ARTICLE

METAGENOME OF INDIAN ONE RUPEE COIN REVEALS PLETHORA OF MICROBIOTA

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ABSTRACT

Background: *Currency coins are widely exchanged inanimate object in the world and thus are potential source of pathogenic microorganisms. Most of the earlier studies focused upon culture dependent screening of currency coins and proved less fruitful.* ***Methods:*** *We studied an Indian One Rupee Coin through metagenomic approach. Metagenomic DNA was isolated and Illumina Mi-seq PCR and sequencing was carried out. Further, denoising, chimera checking, SFF file generation, quality file generation, sequence clustering, taxonomic identification and data analysis were performed.* ***Results:*** *Among the trimmed kingdom, bacteria ranked first (99.81%) and the rest were no hit. Among trimmed phylum and trimmed class, actinobacteria was abundant (90.52% and 90.45%) respectively. Among the trimmed order and family, propionibacteriales and Propionibacteriacea were found to be copious (88.67% and 88.53%) respectively. Among genus, Propionibacterium was found to be abundant (88.53%).* ***Conclusions:*** *Possible pathogenic microorganisms found at species included: Corynebacterium accolens, Corynebacterium kroppenstedtii, Propionibacterium granulosum, Staphylococcus aureus, Finegoldia magna, Listeria monocytogenes and Staphylococcus epidermidis and thus coins are potential source of pathogenic microorganisms.*

KEY WORDS
Coin, Metagenome, Currency, India

INTRODUCTION

Currency coin is a potential source of pathogenic microorganisms. Currency coins are widely exchanged inert object in the world. Most of the studies payed attention upon culture dependent selection of currency coins and proved less productive. To our best knowledge, this scientific communication of metagenomic study of Indian currency coin revealed a plethora of potential pathogenic bacteria that might play a significant role in disease spread and possible antibiotic resistance spread.

MATERIALS AND METHODS

Sample collection and metagenomic DNA isolation

An Indian One Rupee coin was obtained. Metagenomic DNA was isolated from an Indian One Rupee coin by adding 0.25 ml of saline sample to powermag bead plate and further powermag bead solution, lysis solution and RNase A was added. The powermag bead plate was placed in the 96 well plate shaker and later centrifuged. The supernatant was transferred to a clear powermag 1ml collection plate. By using inhibitor removal technology, powermag IRT solution was added and incubated at 4˚C and later centrifuged. 450 µl of supernatant was transferred to a new powermag collection plate and again centrifuged. 450 µl of supernatant was transferred to a kingfisher deep well 96 plate and the metagenomic DNA was isolated with the technical support of Rocio Navarro Garcia and J. Delton Hanson in Research and Testing Laboratory, USA (Research and Testing Laboratory, 4321 Marsha Sharp FWY, Door #2, Lubbock, Texas 79407, USA).

Sequencing of isolated metagenomic DNA

Illumina Mi-seq was performed [1] with the technical support of above mentioned personnel in Research and Testing Laboratory, USA. Illumina Mi-seq PCR technique is an accurate and widely used technique [1]. PCR was performed in a two step process according to Users' manual. The forward and reverse primers namely TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG and GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG were used for first round of PCR. PCR was performed according to Users' manual with the technical support of already mentioned personnel in Research and Testing Laboratory, USA in 25 µl reactions with 1 µl template and 1 ul 5 uM primer on ABI verity thermo cyclers (Applied Biosytems, California, USA) using Qiagen Hot Star Taq master mix (QiagenInc, California, USA) under the following conditions: 95 ˚C for 5 minutes, 25 cycles of 94 ˚C for 30 S, 54 ˚C for 40 S, 72 ˚C for 1 minute, one cycle of 72 ˚C for 10 minutes and 4 ˚C hold. The forward and reverse primers namely AATGATACGGCGACCACCGAGATCTACAC (i5 index)- TCGTCGGCAGCGTC and CAAGCAGAAGACGGCATACGAGA (i7 index)- GTCTCGTGGGCTCGG were used for second round of PCR according to Users' manual. PCR was performed according to Users' manual as before with the technical support of personnel in Research and Testing Laboratory, USA. PCR products were visualized in e-gels according to Users' manual (Life Technologies, New York, USA), pooled in equal molar and all the pooled products were size selected into 2 rounds according to Users' manual using AgencourtAmpure XP (BeckmanCoulter, Indiana, USA) with the technical support of personnel in Research and Testing Laboratory, USA. A 0.7 ratio for both rounds size chosen pool was then quantified using a Quibit 2.0 fluorometer (Life Technologies) according to Users' manual and loaded on an illumina Mi-Seq (Illumina, , California, USA) 2 x 300 flow cell at 10 pM and PCR products were sequenced according to Users' manual with the technical support of personnel in Research and Testing Laboratory, USA.

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Bioinformatics analysis

After completion of sequencing, FASTQ data was segregated as paired and single end. Paired end FASTQ data was merged and was send to converter along with single end FASTQ data. FASTA/ Qual file was generated and denoising [2] , chimera detection [3] was performed. Diversity analysis consisted of SFF file generation [4], quality file generation [5], sequence clustering [6], taxonomic identification [7] using OTU selection (default) / dereplication and data analysis using RDP and USEARCH (default) [8,9,10].

RESULTS AND DISCUSSION

KRONA depiction [Fig. 1] indicated among trimmed kingdom, bacteria ranked first (99.81%) and the rest were no hit [Table 1]. Among trimmed phylum and trimmed class, actinobacteria was abundant (90.52% and 90.45%) respectively. Among trimmed order and family, propionibacteriales and Propionibacteriacea were found to be copious (88.67% and 88.53%) respectively.

Among genus, Propionibacterium was found to be abundant (88.53%). Apart from actinobacteria, firmicutes (6.13%) dominated the trimmed taxa percentage [Table 2]. Bacilli was found to be second position (5.17%) among trimmed class [Table 3]. A slightly significant percentage of bacillales (3.82%), lactobacillales (1.35%) and corynebactetriales (1.30%) were present apart from leading propionibactetriales (88.67%) [Table 4]. Apart from leading genus Propionibacterium (88.53%), other prominent genus included Corynebacterium (1.06%), Staphylococcus (3.45%), Streptococcus (1.00%) [Table 6]. Prominent species found included *Pr*opionibactetrium acnes (88.2%), *Staphylococcus epidermidis* (3.37%) [Table 7].

Among the species found, many of them have been earlier reported to cause various infections: *Actinomyces* sp. (Oral- cervico facial disease) [11], *Corynebacterium accolens* (Pelvic osteomyelitis) [12], *Corynebacterium kroppenstedtii* (Inflammatory breast disease) [13], *Streptomyces* sp. (Mycetoma) [14], *Propionibacterium granulosum* (Septicemia) [15], *Bacteroides* sp. (Root canal infection) [16], *Porphyromonas* sp. (Infection in anatomic cells) [17], *Staphylococcus aureus* (Skin infection, respiratory infection) [18], *Peptostreptococcus* sp. (Brain, liver, abcesses, soft tissue infection) [19], *Veilionella* sp. (Endocarditis) [20], *Finegoldia magna* (Prosthetic infection) [21], *Methylobacterium* sp. (general infection) [22], *Listeria monocytogenes* (Meningitis, sepsis) [23], *Wolbachia* (Infection in ovary) [24] and *Staphylococcus epidermidis* (Infection in heart valves and joints) [25].

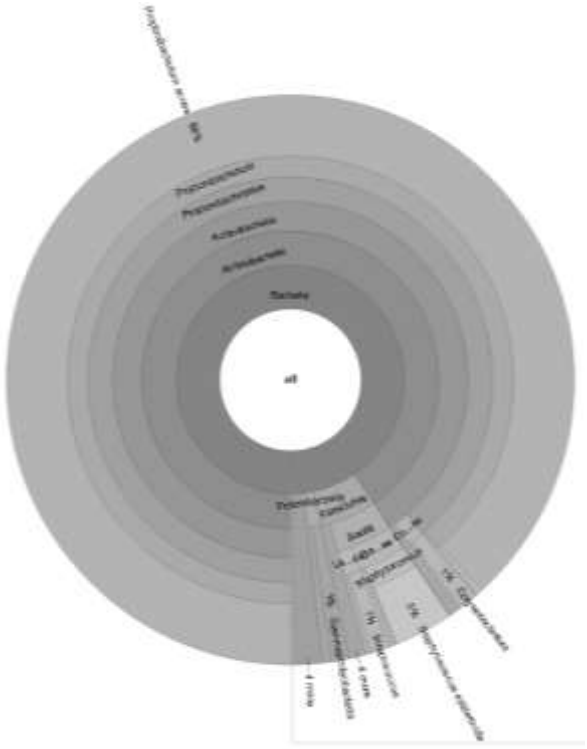

Fig. 1: Krona hierarchical pie chart of One Rupee coin metagenome Among trimmed kingdom, bacteria ranked first (99.81%) and the rest were no hit. Among trimmed phylum and trimmed class, actinobacteria was abundant (90.52% and 90.45%) respectively. Among genus, Propionibacterium was found to be abundant (88.53%).

Table 1: Kingdom percentage

Kingdom	Trimmed Taxa Percentage
Bacteria	99.81
No hit	0.18

Table 2: Phylum percentage

Phylum	Trimmed Taxa Percentage
Actinobacteria	90.52
Bacteroidetes	0.65
Cyanobacteria	0.15
Firmicutes	6.13
Proteobacteria	1.52
Unclassified	0.07
No hit	0.18
Unknown	0.74

Table 3: Class percentage

Class	Trimmed Taxa Percentage
Actinobacteria	90.45
Rubrobacteria	0.01
Bacteroidia	0.35
Flavobacteria	0.03
Sphingobacteria	0.20
Bacilli	5.17
Clostridia	0.54
Negativicutes	0.11
Tissierellia	0.05
Alphaproteobacteria	0.14
Betaproteobacteria	0.36
Gammaproteobacteria	1.00
Unclassified	0.07
No Hit	0.18

Table 4: Order percentage

Order	Trimmed Taxa Percentage
Actinomycetales	0.01
Corynebacteriales	1.30
Geodermatophilales	0.03
Micrococcales	0.15
Propionibacteriales	88.67
Streptomycetales	0.07
Rubrobacterales	0.01
Bacteroidales	0.35
Flavobacteriales	0.03
Sphingobacteriales	0.20
Bacillales	3.82
Lactobacillales	1.35
Clostridiales	0.50
Selenomonadales	0.11
Tissierellales	0.05
Rhizobiales	0.07
Rhodobacterales	0.03
Rickettsiales	0.03
Burkholderiales	0.21
Neisseriales	0.09
Enterobacteriales	0.56
Oceanospirillales	0.01
Pseudomonadales	0.28
Xanthomonadales	0.13
No Hit	0.18

Table 5: Family percentage

Family	Trimmed Taxa Percentage
Actinomycetaceae	0.01
Corynebacteriaceae	1.06
Mycobacteriaceae	0.23
Geodermatophilaceae	0.03
Microbacteriaceae	0.14
Micrococcaceae	0.01
Nocardioidaceae	0.14
Propionibacteriaceae	88.53
Streptomycetaceae	0.07
Rubrobacteraceae	0.01
Bacteroidaceae	0.10
Porphyromonadaceae	0.01
Prevotellaceae	0.23
Chitinophagaceae	0.04
Sphingobacteriaceae	0.16
Bacillaceae	0.27
Listeriaceae	0.09
Staphylococcaceae	3.45
Lactobacillaceae	0.31
Streptococcaceae	1.00
Flavobacteriaceae	0.03
Lachnospiraceae	0.02
Peptostreptococcaceae	0.07
Veillonellaceae	0.11
Peptoniphilaceae	0.05
Methylobacteriaceae	0.05
Xanthobacteraceae	0.02
Rhodobacteraceae	0.03
Anaplasmataceae	0.03
Burkholderiaceae	0.14
Enterobacteriaceae	0.56
Oceanospirillaceae	0.01
Moraxellaceae	0.18
Pseudomonadaceae	0.10
Xanthomonadaceae	0.13
Unknown	0.74
No Hit	0.18

Table 6: Genus percentage

Genus	Trimmed Taxa Percentage
Actinomyces	0.01
Corynebacterium	1.06
Mycobacterium	0.23
Blastococcus	0.03
Microbacterium	0.14
Arthrobacter	0.01
Nocardioides	0.14
Propionibacterium	88.53
Streptomyces	0.07
Rubrobacter	0.01
Bacteroides	0.10
Porphyromonas	0.01
Prevotella	0.23
Segetibacter	0.04
Pedobacter	0.16
Anoxybacillus	0.27
Listeria	0.09
Staphylococcus	3.45
Lactobacillus	0.31
Streptococcus	1.00
Oribacterium	0.02
Peptostreptococcus	0.07

Veillonella	0.11
Finegoldia	0.05
Methylobacterium	0.05
Xanthobacter	0.02
Rubellimicrobium	0.03
Wolbachia	0.03
Burkholderia	0.14
Tepidimonas	0.07
Buchnera	0.06
Escherichia	0.36
Marinomonas	0.01
Acinetobacter	0.08
Psychrobacter	0.09
Pseudomonas	0.10
Stenotrophomonas	0.13
Unclassified	0.07
Unknown	0.74
No Hit	0.18

Table 7: Species percentage

Species	Trimmed Taxa Percentage
Corynebacterium accolens	0.02
Corynebacterium kroppenstedtii	0.50
Corynebacterium sp.	0.17
Microbacterium oleivorans	0.03
Microbacterium phyllosphaerae	0.10
Arthrobacter sp.	0.01
Nocardioides sp.	0.14
Propionibacterium acnes	88.27
Propionibacterium granulosum	0.19
Propionibacterium sp.	0.06
Streptomyces sp.	0.03
Rubrobacter sp.	0.01
Bacteroides sp.	0.10
Porphyromonas sp.	0.01
Segetibacter sp.	0.04
Pedobacter duraquae	0.16
Anoxybacillus sp.	0.27
Listeria monocytogenes	0.09
Staphylococcus aureus	0.07
Staphylococcus epidermidis	3.37
Lactobacillus jensenii	0.31
Streptococcus sp.	0.70
Peptostreptococcus sp.	0.07
Veillonella sp.	0.11
Finegoldia magna	0.05
Methylobacterium sp.	0.01
Rubellimicrobium sp.	0.03
Buchnera aphidicola	0.06
Acinetobacter sp.	0.08
Psychrobacter sp.	0.09
Pseudomonas sp.	0.10
Stenotrophomonas sp.	0.13
No Hit	0.18

Table 8: Possible pathogenic bacteria

Bacteria	Reported disease	Trimmed Taxa Percentage	Reference
Actinomyces	Oral- cervico facial disease	0.01	11
Corynebacterium accolens	Pelvic osteomyelitis	0.02	12
Corynebacterium kroppenstedii	Inflammatory breast disease	0.50	13
Streptomyces sp.	Mycetoma	0.03	14
Propionibacterium granulosum	Septicemia	0.19	15
Bacteroides sp.	Root canal	0.10	16

	infection		
Porphyromonas sp.	Infection in anatomic cells	0.01	17
Staphylococcus aureus	Skin infection, respiratory infection	0.07	18
Peptostreptococcus sp.	Brain, liver, abcesses, soft tissue infection	0.07	19
Veilionella sp.	Endocarditis	0.11	20
Finegoldia magna	Prosthetic infection	0.05	21
Methylobacterium sp.	General infection	0.015	22
Listeria monocytogenes	Meningitis, sepsis	0.09	23
Wolbachia	Infection in ovary	0.03	24
Staphylococcus epidermidis	Infection in heart valves and joints	3.37	25

CONCLUSION

Possible pathogenic microorganisms found at species included: *Corynebacterium accolens, Corynebacterium kroppenstedii, Propionibacterium granulosum, Staphylococcus aureus, Finegoldia magna, Listeria monocytogenes* and *Staphylococcus epidermidis*.

CONFLICT OF INTEREST
There is no conflict of interest.

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AUTHOR CONTRIBUTION
All authors have contributed equally to this work.

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