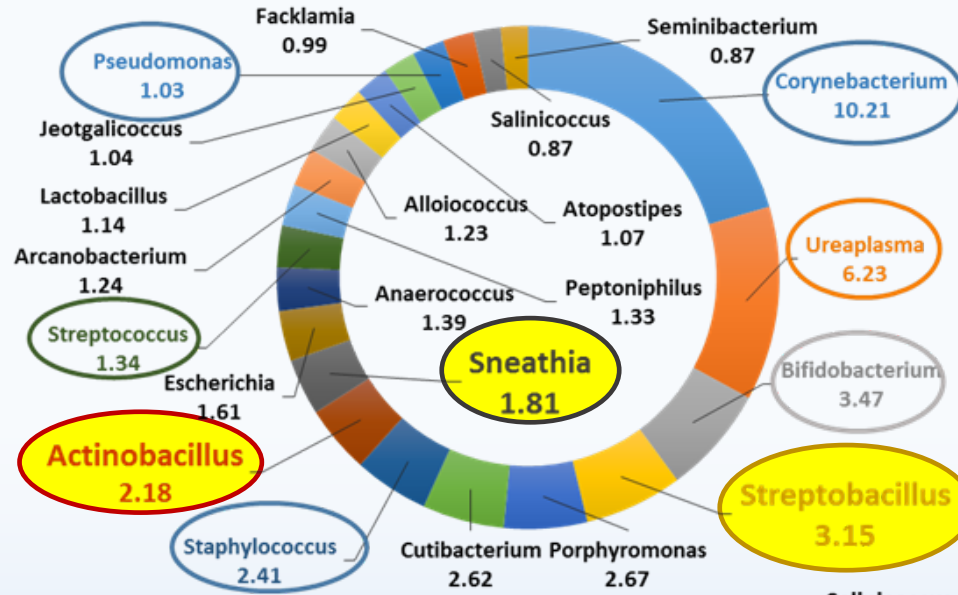
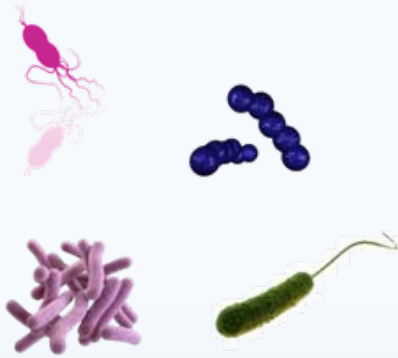


▶ **DESeq2 R package** (*negative binomial distribution*)

▶ *Pairwise comparisons: **log₂FC** of the normalized average counts ratio*

▶ *T test **log₂FC/log₂FCse**. Adjusted **p_values** Benjamini & Hochberg*

RESULTS



EWES

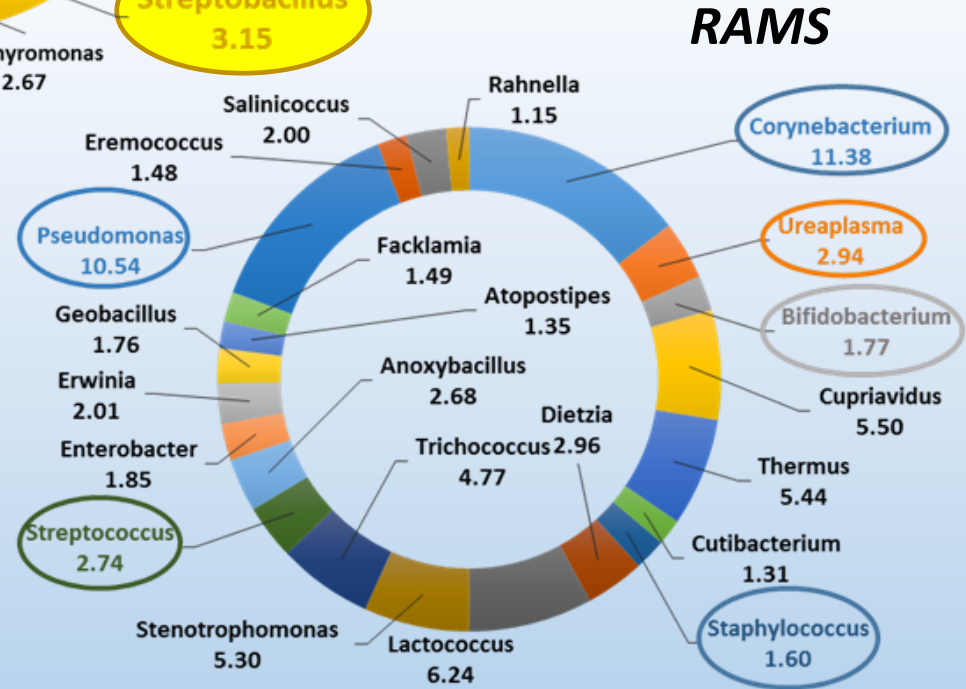


	% of AI success
Ram 1	40%
Ram 2	40%
Ram 3	40%
Ram 4	30%
Ram 5	50%
average rams	40%
average ewes	20%
Herd 1	70%
Herd 2	20%
Herd 3	20%
Herd 4	60%
Herd 5	30%

Moderate differences in AI success among rams

⇒ ⇒ ⇒ *Low AI success in ewes*

High differences in AI success among herds



RESULTS

ANTIBIOTIC effect on microbiota

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Only one GENUS was significant affected by the use of ANTIBIOTIC in the PRIDs

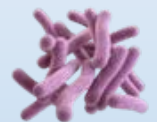
<i>PRIDs non antibiotic vs PRIDs antibiotic</i>	<i>base Mean</i>	<i>log₂FC</i>	<i>p_adj</i>
<i>Leptotrichiaceae; Streptobacillus</i>	509.00	4.424	0.0002

✓ **Streptobacillus** (*Leptotrichiaceae*) **21 times** more abundant in ewes carrying **PRID_{NAB}**



Streptobacillus notomytis **19 times** more abundant in **NP** ewes

(log₂FC = 4.23 p_{adj} = 0.05)



Although any **reproductive effect** have been described for this genus, its abundance seems to affect indirectly the pregnancy rate of ewes

RESULTS

microbiota effect on the PREGNANCY RATE

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Significant differences in GENERA abundance between NON PREGNANT and PREGNANT ewes

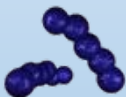
Genera	base Mean	log ₂ FC	p_adj
<i>Corynebacteriaceae; Corynebacterium</i>	2015.90	1.476	0.0002
<i>Enterobacteriaceae; Escherichia</i>	174.66	2.825	0.0007
<i>Porphyromonadaceae; Petrimonas</i>	173.54	3.244	0.0010
<i>Leptotrichiaceae; Sneathia</i>	801.38	3.757	0.0355
<i>Pasteurellaceae; Actinobacillus</i>	1997.55	4.492	0.0051
<i>Pasteurellaceae; Histophilus</i>	215.54	4.663	0.0298

- ✓ **Sneathia** (*Leptotrichiae*) → part of the normal microbiota of genitourinary tracts of men and women, but also has been associated with **bacterial vaginosis** and **spontaneous abortion**

13 times more abundant in **NP** ewes

Sneathia sanguinegens **20 times** more abundant in **NP** ewes

(log₂FC = 4.35; p_adj = 0.05)



RESULTS

microbiota effect on the **PREGNANCY RATE**

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HAP group (*Haemophilus-Actinobacillus-Pasteurella*) **pathogens resistant to most cellular defence mechanisms**

✓ **Histophilus** (*Pasteurellaceae*) **25 times** more abundant in **NP** ewes **Histophilus somni** **x28** in **NP**

($\log_2FC = 5.36$; $p_{adj} = 0.05$)

- Respiratory and **genitourinary** infections in cattle. Ability to produce a **biofilm**

✓ **Actinobacillus** (*Pasteurellaceae*) **22 times** more abundant in **NP** ewes **Actinobacillus seminis** **x26** in **NP**

($\log_2FC = 4.80$; $p_{adj} = 0.03$)

- **Abnormal semen, epididymitis and infertility** in rams and **abortions** in ewes
- **Epididymitis** is a contagious disease which transmission occurs **venereally**, by homosexual activity and even by ewe to lamb, and results in substantial economic losses worldwide due to reproductive failure and culling of breeders
- This microorganisms were **not detected in the AI rams** here used



RESULTS

HERD effect on microbiota

Significant differences in GENERA abundance between HERDS

	% of AI success
Herd 1	70%
Herd 2	20%
Herd 3	20%
Herd 4	60%
Herd 5	30%



Herd	Genera	base Mean	log ₂ Fold Change	p _{adj}
2 vs 1	Enterobacteriaceae; Escherichia	344.25	5.340	0.0003
2 vs 1	Fusobacteriaceae; Fusobacterium	223.83	6.375	0.0011
2 vs 1	Pasteurellaceae; Actinobacillus	1997.55	6.480	0.0003
2 vs 1	Leptotrichiaceae; Sneathia	616.77	7.326	15.7E-06
2 vs 1	Pasteurellaceae; Histophilus	407.45	8.162	24.6E-06
2 vs 4	Actinomycetaceae; Actinomyces	165.04	4.359	0.0323
2 vs 4	Enterobacteriaceae; Escherichia	344.25	4.418	0.0323
2 vs 4	Leptotrichiaceae; Sneathia	616.77	5.045	0.0323
2 vs 4	Leptotrichiaceae; Leptotrichia	208.58	5.196	0.0492
2 vs 4	Pasteurellaceae; Histophilus	407.45	9.501	1.79E-06
2 vs 3	Actinomycetaceae; Actinomyces	165.04	4.919	0.0013
2 vs 3	Leptotrichiaceae; Streptobacillus	455.19	5.729	321.9E-06
2 vs 3	Leptotrichiaceae; Leptotrichia	208.58	6.061	0.0015
2 vs 3	Fusobacteriaceae; Fusobacterium	223.83	6.422	0.0013
2 vs 3	Pasteurellaceae; Histophilus	407.45	7.644	163.2E-06
2 vs 3	Leptotrichiaceae; Sneathia	616.77	8.273	384.5E-09
2 vs 5	Staphylococcaceae; Staphylococcus	413.60	2.476	0.0015
2 vs 5	Actinomycetaceae; Actinomyces	165.04	4.166	0.0063
2 vs 5	Fusobacteriaceae; Fusobacterium	223.83	4.790	0.0274
2 vs 5	Enterobacteriaceae; Escherichia	344.25	6.109	4.6E-06
2 vs 5	Leptotrichiaceae; Streptobacillus	455.19	7.201	209.1E-09
2 vs 5	Leptotrichiaceae; Leptotrichia	208.58	8.550	209.1E-09
2 vs 5	Pasteurellaceae; Actinobacillus	1997.55	9.156	8.3E-09
2 vs 5	Leptotrichiaceae; Sneathia	616.77	9.610	185.0E-12
2 vs 5	Pasteurellaceae; Histophilus	407.45	11.262	185.0E-12
3 vs 5	Pasteurellaceae; Actinobacillus	1997.55	7.885	29.2E-06
4 vs 5	Leptotrichiaceae; Streptobacillus	455.19	4.960	0.0567

CONCLUSIONS



- ▶ *Since only a genus seems to be affected by the **ANTIBIOTIC**, we wonder about the indiscriminate use of **ANTIBIOTICS** as preventives. Concerning about **ANTIBIOTIC RESISTANCE** and human health, we should recommend **reduce antimicrobials** to the minimum necessary and **replace** it with other compounds such as pre and probiotics.*
- ▶ *We have verified that microorganisms of the genera **Histhopilus**, **Actinobacillus** and **Sneathia** have an important role in the fertility of this species and that they must be controlled to prevent its dissemination to the whole herd*
- ▶ *All success highly depends on the “**reproductive health**” of herds in terms of its **MICROBIOTA** composition*

Next Generation Sequencing techniques are a powerful tool to generate a valuable information to study the variability of complex traits, as is the case of **FERTILITY**



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!!! THANKS FOR YOUR ATTENTION !!!

HAVE COLLABORATED



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Profiles can study only at the genus levels, because PCR product for bacteria yields only 400 nt which did not allow a correct identification of the species. Species levels reported previously were shown as the closest found in the database, but we can not be sure since we are studying a really small portion of the genome.

AB	no	no	yes	no	yes	yes	yes	no	no	no	yes	yes	yes	yes	yes	no	no	no	no	yes	yes
Pregnancy	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
ID paper	ewe 1- R5	ewe 4- R5	ewe 10- R5	ewe 12- R3	ewe 19- R3	ewe 29- R2	ewe 30- R2	ewe 31- R1	ewe 34- R1	ewe 35- R1	ewe 36- R1	ewe 37- R1	ewe 38- R1	ewe 39- R1	ewe 42- R4	ewe 43- R4	ewe 44- R4	ewe 45- R4	ewe 47- R4	ewe 50- R4	
Histophilus	0	0	0	0	0	0.288	0.161	0	0	0	0	0	0	0	0	0	0	0.004	0	0.005	0.023
Sneathia	0.115	0	0	0	0.007	1.399	1.788	0	0.057	0.919	0.005	0.008	0.004	0	1.438	0	0.004	0.417	0	0.154	0.316
Actinobacillus	0	0	0.015	0.004	0.207	1.811	3.09	0.01	0	0	0	0	0.004	0	1.354	3.324	0.79	0.289	0.005	0.005	0.545
Mageebacillus	0	0	0	0	0	0	0.216	0	0	0	0	0	0	0	0	0	0	0	0	0	0.011
Streptococcus	3.369	0	0.185	5.252	0.938	1.126	0.443	0.247	1.178	1.783	4.342	0	0.699	2.454	1.33	1.82	4.381	0.373	0.357	0.87	1.557
Staphylococcus	1.669	1.926	0.201	1.361	0.486	2.378	0.77	0.596	0.399	5.694	5.745	0.511	0.784	0.763	5.944	3.541	1.965	1.337	1.457	2.483	2.001
Corynebacterium	8.185	13.211	14.809	4.839	9.739	16.715	6.187	0.533	2.863	2.101	5.952	2.005	6.778	3.051	6.92	3.689	4.02	21.877	3.015	2.741	6.962
Streptobacillus	0.139	0	0.012	0.008	0.021	0.08	0.012	3.922	0.045	0.023	0.156	0	0.017	0.01	4.598	8.624	3.492	0.648	0.009	0.019	1.092

AB	no	no	no	yes	yes	yes	yes	no	no	no	no	yes	yes	yes	yes	no	no	no	no	yes	yes	yes	no	no	yes	no	yes	yes	yes		
Pregnancy	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no		
ID paper	ewe 2- R5	ewe 3- R5	ewe 5- R5	ewe 6- R5	ewe 7- R5	ewe 8- R5	ewe 9- R5	ewe 11- R3	ewe 13- R3	ewe 14- R3	ewe 15- R3	ewe 16- R3	ewe 17- R3	ewe 18- R3	ewe 20- R3	ewe 21- R2	ewe 22- R2	ewe 23- R2	ewe 24- R2	ewe 25- R2	ewe 26- R2	ewe 27- R2	ewe 28- R2	ewe 32- R1	ewe 33- R1	ewe 40- R1	ewe 41- R4	ewe 46- R4	ewe 48- R4	ewe 49- R4	
Histophilus	0	0	0	0	0	0	0	0	0	0.004	0	0.049	0.07	0	1.542	1.73	9.078	0	11.36	2.73	0.135	0.168	0	0.119	0	0	0	0	0.005	0.900	
Sneathia	0	0.006	0	0.025	0	0.004	0	0	0.02	0.014	0	0	0	0.002	0.006	20.562	9.489	2.042	0.005	0.43	2.114	27.516	0.394	21.578	0.004	0	0	0	0.065	2.809	
Actinobacillus	0	0	0	0.143	0	0	0.006	0.004	0.127	10.499	0.011	0	0.062	0.07	0.187	41.098	18.45	12.277	0.185	6.293	5.14	0.179	1.028	0.735	0.014	0	0	0	0.059	1.315	3.263
Mageebacillus	0.004	0	0	0.021	0	0	0	0	0	1.54	0	0	0	0.068	0	0.349	0.241	0.165	0.068	0.548	0.128	0.008	0.054	0.007	0	0.003	0	0	0	0.107	
Streptococcus	7.539	0.653	0.314	1.094	0.755	0.799	2.533	1.796	0.377	0.133	0.093	2.07	0.577	0.164	3.31	0.106	0.407	0.115	0.58	0.241	0.503	0.012	0.621	0.453	4.891	1.929	0.045	2.949	0.838	0.114	1.200
Staphylococcus	1.342	0.822	0.106	2.119	0.975	1.458	0.312	34.158	1.253	0.017	0.064	3.315	0.538	0.766	4.225	1.88	1.808	0.36	4.574	0.28	0.718	0.037	8.679	0.511	3.342	0.288	0.434	4.273	1.111	0.477	2.675
Corynebacterium	7.977	20.89	32.162	15.895	35.962	3.37	17.501	3.554	29.223	0.533	51.909	9.501	1.893	27.399	15.769	1.811	3.091	0.834	28.401	4.885	2.781	0.141	17.111	3.161	5.28	2.508	8.881	7.734	7.408	3.559	12.371
Streptobacillus	0.59	0.003	0	0.002	0.008	0.004	0.012	0.004	0	67.778	0.032	0.051	0.278	0.073	0.006	0.139	0.053	8.921	0.024	14.064	0.082	39.108	0.172	2.717	0	0.088	0	0	0.19	1.405	4.527