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Aerobic Bacterial Flora of Nasal Cavity of Seven Giant Pandas (*Ailuropoda melanoleuca*)

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Abstract: The nasal bacterial flora of seven giant pandas was identified by 16S ribosomal RNA (*rRNA*) gene sequencing analysis. The most widely occurring species were *Staphylococcus* and *Escherichia coli*. Several species not known to be associated with giant pandas were present in the study including *Pantoea agglomerans*, *Corynebacterium auriscanis*, *Kurthia gibsonii* and *Corynebacterium glutamicum*.

Key words: Nasal flora, 16S rDNA, giant panda, *Kurthia gibsonii*, China

INTRODUCTION

Animals harbour a commensal microflora on different mucosal surfaces. The microflora plays an important role in host defense against microbial infection. Colonization resistance implies that micro-organisms established in a body site can prevent colonization by newly introduced strains (Tannock, 1999) and as such artificial colonization with harmless strains belonging to the normal flora could be helpful to prevent infection of the body site by pathogens (Baele *et al*., 2001). Giant pandas, *Ailuropoda melanoleuca* are the national treasure who are praised as living fossil and play an important role in biological diversity. Upper respiratory tract infection is one of the most common diseases of giant pandas. The cases of giant pandas suffered with respiratory tract infection by *Streptococcus pneumoniae* (Chunhua *et al*., 2012), *Staphylococcus aureus* (Luo *et al*., 2005) and *Pseudomonas aeruginosa* (Yunfang *et al*., 2006) have been reported, respectively.

Nasal cavity harbours an important bacterial flora. The researchers developed a study to investigate the normal flora of giant pandas' nasal cavity which could help to clarify the bacterial pathogenesis of upper respiratory tract infection of giant pandas.

MATERIALS AND METHODS

Colletion of specimens: Nasal swabs of seven apparently healthy giant pandas were collected from Bifeng Xia Giant Panda Protection Base in Ya'an, Sichuan province, South-West China. Specimens were collected with sterile swabs with transport medium and kept in lower temperature environment with ice bags until being processed in the laboratory within 4 h.

Bacterial isolation: Specimens were directly inoculated on Tryptic Soy Agar (TSA) supplemented with 5% fetal calf serum (Gibco), MacConkey agar, blood agar (10% concentration of rabbit blood was added to Luria-Bertani plates). All media were incubated at 37°C aerobicly. After 24, 48 and 72 h of incubation, the plates were examined and different colonial types which were identified by colonial morphologies and microscopic examination were subcultured by streaking for isolation. Microorganism sub-cultured several times to obtain a pure culture.

Identification of bacteria: Colonial and celluar morphology were recorded for each strain isolated. Every pure culrure was identified by biochemical tests referred to Bergey's Manual of Determinative Bacteriology and method of 16S ribosomal RNA (*rRNA*) gene sequencing analysis were mainly used which was able to identify the isolate to species level.

DNA preparation: DNA was prepared of the purified cultures. A 1 μL loopful of cells was suspended in 200 μL sterilized double distilled water, the mixture was boiled for 10 min. After centrifugation for 5 min at 12000 r min^{-1} 4°C, the supernatant was directly used as the template for PCR.

PCR amplification of *16S rDNA* gene: In order to firmly identify this isolate, PCR-mediated amplification of the

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16S ribosomal DNA (*rDNA*) gene was performed with universal primers: F: 5'-AGAGTTTGATCCTG GCTCAG-3'; R: 5'-AAGGAGGTGATCCAACCGCA-3' (Mingxing *et al*., 2010); *Escherichia coli* DNA as a positive control and distilled water as a negative control.

The per PCR reaction was performed in a total volume of 50 μL containing 25 μL 2×Taq PCR Master Mix (Takara), primers (10 μmol L^{-1}) each 2 μL, DNA template 3 μL and nuclease-free PCR-grade water was added to 50 μL. A touchdown PCR was done to amplify the purposed DNA fragments. The cycling conditions included an initial denaturation for 6 min at 95°C; 12 cycles of 40 sec at 95°C, 40 sec at 62°C (the temperature went down 0.5°C at every cycle) and 90 sec at 72°C for extension; 23 cycles of 40 sec at 95°C, 40 sec at 55°C and 90 sec at 72°C for extension and a final extension for 7 min at 72°C.

DNA sequencing of the 16S rDNA fragment and sequence analysis: The PCR product was analyzed in 1% agarose in Tris-Borate-EDTA (TBE) buffer gel containing 0.5 mg mL^{-1} EB (Ethidium Bromide). The unique and purposed band (about 1500 bp) was purified by using universal DNA purification kit of TIANGEN Biotech then the the purification was ligated into pMD®18-T vector, the ligation mixture was transformed into *Escherichia coli* DH5α competent cells. The recombinant plasmid DNA was sent to Takara for sequencing. Furthermore, the researchers compared the sequencing *16S rRNA* gene to published sequences retrieved from the GenBank database by using BLAST, phylogenetic tree analysis was done using the Neighbour joining method and similarities were calculated by using Clustal W program.

RESULTS AND DISCUSSION

The results are shown in Table 1. The fact that Staphylococcus was the most frequently isolated species in this study is not surprising as it is well known to widely distributed in the air, drinking water, body surface, Staphylococcus also exists on man and animals' mucosal surfaces, skin, intestinal tract and so on (Lu, 2007).

Table 1: Bacteria were isolated from nasal cavity of seven giant pandas

Pandas	Bacterial species isolated
1	*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Esherichia coli*
2	*Staphylococcus intermedius*, *Staptococcus hyicus* and *Esherichia coli*
3	*Staphylococcus aureus*
4	*Kurthia gibsonii*, *Pantoea agglomerans*, *Corynebacterium glutamicum*, *Streptococcus alactolyticus* and *Staphylococcus aureus*
5	*Bacillus cereus*, *Staphylococcus epidermidis* and *Staphylococcus aureus*
6	*Staphylococcus sciuri* and *Corynebacterium auriscanis*
7	*Acinetobacter* sp., *Esherichia coli*, *Streptococcus pneumoniae*, *Streptococcus dysgalactiae*, *Staphylococcus aureus* and *Staphylococcus chromogenes*

Esherichia coli was isolated from three pandas. It is known to belong to the normal intestinal flora of man and animals and less frequently colonize the upper respiratory tract in a consistent way (Lu, 2007).

The genus *Corynebacterium* contains many species which have long been recognized as pathogens of humans and/or animals. *Corynebacterium auriscanis* is first isolated from clinical specimens from dogs (Collins *et al*., 1999). A clinical case that *Corynebacterium auriscanis* is isolated from a leg wound infection following a dog bite in a previously healthy human patient is described and confirms this organism to be a potential human pathogen (Bygott *et al*., 2008).

Acinetobacter sp. is an important opportunistic pathogen and has strong resistance to adverse environment (Lu, 2007). An adult male giant panda died of being infected by *Acinetobacter* sp. (Chen *et al*., 2001).

Kurthia gibsonii is a non-spore foming gram-negative bacillus, the two other species described in this genus, *K. zopfii* and *K. sibirica* is widely distributed in the environment, often exist in animal wastes and meat products (Chen *et al*., 2010).

Pantoea species are usually isolated from soil, fruit and vegetables, also from diverse geographical and ecological sources such as hudiman feces and the environment. Species in this genera could be an opportunistic bacterium to human. The often yellow pigmented *P. agglomerans* is known to compete successfully with the indigenous flora of a variety of microenvironments (Uche, 2008).

Illness is almost invariably caused by members of host's commensal microflora acting as opportunistic pathogens (Adams, 1999). For example, *Staphylococcus aureus* are part of the microbiota found at various sites in the human body (skin and mucosae) and may serve as sources of infection when the normal defenses of the host organism are impaired by associated diseases (e.g., viral diseases) or when the delicate balance of this microbiota is altered by antimicrobial therapy (Palazzo *et al*., 2005). It has been suggested that the commensal microflora could act as a reservoir of antibiotic resistance genes. Knowledge of bacterial species belonging to this microflora is of importance to study transfer of antibioticresistance genes to pathogens (Baele *et al*., 2001).

CONCLUSION

In this study, opportunistic pathogen would turn to be pathogenic bacterium when the immune system weakens. Normal flora of the seven giant pandas' nasal cavity may lead to infection of the upper respiratory

tract. Feeders should keep the living environment of giant pandas clean and conduct periodic sterilization. The study could make a contribution to the protection of giant pandas in some way.

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