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根據2021年醫管局癌症資料庫顯示，男性頸頸癌（唇，舌，涎腺，口腔，扁桃體、口咽，下咽，其他口腔未列明部位，鼻腔、中耳及副鼻竇，喉）新症患者佔整體約2/3[2]。約70%口咽癌與HPV感染有關[3]

Reference: 1. CDC. Head and Neck Cancers. Available at: https://www.cdc.gov/cancer/headneck/index.htm. Accessed on: 9 Apr 2024.
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The Cover Shot

Jiuzhaigou, named after the nine Tibetan villages, is the national park and nature reserve in the north of Sichuan Province. It features multi-level waterfalls, colourful lakes, snowcapped peaks, and ecosystem biodiversity. It was recognised by UNESCO as a World Heritage Site in 1992 and has been a World Biosphere Reserve since 1997.

This stunning view of Five Colors Lake was captured in October 2016 just before a Magnitude 7 Earthquake struck in August 2017, which caused extensive damages and closure of the park. Fortunately, the park has fully recovered through extensive reconstruction and nature's resilience and is open to visitors from September 2021.

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Editorial

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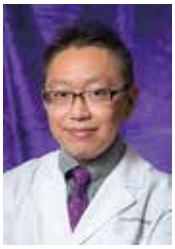



Dr Christopher Koon-chi LAI Dr Ken Ho-leung NG

We are living in an exciting time with a tremendous pace of technological advancement. Being at the forefront of laboratory medicine, we are privileged to have witnessed how the sequencing and proteomics techniques revolutionised the way we work in the clinical laboratories, changing the way we can diagnose, and even treat infections in a way that we could not even dream of twenty years ago. In the past few years, the spotlight was on COVID-19. In this issue, we would like to bring your attention to other aspects of advancement in the field of clinical microbiology and infection.

The article contributed by Prof Gilman Siu neatly guides us through the application of high-throughput sequencing methods for pathogen identification in clinical microbiology laboratories. 16S ribosomal RNA gene sequencing, often used together with PCR enrichment, can greatly improve bacterial detection. In contrast, metagenomic shotgun sequencing can detect all pathogens as well as antimicrobial resistance gene detection. However, the interpretation of the results may not be straightforward, and the cost is relatively high for routine clinical use. The target-enriched metagenomic sequencing techniques, on the other hand, are particularly suitable for the detection of clinically relevant organisms, including samples collected from non-sterile sites and could be the way forward for pathogen detection in patient care in the near future.

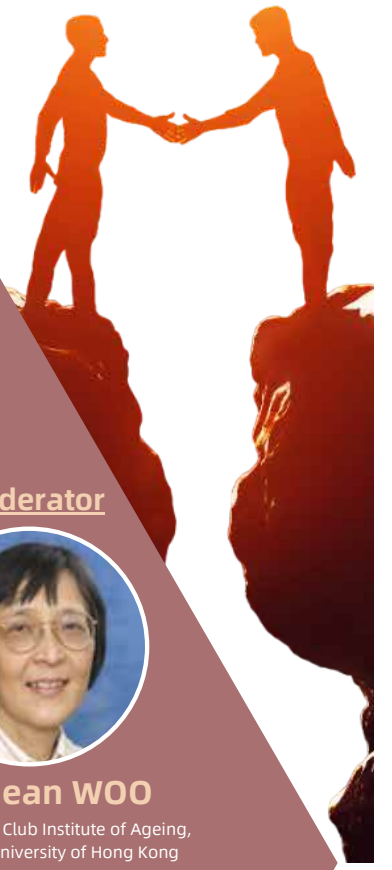
Identification of pathogens alone is necessary but not sufficient in guiding optimal antimicrobial therapy. Dr Teresa Wang gives us an update on the state-of-the-art overview of the antimicrobial susceptibility detection methods. In the past, antimicrobial susceptibility test results could only be available after at least 48 hours to wait for pure growth of the bacteria, and observe for the inhibition of their growth in the presence of antibiotics. Molecular methods are now increasingly being employed to give rapid genotypic diagnosis with a high level of accuracy and concordance with phenotypic results. Newer methods like microfluidics that are on the horizon will likely push forth further to improve the detection of antimicrobial resistance.

Knowledge in medical mycology is also rapidly evolving, hand-in-hand with the evolving technology. In this issue's article written by Dr Li Xin, she gives an update to readers on the latest developments in medical mycology from four different perspectives: epidemiology, host susceptibility, antifungal resistance, and new treatment options, illustrated with important classical fungal pathogens of *Cryptococcus spp.*, and *Aspergillus fumigatus*, and the new kid on the block, *Candida auris*.

High-throughput sequencing technologies allow a comprehensive understanding of the human gut microbiome. In patients with recurrent *Clostridiodes difficile* infection, the gut bacterial community are severely disrupted with significant loss of microbial diversity and is unable to return to its baseline on its own. Faecal material transplantation (FMT) works by quickly normalising the microbial diversity and community structure of the gut microbiota. Dr Rita Ng gives us an overview of the latest updates on FMT, highlighting the clinical efficacy of FMT treatment, international guidelines of the FMT centres worldwide, and the key stages in the FMT preparation process.

We would like to express our sincere thanks to all the colleagues who have contributed to this issue of the Medical Diary. We would also like to thank the Federation of Medical Societies of Hong Kong, the Secretariat team, and the Medical Diary Editorial Board.

賽馬會安寧頌
JCECC
Jockey Club End-of-Life Community Care Project



MIND THE GAP:
MAKING ADVANCE CARE PLANNING AND ACTUAL EXPERIENCES OF END-OF-LIFE CARE

Join us for a thought-provoking webinar as we delve into the importance of effective communication in providing high-quality End-of-Life (EOL) care. In light of the recent legislation on Advance Decision on Life-sustaining Treatment Bill, the implementation of the Advance Medical Directive (AMD) is just around the corner. This signifies an imminent rise in public requests for Advance Care Planning (ACP) during medical visits.

During this webinar, we will focus on two commonly encountered cases related to neurodegenerative diseases. Through an in-depth discussion, we aim to shed light on how we can bridge the gap between the expectations of patients and their families, and the practical implementation of the work undertaken by frontline healthcare professionals.

Moderator

Prof. Jean WOO
Director, Jockey Club Institute of Ageing,
The Chinese University of Hong Kong

Panelists

Prof. Bee WEE CBE
Consultant in Palliative Medicine,
Oxford University Hospitals NHS Trust
Associate Professor, Nuffield Department of Medicine, and Governing Body Fellow,
Harris Manchester College, Oxford University

Dr. David SUN
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The Chinese University of Hong Kong

Dr. Raymond LO
Specialist in Geriatrics and Palliative Medicine
Clinical Professor (Hon),
Department of Medicine and Therapeutics,
The Chinese University of Hong Kong

DATE: 30 July 2024 (Tuesday)
TIME: 4:00 - 5:30 P.M. HK TIME; (9:00 - 10:30 A.M. UK TIME)
FORMAT: Online (Zoom webinar)
MEDIUM: English (with simultaneous interpretation to Cantonese)
TARGET: Healthcare professionals and other interested parties

Registration link: https://bit.ly/3Uic8Pk

REGISTRATION DEADLINE: 15 JULY 2024

CME / CNE: 1.5point
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Clinical Importance and Interpretation of Antimicrobial Susceptibility Testing

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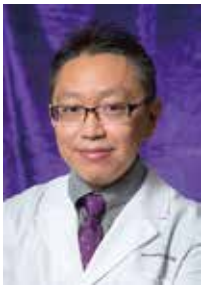

Dr Teresa Kin-fong WANG Dr Christopher Koon-chi LAI

This article has been selected by the Editorial Board of the Hong Kong Medical Diary for participants in the CME programme of the Medical Council of Hong Kong (MCHK) to complete the following self-assessment questions in order to be awarded 1 CME credit under the programme upon returning the completed answer sheet to the Federation Secretariat on or before 31 July 2024.

Antimicrobial susceptibility testing (AST) is a crucial component in the management of infectious diseases. On an individual level, it guides clinicians in selecting the most effective antimicrobial therapy for patients, improving their clinical outcomes[1,2]. AST supports infection control strategies by identifying drug-resistant organisms and allowing implementation of appropriate isolation measures[3] On the public health level, we are increasingly under the threat of multi-drug resistant organisms (MDRO). AST provides information on the emergence of antimicrobial resistance (AMR) in microorganisms of clinical importance, which is essential for public health planning and response[4]. By informing targeted therapy, AST also helps to minimise the use of broad-spectrum antibiotics, thereby reducing the risk of AMR[5,6]. The World Health Organization (WHO) has set up the Global Antimicrobial Resistance and Use Surveillance System (GLASS) to coordinate the collection of AMR data. The WHO and the WHO Collaborating Centres are providing technical support to laboratories to strengthen their capacity in the identification of infectious organisms and performing drug susceptibility at national level[7].

The field of AST is evolving rapidly due to the need for faster and more accurate methods to combat the rise of antibiotic-resistant bacteria and to provide the best treatment to patients. Besides conventional phenotypic testing, genotypic testing for drug resistant genes is gaining increasing importance in AST. The focus of this article is to review the approaches of AST in the laboratory and the clinical relevance of the results.

TRADITIONAL AST METHOD: DISK DIFFUSION TEST AND MINIMAL INHIBITION CONCENTRATION

Disk diffusion test is a widely used method for determining AST that has a long history spanning several decades since the discovery of penicillin by Sir Alexander Fleming[8]. In this test, paper disks impregnated with specific antimicrobial agents are placed onto an agar plate inoculated with the microorganism of interest. As the agar plate incubates, the antimicrobial agents diffuse into the surrounding medium to create a concentration gradient. If the microorganism is susceptible to the antibiotic, it will have a clear zone of inhibition of growth around the paper disk with a particular antibiotic. The size of this zone is measured and compared to standardised interpretive criteria provided by organisations like the Clinical and Laboratory Standards Institute (CLSI)[9] or the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[10].

The minimal inhibition concentration (MIC) test provides a quantitative measure of the susceptibility of an organism to an antimicrobial agent. In the past, MIC was determined by putting a standardised inoculum of the microorganism to be tested into a set of tubes with culture broth mixed with antimicrobial agents at different concentrations. The lowest concentration of the antimicrobial agent that completely inhibits visible growth is considered the MIC. Nowadays, MIC can be determined by various methods, including microbroth dilution, E-test strips and automated systems like VITEK® 2 system by bioMérieux, Phoenix™ Automated Microbiology System developed by BD (Becton, Dickinson and Company), MicroScan WalkAway® System manufactured by Beckman Coulter and Sensititre® Automated System developed by Thermo Fisher Scientific[11]. These systems offer predefined panels and some also have custom-designed panels to facilitate the testing of antimicrobial agents best suited for the needs of the institutes. In addition, these systems reduce hands-on time and provide auto-interpretation of susceptibility results according to criteria entered into the systems, usually with reference to CLSI or EUCAST.

OTHER NON-GENOTYPIC METHODS OF AST

Disk diffusion test and MIC test are reliable AST, with well-established guidelines to guide the procedures and interpret the results. However, the turnaround time of these tests is relatively long, requiring up to two days. This is suboptimal in terms of providing targeted treatment to patients, identifying drug-resistant infections and early implementation of appropriate infection control measures to prevent the spread of multi-drug resistant organisms. Thus, there is a need to develop AST that could provide results timely. The following technologies are some newer non-genotypic AST methods developed[12].

1. **Mass Spectrometry**
   Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF) mass spectrometry is a technology now broadly used in clinical microbiology laboratories for the identification of bacteria. By analysing the patterns of protein expression or the detection of specific degradation products after mixing with particular antibiotics, MALDI-TOF can also be used to assess the susceptibility of bacteria[11,13,14].

2. **Microfluidics and Lab-on-a-Chip Devices**
   Microfluidic devices allow for the manipulation of tiny volumes of fluids and can conduct AST within microchannels[15]. Some of these systems incorporate molecular assays, which can even support the use of direct human samples without pretreatment or pre-cultivation for AST[16]. These devices significantly reduce the time and the amount of reagents required for testing.

3. **Biosensors and Nanotechnology**
   Biosensors, which can be optical[17], electrochemical[18], or mechanical[19], detect bacteria and their susceptibility to antibiotics by sensing changes in the sensor in response to bacterial metabolism, or growth directly under the influence of antibiotics, or using specific bacteriophage[20]. Nanoparticles and nanomaterials are also being explored for their potential to detect resistance[21].

4. **Flow Cytometry**
   Flow cytometry can be used for AST by assessing the bacterial response to antibiotics in real-time, based on changes in cell sise, complexity, or uptake of fluorescent markers[22,23].

5. **Digital Imaging and Machine Learning**
   Digital imaging of bacterial growth in the presence of antibiotics, combined with machine learning algorithms, allows for the automation of growth curve analysis and interpretation, speeding up the AST process[24,25].

6. **Electrical Impedance**
   By measuring changes in electrical impedance caused by bacterial growth in the presence of antibiotics, this technique can quickly determine bacterial resistance or susceptibility within an hour[26].

7. **Time-Lapse Microscopy**
   Sophisticated time-lapse microscopy can monitor bacterial growth and death in the presence of antibiotics. When used together with microfluidic devices, it can perform AST at a single-cell level, which may reveal resistance patterns more accurately[27]. It can also be used to assess the efficacy of antibiotic combinations against drug-resistant MDRO[28,29].

These methods are developed to enhance our ability to perform AST with reduced time needed. However, these methods are not yet readily available and are not used routinely in clinical laboratories.

MOLECULAR METHODS: DETECTION OF RESISTANT GENES

Conventional AST requires the demonstration of evidence of growth or metabolism of bacteria, in order to confirm the effectiveness of an antimicrobial agent. On the other hand, we now know that antimicrobial resistance is associated with the presence of resistant genes found on bacterial chromosomes or carried by plasmids[30]. Nucleic acid amplification technologies (NAATs), detecting targeted resistant genes, can generate results within 15 minutes to 7 hours. Together with chromogenic agars, they are used most commonly in MDRO detection, such as vancomycin-resistant enterococci[31] and carbapenemase-producing enterobacterales[32] commercial kits have been developed and are widely used in clinical microbiology laboratories.

Besides targeted resistant gene detection, whole genome sequencing (WGS) is also increasingly being applied to AST[33]. WGS can provide comprehensive data on resistance genes and mutations, potentially predicting susceptibility to various antibiotics, especially when the mechanism of resistance is not yet known. With the availability of continuously updated antimicrobial resistance databases, the analysis of WGS data is simplified and can be done much faster than before[34] .Besides resistance detection, performing pan-genomic nucleotide-to-nucleotide comparisons between strains of same species may help to understand the epidemiology relationship in nosocomial infection and outbreaks caused by MDRO[35].

Molecular technology allows the direct detection of pathogens and resistant genes from clinical samples at the same time, without the need for conventional cultures.

INTERPRETATIONS OF AST RESULTS

When clinicians receive culture reports with AST, they seldom get to know which methods are used by the laboratory for AST. It is only in exceptional circumstances that the exact figure of MIC of a drug against a bacterium is reported. Based on CLSI and EUCAST, the disk diffusion test results and the MIC test results are classified as susceptible (S), intermediate (I), or resistant (R)[9,10]. These criteria are developed based on extensive research, clinical trials, and epidemiological data. Factors including pharmacokinetics, pharmacodynamics, clinical outcomes, and resistance mechanisms are all taken into account[8]. CLSI has published rationale documents, which are accessible freely on the internet, that provide the scientific reasons for the institute's methods used to determine breakpoints[36]. Those organisms being tested susceptible to a certain antimicrobial agent can be interpreted as having a high likelihood of treatment success with this agent given at standard doses. For those tested intermediate, they exhibit uncertain responses to treatment of this antimicrobial agent and clinical decisions should be made with reference to the site of infection, drug concentrations achievable at the site of infection and patient-specific factors. In 2014, CLSI introduced a new category of interpretation

as susceptible-dose dependent (SDD) - a category that implies that the susceptibility of an isolate is dependent on the dosing regimen that is used in the patient[37]. Similarly, EUCAST, in 2019, redefined I to mean "increased exposure" and introduced the "area of technical uncertainty" (ATU) category, to account for testing variability[37,38]. It is believed that higher drug exposure than the dose that was used to establish the susceptible breakpoint, achieved by higher doses, more frequent doses, or both with reference to the maximum approved dosage of the antimicrobial agent, is likely to be clinically effective. CLSI reviews the criteria annually and SDD category is increasingly being assigned when doses well above those used to calculate the susceptible breakpoint are approved and used clinically, and where sufficient data to justify the designation[9,37]. Finally, for a bacterium being resistant to a certain antimicrobial agent, means that it is not inhibited by the usually achievable concentration of the agent with normal dosage schedules.

It is not uncommon for clinicians to ask for additional AST when the microorganisms demonstrate resistance to drugs tested. However, these additional testing may not be useful, and sometimes may be misleading. For instance, cephalosporins are not routi*nely tested against Enterococcus sp. because* Enterococcus generally exhibits intrinsic resistance to cephalosporins due to their naturally modified penicillin-binding proteins that have lower infinity for these groups of antibiotics[39]. Another example is AST of penicillins or cephalosporins without anti-pseudomonal effect to be tested on *Pseudomonas aeruginosa*. *P. aeruginosa* has reduced permeability to amino-penicillins, alone or combined with clavulanic acid, as well as to most of the older cephalosporins, including the third-generation cephalosporins cefotaxime and ceftriaxone. It also produces an inducible AmpC β-lactamase and thus is intrinsically resistant to these antibiotics[40,41]. Because of these known mechanisms of resistance, there are no interpretation criteria for cephalosporins in Enterococcus sp. and non-anti-pseudomonal β-lactams in *Pseudomonas aeruginosa* in CLSI and EUCAST guidelines. The test results, in terms of the sise of the zone of inhibition by disk diffusion test and the absolute value of MIC, cannot be interpreted in the relevant clinical context. On the other hand, for certain organisms, we may choose to test for a representative drug and may infer resistance to the other drugs in the same group. This is the case seen in *Staphylococcus aureus*, using cefoxitin to confirm *mecA*-mediated oxacillin resistance and reported as "methicillin-resistant *S. aureus*" (MRSA), which can infer resistance to all other β-lactams other than ceftaroline. Testing on these drugs should be rejected[42].

Furthermore, some of the AST only applied to isolates identified from certain clinical sites. For example, there are interpretation criteria for fosfomycin in the table of zone diameter and MIC breakpoints for Enterobacterales in CLSI guidelines[9]. However, it is explicitly mentioned in the table that the disk diffusion and MIC breakpoints of fosfomycin apply only to *E. coli* urinary tract isolates and should not be extrapolated to other species of Enterobacterales.

The development and integration of genotypic AST into clinical practice aim to provide faster and more precise AST results, allowing for timely and targeted antibiotic therapy, which is critical in the fight against antibiotic resistance.

However, one must also be aware of its limitations. Genotyping tests can provide information on the presence or absence of resistance-associated genes or mutations, but they do not assess the expression or function of these genes. Consequently, genotypic results may not always correlate with actual antimicrobial resistance phenotypes, leading to potential misinterpretation of AST. On the other hand, for the commonly used genotypic tests, they often focus on specific resistance genes more commonly encountered. Emerging or novel resistance mechanisms may not be picked up and may potentially lead to incomplete or inaccurate susceptibility profiles. Henceforth, a holistic approach that combines genotypic and phenotypic testing remains crucial for robust and reliable AST[43].

CHALLENGES IN AST INTERPRETATION AND FUTURE DEVELOPMENTS

Even though AST is useful in guiding the choice of antibiotics in managing patients with infection, we may still encounter treatment failure when the tested "susceptible" antibiotics are used. The interpretation of AST must be contextualised within the clinical scenario, considering patient factors such as the site of infection, the immune status of the patient, and the pharmacokinetic and pharmacodynamic properties of the antimicrobial agents[44].

There are several factors that may complicate the interpretation of AST results:

1. Heteroresistance: Some bacterial populations may contain subpopulations with different susceptibility profiles, complicating the interpretation[45].

2. Phenotypic variability: The expression of resistance can be influenced by environmental factors, leading to variability in test results[46].

3. Emerging resistance mechanisms: Novel resistance mechanisms may not be detected by standard AST methods.

4. Host Factors: Patient-specific factors like immune status, comorbidities, and genetic factors may affect the efficacy of antimicrobials despite in vitro susceptibility.

Phenotypic testing with disk diffusion and MIC test are still the mainstay methods of AST. However, molecular tests are increasingly being used routinely in clinical microbiology laboratories. This is particularly useful when slow-growing organisms or highly contagious pathogens are encountered. In *Mycobacterium tuberculosis,* as special laboratory facilities are needed and prolonged incubation is needed for phenotypic AST, genotypic AST is gradually replacing the traditional phenotypic AST and global standard for interpreting the genotypic AST results of *M. tuberculosis* has been established[47].

Emerging technologies like whole-genome sequencing (WGS), next-generation sequencing (NGS) and machine learning are poised to revolutionise AST. When run with conventional phenotypic AST, these new testing methods can help us to understand the resistant mechanisms and identify genetic targets to be tested. WGS is also increasingly being used in the detection and investigation of heteroresistance[48]. It is foreseeable that the costs of WGC and NGS will keep on reducing and becoming more affordable to be used in clinical settings. When WGS databases are getting more and more comprehensive and easily available, they could also predict resistance patterns and inform treatment decisions more rapidly and accurately than traditional methods[49].

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MCHK CME Programme Self-assessment Questions

Please read the article entitled "Clinical Importance and Interpretation of Antimicrobial Susceptibility Testing" by Dr Teresa Kin-fong WANG and Dr Christopher Koon-chi LAI and complete the following self-assessment questions. Participants in the MCHK CME Programme will be awarded CME credit under the Programme for returning completed answer sheets via fax (2865 0345) or by mail to the Federation Secretariat on or before 31 July 2024. Answers to questions will be provided in the next issue of The Hong Kong Medical Diary. (Address: Duke of Windsor Social Service Bldg., 4/Fl., 15 Hennessy Rd., Wan Chai. Enquiry: 2527 8898)

Questions 1 - 10: Please answer T (true) or F (false)

1. **Antimicrobial susceptibility testing (AST) is used for infection control strategies.**
2. **The World Health Organization (WHO) is responsible for coordinating the collection of antimicrobial resistance (AMR) data through the Global Antimicrobial Resistance and Use Surveillance System (GLASS).**
3. **Genotypic testing for drug-resistant genes is gaining importance in AST.**
4. **The disk diffusion test measures the minimum inhibitory concentration (MIC) of an antimicrobial agent.**
5. **Minimum inhibitory concentration (MIC) can be determined by methods such as microbroth dilution, E-test strips, and automated systems.**
6. **The turnaround time for traditional AST methods is relatively short, usually requiring just 7 hours.**
7. **Automated systems like VITEK® 2 and MicroScan WalkAway® provide auto-interpretation of susceptibility results based on predefined panels.**
8. **Mass spectrometry is a non-genotypic method of AST that can assess the susceptibility of bacteria by analyzing protein expression patterns or specific degradation products.**
9. **Nucleic acid amplification technologies (NAATs) can detect targeted resistant genes and generate results within a short timeframe in less than an hour.**
10. **The use of genotypic testing methods in antimicrobial susceptibility testing (AST) is decreasing in importance compared to phenotypic testing methods.**

ANSWER SHEET FOR JULY 2024

Please return the completed answer sheet to the Federation Secretariat on or before 31 July 2024 for documentation. 1 CME point will be awarded for answering the MCHK CME programme (for non-specialists) self-assessment questions.

Clinical Importance and Interpretation of Antimicrobial Susceptibility Testing

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Answers to June 2024 Issue

Precision Medicine in Diabetes - What Is It and Why Do We Need It?

1. T 2. F 3. F 4. T 5. T 6. F 7. F 8. F 9. T 10. F

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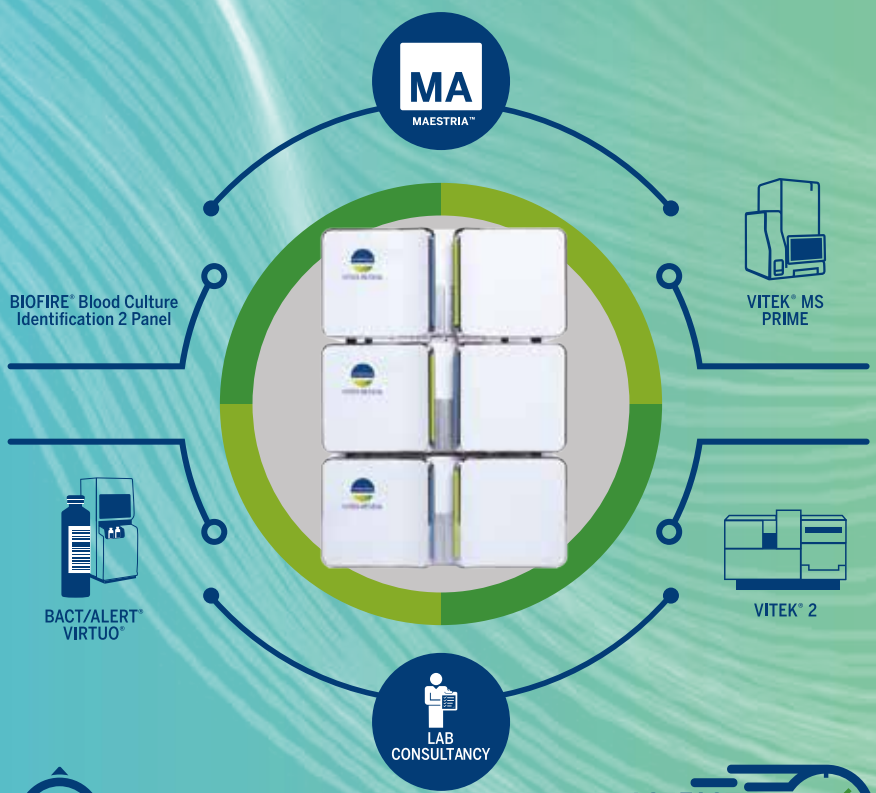

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The Application of High-Throughput Sequencing Methods for Pathogen Identification in Clinical Microbiology

Prof Gilman Kit-hang SIU

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INTRODUCTION

Since its invention in the 1990s, next-generation sequencing (NGS) has revolutionised microbiology research, significantly deepening our understanding of microbial genomics[1]. In the past decade, its application has extended to public health and food safety laboratories, making it an indispensable tool for microbial surveillance and outbreak investigation[2]. Recently, reductions in reagent costs and simplifications in library preparation for both NGS and Oxford Nanopore Technologies (ONT) sequencing have encouraged further exploration of their utilities for diagnosing infectious diseases. These high-throughput sequencing techniques have been shown to dramatically enhance the detection and identification of pathogens that previously eluded detection or were misidentified, effectively addressing unmet clinical needs[3]. Despite their considerable potential and proven value, the integration of NGS into real-life clinical practice presents substantial challenges. These challenges are not limited to individual laboratories but extend across the entire field.

The implementation of high-throughput sequencing in clinical settings is complicated by various factors. The diversity of sequencing strategies, such as whole-genome sequencing (WGS), targeted sequencing (tNGS), and metagenomic sequencing (mNGS), often leave laboratories uncertain about the best choice of method. The technical complexity, lack of standardised interpretation guidelines, and the extensive resources required for bioinformatic analysis pose significant hurdles[4]. This chapter will delve into the selection of appropriate sequencing methods for pathogen identification, taking into account the variations in sample types, and discuss the criteria for interpreting the results.

16S RIBOSOMAL RNA (16S rRNA) GENE SEQUENCING

In clinical microbiology laboratories, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is widely used for bacterial identification. However, its effectiveness can be limited by the high similarity of mass spectra among closely related species or the absence of reference spectra in the database[5]. In such cases, 16S rRNA gene sequencing serves as a reliable alternative for taxonomic identification. Traditionally, this involves PCR amplification of the 16S rRNA gene from cultured samples, followed by Sanger sequencing. A significant advancement in this method is the adoption of long-read ONT sequencing, which allows for the sequencing of the entire 16S rRNA gene, thereby improving taxonomic resolution at the species level. A local study by Lao et al. showed that the diagnostic accuracy of the ONT protocol reached 96.36%, comparable to Sanger sequencing and superior to the Illumina protocol, which exhibited an accuracy of 71.52% for species-level identification in isolated cultures[6]. Other studies have also confirmed that ONT sequencing provides better taxonomic resolution than Illumina for 16S rRNA gene sequencing[7].

Extending the application to direct samples, 16S rRNA gene sequencing can rapidly identify bacteria from infected body fluids collected from sterile sites. PCR enrichment of the 16S rRNA gene helps to minimise interference from human DNA during sequencing analysis[8]. However, it is important to note that direct 16S rRNA sequencing is susceptible to contamination. Contaminating microbes are ubiquitous and may be present in reagents or labware, the environment[9], or normal human flora[10]. Due to the high sensitivity of direct 16S rRNA sequencing, even minute amounts of contaminating microbes can be present in the sequencing data. As a critical quality assurance measure, nuclease-free water should be used as a non-template control (NTC) in each sequencing batch to monitor for environmental and kit contaminants[7]. Reads detected in the NTC are considered contaminants and excluded from sample analysis.

The interpretation of test results is a new challenge. It is common to find a list of bacterial taxa with low read counts in sequencing data, even in samples collected from patients with clear non-infectious diagnoses. Such background noise is often attributed to contaminating microbes or sequencing artefacts. Therefore, it is essential to establish a threshold for the read count of detected organisms relative to the total number of reads in a sample. This threshold helps distinguish a true call from background noise. Recent studies have shown that setting a threshold of relative abundance at 0.058 enables the ONT 16S assay to achieve an accuracy of 97.7% in monomicrobial samples and 81.7% in polymicrobial body fluid samples, compared to a composite laboratory reference[11].

METAGENOMIC SHOTGUN SEQUENCING (mNGS)

mNGS revolutionises pathogen detection through its unbiased, hypothesis-independent shotgun sequencing

approach. This method analyses all genetic material in a sample without presuppositions about its contents. Unlike 16S rRNA sequencing, which is limited to bacterial identification, mNGS can detect a broad spectrum of organisms, including viruses, fungi, and parasites, as well as genes associated with antimicrobial resistance (AMR)[12]. mNGS achieved higher positivity rates (85.0%) in patients with pneumonia than conventional microbiological tests (62.2%)[13]. The target agnostic approach also enables the detection of unexpected pathogens[14] or even the discovery of new organisms[15].

A significant disadvantage inherent to mNGS is the overwhelming presence of human host DNA in most samples, where typically more than 99% of the reads are derived from the human host. This dominance can significantly hinder pathogen detection due to the relative scarcity of microbial reads. This issue can be partially addressed through host depletion methods or targeted enrichment strategies.

Host depletion techniques reduce the proportion of human sequences in mNGS data. While computational subtraction of human host sequences is possible during bioinformatics analysis, physically removing human DNA or RNA during sample preparation is more cost-effective, as it prevents the sequencing of irrelevant human reads. Methods include using saponin or other chemical agents to selectively lyse human white blood cells, followed by treatment with deoxyribonuclease (DNase) to degrade released human genomic material, thereby enriching microbial DNA that is protected within viral capsids or microbial cell walls[16]. Another approach involves targeting low-molecular-weight cell-free nucleic acids and removing high-molecular-weight genetic material predominantly associated with human DNA, achieved by separating cellular from cell-free compartments in clinical samples, such as through centrifugation. While these techniques might reduce microbial reads from intracellular organisms (e.g., Listeria monocytogenes), they generally result in an overall enrichment of pathogen reads relative to human reads[17].

Moreover, mNGS often encounters challenges related to microbial contaminants in samples, reagents, or the lab environment, complicating analysis, and interpretation of results. Contamination can occur during sample collection from presumed sterile sites, such as skin flora during fine needle aspiration. For instance, reads mapping to Cutibacterium acnes are frequently observed in mNGS datasets as a result of such contamination[10]. Rigorous adherence to quality control procedures in reagent handling and workflow execution, including the use of negative controls, is crucial for maintaining a sterile testing environment and minimising extraneous nucleic acids. Additionally, normalising detection thresholds to background levels by dividing the read count observed in the sample by that in negative or no-template controls (NTC) helps set a threshold for reporting detected organisms and prevent false positives[18].

In clinical reporting, mNGS typically presents the composition of microorganisms in a sample in descending order of microbial sequence counts. A variety of open-source software packages are available for detecting and characterising microbial sequences from mNGS data. Notable examples include Sequence-based Ultrarapid Pathogen Identification (SURPI)[19], Kraken[20] ,and MegaPath[21]. These bioinformatics pipelines generally follow a structured process: (i) preprocessing of sequencing reads to remove adapters and filter out low-quality and low-complexity regions; (ii) optional alignment to the human genome for computational host subtraction; (iii) alignment of the processed, non-human sequencing reads to a curated pathogen database, followed by the assignment of taxonomic classification to each sequence; and (iv) organisational and statistical analysis of the resultant data, often accompanied by visualisation in a graphical user interface.

While invaluable for identifying pathogens in samples from sterile sites, mNGS can complicate diagnoses and lead to inappropriate treatment when non-pathogenic resident flora are reported in samples from body parts with normal microbial colonisation, such as the respiratory tract. Thus, combining targeted enrichment with mNGS may be especially beneficial for analysing non-sterile specimens, like those from bronchoalveolar lavage, stool, or polymicrobial abscesses.

TARGET-ENRICHED METAGENOMIC SEQUENCING

The hybridisation-capture-based approach is increasingly recognised for targeting enriched mNGS of non-sterile samples in clinical settings[18]. This method employs biotinylated oligonucleotide probes specifically designed to target genomes of interest. These probes hybridise with DNA libraries and are subsequently enriched using streptavidin-coated beads. This targeted capture technique facilitates the enrichment of specific microbial sequences, enabling a more focused sequencing analysis. In contrast to broader mNGS techniques, which typically require extensive sequencing output of over 10 million reads per sample and sophisticated bioinformatics to navigate vast amounts of microbiome and host DNA data, the hybridisation-capture approach is more suitable for development into decentralised tests that can be implemented in resource-limited laboratories. It supports the precise detection of clinically relevant organisms and is compatible with low throughput sequencing instruments, which typically generate fewer than three million reads per sample[18].

Recently, a commercial hybridisation probe-based enrichment kit has been introduced into the market, capable of detecting more than 200 organisms and over 1,000 AMR genes from respiratory specimens. The targeted organisms are categorised into three groups: primary pathogens (Group 3), opportunistic pathogens (Group 2), and resident microbiota (Group 1)[18]. The interpretation of results is based on read counts assessed on an organism-by-organism basis. For instance, the criteria prioritise reporting Group 3 organisms when their read counts surpass random noise levels. For opportunistic pathogens (Group 2 organisms), they are considered causative agents only if their read counts surpass those of the resident microbiota (Group 1), except when they are the only organisms detected. This

methodology aligns with the interpretative guidelines used in semi-quantitative cultures of respiratory samples, where only predominately growing organisms are reported[22]. Therefore, a Group 2 organism is reported as a pathogen if its read count is at least five times higher than that of the resident microbiota, provided it is not the only organism identified in the sample. A recent extensive evaluation of this kit demonstrated an overall accuracy of 65.6% when benchmarked against a composite clinical standard that includes provider-ordered microbiology tests, chart reviews, and orthogonal testing[18].

CONSIDERATIONS FOR HIGH-THROUGHPUT SEQUENCING TESTING

From a clinical application perspective, several expert consensuses have been published in China since 2019, summarising the main indications for high-throughput sequencing in clinical microbiology. These indications include: (i) severe infections; (ii) immune dysfunction; (iii) refractory infections or infections with poor previous treatment efficacy; (iv) pathogens that cannot be detected using traditional methods; and (v) suspected infections caused by new pathogens[23]. Unbiased mNGS can be the preferred option for central nervous system infections, where the range of potential pathogens is broad and the causative pathogens, in some cases are missed despite extensive diagnostic testing. Numerous case reports have documented the identification of viruses, bacteria, fungi, and parasites using mNGS from cerebrospinal fluid and brain tissue[24]. The workflows and the estimated time-to-report of different high-throughput sequencing methods are illustrated in Fig. 1. For non-sterile site infections, mNGS coupled with a target enrichment approach may be more appropriate. However, due to the high reagent costs, complicated library preparation procedures, and the lack of official approval by any governing bodies for specific indications, mNGS is recommended as an adjunctive test, used in conjunction with traditional microbiological and molecular methods[18]. The advantages and disadvantages of different sequencing methods are summarised in Table 1.

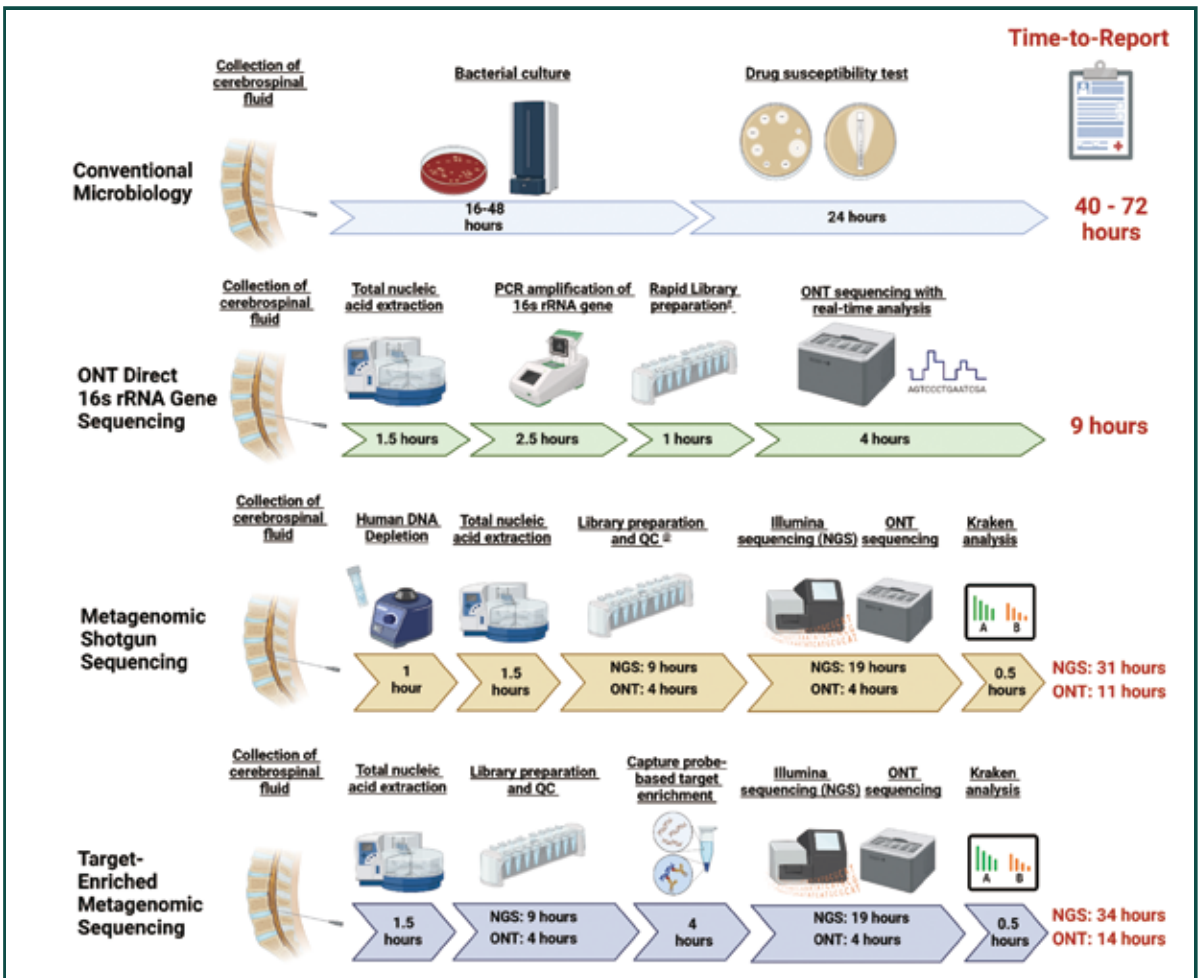

Fig. 1: The workflows and the estimated time-to-report of different high throughput sequencing methods in comparison to conventional microbiology. (Developed by Prof. Gilman SIU using Biorender, https://www.biorender.com/).
 # Rapid library preparation includes adapter ligation, magnetic bead purification and Qubit quantification
@ Library preparation includes DNA synthesis, tagmentation, indexing, magnetic bead purification and PCR quantification.

Table 1: Comparison of high-throughput sequencing methods for clinical microbiology (Developed by Prof. Gilman SIU using Biorender, https://www.biorender.com/).

Sequencing method	Application in clinical microbiology	Advantages	Disadvantages
Whole-genome Sequencing	Typing of organisms for genomic survillance and outbreak investigation	Highest resolution for linking cases in an outbreak, determining transmission pathways, and distinguishing between related and sporadic cases.	Inconsistent guidelines for defining genetic relatedness thresholds.
		Facilitating comparison across different times and geographic locations	Requires sophisticated bioinformatics tools and expertise for data analysis
	Characterization of unknown microorganisms	Allows detailed investigation of microbial genomes; profiling virulence factors and antimicrobial resistance (AMR) genes	Organisms must be isolated and cultured before extraction and sequencing
16S rRNA gene sequencing	Species-level identification of cultured isolates that failed identification by MALDI-ToF-MS	Standardized workflow and real-time bioinformatic analysis software for Oxford Nanopore Technologies (ONT) 16S rRNA sequencing	Limited to bacterial pathogen only
			Cannot differentiate closely-related species with highly similar 16S rRNA sequences.
	Direct identification of bacterial pathogens from sterile body fluids	Low interference from human DNA, making it suitable for sterile site infections suspected to be bacterial	Prone to contamination with environmental species
			Lack of standardised interpretation criteria to differentiate causative organisms from background noise
Metagenomic shotgun sequencing	Unbiased and hypthesis-free pathogen identification from sterile body fluid	Detects a broad spectrum of organisms, including viruses, fungi, and parasites, alongside AMR genes.	Dominance of human DNA reduces sensitivity
		Discovery of new or unexpected organisms	Prone to contamination with environmental species
		Less extensive library preparation; lower cost for the library preparation kits without target-capture reagents	Lack of standardised interpretation criteria to report causative organisms from background noise
			Greater depth (goal of 10 million reads/sample), limiting the multiplexing capacity in each sequencing run
			Higher sequencing cost per sample
Target-enriched metagenomc sequencing	Detection of a panel of organisms in both sterile and non-sterile infection sites	Shallower depth (goal of 3 million reads/sample); lower sequencing cost per sample	More extensive library preparation; additional time and higher reagent cost
		Reduced interference from human DNA	Prone to contamination with environmental species
		Less bioinformatic resources involved	Lack of standardised interpretation criteria to differentiate causative organisms from resident flora in non-sterile sites

From a laboratory technology perspective, there is currently no unified standard or quality control principle for mNGS testing, leading to inconsistencies in testing standards and results between different institutions. To improve this situation, it is proposed that (i) industry-specific testing quality norms, qualified testing sample standards, and scientific reporting formats should be established; (ii) quality control data such as sequencing quality, sample data volume, read length, microbial sequence numbers, and relative abundance should be documented. Moreover, quality control materials for proficiency testing should be established for scientific evaluation of the capabilities of testing institutions and facilitate interlaboratory comparisons.

The interpretation of test results is a significant challenge in the clinical application of mNGS technology. Such interpretation requires detailed analysis by clinicians considering the patient's overall condition[18]. The principles of interpreting results vary depending on the source and type of sample, and there is a lack of standardised guidelines for interpreting test results, including detection thresholds and unified clinical standards. This gap necessitates the involvement of professionals from multiple disciplines, such as laboratory medicine, infectious diseases, specialty diseases, bioinformatics, and artificial intelligence. Establishing standards for interpreting test results is critical, and relevant data should be continuously collected in clinical practice to conduct research on the impact of test results from different sources on clinical diagnosis. This research can help discover the underlying logic and patterns in interpretation, maximising the clinical value of test results. There is also a need to enhance the ability of clinical physicians, especially those in infection-related departments, to interpret sequencing test reports. Exploring the application of artificial intelligence in interpreting test results, which combines data on microbial composition, patient symptoms, infection markers, imaging features, and more, could lead to the development of diagnostic models for the automated interpretation of test reports[25].

CONCLUSION

High-throughput sequencing technologies, such as 16S rRNA gene sequencing and mNGS, offer a revolutionary approach to pathogen identification,

surpassing the limitations of traditional methods. However, their integration into clinical practice requires careful consideration of various factors.

The choice between different sequencing methods depends on the sample type and the desired level of resolution. While 16S rRNA sequencing excels in bacterial identification, mNGS provides a broader spectrum of detection, encompassing viruses, fungi, and parasites. However, challenges such as contamination, host DNA interference, and the lack of standardised interpretation guidelines necessitate stringent quality control measures and the development of robust bioinformatic tools.

Targeted enrichment strategies, like hybridisation capture, offer a promising solution for specimens collected from non-sterile sites. This approach enhances pathogen detection while minimising background noise and resource requirements.

Moving forward, establishing industry-wide standards for quality control, data interpretation, and reporting is crucial. Collaborative efforts involving clinicians, laboratorians, and bioinformaticians are essential to unlock the full potential of high-throughput sequencing and ensure its effective application in clinical settings. Continuous research and the integration of artificial intelligence will further refine diagnostic accuracy and pave the way for personalised treatment strategies. As these technologies evolve, they hold the potential to revolutionise infectious disease diagnostics and significantly improve patient care.

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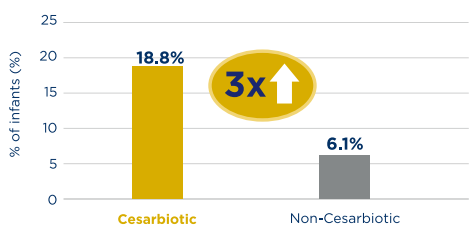

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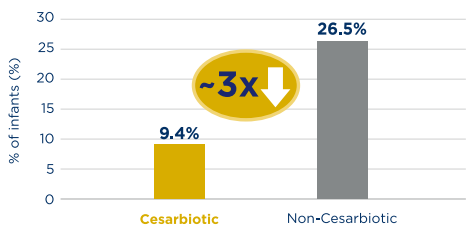
≥3 illness events



Total sample size: Cesarbiotic, n=32; Non-cesarbiotic, n=49.

※ 根據本地大學針對剖腹產嬰兒的餵哺方式與健康成長需要的研究。資料來源：於2024年5月24日相關研究的新聞發布會。成分指專研配方中的益生致親組合。
▲ 根據Kantar香港2021的調查結果，調查對象為醫生（婦產科/兒科專科），樣本數量為73。

References: 1.Data on file. According to research findings of the study form The Hong Kong Polytechnic University. Data presented on 8 May 2024, The Cross-Strait Medical Symposium: The Future Practices for C-section- Integrating Research Findings.

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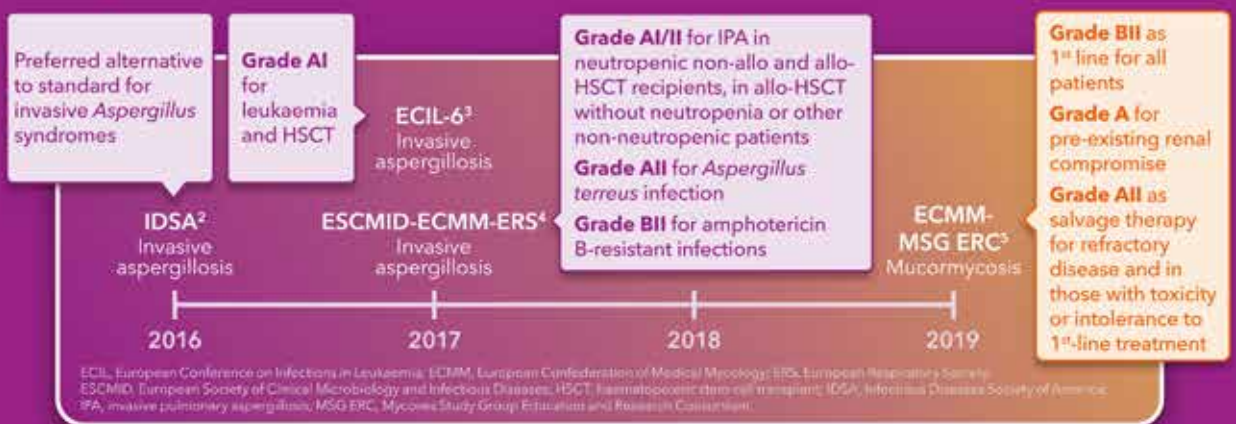

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References: **1.** CRESEMBA® (isavuconazole) Prescribing Information. Pfizer Corporation Hong Kong Limited; Version Jul 2022. **2.** Patterson TF, et al. *Clin Infect Dis* 2016;63:e1-e60. **3.** Tissot F, et al. *Haematologica* 2017;102:433-444. **4.** Ullmann AJ, et al. *Clin Microbiol Infect* 2018;24(Suppl 1):e1-e38. **5.** Cornely OA, et al. *Lancet* 2019;3099:1-17. **6.** Maertens JA, et al. *Lancet* 2016;387:760-769. **7.** Mercier T, Maertens J. *J Antimicrob Chemother* 2017;72:i29- i38. **8.** Marty FM, et al. *Lancet Infect Dis* 2016;16:828-837. **9.** Wilson DT, et al. *Ther Clin Risk Manag* 2016;12:1197-1206. **10.** Groll AH, et al. *Clin Pharmacol Drug Dev* 2017;6:76-85. **11.** Natesan SK, Chandrasekar PH. *Infect Drug Resist* 2016;9:291-300.

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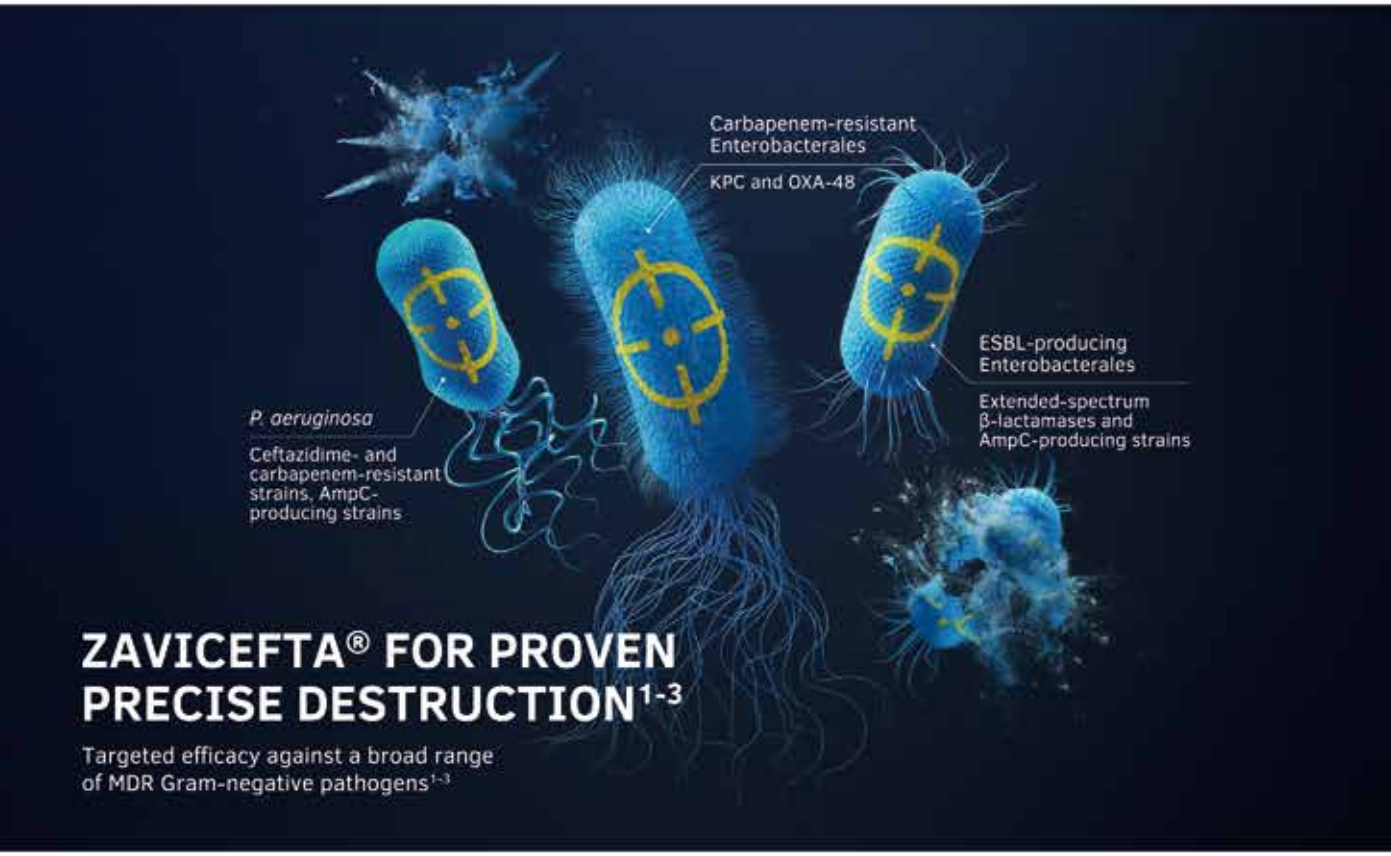

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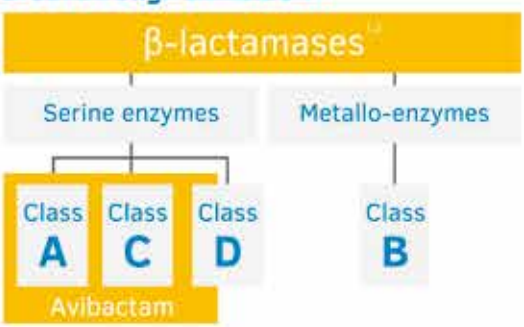

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ESBL, extended-spectrum β-lactamase;
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References:

1. Zavicefta (Ceftazidime-avibactam) Prescribing Information. Pfizer Corporation Hong Kong Limited Version August 2022.
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3. Zhang W, *et al. Antimicrob Resist Infect Control* 2018;7:142.

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Faecal Microbiota Transplantation

Dr Rita Wai-yin NG

MBChB(CUHK), MRCP(UK), PDipID(HKU), FRCPath(UK), FHKCPath, FHKAM(Pathology)

Dr Rita Wai-yin NG

INTRODUCTION

Clostridiodes difficile infection (CDI) is an important cause of nosocomial diarrhoea related to antibiotic use and it poses a major health burden worldwide. As a Gram-positive, anaerobic, spore-forming bacillus, *Clostridiodes difficile* is associated with gastro-intestinal manifestations ranging from mild watery diarrhoea to severe complications including pseudomembranous colitis and toxic megacolon[1]. *Clostridiodes difficile* is found in water, soil, meats and vegetables and is especially important in health care environments. The organism can survive in environments under aerobic conditions by forming spores. Patients are exposed to Clostridiodes difficile spores in the health care setting due to contact with the hospital environment and health care personnel. The commonest risk factor for CDI is exposure to broad-spectrum antibiotics, especially with the usage of third-generation cephalosporins, fluroquinolones and clindamycin. On the other hand, old age, severe underlying medical diseases and prior hospitalisation are the other risk factors for CDI[2].

The indigenous gut microbiota is thought to play a number of important roles in host homeostasis and pathogenesis of CDI. Disruption of gut microbiota can lead to the development of CDI. The ability of *Clostridiodes difficile* to cause colitis depends on a range of virulence factors, including toxins (toxin A and toxin B) that primarily target intestinal epithelial cells leading to necrosis and activation of host inflammatory response. Antibiotic that precedes CDI substantially alters the intestinal microbiome, creating a more hospitable environment for the growth of the organism, for example, through depletion of commensal bacteria leading to the abundance of sialic acid and succinate which enhances the growth of *Clostridiodes difficile*[3].

Despite antibiotic treatment, recurrence of CDI occurs in 25% following discontinuation of therapy after an initial episode of disease. The risk of recurrence increases with each episode, accounting for significant morbidity and mortality in this population of patients. Patients with recurrent CDI exhibit decreased microbial diversity of their indigenous gut microbes[4]. It is presumed that patients will be at increased risk of reinfection or regrowth of residual *Clostridiodes difficile* after antibiotic therapy if the gut microbiota is unable to return to its baseline condition. This has led to the exploration of the administration of faecal microbiota transplantation (FMT) from normal donors to restore the gut microbiota diversity in recurrent CDI.

MECHANISMS OF FMT

FMT refers to the transplantation of gut microbiota through the administration of healthy donor faecal material with the aim to cure disease or improve patient's conditions by normalising microbial diversity and community structure. In patients with CDI, there is loss of microbial diversity with an increase of relative abundance of *Proteobacteria* and a decrease in the relative abundance of *Bacteroidetes* and *Firmicutes*. FMT normalises microbial diversity & community structure of a patient's gut microbiota, and this normalisation is observed as early as 24 hours after FMT[5].

Two broad, not mutually exclusive, mechanistic categories exist for the effectiveness of FMT that can be considered. First, commensal microbiota delivered by FMT can directly compete with *Clostridiodes difficile* for nutritional and colonisation resources, resulting in interference with its virulence factors and death of *Clostridiodes difficile* bacteria. Second, the gut microbiota can activate various host immune defences and constrain *Clostridiodes difficile* through secondary bile acids, which can be inhibitory of germination and vegetative growth of *Clostridiodes difficile*[6]. The secondary bile acids are generated following deconjugation of taurine and glycine, and 7α dehydroxylation by some bacteria, yielding deoxycholic and lithocholic acids from cholic and chenodeoxycholic acids, respectively. Antibiotic treatments used to treat *Clostridiodes difficile* infection inhibit bacteria involved in secondary bile acid metabolism, and secondary bile acids are essentially absent in patients with refractory *Clostridiodes difficile* infection syndrome[7].

CLOSTRIDIODES DIFFICILE

FMT given in faecal enema was first documented in a case report in curing patients suffering from five episodes of CDI failing to respond to antibiotic treatment, resulting in prompt and complete normalisation of bowel function[8]. There was a booming of FMT practices in the past two decades due to the increasing incidence of CDI and a better understanding of the gut microbiota. The first randomised controlled trial (RCT) of FMT in treating recurrent CDI was conducted in 2013. It showed that infusion of donor faeces was significantly more effective for the treatment of recurrent CDI than the use of vancomycin, with 81% and 31% of the patient response respectively[9].

The primary cure rate of FMT in treating CDI was 91.2%

in a systematic review including 18 observational studies with 611 patients[10]. FMT is recommended as standard treatment for second CDI recurrence in the updated guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America IN 2021 (SHEA)[11]. In another meta-analysis including 7 RCT and 30 case series, FMT was more effective than vancomycin in resolving recurrent and refractory CDI with 92% clinical resolution rate[12].

KEY STAGES IN THE FMT PROCESS

There are two main models to supply stool for FMT, including patient-selected donors and stool banking. Under the patient-selected donor model, the patient or their guardian identifies their own stool donor candidate, who is screened by the treating physician and the donor's stool is processed by the medical team. The other model is the stool bank, which addresses the logistics limitations inherent to the patient-selected donor model, including the safety risks caused by variable screening standards. A stool bank is a centralised facility that screens donors, processes stool, stores FMT preparations, fulfils clinicians' and researchers' requests for FMT and monitors safety and efficacy of FMT material[13]. Centralised processing is the most cost-efficient and controlled process in which qualified donors provide FMT material that can be used to treat many patients, in a purpose-built facility that allows stringent manufacturing quality standards.

Although there are variations of regulatory requirements in different countries, FMT stool banks generally adhere to standard methodology, including donor recruitment, donor evaluation with stringent screening questionnaire and laboratory tests for infectious agents, manufacturing of stool formulation, donor health monitoring and release of FMT material, provision of FMT material to clinicians and researchers and evaluation of safety and quality of FMT material.

Donor evaluation is a key part of FMT stool bank to ensure the quality of FMT. Potential donor candidates are required to complete an in-depth donor health questionnaire, which includes questions about gastro-intestinal comorbidities, metabolic conditions, neuro-psychiatric comorbidities, infectious diseases, autoimmune diseases, atopy, asthma and allergies, malignancy, surgeries or other medical history, current symptoms, medications, diet, social history, and family history. Candidates who pass the in-person clinical assessment undergo a three-part laboratory screening including blood, stool and respiratory samples. Candidates who have passed both the health questionnaire and laboratory screening tests are accepted into the stool donation programme.

INTERNATIONAL GUIDELINES OF FMT CENTreS WORLDWIDE

In 2016, the U.S. Food and Drug Administration (FDA) exercised enforcement discretion of investigational new drug (IND) designation for FMT for the treatment of recurrent and refractory C. difficile infections (rCDI). In 2014 in the UK NICE supported the use of FMT in rCDI who have two or more previous episodes with suggestions for clinical governance, patient screening, and management of patients. Subsequently, the MHRA have licensed some U.K. stool banks to provide FMT as a medicinal product to third parties under their special licensing procedures[14]. Different stool banks in different parts of the world have also shared their experience in the standard operating procedures, including the Netherlands[15], Taiwan[16] and Australia[17]. In Hong Kong, the healthy donor stool bank with pre-screened frozen stools was established in 2017. Real-world data over eight years from the Hong Kong FMT Registry have demonstrated excellent long-term safety outcomes of 123 subjects who underwent 510 FMTs with prospective follow-up[18].

However, there remains much variability regarding the selection procedures and regarding the specific methodology for banking and producing a safe FMT product to ensure successful regulation of this emerging health intervention, hence limiting the establishment and supply of FMT. A recent systematic review[19] was conducted, including 33 clinical studies and 11 consensus documents were compared along with the local donor screening procedure in Hong Kong. A significant proportion of clinical studies (n= 14/33) and consensus guidelines (n= 6/11) on FMT are reported from the WHO European region. (Fig. 1) There is a wide variation in the investigations and the screening methods selected for donor acceptance from different geographical regions.

SAFETY CONSIDERATIONS OF FMT

The screening of asymptomatic faecal donors for FMT is of critical importance to ensure the safety of FMT. In 2019, two patients in whom extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli bacteremia occurred after they had undergone FMT in two independent clinical trials. Both cases were linked to the same stool donor by means of genomic sequencing[20]. In addition, the changing landscape of emerging infectious diseases, including SARS-CoV-2 and multi-resistant organisms, means that stool biobanks need to have constant updates of the literature and evidence-based screening strategy of faecal donors to ensure the safety and quality of FMT.

CONCLUSIONS

FMTs are increasingly utilised in the treatment of relapsing CDI and as an investigational modality for the treatment of a wider range of medical conditions. The repertoire of the optimal testing methods for infective agents is rapidly changing due to the advancement of technology and our increased understanding of the risks associated with FMTs. With the rapidly increasing numbers of FMT biobanks established worldwide, there is a need for a working consensus perhaps of a minimal set of screening questionnaires and laboratory test requirements for donor selection (Table 1). Additional consideration made to specific conditions and tests will be based, according to a risk-based assessment, depending on the geographical prevalence of disease and other cultural and medicine licensing requirements and risk-benefit factors in their region.

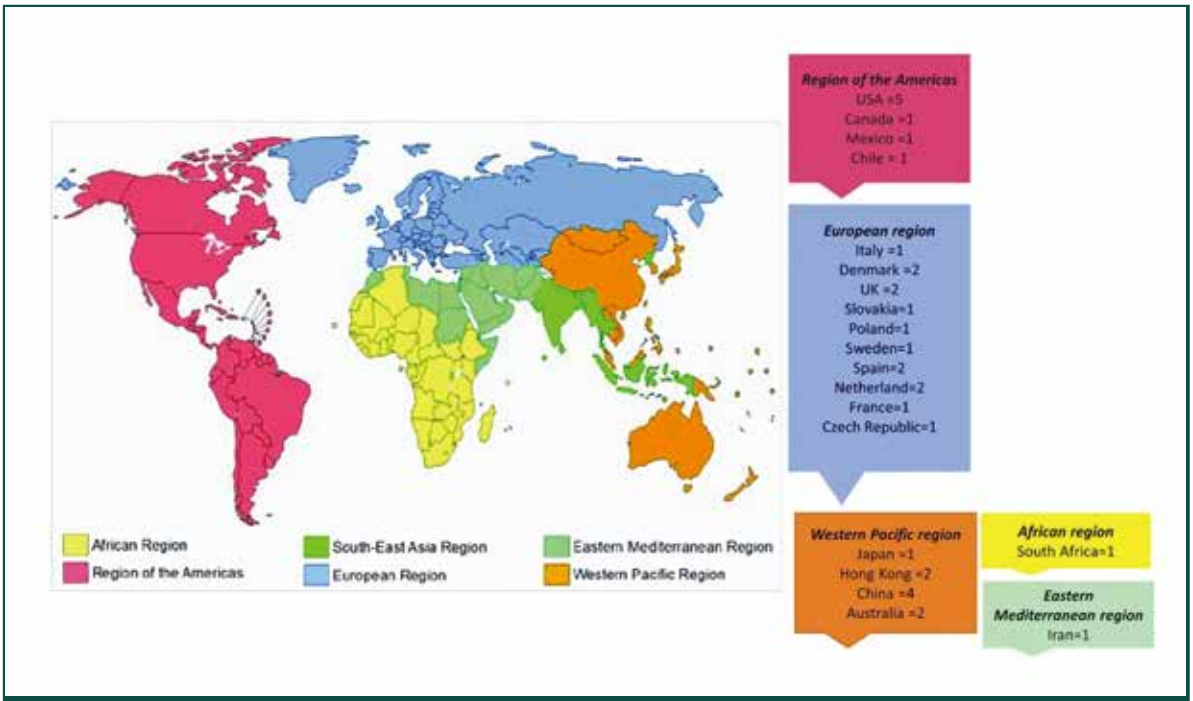

Fig. 1: Included study stratification according to the WHO regions.(Adapted from https://pubmed.ncbi.nlm.nih.gov/37142392/#&gid=article-figures&pid=figure-1-uid-0)

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Table 1: Recommended minimum list of Questionnaire, blood and stool test for rigorous FMT donor screening procedure. (Adapted from https://pubmed.ncbi.nlm.nih.gov/37142392/#&gid=article-figures&pid=figure-1-uid-0)

Pre-screening data	Test Method
Risk of infectious agents	
Known HIV, Hepatitis B or C infections	Questionnaire
High-risk sexual behaviours	Questionnaire
Use of illicit drugs	Questionnaire
Travel (within the last 6 months) to high risk countries with travellers' diarrhea	Questionnaire
Recent needle stick accident	Questionnaire
Gastrointestinal co-morbidities	
History of inflammatory bowel disease	Questionnaire
History of irritable bowel syndrome, idiopathic chronic constipation, or chronic diarrhea	Questionnaire
History of gastrointestinal malignancy or known polyposis	Questionnaire
Factors could affect the composition of Gut microbiota	
Antibiotics within the preceding 3 months	Questionnaire
Major immunosuppressive medications	Questionnaire
Other factors	
History of major gastrointestinal surgery	Questionnaire
Metabolic syndrome	Questionnaire
Systemic autoimmunity (multiple sclerosis, connective tissue disease)	Questionnaire
Atopic conditions (Asthma, atopic dermatitis, Eczema)	Questionnaire
Obesity	Questionnaire
Depression	Questionnaire
Schizophrenia or delusion disorder	Questionnaire
Blood Tests	**Test Method**
Testing for viruses	
Hepatitis A	HAV-IgM
Hepatitis B	HbsAg, Anti-Hbc
Hepatitis C	Anti-HCV
Hepatitis E	Anti-HEV IgM
HIV I and II	Anti-HIV
Human T-cell lymphoma virus	Anti-HTLV
Testing for bacteria	
Treponema pallidum	Screening test (for example RPR, VDRL, EIA)
Other tests	
Complete blood count	NA
C-reactive proteins	NA
Renal function test	NA
Liver function test	NA
Stool tests	**Test Method**
Testing for viruses	
Rotavirus	EIA
Norovirus	PCR
Testing for bacteria	
Salmonella species	Culture +/-PCR
Shigella species	Culture +/-PCR
Campylobacter species	Culture +/-PCR
Vibrio species	Culture +/-PCR
C. difficile	PCR
H. pylori	Stool antigen
MDR Bacteria	
ESBL producing Enterobacteraecae	Culture
VRE	Culture
CRE (KPC, NDM, OXA 48)	Culture
MRSA	Culture
Testing for parasites	
Cyclospora species	Microscopy +/-antigen
Isospora species	Microscopy +/-antigen
Giardia species	Microscopy +/-antigen
Cryptosporidium species	Microscopy +/-antigen
Entamoeba histolytica	Microscopy +/-antigen
Light microscopy for ova, cysts	Microscopy

Footnote: rapid plasma regain (RPR), venereal disease research laboratory test (VDRL), enzyme immunoassay (EIA)

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VEKLURY® (n=541) significantly reduced time to recovery by a median of 5 days compared with placebo (n=521) in the overall study population (rate ratio for recovery 1.29; 95% CI 1.12–1.49; p<0.001).*[1,2]

* Study design: A placebo-controlled, randomized, double-blind, parallel-group clinical trial was conducted in patients aged ≥ 18 years with SARS-CoV-2 infection. Subjects received VEKLURY® 200 mg IV once daily on Day 1 followed by VEKLURY® 100 mg IV once daily on Days 2 to 10 or placebo. If the subject was discharged from the hospital, the study drug was discontinued. In addition to study drug, standard of care was allowed in accordance with local guidelines for the treatment of infections with SARS-CoV-2. The primary endpoint was time to recovery within 28 days after randomization defined as not hospitalized, no limitation on activities; not hospitalized, limitation on activities and/or requiring home oxygen; hospitalized, not requiring supplemental oxygen – no longer required ongoing medical care.[2]

CI=confidence interval; COVID-19=coronavirus disease 2019; IV=intravenous; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

References: 1. Beigel JH, et al. N Engl J Med. 2020;383:1813–1826. **2.** VEKLURY® Hong Kong Prescribing Information. [HK-MAY22-JP-MAR22-EU-AUG21]

VEKLURY® Abbreviated Prescribing Information (Version: HK-MAY22-JP-MAR22-EU-AUG21)
Presentation: Veklury powder for concentrate for solution for infusion 100 mg: Each vial contains 100 mg of remdesivir. White to off-white to yellow solid. **Indications:** SARS-CoV-2 Infection. In principle remdesivir should be used for SARS-CoV-2 infections in patients who do not require oxygen support and are considered to need treatment with VEKLURY because of their risk factors for disease progression of SARS-CoV-2 infection etc., or patients with pneumonia who are infected with SARS-CoV-2. Also, refer the latest guideline on the treatment target of VEKLURY. **Dosage:** Adults and pediatrics with body weight ≥40 kg: Single dose of remdesivir 200 mg IV injection on Day 1 followed by once-daily doses of remdesivir 100 mg IV injection from Day 2. Pediatrics with body weight between 3.5 kg and <40 kg: One dose of remdesivir 5 mg/kg IV injection on Day 1 followed by remdesivir 2.5 mg/kg IV injection from Day 2. Treatment duration: The treatment course of VEKLURY should be initiated as soon as possible after symptom of SARS CoV-2 infection has developed, VEKLURY is administered until Day 3. As a guide, VEKLURY is administered until Day 5 for patients with pneumonia who are infected with SARS-CoV-2, VEKLURY is administered until Day 10 if patients do not obtain improvement. Renal impairment: Not recommended for adults, infants, children and older children with eGFR <30 mL/min/1.73m^2 and term newborns (7 to 28 days) with serum creatinine levels of ≥1 mg/dL. Hepatic impairment: Not recommended for patients with ALT levels ≥5 times the Upper Limit of Normal Range. **Contraindications:** Hypersensitivity to the active substances or to any of the excipients. **Warnings and Precautions:** Kidney and liver function tests should be performed daily before and during administration and the patient's condition should be carefully monitored. The patient's condition should be carefully monitored for infusion reactions and anaphylactic reactions. Administration should be immediately discontinued and appropriate measures should be taken if any abnormalities are observed. **Adverse reactions:** Clinically significant adverse reactions include acute renal impairment, hepatic impairment and hypersensitivity (Including infusion reactions and anaphylactic reactions: hypotension, hypertension, tachycardia, bradycardia, hypoxia, fever, dyspnea, wheezing, angioedema, rash, nausea, vomiting, diaphoresis, and shivering). Refer to package insert for other adverse reactions. **Drug interactions:** *In vitro* studies have shown that remdesivir is a substrate for CYP2C8, CYP2D6 and CYP3A4, as well as OATP1B1 and P-gp, and, in addition, is an inhibitor of CYP3A4, OATP1B1, OATP1B3, BSEP, MRP4 and NTCP. No clinical drug-drug interaction studies have been conducted. This drug should be administered with caution when co-administered with hydroxychloroquine sulfate or chloroquine.

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Advances in Medical Mycology in the Past Decade

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INTRODUCTION

The field of medical mycology has seen significant advancements in the past decade, owing to rapid development in phylogenetic profiling, diagnostic innovations, international collaboration in surveillance efforts, and expansion in the therapeutic armamentarium. Compared to their bacterial and viral counterparts, fungal infections derive relatively less attention from both the public health and research perspectives, yet they often pose formidable challenges, even to the experienced clinicians. In late 2022, the World Health Organization (WHO) published the first-ever fungal priority pathogens list, which systematically prioritises fungal pathogens based on their unmet research and development (R&D) needs and perceived public health importance[1]. This is a game changer that brings to the spotlight a group of fungal pathogens that are on the rise globally, capable of causing significant human morbidity and mortality, and with unmet gaps that are often neglected. Among the critical priority pathogen list are *Cryptococcus neoformans, Aspergillus fumigatus, Candida auris, and Candida albicans*, all of which are no strangers to many health professionals in Hong Kong. In this article, we wish to provide the readers with an update on the latest development in medical mycology that centred around four areas of significance – epidemiology, host susceptibility, antifungal resistance, and new treatment options, that are related to the critically important fungal pathogens.

EPIDEMIOLOGY OF *CANDIDA AURIS*

Antimicrobial resistance (AMR) has been gaining increasing attention from both researchers and policymakers. The emergence and spread of multidrug-resistant organisms (MDROs) threaten the effectiveness of existing antimicrobials, jeopardise patients' well-being and survival, and lead to a tremendous economic burden on healthcare systems. Among the fungal pathogens capable of causing invasive human infections, *C. auris* stands out for its multidrug resistance nature (almost half are resistant to at least two classes of antifungals), high outbreak potential, and high mortality associated with invasive infections, which have made *C. auris* the most significant fungal MDRO of global significance.

Since its first report in Japan in 2009, *C. auris* has spread to over 30 countries and on all continents except Antarctica. The study conducted by the US Centers for Disease Control and Prevention (CDC) in 2017 suggested that antifungal-resistant *C. auris* likely had emerged rather recently, independently, and almost simultaneously on different continents[2]. Based on phylogenetic analysis, *C. auris* isolates can be classified into different clades with strong geographic clustering. The results also raised concerns that *C. auris* may spread within the hospital settings, evidenced by the presence of nearly identical isolates in two hospitals in South Asia. This was also supported by the fact that most patients with *C. auris* infections had risk factors such as diabetes mellitus, immunosuppression, recent surgical procedures, and/or had indwelling catheters such as central venous catheters or urinary catheters[2].

The ability of *C. auris* to cause tenacious hospital outbreaks and clonal dissemination over large distances within countries and continents means that regions with high population density and international travel are at high risk of *C. auris* outbreaks[3]. The first imported case of *C. auris* in Hong Kong was reported in 2019. Since then, more than 800 cases have been recorded in Hong Kong, the great majority being asymptomatic carriers[4]. In view of this, the Hospital Authority has been adopting a risk-based strategy, focusing on proactive surveillance and stringent implementation of transmission-based precautions. Meanwhile, the antimicrobial stewardship programme (ASP) has been reinforced. Like other AMR pathogens, *C. auris* must be looked at from the One Health perspective, calling for collaborative efforts from various disciplines to better understand the epidemiology and to control their spread[5].

HOST SUSCEPTIBILITY TO CRYPTOCOCCOSIS

Cryptococcus spp. are opportunistic fungal pathogens that occupy unique environmental niches. The clinical manifestation of cryptococcosis ranges from asymptomatic pulmonary infection to disseminated disease with the predominant involvement of the central nervous system (CNS). The *C. neoformans/gattii* species complex is the predominant *Cryptococcus* species causing human infections. Earlier studies have demonstrated substantial differences in the ecology, epidemiology, and clinical associations between *C. neoformans* and *C. gattii*[6]. *C. gattii*, which accounts for 11-33% of cryptococcosis cases globally[7], was initially thought to be prevalent only in tropical and subtropical areas, but the subsequent spread to and outbreaks in British Columbia and the Pacific Northwest have clearly indicated an expansion in its ecological niche[8]. In contrast to *C. neoformans*, which is highly

abundant in avian guano, decomposing wood in tree hollows, especially the red gum species of eucalypt, were identified as the major environmental source of *C. gattii*[6], though later molecular epidemiological studies have identified a much larger environmental reservoir. From a clinical perspective, *C. gattii* is overall less likely to cause CNS disease (though vary by genotype), but when it does, there is a higher incidence of imaging abnormalities, neurological complications, mass lesions, and hydrocephalus[7]. *C. gattii* dominated in cryptococcosis in apparently immunocompetent hosts[6], and infection is generally associated with lower mortality than *C. neoformans*[7].

Cryptococcosis has become a major global health concern since the start of the human immunodeficiency virus (HIV) pandemic in the 1980s. A recent modelling study estimated that there were 152,000 cases of cryptococcal meningitis occurring among people with HIV per year, resulting in 112,000 cryptococcosis-related deaths[9]. Besides HIV infection, other risk factors include solid organ or haematopoietic stem cell transplantation, haematological malignancies, organ failure, sarcoidosis, immunosuppressants such as corticosteroid therapy, primary immunodeficiencies affecting the cell-mediated immunity, and autoantibodies against the granulocyte-macrophage colony-stimulating factor (GM-CSF). Anti-GM-CSF autoantibodies were first described in patients with autoimmune pulmonary alveolar proteinosis (PAP), in whom inhibition of the alveolar macrophage function leads to defective catabolism and subsequent accumulation of surfactant proteins in the pulmonary alveoli[10]. In recent years, anti-GM-CSF autoantibodies have been recognised to predispose previously healthy individuals to cryptococcosis. Most anti-GM-CSF autoantibody-positive cryptococcosis cases had CNS involvement, and the majority were caused by *C. gattii*[11]. Compared with patients who were autoantibody-negative, case patients had higher initial and peak levels of serum cryptococcal antigen and longer duration of antigenaemia[12]. In addition, the increasing use of biologics in haematology, rheumatology, and transplantation also puts increasing number of patients at risk of cryptococcosis. Increased incidence of infection has been observed in patients receiving tumour necrosis factor (TNF)-α antagonists, ibrutinib, JAK/STAT inhibitors, and fingolimod. With the wider availability of biologics, expanding from the treatment of autoimmune diseases and neoplasms to novel therapeutics for atopy and metabolic diseases, clinicians must be vigilant of the risks of infection as delayed diagnosis may lead to poorer outcomes.

ANTIFUNGAL RESISTANCE IN *ASPERGILLUS FUMIGATUS*

Based on a recent evidence-based global estimate, over two million people develop invasive aspergillosis (IA) per annum in the context of chronic obstructive pulmonary disease, intensive care, lung cancer, or haematological malignancies, with a crude annual mortality of 1.8 million (85.2%)[13]. The strikingly high mortality was largely attributed by undiagnosed and, therefore, untreated cases. In recent years, the incidence and mortality of IA in patients with haematological malignancies have decreased because of the implementation of antifungal prophylaxis and proactive diagnostic approaches. However, the development of azole resistance may set back the mortality reduction to the pre-azole era[14].

Azole resistance in clinical *A. fumigatus* isolates has emerged since the 1990s[15]. In addition to their isolation from treatment-experienced patients, azole-resistant strains of *A. fumigatus* have been recovered from patients who had not been exposed to medical azoles, indicating an environmental source of acquisition[16]. Agricultural antifungal exposure is a believed to have driven azole resistance in environmental aspergilli. This was first reported in the Netherlands where a signature mutation in the *CYP51A* gene (TR34/L98H), which encodes the drug target - cytochrome P450-dependent enzyme lanosterol 14α-demethylase, was found to be the dominant resistance mechanism that confers pan-azole resistance[17]. The TR34/L98H mutant and other mutations signalling environmental resistance have been identified in azole-resistant *A. fumigatus* isolates from different continents, suggesting a global dissemination of aspergilli with environmental azole resistance[18]. Since Asian countries accounted for a large proportion of the global fungicide consumption[19], fungicide-induced azole resistance cannot be overlooked. Chen *et al.* collected a total of 317 clinical and 144 environmental *A. fumigatus* isolates from 12 provinces in China between 2014 and 2015, and identified azole resistance in 2.5% of clinical and 1.4% of environmental isolates[20]. In a more recent study from Taiwan, azole resistance was present in 4% of *A. fumigatus* clinical isolates, which mainly emerged from the environment based on genetic charaterisation[21].

In a multi-centre retrospective cohort study conducted in the Netherlands, where the highest rate of azole resistance was found, IA caused by voriconazole-resistant isolates was associated with a 21% increase in mortality on day 42 and 25% increase on day 90[22]. Despite intensive resistance screening of culture isolates in these centres, escalation to appropriate antifungal therapy occurred after a median of 10 days, which was associated with excess mortality. In light of this evidence, the latest European guideline on the diagnosis and management of aspergillosis recommended that, in settings with environmental azole resistance rate of more than 10%, first-line therapy with alternative antifungal (liposomal amphotericin B) or combinational antifungals should be considered for invasive disease[23]. However, resistance data is lacking in many regions of the world, and due to the likely environmental source, there are no risk factors that can help identify patients at high risk of azole-resistant IA. To overcome the gap, surveillance studies should be further expanded with the inclusion of molecular characterisation, and diagnostic methods that allow early detection of resistance should be introduced to clinical laboratories serving populations at risk of azole-resistant IA.

NEW TREATMENT OPTIONS FOR INVASIVE CANDIDIASIS

About 1.6 million people develop *Candida* bloodstream infection or other forms of invasive candidiasis (IC) each year[13]. *Candida* spp. is part of the normal human

microbiome (including the gastrointestinal tract, female genital tract, and skin). Risk factors for IC include prolonged neutropenia, haematologic malignancies, immunosuppressive treatment, indwelling medical devices, total parenteral nutrition, and surgical interventions involving the gastrointestinal tract. More than 90% of IC are caused by five species of *Candida - C. albicans, C. parapsilosis, C. tropicalis, C. glabrata* (now named *Nakaseomyces glabratus*), and *C. krusei* (now named *Pichia kudriavzevii*). Though *C. albicans* is often the leading cause of IC, the *Candida* species distribution varies by the geographic location and patient population[24]. Echinocandins are the recommended first-line therapy for IC, through resistance in several species has been on the rise. A similar trend is also observed with azoles, the only available antifungal class for oral transition[25]. The mean total cost per patient with candidaemia and IC ranged from $48,487 to $157,574, with hospitalisation being the main cost driver[26]. Hence, there is an urgent need to develop and evaluate antifungal agents with improved pharmacokinetics and/or resistance profiles to overcome these obstacles.

There are several promising candidates. Rezafungin is a newer echinocandin with extended half-life allowing once-weekly dosing. Based on the pooled data from the phase 2 STRIVE and phase 3 ReSTORE trials on patients with candidaemia and IC, the all-cause mortality rates at day 30 were comparable for the rezafungin group and for the comparator caspofungin group[27]. In addition, rezafungin may be associated with more rapid mycological eradication, though further studies are needed to see whether this can reduce the emergence of resistance during treatment. Fosmanogepix, a first-in-class antifungal targeting the fungal enzyme Gwt1 with both intravenous and oral formulations, has also demonstrated clinical efficacy in the treatment for candidaemia in phase 2 trials, including in patients with candidaemia caused by *C. auris*[28,29]. Ibrexafungerp is a triterpenoid antifungal and the first non-azole agent approved by the US Food and Drug Administration (FDA) for the treatment of vulvovaginal candidiasis. The phase 3 MARIO trial evaluating the use of oral ibrexafungerp following intravenous echinocandin for patients with IC is ongoing. With the possibility of less frequent parenteral administration or even earlier switch to the oral agent with comparable efficacy, the length of hospital stay, and thus overall cost might be reduced.

CONCLUSION

In summary, knowledge in medical mycology is rapidly evolving. Fungal pathogens represent a complex entity and a perfect embodiment of the One Health concept linking humans, animals, plants, and the environment. Increased awareness and research priorities are needed to better understand the epidemiology, pathogenesis, host immunity, resistance, and therapeutics of fungal infections to better inform clinical management and health policy.

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A Must-visit Place - Hulunbuir Grassland

Mr Raymond LEUNG

AIBMS(UK), HTC(HK)

Mr Raymond LEUNG

From the moment I set foot on the Hulunbuir Prairie, I entered a completely different world. The vast grassland stretches to the sky, joining the blue sky and white clouds in the distance. The breeze blew in my face, carrying the fragrance of the grassland and the breath of freedom.

We used the most popular travel method in China: chartering a private vehicle with a driver, and the five of us set off on a beautiful day in July.

HULUNBUIR 呼倫貝爾草原

Hulunbuir, known as the world's largest prefecture-level city, has 126,000 km² of forests, 100,000 km² of grasslands, 20,000 km² of wetlands, more than 500 lakes and 3,000 rivers, forming the largest and most complete ecosystem in China. It is the only key development area for grassland tourism in the country. Chinese travel magazines have recommended Hulunbuir as one of the top ten must-visit destinations in China.

Not long after leaving the urban area of Hailar, we arrived at the hinterland. The Mozhgrad River meanders into the distance, wrapping around the mountains like a blue ribbon. The river is not wide; the widest point is only five or six meters outside of the flooding season. It is formed by the convergence of springs, and the areas it passes through are mostly uninhabited mountains and grasslands, so the water is exceptionally clean and completely unpolluted.

I imagined lying on the grassland, looking up at the sky, and then drifting off to peaceful sleep with herds of cattle, sheep, and horses nearby. What a stark contrast from the glitz of cities! I tried various photography techniques, but nothing truly captured the essence of what I saw and how I felt.

Fig. 2 (Personal collection)

We drove to our next destination, the Ergun Wetland. It is the largest wetland in Asia, and it is the passage for migrating birds when flying south. Once we reached the top of the hill, we saw a picture of lush forests, abundant grassland, vibrant-coloured flowers, dense springs, and crisscrossing rivers. This magnificent scenery has silently revealed itself through the cycle of seasons for hundreds of millions of years. Seeing this alone made our trip worthwhile.

There is a county road on the Hulunbuir Grassland, also known as the Sino-Russian Border Highway, stretching over 140 km. From north to southwest, the highway passes through Chinese cities such as Shiwei, Jiuka, Qika, Wuka, and Heishantou, with Russia on the other side.

Here are a few highlights from our trip:

GENHE CITY 根河市

Genhe is known as China's "Pole of Cold" for its subarctic climate and record-holding low temperature,

Fig. 1 (Personal collection)

-58°C. The annual average temperature in Genhe is -5.3°C, with an astonishing freezing period of over 210 days a year.

The name Genhe City came from the Root River that flows through the city. It has a long history and offers many cultural and natural attractions, including the Aoluguya Ewenki Deer Tribe, the last hunting tribe in China. This tourist attraction showcases the culture and lifestyle of the Ewenki people. It is the home to hundreds of reindeer, making it the only reindeer population in Asia.

Fig. 3 (Personal collection)

IRON POT STEW / "Tiěguo dùn" 鐵鍋炖

Iron Pot Stew is a traditional cooking method in Northeastern China and some rural areas. Chicken, fish, ribs or meat are slow-cooked in an iron pot on a wood-burning stove. This technique helps preserve the flavour and nutrients of the ingredients. The stew may look like a random mix of ingredients, I instantly fell in love with its taste upon trying it.

Fig. 4 (Personal collection)

SHIWEI PORT 室韋口岸

The port is located in Mengwu Shiwesumu and is connected to Olochi Port in Russia. The connecting cross-border bridge is one of the most visited tourist attractions in Mengwu Shiwesumu. The two cities (countries) are separated by a grassland where tourists can ride horses back and forth across the border, making this an extraordinary experience for riders.

Fig. 5 (Personal collection)

HULUN LAKE 呼倫湖

The lake, from which Hulunbuir gets a part of its name, is one of the largest freshwater lakes in Asia. It is hard to tell where the sky ends and the lake begins. I sat by the lake quietly, with the sun shining over me, a light breeze on my face, and brightly coloured blossoms surrounding me. I was in awe of nature's serenity and beauty.

Fig. 6 (Personal collection)

The yurts on the Hulunbuir Prairie are the traditional residences of the Mongolians. They maintain stable living conditions using ventilation and insulation. In the summer, the yurt is cool and pleasant; in the winter, it provides warmth and shelter from the harsh winter conditions of Inner Mongolia. We stayed in a modern yurt in the Heishantou Pastoral Area. Inside its traditional façade, the yurt provided all the comforts of a modern hotel room. The biggest reward was gazing at the stars shining brightly in the sky that night. I even took photos of the Milky Way. It was pure bliss.

Fig. 7 (Personal collection)

MANZHOULI CITY 滿州里

Manzhouli City is a famous Sino-Russian border city and a fusion of Chinese and Russian cultures. The main building in the famous Matryoshka Square is a large 30m-tall matryoshka doll housing a Russian restaurant and a performance hall.

The matryoshka dolls in the Square represented China, Russia, Mongolia, and other regions around the world. Surrounding the music fountain are twelve zodiac signs representing Chinese culture and twelve zodiac signs of Western astrology. At night, the square lights up like a fairytale world.

Fig. 8 (Personal collection)

Inner Mongolia, particularly the Hulunbuir Prairie, is a charming destination with exceptional scenery. There, I witnessed the power and tranquillity of nature and experienced the enthusiasm and perseverance of the Mongolians. This trip gave me a deeper understanding of grassland culture and left me with indelible memories. I hope one day I can set foot on this beautiful grassland again to appreciate nature and feel the power of life once more.

Certificate Course for General Practitioners, Nurses and Health Care Providers

• Course No. C410 • CME/CNE Course

Certificate Course in

Cardiology 2024

(Video Lectures)

Jointly organised by

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Objectives:

This course is designed for General Practitioners, Nurses and Health Care Providers who are interested in Cardiology. A series of lectures covering up-to-date cardiology knowledge and skill in day-to-day clinical practice.

Date	Topics	Speakers
25 Jul 2024	1. Exercise Recommendation in Patients with Cardiovascular Diseases	Dr. Chan Kit, Jacky Specialist in Cardiology
	2. ECG Interpretation in Athletes from International Consensus to Case Studies	
1 Aug 2024	1. Clinical Approach to Common Cardiovascular Symptoms and Overview of Cardiac Investigations	Prof. Fung, Erik Yee Mun George Assistant Professor The Chinese University of Hong Kong
	2. Update on Medications for Heart Failure, Dyslipidaemia and Obesity from the Cardiovascular Perspective	
8 Aug 2024	Management Algorithm for Chronic High Blood Pressure	Dr. Ko Kwok Chun, Jason Specialist in Cardiology
15 Aug 2024	Management of Common Cardiac Arrhythmias and ECG Interpretation	Dr. Chu Man Wah, Amy Associate Consultant United Christian Hospital
	ECG Cases Sharing	Dr. Lo Ka Yip, David Specialist in Cardiology
22 Aug 2024	Noninvasive Imaging of Ischemic Heart Disease	Dr. Ching Shing Associate Consultant United Christian Hospital
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	Screening of Coronary Artery Disease in Asymptomatic Populations: Pros and Cons	Dr Lo Ka Yip, David Specialist in Cardiology

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Duration of Session : 1.5 hours (6 sessions)
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Dermatology Quiz

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Specialist in Dermatology & Venereology

Dr Lai-yin CHONG

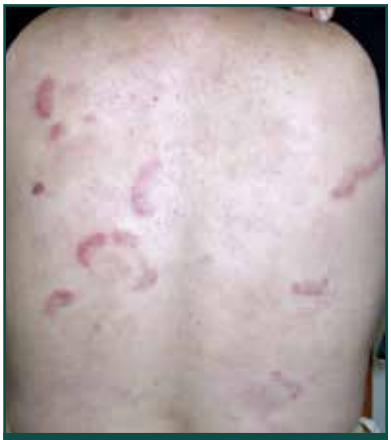

Fig. 1: Asymptomatic polycyclic rash at the back.

This 40-year-old female had developed these asymptomatic non-scaling polycyclic erythematous rash over her back (Fig.1) for three months. Other parts of the body were spared. She did not have any systemic symptoms except mild arthralgia over her fingers. She had been treated as tinea corporis with the antifungal agent without effect. Her past health was good. There was no significant drug history.

Questions

1. What are your differential diagnoses?
2. How would you establish the diagnosis?
3. How do you treat this patient?
4. What is the prognosis of this disease?

(See P.37 for answers)

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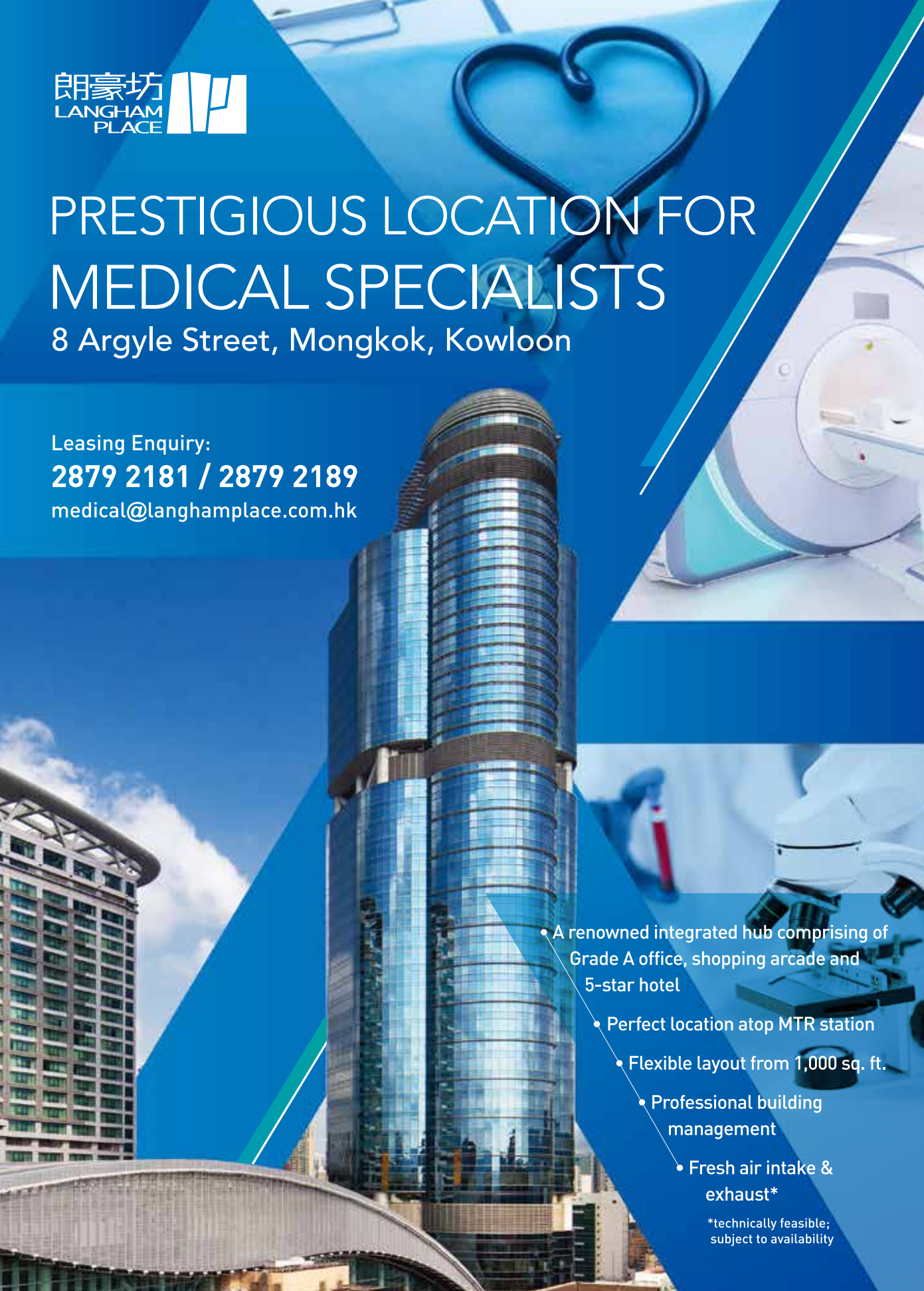

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- Perfect location atop MTR station
- Flexible layout from 1,000 sq. ft.
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*technically feasible; subject to availability

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	1	★In-person / Zoom HKMA-HKSH CME Programme 2023-2024 Topic: PET Scan in Dementia 2	★Certificate Course in Allergy 2024 (Video Lectures) 3	★Certificate Course on Mental Health 2024 (Video Lectures) 4	★Hong Kong Primary Care Conference 2024 - "Family Medicine in the Community: Strengthening Connections" 5	★In-person HKMA Medico-legal Conference 2024 ★Hong Kong Primary Care Conference 2024 - "Family Medicine in the Community: Strengthening Connections" 6
★Hong Kong Primary Care Conference 2024 - "Family Medicine in the Community: Strengthening Connections" 7	★Zoom Latest 2023 ESH Guideline of Arterial Hypertension Management and Share Clinical Practice of Beta-blockers 8	9	★In-person / Zoom HKMA-CUHK Medical Centre CME Programme 2024 Men's Health - Topic: Update On Anal fistula ★Certificate Course in Allergy 2024 (Video Lectures) ★The Hong Kong Neurosurgical Society Monthly Academic Meeting - To be confirmed 10	★Zoom Understanding Our "Second Brain": Gut-Brain Communication and The Role of The Gut Microbiome ★Certificate Course on Mental Health 2024 (Video Lectures) 11	★Zoom Antiplatelet Therapy and Bleeding Risk: Balancing Efficacy and Safety in Primary Care 12	13
14	15	★In-person / Zoom HKMA-GHK CME Programme 2024 Topic: TBC 16	★Zoom Recent Advancement of Gut Microbiome Research in Atopic Eczema Management ★Certificate Course in Allergy 2024 (Video Lectures) 17	★FMSHK Foundation Meeting & Executive Committee Meeting 18	★Zoom Topic:Management of Urinary Stone, Diagnosis and Management Approach in Primary Care 19	20
21	22	23	★In-person The HKMA CME Lecture for District Health Network CME Programme in Physical Attendance Mode Topic: Early Glycemic Control in the Management of Type 2 Diabetes: Optimizing Patient Outcomes Through Timely Intervention ★Certificate Course in Allergy 2024 (Video Lectures) 24	★Certificate Course in Cardiology 2024 (Video Lectures) 25	★Zoom Current management of Allergic Rhinitis and Urticaria 26	27
28	★Zoom Child and Adolescent Mental Health: Latest Research Findings and its Relations with Microbiota-Gut-Brain Axis 29	30	★In-person The HKMA CME Lecture for District Health Network CME Programme in Physical Attendance Mode Topic: RSV Prevention on Elderly with The Latest Recombinant Adjuvanted Vaccine (To-be-confirmed) ★Certificate Course in Allergy 2024 (Video Lectures) 31			

Date / Time	Function	Enquiry / Remarks
2 TUE 1:00 PM	**In-person / Zoom** **HKMA-HKSH CME Programme 2023-2024** **Topic: PET Scan in Dementia** Organiser: The Hong Kong Medical Association and The Hong Kong Sanatorium & Hospital Speaker: Dr LEUNG Yim-lung Eric Venue: The HKMA Wanchai Premises, 5/F, Duke of Windsor Social Service Building, 15 Hennessy Road, Wanchai, Hong Kong	HKMA CME Dept. Te: 3108 2507 1 CME Point
3 WED 7:00 PM	**Certificate Course in Allergy 2024 (Video Lectures)** Organiser: The Federation of Medical Societies of Hong Kong Speaker: Dr Alson CHAN	Ms ToTo CHAN Tel: 2527 8898
4 THU 7:00 PM	**Certificate Course on Mental Health 2024 (Video Lectures)** Organiser: The Federation of Medical Societies of Hong Kong Speaker: Dr Rommel HUNG	Ms ToTo CHAN Tel: 2527 8898
5 FRI 7:00 PM	**Hong Kong Primary Care Conference 2024 - "Family Medicine in the Community: Strengthening Connections"** Organiser: The Hong Kong College of Family Physicians Speaker: Dr David CK LUK Venue: HKAM Jockey Club Building, 99 Wong Chuk Hang Road, Aberdeen, Hong Kong	Ms Ally Chan/ Ms Nana Choy (Registration) Ms Carol Pang (Other information) Te: 2871 8899 Fax: 2866 0616
6 SAT 12:00 PM	**In-person** **HKMA Medico-legal Conference 2024** **Topics:** **1) MCHK, PIC and Inquiry Panel** **2) Gross Negligence Manslaughter in The Medical Context** **3) Managing Patient Complaints – An Overview** **4) Effective Record Keeping as A Way to Manage Patient Complaints** **5) Managing Patient's Complaints Effectively, Giving an Apology** **6) The Impact of Patient Complaints on Medical Practitioners** Organiser: The Hong Kong Medical Association Speaker: Dr CHENG, Chi-man, Ms Vinci LAM SC, Ms Christine TSANG, Mr Woody CHANG, Dr David KAN Kam-fai and Dr Anthony Shu-yan, FUNG Venue: The HKMA Central Premises, Dr. Li Shu Pui Professional Education Centre, 2/F, Chinese Club Building, 21-22 Connaught Road Central, Hong Kong	HKMA CME Dept. Te: 2527 8452 3 CME Point
1: 30 PM	**Hong Kong Primary Care Conference 2024 - "Family Medicine in the Community: Strengthening Connections"** Organiser: The Hong Kong College of Family Physicians Speaker: Ms Josephine YC LEE, Dr Pamela PY LEUNG, Prof Helen FK CHIU, Dr SHUM Chun-keung, Dr Johnny KS LAU, Dr Patrick SK CHONG, Prof Martin CS WONG, Prof Joshua WK HO, Dr YANG Jian, Dr Irene WK KAM & Ms Annette KK LAM Venue: HKAM Jockey Club Building, 99 Wong Chuk Hang Road, Aberdeen, Hong Kong	Ms Ally Chan/ Ms Nana Choy (Registration) Ms Carol Pang (Other information) Te: 2871 8899 Fax: 2866 0616
7 SUN 9:00 AM	**Hong Kong Primary Care Conference 2024 - "Family Medicine in the Community: Strengthening Connections"** Organiser: The Hong Kong College of Family Physicians Speaker: Dr LOK Chi-wing, Ms YAN Ka-wai, Dr NG Man-yuk, Dr Bosco HM MA, Ms CHIANG Sau-chu, Dr Eric KP LEE, Dr Anastasia S MIHAILIDOU, Prof DONG Dong, Dr CHAN Kwok-wai & Dr LAI Wai-wah Venue: HKAM Jockey Club Building, 99 Wong Chuk Hang Road, Aberdeen, Hong Kong	Ms Ally Chan/ Ms Nana Choy (Registration) Ms Carol Pang (Other information) Te: 2871 8899 Fax: 2866 0616
8 MON 2:00 PM	**Zoom** **Latest 2023 ESH Guideline of Arterial Hypertension Management and Share Clinical Practice of Beta-blockers** Organiser: The Hong Kong Medical Association Speaker: Prof MANOLIS, Athanasios J.	HKMA CME Dept. Te: 3108 2507 1 CME Point
10 WED 2:00 PM	**In-person / Zoom** **HKMA-CUHK Medical Centre CME Programme 2024** **Men's Health - Topic: Update On Anal fistula** Organiser: The Hong Kong Medical Association The CUHK-Medical Centre Speaker: Dr NGO Kwok-yu Venue: The HKMA Wanchai Premises, 5/F, Duke of Windsor Social Service Building, 15 Hennessy Road, Wanchai, Hong Kong	HKMA CME Dept. Te: 3108 2507 1 CME Point
7:00 PM	**Certificate Course in Allergy 2024 (Video Lectures)** Organiser: The Federation of Medical Societies of Hong Kong Speaker: Dr Agnes LEUNG	Ms ToTo CHAN Tel: 2527 8898
	The Hong Kong Neurosurgical Society Monthly Academic Meeting - To be confirmed Organiser: Hong Kong Neurosurgical Society Speaker: Dr Ben LUK Kin-long Venue: Seminar Room, G/F, Block A, Queen Elizabeth Hospital; or via Zoom meeting	Dr Jason Chow Tel: 2595 6456 Fax: 2965 4061 1.5 CME points
11 THU 2:00 PM	**Zoom** **Understanding Our "Second Brain": Gut-Brain Communication and The Role of The Gut Microbiome** Organiser: The Hong Kong Medical Association; CUHK-Medical Centre Speaker: Prof. NG, Siew Chien	HKMA CME Dept. Tel: 3108 2507 1 CME Point
7:00 PM	**Certificate Course on Mental Health 2024 (Video Lectures)** Organiser: The Federation of Medical Societies of Hong Kong Speaker: Dr Calvin CHENG	Ms ToTo CHAN Tel: 2527 8898
12 FRI 2:00 PM	**Zoom** **Antiplatelet Therapy and Bleeding Risk: Balancing Efficacy and Safety in Primary Care** Organiser: The Hong Kong Medical Association Speaker: Dr FU Chiu-lai	HKMA CME Dept. Tel: 3108 2507 1 CME Point
16 TUE 2:00 PM	**In-person / Zoom** **HKMA-GHK CME Programme 2024** **Topic: TBC** Organiser: The Hong Kong Medical Association and Gleneagles Hong Kong Hospital Speaker: Dr Vince LAU Wing-hang Venue: The HKMA Wanchai Premises, 5/F, Duke of Windsor Social Service Building, 15 Hennessy Road, Wanchai, Hong Kong	HKMA CME Dept. Tel: 3108 2507 1 CME Point
17 WED 2:00 PM	**Zoom** **Recent Advancement of Gut Microbiome Research in Atopic Eczema Management** Organiser: The Hong Kong Medical Association Speaker: Prof Martin WONG Chi-sang	HKMA CME Dept. Tel: 3108 2507 1 CME Point

Date / Time	Function	Enquiry / Remarks
17 WED 7:00 PM	**Certificate Course in Allergy 2024 (Video Lectures)** Organiser: The Federation of Medical Societies of Hong Kong Speaker: Dr Marco HO	Ms ToTo CHAN Tel: 2527 8898
18 THU 7:00 PM	**FMSHK Foundation Meeting & Executive Committee Meeting** Organiser: The Federation of Medical Societies of Hong Kong; Venue: Council Chamber, 4/F, Duke of Windsor Social Service Building, 15 Hennessy Road, Wanchai, Hong Kong	Ms Nancy CHAN Tel: 2527 8898
19 FRI 2:00 PM	**Zoom** **Topic:Management of Urinary Stone, Diagnosis and Management Approach in Primary Care** Organiser: The Hong Kong Medical Association Speaker: Dr LO Ting-kit	HKMA CME Dept. Tel: 3108 2507 2 CME Point
24 WED 2:00 PM	**In-person** **The HKMA CME Lecture for District Health Network CME Programme in Physical Attendance Mode** **Topic: Early Glycemic Control in the Management of Type 2 Diabetes: Optimizing Patient Outcomes Through Timely Intervention** Organiser: The HKMA District Health Network Speaker: Dr WONG Cheuk-lik Venue: The Cityview, Crystal Ballroom A&B, 2/F, 23 Waterloo Road, Kowloon, Hong Kong	Mr Peter HO Tel: 3108 2514 1 CME Point
7:00 PM	**Certificate Course in Allergy 2024 (Video Lectures)** Organiser: The Federation of Medical Societies of Hong Kong Speaker: Dr Gilbert CHUA	Ms ToTo CHAN Tel: 2527 8898
25 THU 7:00 PM	**Certificate Course in Cardiology 2024 (Video Lectures)** Organiser: The Federation of Medical Societies of Hong Kong Speaker: Dr CHAN Kit, Jacky	Ms ToTo CHAN Tel: 2527 8898
26 FRI 2:00 PM	**Zoom** **Current management of Allergic Rhinitis and Urticaria** Organiser: The Hong Kong Medical Association Speaker: Dr CHONG Chun-yin	HKMA CME Dept. Tel: 3108 2507 1 CME Point
29 MON 2:00 PM	**Zoom** **Child and Adolescent Mental Health: Latest Research Findings and its Relations with Microbiota-Gut-Brain Axis** Organiser: The Hong Kong Medical Association Speaker: Dr Oscar WONG Wing-ho	HKMA CME Dept. Tel: 3108 2507 1 CME Point
31 WED 2:00 PM	**In-person** **The HKMA CME Lecture for District Health Network CME Programme in Physical Attendance Mode** **Topic: RSV Prevention on Elderly with The Latest Recombinant Adjuvanted Vaccine (To-be-confirmed)** Organiser: The HKMA District Health Network Speaker: To-be-confirmed Venue: Lei Garden Restaurant, Shop 1130 - 1143, 1/F, Phase 1, Yoho Mall	Mr Peter HO Tel: 3108 2514 1 CME Point
7:00 PM	**Certificate Course in Allergy 2024 (Video Lectures)** Organiser: The Federation of Medical Societies of Hong Kong Speaker: Ms Sabrina MOK	Ms ToTo CHAN Tel: 2527 8898

Answers to Dermatology Quiz

Answers:

1. The differential diagnoses include Subacute cutaneous lupus erythematosus (SCLE), Erythema annulare centrifugum, Mycosis fungoides, Polymorphic light eruption and Sarcoidosis. Other common differential diagnoses, such as Tinea corporis and Annular psoriasis are unlikely due to absence of scaling, unless they are partially treated with topical steroid.

   Drug aetiology should be excluded as it may exacerbate or induce SCLE up to 20-30% cases. The well-documented culprits include hydrochlorothiazide, terbinafine, TNF antagonists, antiepileptics, and proton pump inhibitors.

2. In this patient, laboratory tests such as antinuclear antibody (ANA) (1:320 titre), anti-dsDNA (Negative), anti-Ro and anti-La (Both were strongly positive) had been done. Skin scraping for fungal study was negative. Complete blood cell, C-reactive protein, Renal function test and Complement level (C3 and C4) were all normal. Skin biopsy was consistent of the diagnosis of lupus erythematosus. The clinical features, together with these investigations, confirmed the diagnosis of SCLE.

   Subacute cutaneous lupus erythematosus (SCLE) is an autoimmune disease with female predominance (female to male: 4:1). It is a non-scarring, non–atrophy photosensitive dermatosis, that commonly develops in sun-exposed areas such as the neck, upper trunk, shoulders, and extensor of arms. Unlike systemic lupus erythematosus (SLE) and discoid lupus erythematosus (DLE), the face and scalp in SCLE are usually spared. If the face is involved, it is often the lateral face with sparing of central and malar area. The lesions can present as two forms: either papulo-squamous /psoriasiform or annular / polycyclic lesions (Fig.1). In contrast to DLE, they heal without scarring or atrophy, but may result in prominent residual dyspigmentation. Accompanying symmetrical polyarthralgia at small joints is common.

   Serological tests of autoantibodies are mandatory in establishing the diagnosis of SCLE. Anti-Ro (SS-A) are present in a high percentage (80-90%), while anti-La (SS-B) are often present in a lower percentage (< 50%). ANA was found in 70% of patients, while anti-dsDNA only present in 5% of patients.

   Skin biopsy shows characteristic histopathological features, together with a positive direct immunofluorescent study in 60% of cases.

3. Sun protection is crucial. In most patients, potent topical steroids and antimalarial agents are the mainstay of treatment. In drug-induced SCLE, withdrawal of the culprit drug in conjunction with medical therapy is necessary. In severe cases, other systemic immunosuppressants may be needed.

4. Subacute cutaneous lupus erythematosus (SCLE) without SLE has a favourable prognosis. Some patients may even end up in spontaneous remission, though most of them run a wax-and-wane course. Up to 10% of patients may have SLE with severe systemic complications such as central nervous system involvement, vasculitis, or nephritis. SCLE is also the most common subtype of cutaneous lupus erythematosus associated with Sjögren syndrome. It is therefore important to have regular follow-up of SCLE.

Dr Lai-yin CHONG
MBBS(HK), FRCP(Lond, Edin, Glasg), FHKCP, FHKAM(Med)
Specialist in Dermatology & Venereology

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Pneumococcal 15-valent
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TARGET OUR BURDEN

CHP SCVPD recommended PCV15 over other PCVs[1*]

Included in Hong Kong **Vaccination Subsidy Scheme**[2]

Serotype 3 IPD rebounded in 2023 at **44%**[3]

* under the Hong Kong Childhood Immunisation Programme and the Government Pneumococcal Vaccination Programme

INFANTS

According to a Phase 3 clinical trial in healthy infants aged 42 to 90 days (n=1,720),

When compared to PCV13, Vaxneuvance® induced

~73%

increases IgG GMC against SEROTYPE 3.

GMC Ratio = 1.73 (95% CI: 1.61-1.87)

Superiority criteria: lower bound of the 2-sided 95% CI for the IgG GMC ratio (V114/PCV13) >1.2

ADULTS

When compared to PCV13, Vaxneuvance® induced

~60%

increases IgG GMC against SEROTYPE 3.

Vaxneuvance® (PCV15) was noninferior[4a,5a] to PCV13 for all 13 shared serotype[4,5]

Vaxneuvance® (PCV15) was **SUPERIOR**[4b,5c] to PCV13 for **unique serotypes 22F** and **33F**[4,5]

Gov Press Release

SCVPD recommendation

Safety Result: Adults: The majority of participants experienced at least 1 adverse event (67.5% after V114 and 58.2% after PCV13). The most frequently reported AEs (>5% of participants in either group) were the solicited events of injection-site pain, injection-site erythema, injection-site swelling, arthralgia, fatigue, headache, and myalgia.[4]

Children: The majority of participants experienced at least 1 adverse event (93.8% after V114 and 92.4% after PCV13). The overall proportions of participants with injection site, systemic, vaccine-related, and serious AEs were generally comparable between treatment groups. The most common AEs were those solicited in the trial, with the 3 most frequently reported AEs being irritability, somnolence, and injection-site pain.[5]

Abbreviations: Adults: Adults ≥ 50 years old; CHP: Centre for Health Protection; CI: confidence interval; GMC: geometric mean concentration; GMT: geometric mean titer; Infants: Healthy infants; IPD: invasive pneumococcal disease; OPA: opsonophagocytic activity; PCV13: 13-valent pneumococcal conjugate vaccine; PCV15: 15-valent pneumococcal conjugate vaccine; SCVPD: Scientific Committee on Vaccine Preventable Diseases

Vaxneuvance Selected Safety Information:

Before prescribing, please consult the full prescribing information.

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