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Identification and Antimicrobial Resistance Profile of Major Gastrointestinal Bacteria in Monkey (Olive Baboon)

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Abstract

All higher animals are associated with a diverse microbial community, mainly composed of bacteria. Diversity of gut micro-biota and health depends upon the food and environment. Zoo provides unintentionally diverse population exposure of microbes to animals as well as humans. About 60% of all human diseases and approximately 75% of emerging infectious diseases are zoonotic. Fecal samples were collected of Olive Baboon kept at Lahore zoo. Samples were collected by using sterilized polythene bags and shifted to the laboratory of Microbiology of IMBB Department of University of Lahore immediately. Primary isolation was done on Nutrient agar and resulting growth was shifted on Differential media by streaking method. Gram staining was performed for the confirmation of bacteria. Pure colonies of bacteria were isolated through selective media. Different biochemical tests were performed for the further confirmation of genus of bacteria. Different bacteria such as E. coli, Staphylococcus aureus, Shigella, Klebsiella spp., and Bacillus cereus were isolated. Antimicrobial resistivity of isolated bacteria was checked by using different antimicrobial discs and the zone of inhibition was measured in mm.

Keywords: Diverse microbial community • Gut micro-biota • Olive Baboon • Biochemical tests • zone of inhibition • Molecular level

Introduction

Primates have been counted and studied for over five decades as part of scientific research for their ecology, behavior, and conservation. Primates occupy a wide range of habitats even though they are a relatively small order [1]. Zoological parks provide essential reservoir of genetic materials for such species through captive breeding and re-introduction programs. While living in captivity, zoo animals are often exposed to a variety of pathogens that pose immediate risks on the survival of threatened species, and the situation is exaggerated by climatic changes that are leading to substantial loss in biodiversity [2].

Olive baboons (Papio anubis) are close to human, the member of Class Mammalia belong to Order Primates. They are the largest monkey of the world. Olive baboons are distributed in different regions of Africa and Arabia, being extremely adaptable, they occupy a wide variety of habitat ranging from mountains to riverine areas. They do not have gripping tail like old world monkey but are capable to climb when it is required. Olive baboons have strong jaws, bulky fur, sharp edged canine, and dog like nose [3].

Bacillus spp. is a Gram-positive aerobic or potentially anaerobic rod-shaped endospore-forming bacterium that is a major cause of food-borne disease in humans and is often implicated in food-borne outbreaks [4]. Bacillus species are widespread in the ecosystem because of their spore-forming ability, nutritional flexibility, and contamination of the food [5]. B. cereus has been shown to be responsible for eye and wound infections and systemic infections [4].

Staphylococcus aureus is a Gram-negative anaerobic cocci bacterium. It produces infections in food animals and humans. It mostly lives in respiratory, upper dietary, mucosal, and urogenital tract skin surfaces. These bacteria cause large number of superficial skin problems and life threating infections including toxic shock syndrome, endocarditis, and pneumonia [7]. Different types of extracellular enzymes and toxins are also produced by this bacterium which causes food poisoning and therapeutic problems. To study distribution of S. aureus in many animal species including exotic one, zoological parks plays an important role in providing the easy access to highly classified habitats [2]. From previous studies it has come to know that diet play vital role in shaping the diversity and composition of gut microbiota in humans and nonhuman primates. Bacterial diseases have significant effect on public health and these effects are because of the continuous increase in antibiotic resistance of

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bacterial pathogens. In 1972, to cure methicillin resistance in S. aureus and coagulase negative staphylococci, vancomycin antibiotic was used for clinical practice [6].

Sequencing of the bacterial genome has greatly increased the understanding of the genetics of a large number of bacterial pathogens and identifies novel antibiotics targets. Two decades ago, since the incarnation of genome sequencing, about 1800 bacterial genomes sequenced. Conservation of 16S rRNA gene sequence was noted in Bacillus spp., in 1960s [1]. Extensive use of this gene sequence was followed as premier work by Woese, who explained important properties [1].

It is contrast to genes which are needed to make enzymes. In such gene's mutations can be handled more frequently since they may affect structures not as peculiar and of outmost importance as rRNA (A bacterium can use sugar or protein as energy source as alternative if it does not have gene to make the enzymes which are required to utilize lactose). Therefore, few genes are conserved as highly as the 16S rRNA gene. 16S rRNA shows the relatedness of organisms as well as gaps in the evolutionary history of organisms since the complete change in the gene sequence of 16S rRNA with respect to time is not recognized [2].

Materials and Methods

The aim of this study was to isolate the abundant bacteria from fecal sample of olive Baboon kept at Lahore zoo. For this purpose, following procedure was carried out as: This experiment was conducted at laboratory of IMBB department, The University of Lahore, Pakistan.

Collection of samples

Fecal samples were collected from the cages of olive baboons at Lahore zoo. Twelve samples were collected using polythene bags after a week. Bags were sterilized with 9% Normal saline solution before sample collection to reduce the contamination chances. These samples after collection were shifted immediately to laboratory.

Laboratory procedure

For further study following laboratory procedure was carried out as:

Preparation of media

All the glassware (Petri plates, flasks, measuring cylinders, etc.) and plastic wears were washed out thoroughly using household detergent by tape water. Petri plates were incubated at 180°C for 2 hours for their sterilization before pouring of media to them. Laminar air flow cabinet (LAFC) was sterilized using ethanol by outward flow before working in it. For nutrient ager, the standard calculation was calculated. Hence media was prepared according to these standards in distill water according to required volumes.

Pouring of media

Nutrient agar media was sterilized by autoclaving at 121°C for 1 hour. After autoclaving of media, it was checked out for any contamination by making control as 20ml of media on each separate sterilize petri plate and then incubating them for 24hours as for Nutrient ager. When no contamination on control was found, then media was poured out on sterilized plates as 20ml for each plate and waited for media solidification for 5-10 minutes. This process was repeated for each sample.

Microbial isolation

Swabbing of fresh fecal sample was done on Petri plate carefully in Laminar air flow cabinet (LAFC) or near the flames to reduce the chances of contamination from environment. Petri plates were placed in incubator at 37°C for 24hrs. After 24hrs plates were analyzed for both bacterial contamination and colony formation.

Differential media

Selective media (such as MacConkey agar, Eosin Methylene Blue agar, CLED agar, Salmonella Shigella agar, Mannitol salt agar, Blood Agar, Pseudomonas Citramide agar and Citramide agar) were prepared and sterilized by autoclaving at 121°C for 1 hour. Media was checked out for any already present contamination by making control as 20ml of media on each separate sterilized petri plate and then incubating them for 24hours. When no contamination on control plate was found, then media was poured out on sterilized plates as 20ml for each plate using laminar air flow cabinet and waited for media solidification for 5-10 minutes. This process was repeated for each sample. After that, bacterial colonies were inoculated on differential media by streaking method. Petri plates were placed in incubator for differential media (for isolation of gram negative and gram-positive bacteria) at 37°C for 24hrs. After 24hrs plates were observed for gram negative and gram-positive bacteria.

Determination of bacterial morphology

The structural and functional attributes of bacteria were examined under light microscope. Following staining techniques and test were performed.

Gram staining technique

Gram staining technique was performed to determine gram characteristics of bacteria. After fixing the bacterial smear, crystal violet was applied first for 30 second and rinsed with water. It was followed by addition of mordant iodine for 1 minute which fixed the stain. After rinsing with water, the slide were washed with alcohol for 30 seconds. The bacterial slides were subsequently stained with the safranin dye counterstained for 45 seconds and rinsed again. The slide was blot dried and observed under light microscope.

Biochemical test

Biochemical tests (Simon citrate, Indole, Catalase, Coagulase, Triple sugar iron, SIM, Coagulase and Oxidase) were performed to identify bacterial genus based on their biochemical activities.

Antibacterial resistance

Mueller-Hinton agar (MH agar) was prepared and inoculated with 50 µl of dilute bacterial culture by spreading it over agar surface with the help of sterile cotton swabs. Filter paper discs were impregnated and placed on the inoculated agar surface using sterilized forceps.

Then Muller Hinton agar containing plates were incubated for 24 hours at 37°C. The concentration of bacterial suspensions was adjusted to 108 colony forming units (108 cfu/ml) in Muller Hinton Agar. Thereafter plates were incubated at 37°C in an incubator for 16-18 hours. The diameter of inhibition zone i.e., clear zone around the disc resulting from the inhibition of bacterial growth was measured.

Molecular characterization

Two bacterial stain were sent for molecular characterization.

Preservation of bacterial culture

A single colony was inoculated in liquid culture and incubated overnight. 500 µL of the overnight culture was added to 500µL of 50% glycerol (sterile) in a 2 mL screw top tube or cryovial and was gently mixed by vertexing or pipetting. The stock can be stable for years, if it is kept at -80°C.

Result

Fecal samples were collected from the Zoo. E. coli, Klebsiella, Shigella, Bacillus cereus, S. aureus, were isolated from the fecal samples of Baboon. Frequency and percentage of bacteria are reveled in (Figure 1,Figure 2).

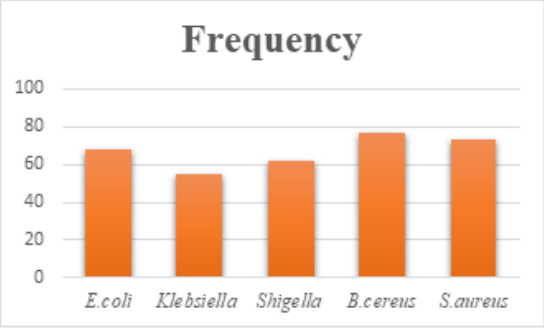

Figure 1. Frequency.

Figure 2. Sample collections.

Discussion

From this study bacteria were isolated from the fecal sample of Olive baboon from Lahore zoo. Different bacteria such as E. coli, Shigella, Klebsiella spp., Staphylococcus spp., and Bacillus cereus were found by chemical characterization. From the study S. aureus was isolated abundantly. Yellow mucoid colonies on MSA agar. It stained purple color on performing gram staining. Microscopy reveled that S. aureus is gram positive round shaped (cocci) bacteria.

Drougka, who investigated clonal spread of S. aureus between animals and working staff, supports this study. About 32 mammals, 11 birds and eight humans, samples were obtained from colonized and polluted sites. Seven isolates of S. aureus have been recovered from livestock. In several animal species, Staphylococcus aureus has been found the part of the flora of the microbiota. Febler in 2018 isolated S. aureus isolates mostly of bacterial infections from wild and zoo animals. Biochemical tests were performed for further confirmation. It gave Catalase, citrate, coagulase, MR, positive and oxidase negative.

During the study B. cereus was found in large amount repeatedly. Pure colonies of B. cereus were obtained from nutrient agar. On performing gram staining technique, it stained pink color as shown in figure no. Microscopy showed that it is gram-positive rod-shaped bacteria. Similarly, Lugano in 2018 identified Species of Gram-positive cocci, Gram-positive and Gram-negative rods, from wild and captive baboon fecal samples. E. coli, B. cereus and B. firmus were found to be the most common isolates in both groups of animals [3]. Different biochemical tests were performed for the confirmation. Catalase, citrates, test were positive and indole, MR were negative for Bacillus cereus.

Naas, similarly, showed that B. cereus isolates showed resistance to amoxicillin, amoxicillin / clavulanic acid, ampicillin, and cefotaxime. However, gentamicin, tobramycin, vancomycin, ciprofloxacin, and tetracycline were susceptible to more than 80 percent of the isolates. Both tested isolates were successful against Levofloxacin and doxycycline [4]. B. cereus was characterized at the molecular level to ascertain the species as well as genetic diversity. It was differentiated based on 16SRNA, the sequence of bacteria that was sent by Macrogene company has been entered in NCBI softer ware and comparison with the already been registered database. The phylogenetic trees and a graphic summary of B. cereus and Staphylococcus aureus which were obtained after BLAST. The Accession number of B. cereus MN192138.1, max score 1254, total score 1254, query cover100 percent, E value 0.00, Percent identity 99.85 percent, and Accession number of Staphylococcus aureus is NR. 037007.2, max score 1458, total score 1458, query cover100 percent, E value 0.00, Percent identity 100.00 percent. Weisburg used universal 253 primers fD1 and rD1 the 16SrRNA gene has been 255 amplified [5]. The 16SrRNA gene sequences obtained were identified by 256 matching sequences with Genbank database sequences using NCBI BLAST (Basic Local 257 Alignment Search Tool) and deposited with accession 258 MK426815-MK426832 numbers to Genbank and phylogenetic trees were constructed [6, 7].

Staphylococcus aureus is characterized at molecular level for the first time. No previous study is found about Staphylococcus aureus especially at molecular level in fecal sample.

Conclusion

S. aureus and B. cereus are found abundantly during study. Imipenem, Gentamicin, and Amikacine are effective antibiotics against these bacteria. Sequencing of S. aureus has given max score1458, total score 1458, query cover100 percent, E value 0.00, Percent identity 100 percent, Accession number NR.037007.2. And B. cereus has given max score 1254, total score 1254, query

cover100 percent, E value 0.00, Percent identity 99.85 percent, Accession number MN192138.1.

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References

1. Koyyati, Rama, Kudle Karunakar Rao, and Padigya P Rudra Manthur. "Evaluation of Antibacterial and Cytotoxic Activity of Green Synthesized Cobalt Nanoparticles using Raphanus Sativus Var. Longipinnatus Leaf Extract." *Int J Pharmtech Res* 9 (2016): 466-472.
2. Sowka, E, Leonowicz M, Andrzejewski B, and Pomogailo A D, et al. "Cobalt Nanoparticles Processed By Thermal Decomposition of Metal-Containing Monomers." *Mater Sci Wroclaw* 24 (2006): 311.
3. Gardea-Torresdey, J L, Parsons J G, Gomezv, and Peralta-Videa J, et al. "Formation and Growth of Au Nanoparticles inside Live Alfalfa Plants." *Nano Lett* 2 (2002): 397-401.
4. Gardea-Torresdey, Jorge L, Gomez Eduardo, Peralta-Videa Jose R, and Parsons Jason G, et al. "Alfalfa Sprouts: A Natural Source for the Synthesis of Silver Nanoparticles." *Langmuir* 19 (2003): 1357-1361. Ledo-
5. Suárez, A, Rodríguez-Sánchez L, Blanco M C, and López-Quintela M A. "Electrochemical Synthesis and Stabilization of Cobalt Nanoparticles." *Phys Stat Solidi* (a) 203 (2006): 1234-1240.
6. Shankar, S Shiv, Rai Akhilesh, Ahmad Absar, and Sastry Murali. "Rapid Synthesis of Au, Ag, and Bimetallic Au Core–Ag Shell Nanoparticles Using Neem (Azadirachta Indica) Leaf Broth." *J Colloid Int Sci* 275 (2004): 496-502.
7. Chung, Bing-Xian, and Liu Chuan-Pu. "Synthesis of Cobalt Nanoparticles by DC Magnetron Sputtering and the Effects of Electron Bombardment." *Mate Lett* 58 (2004): 1437-1440.

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