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Antibiotic resistance among bacteria from Antarctic and Tropics

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Abstract

The emergence of antibiotic resistant strains of environmental bacteria and human pathogens is a natural phenomenon that happens when bacteria are constantly exposed to sub-inhibitory concentration of antibiotics. As a result, many environmental bacteria develop resistance to multiple antibiotics. These antibiotic determinants can be transferred to other bacteria as well as human pathogens through horizontal gene transfer. A better understanding of the extent of multiple antibiotic resistance determinants among environmental bacteria may help to predict and counteract the emergence and future evolution of resistance. Hence, this research is undertaken to determine the antibiotic resistance profiles of 14 bacterial strains from the Antarctic and 11 bacterial strains from the tropics. All bacterial strains were exposed to 13 different types of antibiotics. The results showed that all tropical bacteria were sensitive to most of the 13 antibiotics tested. Meanwhile, most of the Antarctic bacteria were resistant to multiple antibiotics and sensitive to only few antibiotics such as imipenem, metronidazole and novobiocin. Bacterial strains that were resistant to a significant number of antibiotics, such as AP1, AP3, AP4, and AP6 were identified based on their 16S rDNA sequences. Isolates AP1, AP3, and AP4 were identified as *Pedobacter* spp. while AP6 was identified as *Arthrobacter* sp. All bacteria were checked for presence of plasmid. It was found that only bacterial strain DL5 from Antarctica possessed a plasmid.

Introduction

Since the discovery of penicillin in 1929, antibiotics have been continuously used to cure diseases caused by bacterial infections. The use of antibiotics has somehow increased the survival rate of human suffering from diseases caused by bacterial infections. Initially, many believed that antibiotics may cure and eliminate infectious diseases. However, diseases that are caused by bacteria such as diarrhea, pneumonia, tuberculosis and other nosocomial infections still remain until today as the bacteria continue to evolve to acquire resistance to the effects of antibiotics (St. Clair and Vissick, 2010).

Bacteria that are resistant to antibiotics are commonly found in the natural environment (Kummerer, 2009). Antibacterial resistance that develops in both human pathogens and other microorganisms is sometimes due to the regular exposure to antibiotics or the transmission of resistance traits within and between microbes (Guillemont, 1999). In general, resistance towards antibiotics can be divided into two categories, intrinsic and acquired resistance. Intrinsic resistance occurs naturally in a species and is not due to exposure to antibiotics. Acquired resistance occurs in species that are originally sensitive to an antibiotic, but later develop the resistance. Bacteria can develop antibiotic resistance through mutation or exchange of genetic material among similar or

closely related species. In order to get a better picture of the progression of resistance, it is important to gather more information on the antibiotic resistance capabilities of bacteria from clinical and non-clinical environments (Martinez, 2008).

The emergence of antibiotic resistant strains of environmental bacteria and human pathogens is a natural phenomenon that happens when bacteria are constantly exposed to sub-inhibitory concentrations of antibiotics. As a result, many environmental bacteria develop resistance to multiple antibiotics. The increasing usage of antibiotics and consumption without following the prescriptions among patients, and in the food industry are among the main factors that lead to antibiotic resistance.

Antibiotic resistance genes that occur naturally in environmental bacteria can potentially be transferred to human pathogens (Martinez, 2008; Martinez, 2009). Environmental bacteria could be a reservoir for antibiotic resistant genes. Despite these, there are relatively limited numbers of studies on the presence of resistance determinants in environmental bacteria, especially those from the tropics and polar regions. Hence, this project was set out to determine the antibiotic resistant profiles of bacteria from the tropics and Antarctic.

Methodology

Bacterial Samples Origin

Thirteen bacteria from the Schirmacher Oasis and King George Island, Antarctica and ten bacterial strains from Universiti Malaysia Sabah were used in this study.

Morphology and Physiological Observation

The morphological and physiological properties of all bacterial isolates were determined by basic Gram staining and motility test using motility agar techniques. Physical properties of the bacterial colonies were also recorded.

Antibiotic Susceptibility Test (AST)

AST was carried out using Oxoid™ Antibiotic Susceptibility Disks (Thermo Fisher Scientific Inc.). All bacterial strains were tested against 13 types of antibiotics, namely nitrofurantoin 100 µg, novobiocin 5 µg, rifampicin 5 µg, metronidazole 10 µg, compound sulphonamides 300 µg, ciprofloxacin 5 µg, lincomycin 15 µg, ceftadizime 30 µg, vancomycin 30 µg, vancomycin 30 µg, imepenem 10 µg, streptomycin 10 µg, and trimethroprim 5 µg. The bacteria were grown in broth medium and spread evenly onto the Mueller-Hinton (MHA) agar medium. Antibiotic disks were placed on the inoculated agar and incubated overnight. The diameter of the inhibition zone was measured and the result was scored by referring to the Kirby-Bauer chart.

DNA Genomic Isolation

Genomic DNA was extracted from the bacterial isolates according to the methods described by Nishiguchi *et al.* (2002).

Plasmid extraction

Alkaline lysis method was used for plasmid extraction.

16S rDNA Amplification

Amplification of 16S rDNA was carried out using primers BSF8/20 (5'-aga-gtt-tga-tcc-tgg-ctc-ag-3') and BSR1541/20 (5'-aag-gag-gtg-atc-cag-ca-3'). The PCR mix consisted of 5µl DNA, 10µl 5x buffer, 3µl $MgCl_2$, 1µl dNTPs, 1µl forward and reverse primer, 0.25µl polymerase and sterilized distilled water. The PCR conditions were pre-denaturation step for 2 mins at 96°C followed by 30 cycles of 30 sec at 96°C, 40 sec at 52°C, 1 min at 72°C and ended with final extension step at 72°C for 10 min. The PCR product was analyzed using a 0.8% agarose gel, stained with ethidium bromide and visualized under UV trans-illumination.

Cloning

Purified PCR products were cloned using the TOPO-TA™ cloning kit (Invitrogen) according to the manufacturer's protocol (Thermo Fisher Scientific, Inc.) and sequenced using a Sanger sequencer.

Sequencing and DNA Analysis

The DNA sequences were aligned using the basic local alignment search tool (BLAST) (https://www.ncbi.nlm.nih.gov/blast) software at National Centre for Biotechnology Information website. Alignments were carried out using ClustalW of MEGA software version 6 (Tamura *et al.*, 2013).

Results and Discussion

Based on the antibiotic susceptibility test, most of the 14 Antarctic bacteria were resistant to 1 or more antibiotics while all tropical bacteria were sensitive or intermediately sensitive to the 13 antibiotics tested. All Antarctic and tropical bacteria were sensitive to rifampicin and imipenem. The highest frequencies of resistant strains among the Antarctic isolates were observed for metronidazole (92.8%), nitrofurantoin and vancomycin (64.2%) and ceftadizime (42.8%). As for the tropical isolates, most of them were resistant to trimethroprim (90.9%) and metronidazole (81.8%). Four bacterial isolates, AP1, AP3, AP4, and AP6 that were resistant to between 7 to 9 antibiotics, were identified. Isolates AP1, AP3, and AP4 were *Pedobacter* spp. while AP6 was an *Athrobacter* sp. In general, bacteria from the tropical regions were more exposed to human activities but strangely, they were resistant to lower numbers of antibiotics. In contrast, Antarctic bacteria which had less exposure to human activities were resistant to multiple antibiotics. Miller *et al.* (2009) reported that the frequency of multiple antibiotic resistances among bacterial isolates was the highest at the site nearest to Palmer Station, Antarctica and decreased with the distance from it. They concluded that multidrug resistance is low among native Antarctic bacteria but increases with the increase of human activities. The emergence of antibiotic resistant bacteria has been considered as a human impact on the environment

(Ushida *et al.*, 2010; Cabello, 2006; Chee-Sanford *et al.*, 2001; Goni-Urriza *et al.*, 2000). It is not clear on why there were numerous Antarctic bacteria with multiple resistance to the antibiotics in this study, considering that the locations in Antarctica where these bacteria were isolated are relatively pristine.

Screening for the presence of plasmid DNA was conducted on all bacterial strains. Only *Pedobacter steynii* DL5 from Antarctica possessed a circular DNA which co-migrated with the 6 kb linear marker on the agarose gel. The AST result showed that *P. steynii* DL5 was only resistant to 4 antibiotics when compared to other Antarctic bacteria that were resistant up to 6 and 9 antibiotics but did not harbor a plasmid. The antibiotic resistance genes have always been associated with horizontal gene transfer (HGT) of genetic materials such as plasmids, transposons and integrons. The bacterial plasmids usually confer antibiotic resistance traits and therefore it will be interesting to find out if this is the case for *P. steynii* DL5 in the future.

There are 4 Antarctic bacterial isolates, ER3, DM3, DL4 and DL5, and 3 tropical bacterial isolates, SST1, SST2 and SST7, that had pigmentations. All pigmented bacterial isolates were resistant to 1 to 4 antibiotics. Pigmented bacteria are commonly found to be resistant to both antibiotics and metals when compared to non-pigmented bacterial strains (De Souza *et al.*, 2006). De Souza *et al.* (2006) reported a high percentage of pigmented bacterial isolates that exhibited resistance to both antibiotics and metals. Nevertheless, Moskot *et al.* (2012) suggested that there was no obvious relation between resistance and the presence of plasmids or pigmentation. In their report, they summarized that there were no compelling results that support the evidence of pigmentation related to antibiotic resistance.

Conclusion

Antarctic bacteria which have less exposure to human activities were resistant to multiple antibiotics compared to their tropical counterparts. The emergence of antibiotic resistant bacteria has been considered as a human impact on the environment although it is unknown why Antarctic bacteria could possess multiple resistances towards commercially available antibiotics even though they are located in the most secluded location on earth with minimal human inhabitants.

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