

SCREENING OF PATHOGENIC MICROBIOTA
HARBOURING ANTIBIOTIC RESISTANCE
GENES FROM HEALTHCARE WASTES IN
MALAYSIA: A HIGH-THROUGHPUT
AMPLICON SEQUENCING APPROACH

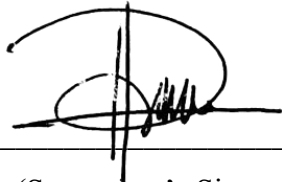
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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Pembuangan sisa klinikal dari hospital tanpa pembasmian patogen dan bahan pencemar berbahaya pastinya membawa kesan negatif terhadap alam sekitar dan kesihatan awam. Pada masa kini, ujian mikrobiologi untuk sisa klinikal menggunakan pendekatan kultur yang klasik dan menyebabkan proses pengecaman bakteria terhad kepada *Bacillus* sp. dalam sampel yang dirawat. Keberkesanan gelombang mikro dalam rawatan sisa klinikal berbahaya amatlah rendah kerana kurang efektif dan tidak membunuh patogen secara berkesan. Tambahan lagi, sangat sedikit data yang dilaporkan mengenai komuniti mikrob yang lengkap dalam sisa klinikal dari hospital yang telah dirawat dengan gelombang mikro menggunakan teknologi penjujukan generasi seterusnya. Kajian ini bertujuan untuk mengenal pasti komuniti mikrob secara lengkap, bakteria yang kebal terhadap antibiotik yang masih hidup di dalam sisa klinikal setelah dirawat dengan gelombang mikro yang terdapat di tiga pengendali sisa berbeza (FC, FV, dan FA) di Semenanjung Malaysia, dan mencari gen patogenik dalam mikroorganisma terpencil. Komposisi bakteria dan kulat ditentukan melalui penjujukan amplicon dengan mensasarkan keseluruhan gen 16S rRNA dan sebahagian gen 18S rRNA dari *Internal Transcribed Spacer* (ITS) 1 dan ITS2. Kultur bakteria dilakukan untuk mengenal pasti bakteria yang hidup di dalam sisa klinikal. Bacteria yang kebal terhadap antibiotik yang diasingkan telah dianalisis menggunakan *whole genome sequencing*. Bacteria patogenik ini telah diuji kerintangan antibiotik menggunakan teknik resapan cakera. Berdasarkan hasil **objektif 1**, komposisi bakteria dalam sampel FC didominasi oleh genera *Aerococcus*, *Comamonas*, dan *Pseudomonas*, sementara FV dan FA didominasi oleh *Bacillus*, *Paenibacillus*, dan *Bacilli* yang tidak dikelaskan. Ketiga-tiga set sampel menunjukkan perbezaan yang ketara dalam kepelbagaian bakteria, dengan kepelbagaian alpha- (nilai $p = 0.048$) dan beta-diversity (nilai $p < 0.006$). Komposisi kulat berbeza secara ketara antara tiga set sampel, seperti yang dibuktikan oleh nilai alpha- ($p = 0.045$) dan kepelbagaian beta (nilai $p < 0.002$). Analisis bioinformatik mendalam mengesahkan kehadiran *bla*_{TEM-1} dan *penP*, yang dikaitkan dengan pengeluaran jalur rintangan beta-laktamase dan beta-laktam kelas A. Berdasarkan **objektif 2**, bakteria yang hidup dikenalpasti melalui kultur adalah dari genera *Proteus*, *Stenotrophomonas*, dan *Pseudomonas*, dengan kepelbagaian beta yang ketara (nilai $p = 0.003$). Berdasarkan hasil BLASTN, bakteria kebal antibiotik yang diasingkan dari sampel VFC, VFV, dan VFA adalah *Proteus mirabilis*, *Stenotrophomonas maltophilia*, dan spesies *Pseudomonas*. Oleh kerana tidak ada spesies *Pseudomonas* tertentu yang dikenal pasti dari pangkalan data, bakteria ini berpotensi hadir sebagai bakteria baru. Untuk **objektif 3**, *P. mirabilis* dan *S. maltophilia* didapati mengandungi gen yang berkaitan dengan fungsi virulensi. Gen rintangan antibiotik *bla*_{OXA-10} dan *sul1* ditemui dalam *P. mirabilis* dan *S. maltophilia*, mengakibatkan kekebalan terhadap beta-laktam dan kelas antagonis laluan folat antibiotik. Ujian rintangan antibiotik menunjukkan bahawa *P. mirabilis* dan *S. maltophilia* adalah bakteria perintang pelbagai dadah, menunjukkan rintangan terhadap banyak kelas antibiotik, termasuk karbapenem. Kesimpulannya, mikroorganisma dan bahan cemar berfungsi sebagai petunjuk putatif dalam penilaian rawatan sisa klinikal dari hospital, menunjukkan ketidakberkesanan pembasmian mikrob menggunakan kaedah pensterilan gelombang mikro. Penemuan kami menunjukkan bahawa mikroorganisma berkaitan klinikal, pencemar antibiotik dan gen rintangan antibiotik akan mencemarkan alam sekitar dan memudaratkan kesihatan manusia apabila dilepaskan ke tapak pelupusan melalui pemindahan gen mendarat sekiranya tidak dibendung dengan baik.

ABSTRACT

The disposal of healthcare waste without prior elimination of pathogens and hazardous contaminants has negative effects on the environment and public health. In past research the microbiological assessment of healthcare wastes employed a culture approach that resulted in the identification of *Bacillus* sp. in a sample of treated solid healthcare wastes. The effectiveness of microwave in hazardous waste treatment studied based on the survival of tested microorganisms using the culture method may overlook the presence of other pathogens after treatment. Yet, there is scarce data reported on the complete microbial community in microwave-treated healthcare waste using next-generation sequencing technology. This study aimed to profile the complete microbial community, identify viable antibiotic-resistant bacteria in microwave-treated healthcare wastes collected from three different waste operators (FC, FV, and FA) in Peninsular Malaysia, and characterize pathogenic gene markers in isolated organisms. The samples were subjected to bacterial and fungal amplicon sequencing for microbial community characterization, by targeting the full-length 16S ribosomal RNA (rRNA) gene and partial 18S rRNA gene with full-length internal transcribed spacer (ITS) 1 and ITS 2 regions, respectively. Bacterial cultivation was performed to identify viable bacteria in healthcare wastes. The isolated antibiotic-resistant bacteria were subjected to species identification and whole genome sequencing for complete genome characterization. In addition, antibiotic susceptibility testing was performed on the confirmed isolates using the disk diffusion technique to determine the antibiotic resistance patterns. Based on the results of **objective 1**, the bacterial composition in FC samples was dominated by the *Aerococcus*, *Comamonas*, and *Pseudomonas* genera, while FV and FA were dominated by *Bacillus*, *Paenibacillus*, and unclassified *Bacilli*. All three sets of samples showed significant differences in bacterial diversity, as evidenced by the alpha- (p -value = 0.048) and beta-diversity (p -value < 0.006) analyses. The fungal composition differed significantly between three groups of samples, as evidenced by the alpha- (p -value = 0.045) and beta-diversity (p -value < 0.002). The deep bioinformatic analysis confirmed the presence of *bla*_{TEM-1} and *penP*, which are associated with the production of class A beta-lactamase and beta-lactam resistance pathways. Based on **objective 2**, the viable bacteria in VFC, VFV, and VFA samples were represented by *Proteus*, *Stenotrophomonas*, and *Pseudomonas* genera, respectively, with significant beta diversity (p -value = 0.003). Based on the BLASTN results, the primary antibiotic-resistant bacteria isolated from VFC, VFV, and VFA samples were *Proteus mirabilis*, *Stenotrophomonas maltophilia*, and *Pseudomonas* sp., respectively. As no specific *Pseudomonas* species were identified from the database, this bacterium is potentially present as a novel bacterium. For **objective 3**, *P. mirabilis* and *S. maltophilia* were discovered to contain genes associated with virulence function and transposable element expression. The antibiotic resistance genes *bla*_{OXA-10} and *sul1* were identified in *P. mirabilis* and *S. maltophilia*, conferring resistance to beta-lactam and folate pathway antagonist antibiotics. The antibiotic susceptibility tests revealed that *P. mirabilis* and *S. maltophilia* were multidrug-resistant bacteria, exhibiting resistance to drugs from multiple classes, including carbapenem. In conclusion, microorganisms and contaminants, which serve as putative indicators in healthcare waste treatment evaluation, revealed the limitations of the microwave sterilization method in microbial inactivation. Our findings suggested that the occurrence of clinically relevant microorganisms, antibiotic contaminants, and associated antibiotic resistance genes represents environmental and human health hazards when released into landfills *via* horizontal gene transfer.

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