Original Article

Journal of Microbiology and Related Research
Volume 10 Number 1, January - June 2024
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.10124.3

Blood Metagenomic Sequence Analysis for Evaluation of Chronic Systemic Infections

Mohammed Shakeel[1], Ayla Sanjay[2], Zainab Kausar[3], Vadlamudi Nikhil[4], Chand Pasha[5]

How to cite this article:

Mohammed Shakeel, Ayla Sanjay, Zainab Kausar *et al.* Blood Metagenomic Sequence Analysis for Evaluation of Chronic Systemic Infections. J Microbiol Relat Res. 2024;10(1):21–27.

Abstract

Blood systemic infections (BSIs) are major threat in hemodialysis patients. BSIs are diagnosed by blood culturing for bacteria and serology for virus infections. These two methods are time taking and expensive and not covering all BSI microbes. An attempt is made to compare microbial culturing and viral screening with serology by ELISA with blood metagenomic sequencing and sequence analysis. In blood agar media culturing 7 microbes (6 bacterial and 1 fungus), in ELISA screening 0 viruses and in metagenomic sequence analysis 24 microbes (19 bacteria and 1 fungus, 3 viruses, 1 mycoplasma) were detected. Unculturable bacteria are also detected by metagenomic sequence analysis. Hence metagenomic sequence analysis can be best as method for effective detection of BSI's.

Keywords: Meta genomic DNA sequence analysis; Systemic infections; Dialysis; Uncultured Bacteria; Blood infection.

Author Affiliation: [1]Research Student, Department of Microbiology, Mumtaz degree and PG College, Malakpet, Affiliated to Osmania University, Hyderabad, Telangana, [2-4]Research Student, Department of Microbiology, Nizam College, Osmania University Hyderabad 500001, Telangana, [5]Senior Assistant Professor & Head, Department of Microbiology, Nizam College, Osmania University Hyderabad 500001, Telangana, India.

Corresponding Author: Chand Pasha, Senior Assistant Professor & Head, Department of Microbiology, Nizam College, Osmania University Hyderabad 500001, Telangana, India.

E-mail: cpasha21@gmail.com

Received on: 21.08.2024

Accepted on: 08.10.2024

INTRODUCTION

Chronic systemic infections or Blood Stream Infections are life-threatening and are responsible for up to 20% of deaths worldwide (Rudd *et al.* 2020). They affect the entire body rather than being localized to one specific area. These infections can persist for months or even years, due to the causative agents such as bacteria, viruses, fungus, or parasites. Prompt and timely diagnosis is essential for effective and timely treatment. Clinical laboratories are crucial in controlling systemic infectious diseases, typically conducting microscopic examinations, blood cultures, blood biomarkers and NAA assay (Yu *et al.* 2024). The limited detecting capabilities and sensitivity of these techniques contribute to loss in identifying pathogens in significant cases (Forbes JD *et al.* 2017).

BSIs can originate from various sources, including surgical incisions, catheter-related, lung, blood coming in contact with equipment and atmosphere like dialysis and peritoneal infections. Blood culturing is primary technique for detecting and identifying bacteria and fungus in sepsis, helping to optimize antimicrobial treatment and assess effectiveness (Garcia RA *et al.* 2015). However, it typically takes twenty-four hours and gives false results as positive and negative. A Current culture methods identify only 30–50% cases within the first 2 days, with some species taking up to five days (Gupta *et al.* 2016) and has lower yield detecting only culturable microorganisms, missing nonculturable bacteria.

In recent times, metagenomic sequencing have gained significant focus on detection of pathogens. Metagenomic shotgun sequencing is culture independent technique which analyses genetic material in sample and has shown promising results in clinical practice by detecting microorganisms undetected by conventional tests and by identifying previously unrecognized pathogens (Vijayvargiya P *et al.* 2019). Since the first use of metagenomic sequence for diagnosing a patient in 2014 suffering from an infection (Wilson MR *et al.* 2014), this innovative method has progressively accepted and incorporated in the clinical practice. The major steps of metagenomic sequence involve preprocessing of sample, extracting nucleic acid, preparation of library, sequencing and bioinformatics evaluation (Li N *et al.* 2021). In patients who are hospitalized and assumed of having sepsis, metagenomic sequence outperformed blood culture, especially in those with moderate symptoms, prior to use of antibiotics and early infection (Zuo YH *et al.* 2023). However, metagenomic sequence has drawbacks (Lamy *et al.* 2020) including complex sample preparation, interference from host DNA and cumbersome sequencing procedures. It is one of the promising methods which detects pathogens from complex samples with a broad spectrum, high sensitivity and minimum time, making it effective to identify rare, unknown and abnormal causes. The current study aims to analyse and for comparing the results of metagenomic sequence for detecting organisms not identified by conventional blood culture methods in chronic systemic infections of dialysis patients.

MATERIALS AND METHODS

Patient and sampling

The male patient of age 40 with fistula for the Dialysis located at Dialysis center, Vikarabad. Patient weighted 66 kg before dialysis and after dialysis 62kg. No abnormalities in vitals like BP, respiration rate, pulse and temperature were noted and diagnosed with diabetes mellitus positive recently. The study was permitted by the institutional ethics committee, written consent was taken from patient for clinical research. During dialysis early 10ml Blood was collected as sample.

Bacterial isolation and growth conditions

Samples

Cultured the blood sample on the blood agar by the spread plate technique and further incubated at 37^{o}C for 24 hours.

Characterization

Morphology: The colony and cellular morphology after gram staining was observed under microscope and further biochemical test were performed to identify the specific organism.

Biochemical test: The following biochemical tests were performed to identify the organisms.

Catalase test, Oxidase test, Coagulase test, IMViC Tests, Bile esculin, Germ tube.

Catalase test: Used to detect catalase enzyme presence in bacteria. Positive result is identified by producing visible oxygen by breaking down hydrogen peroxide.

Oxidase test: Used to detect cytochrome c oxidase presence, It is tested by reacting on N,N,N,N-tetramethyl-p-phenylenediamine dihydrochloride substrate.

Coagulase test: Used to identify organisms based on the ability to produce coagulase, the bacterial suspensions are mixed in plasma to check for coagulase activity of coagulating plasma.

Indole test: Used to test the ability of bacteria to produce indole from tryptophan aminoacid.

Methyl Red test: Detects the production of acids from glucose fermentation.

Voges-Proskauer Test: Used to detect the presence of acetoin, produced by fermentation of glucose in bacterial species.

Citrate Utilization test: Used to test the ability of the organism to use citrate as whole carbon source.

Bile esculin: The test used to identify bacteria based on their ability to hydrolzye esculin in presence of bile.

Germ tube: Test used to differentiate between candida species.

Antibiotic Sensitivity assay

The Antibiotic susceptibility test was carried out on Mueller Hinton agar plates after spreading 8 different bacterial cultures and placing HIMEDIA antibiotic discs. Ampicillin, Cephalosporin, Tetracycline, Carbapenem, Monobactam, Sulfonamide, Nitroimidazole, Macrolide, Chloramphenicol, Rifamycin, Fluoroquinolone, Ceftazidime, Elfamycin, Cefepime, Norfloxacin, Levofloxacin, Methicillin, Streptomycin, Augmentin, Kanamycin, Pencillin – G, Gentamycin and Vancomycin antibiotic discs were used. Augmentin antibiotic solution was prepared by adding 20mg Amoxicillin in 10mg potassium clavulanate, the Whatman discs were suspended in solution and dried. Plates were incubated overnight at 37°C and zone of inhibition were measured in mm.

Viral screening by ELISA

Blood sample for HIV, HBV and HCV viruses were screened by ELISA (Ma *et al.,* 2011).

Isolation of DNA

The collected blood sample of the dialysis patient was undergone 2000 rpm centrifugation for 5 minutes and the supernatant was collected in the sterile Eppendorf. Supernatant (Containing microbial cells and cell free DNA, free of all human cells) was used for genomic DNA isolation using QIAamp BIOstic (QIAGEN Germany) Genomic DNA isolation kits as per manufacturer's instructions.

Library preparation and metagenomic sequencing

Library preparation was carried out by using Nextera XT DNA Library preparation kit (Illumina, USA). DNA was prepared, partial cleaved and tagged using the pUC18 plasmid within the Nextera XT Kit (Moghnia *et al.,* 2015). Separate adapters were assigned to each sample for labelling purposes. A 12-cycle PCR reaction was conducted to amplify DNA fragments, incorporating pUC18 primers and indices for dual-indexed sequencing of pooled libraries. Subsequent to sample normalization, pooling was carried out, followed by 300-base paired-end reads sequencing on the Illumina (Novaseq 6000), 150PE instrument (Moghnia *et al.,* 2015). All steps, from preparation to sequencing, adhered strictly to the manufacturer's instructions.

Organism Identification

The obtained nucleotide sequences from metagenomic sequencing were identified in the NCBI portal by running BLASTn (Chen Y *et al.,* 2015).

Statistical Analysis

Experiments were repeated thrice in triplicate (n=9) and value with standard deviation is presented.

RESULTS

The bacterial cultures isolated from the dialysis patient blood samples were identified by colony morphology, Microscopy and biochemical tests as *Pseudomonas aeruginosa, Salmonella enterica serovar, Staphylococcus aureus, Bacillus paranthracis, Escherichia coli, Streptococcus pyogenes* and *Candida dubliniensis* (Table 1)

Table 1: Morphological, Microscopic and Biochemical results of isolated organisms

S.No	Organism	Colony morphology	Microscopy	Biochemical Tests
1.	*Pseudomonas aeruginosa*	Round with a fluorescent greenish colour	Gram-negative	Oxidase positive, Catalase positive
2.	*Salmonella enterica serovar typhi*	Rod-shaped enterobacterium	Gram- negative	Blackening in (H_2S production) TSI agar
3.	*Staphylococcus aureus*	Circular, smooth, convex	Gram-positive	Beta-hemolysis positive
4.	*Bacillus paranthracis*	Circular colonies	Gram- positive	Voges-Proskauer Test positive,
5.	*Escherichia coli*	Rough or a smooth	Gram- negative	IMViC ++--
6.	*Streptococcus pyogenes*	Dome-shaped, smooth	Gram- positive	Bile esculin-negative
7.	*Candida dubliniensis*	Dark green	Gram-positive	germ tube-positive

Antibiotic susceptibility assay:

All seven cultured microbes were found to be resistant to majority of antibiotics tested.

Table 2: Antibiotic susceptibility assay of isolated organisms against various antibiotics and zone of inhibitions (mm)

Antibiotics	Pseudomonas aeruginosa (mm)	Salmonella enterica serovar typhi (mm)	Staphylococcus aureus (mm)	Bacillus paranthracis (mm)	Escherichia coli (mm)	Streptococcus pyogenes (mm)	Candida dubliniensis (mm)
Ampicillin	06±0.02	04±0.02	15±0.45	13±0.43	06±0.15	16±0.5	18±0.6
Cephalosporin	07±0.16	11±0.37	27±0.84	05±0.09	30±0.8	32±0.9	16±0.6
Macrolide	11±0.45	14±0.2	16±0.8	19±0.7	17±0.5	31±0.85	15±0.57
Monobactam	11±0.3	13±0.4	11±0.47	16±0.6	18±0.7	09±0.45	04±0.03
Carbapenem	27±0.9	14±0.53	12±.0.44	13±0.47	26±0.98	11±0.47	14±0.43
Sulfonamide	05±0.119	04±0.14	11±0.36	04±0.14	02±0.74	13±0.45	15±0.48
Nitroimidazole	16±0.04	18±0.52	11±0.47	13±0.48	15±.0.44	17±0.47	21±0.89
Rifamycin	14±0.11	11±0.21	04±0.07	16±0.5	12±0.4	14±0.3	12±0.4
Fluoroquinolone	26±0.86	06±0.26	18±0.65	12±0.45	06±0.15	14±0.55	13±0.54
Elfamycin	14±0.44	16±0.35	11±0.32	19±0.77	15±0.55	19±0.41	16±0.32
Ceftazidime	28±0.61	05±0.04	14±0.44	18±0.44	16±0.44	14±0.32	15±0.52
Cefepime	18±0.56	14±0.32	04±0.08	17±0.54	06±0.15	20±0.08	19±0.04
Norfloxacin	10±0.32	12±0.42	21±0.52	21±0.31	06±0.15	19±0.14	14±0.65
Levofloxacin	15±0.15	16±0.32	18±0.21	11±0.41	16±0.65	20±0.98	18±0.77
Chloramphenicol	13±0.45	28±0.98	14±0.5	29±0.74	16±0.75	17±0.45	18±0.45
Tetracycline	08±0.12	03±0.065	06±0.22	31±1.12	07±0.14	03±0.06	26±0.885
Streptomycin	14±0.32	04±0.12	15±0.55	17±0.52	15±0.42	19±0.74	12±0.22
Augmentin (Amoxicillin & Potassium Clavulanate)	12±0.35	18±0.52	15±0.62	21±0.74	19±0.69	23±0.52	15±0.23
Kanamycin	15±0.45	15±0.32	14±0.21	16±0.65	19±0.72	11±0.12	09±0.98
Pencillin – G	11±0.32	16±0.2	15±0.15	04±0.06	16±0.95	18±0.85	14±0.52
Gentamycin	18±0.52	14±0.45	14±0.39	30±0.97	18±0.66	19±0.71	16±0.22
Vancomycin	15±0.42	03±0.08	30±0.06	26±0.96	29±0.85	14±0.65	19±0.74
Methicillin	07±0.21	21±0.95	05±0.12	10±0.32	15±0.41	18±0.32	18±0.35

Seven bacterial isolates were tested for antibiotic susceptibility against 23 antibiotics, *Pseudomonas aeruginosa* was sensitive to Carbapenem, fluoroquinolone, ceftazidime. *Salmonella enterica serovar typhi* was sensitive to chloramphenicol. *Staphylococcus aureus* was found to be sensitive for Cephalosporin and vancomycin. Chloramphenicol, Tetracycline, Gentamycin and Vancomycin were effective against *Bacillus paranthracis. E. coli* bacteria was sensitive to Cephalosporin, carbapenem and vancomycin. *S. pyogenes* was sensitive to Cephalosporin and macrolide. *Candida dubliniensis* was resistant to all antibiotics tested. All bacteria were resistant to Ampicilllin, Monobactam, Suldonamide, Nitroimidazole, Rifamycin, Elfamycin, Cefepime, Augmentin, Pencillin-G and Methicillin.

Viral screening by ELISA

Viral Serological screening by ELISA testing for HIV, HbSAg and HCV were not reactive.

Meta genomic sequencing Organism identification

With metagenomic sequencing 19 bacteria and 1 fungus, 3 viruses, 1 mycoplasma consisting of 24 organism sequences were identified (Table 3). Only 4 Un culturable bacteria were identified but on blood agar only 6 bacteria were isolated. Out of 19 bacteria 4 are unculturable hence 15 are culturable. But only 6 bacteria are growing on blood agar in lab conditions. Remaining bacteria can be grown by changing media or growth conditions.

Table 3: Organisms identified by metagenomic sequencing and type of organism

Organism identified by mNGS	Organism type
SARS Cov-2	Virus
Pseudomonas aeruginosa	Bacteria
Corynebacterium pseudokroppenstedtii	Bacteria
Shigella flexneri aerobactin	Bacteria
Salmonella enterica subsp. enterica serovar	Bacteria
Staphylococcus aureus	Bacteria
Bacillus paranthracis	Bacteria
Escherichia coli	Bacteria
Streptococcus pyogenes	Bacteria
Hepatitis C virus	Virus
Uncultured *Citrobacter sp*	Uncultured Bacteria
uncultured *Firmicutes* bacterium	Uncultured Bacteria
uncultured bacterium	Uncultured Bacteria
Caudoviricetes sp.	Virus
Candidatus Enterousia intestinigallinarum	Bacteria
uncultured bacteria	Uncultured Bacteria
Mycobacterium tuberculosis	Bacteria
Candida dubliniensis	Fungus
Mycoplasma conjunctivae	Mycoplasma
Burkholderia pseudomallei	Bacteria
Veillonella parvula	Bacteria
Enterococcus faecium	Bacteria
Moraxella osloensis	Bacteria
Coxiella burnetii	Bacteria

DISCUSSION

In recent advances, blood metagenomics sequencing has been introduced as an effective method for detecting systemic infectious organisms. This study used dialysis patient blood sample to identify pathogens causing BSIs, comparing the result with blood cultures and ELISA viral screening. Peritoneal dialysis-associated peritonitis (PDAP) a complicated issue in dialysis for peritoneum that can impact treatment and endanger patient lives. Its occurrence is linked to factors like improper fluid replacement; infections related to catheter, intestinal bacteria displacement and reduced function of peritoneum (Guo *et al.* 2024). Additionally, high-risk procedures that may lead to peritonitis are common. In current study, investigated systemic infections in dialysis patient. Using metagenomics sequencing 24 microbes were identified, while the blood culture method detected only 7microbes and 0 viruses by ELISA screening. Through this, our findings show that metagenomics sequencing outperforms conventional tests in detecting systemic pathogens. It is demonstrated that metagenomics sequencing has higher rate of detection, greater sensitivity and a wider range of detecting pathogens when comparison with blood cultures and ELISA. In clinical practice, blood is a commonly used sample source for culturing to detect systemic infections but metagenomics sequencing is getting a momentum now. Studies show usage of human blood samples for meta genomic analysis in clinical diagnosis (Yu *et al.* 2024, Qian *et al.* 2023, Moragues-Solanas *et al.* 2024, Lu *et al.* 2023).

The study conducted by Lu *et al.* (2023) found that metagenomics sequencing identified 67 out of 79 patients with positive results, whereas conventional tests only detected 34 positive cases. In another study by Guo *et al.* (2024) showed metagenomic sequencing detected 29 pathogens (which were 24 bacteria, 1 fungus and 4 viruses) while bacterial blood culture identified 10 pathogens (9 bacteria and 1 fungus) and studies by Geng *et al.* (2021) reported 26 positive cases of samples out of 63 while only five were reported in blood culture, whereas our study concludes a total of 24 microbes were detected by metagenomic sequence analysis and 6 bacteria and 1 fungus by blood culture. This indicates that metagenomics sequencing has significant positive rate of detection compared to traditional techniques.

DNA was extracted from blood by cDNA preparation (Yu *et al.* 2024) and Liu *et al.* (2023). The improved DNA extraction procedure of blood samples preserved bacterial DNA at clinical significant amount (Moragues-Solanas *et al.* 2024) and similar results were observed in present study. The blood samples were indused with four primary species responsible for BSI were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Enterococcus faecalis* in the studies of Moragues-Solanas *et al.* (2024). *K. pneumoniae, E. faecium, Pseudomonas aeruginosa* were detected in studies of Yu *et al.* (2024) whereas in our study a total of 24 species were identified, few of which were same as in previous studies. *Candida albicans* was also identified in the studies of Lu *et al.* (2023) along with bacteria, Whereas in present study found *Candida dubliniensis* along with bacteria. Geng *et al.* (2021) reported *Acinetobacter baumannii, S. aureus, K. pneumoniae* and *Candida*. Jerome *et al.* (2019) studied the patients who were diagnosed with hepatitis spp, while in our studies Hepatitis C virus was diagnosed by metagenomics sequencing even it is serologically negative. The samples

were tested positive for HIV virus by Somasekar *et al.* (2017). Grundy *et al.* (2023) reported *Coxiella spp., M. Tuberculosis, Salmonella spp., S. aureus, P. aeruginosa, K. pneumoniae, E. coli,* HIV, Hepatitis B virus, Hepatitis E virus while in the current study *Salmonella enterica subsp. enterica serovar, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, Hepatitis C virus, *Mycobacterium tuberculosis*, *Coxiella burnetii* were reported. Studes by Jerome *et al.* (2019) other strains diagnosed were Dengue virus, Chikungunya virus, mumps virus, Ebola virus, human pegivirus, *Plasmodium falciparum, Plasmodium malariae* whereas in our study the other microbes detected were *Enterococcus faecium, Mycoplasma conjunctivae, Corynebacterium pseudokroppenstedtii,* SARS CoV-2*, Shigella flexneri aerobactin, Veillonella parvula, Burkholderia pseudomallei.* Nie *et al.*(2023) recommends metagenomics sequencing to patients with Peritoneal dialysis in patients who have previously been treated with antibiotics. For individuals who have not undergone treatment with antibiotics, metagenomic sequencing and culture techniques can be used together for detection of pathogens. The positivity rate was 4% with the culture method and 31% with metagenomics sequencing, showing a significant difference between the two methods. Viruses are not detected by culturing and serology methods hence metagenomic sequencing is the best choice of diagnosis.

Summary

To a dialysis patient with systemic infections, compared microbial culturing and viral screening with ELISA with blood metagenomic sequencing and sequence analysis for diagnosis of infective microbe. In blood agar media culturing, 7 microbes (6 bacteria and 1 fungus), in serology by ELISA 0 viruses and in meta genomic sequence analysis 24 pathogens (19 bacteria and 1 fungus, 3 viruses, 1 mycoplasma) were diagnosed. 4 unculturable bacteria were also diagnosed. Hence metagenomic sequence analysis can be one of the best methods for effective detection of BSI's.

REFERENCES

1. Chen, Y., Ye, W., Zhang, Y., & Xu, Y. (2015). High speed BLASTN: an accelerated MegaBLAST search tool. Nuc aci res, 43(16), 7762-7768. doi: https://doi.org/10.1093/nar/gkv784
2. Forbes, J. D., Knox, N. C., Ronholm, J., Pagotto, F., & Reimer, A. (2017). Metagenomics: the next culture-independent game changer. Front. Microb., 8, 1069.doi: https://doi.org/10.3389/fmicb.2017.01069
3. Gan, S. D., & Patel, K. R. (2013). Enzyme immunoassay and enzyme-linked immunosorbent assay. Jou of Inv Derm, 133(9), 1-3.doi: https://doi.org/10.1038/jid.2013.287
4. Garcia, R. A., Spitzer, E. D., Beaudry, J., Beck, C., Diblasi, R., Gilleeny-Blabac, M., & Torregrosa, E. (2015). Multidisciplinary team review of best practices for collection and handling of blood cultures to determine effective interventions for increasing the yield of true-positive bacteremias, reducing contamination and eliminating false-positive central line–associated bloodstream infections. Amer. J. inf. Cont., 43(11), 1222-1237.doi: https://doi.org/10.1016/j.ajic.2015.06.030
5. Geng, S., Mei, Q., Zhu, C., Fang, X., Yang, T., Zhang, L., ... & Pan, A. (2021). Metagenomic next-generation sequencing technology for detection of pathogens in blood of critically ill patients. Int. J. Inf. Dise., 103, 81-87.doi: https://doi.org/10.1016/j.ijid.2020.11.166
6. Grundy, B. S., Parikh, H., Jacob, S., Banura, P., Moore, C. C., Liu, J., & Houpt, E. R. (2023). Pathogen detection using metagenomic next-generation sequencing of plasma samples from patients with sepsis in Uganda. Microb. Spe., 11(1), e04312-22.doi: https://doi.org/10.1128/spectrum.04312-22
7. Guo, S. S., Fu, G., Hu, Y. W., Liu, J., & Wang, Y. Z. (2024). Application of metagenomic next-generation sequencing technology in the etiological diagnosis of peritoneal dialysis-associated peritonitis. Op. Lif. Sci., 19(1), 20220865.doi: https://doi.org/10.1515/biol-2022-0865
8. Gupta, S., Sakhuja, A., Kumar, G., McGrath, E., Nanchal, R. S., & Kashani, K. B. (2016). Culture-negative severe sepsis: nationwide trends and outcomes. Che., 150(6), 1251-1259.doi: https://doi.org/10.1016/j.chest.2016.08.1460
9. Jerome, H., Taylor, C., Sreenu, V. B., Klymenko, T., Filipe, A. D. S., Jackson, C., ... & Thomson, E. C. (2019). Metagenomic next-generation sequencing aids the diagnosis of viral infections in febrile returning travellers. J. Inf., 79(4), 383-388.doi: https://doi.org/10.1016/j.jinf.2019.08.003
10. Li, N., Cai, Q., Miao, Q., Song, Z., Fang, Y., & Hu, B. (2021). High-throughput metagenomics for identification of pathogens in the clinical settings. Sm. Metho., 5(1), 2000792.doi: https://doi.org/10.1002/smtd.202000792
11. Lu, D., Abudouaini, M., Kerimu, M., Leng, Q., Wu, H., Aynazar, A., & Zhong, Z. (2023). Clinical evaluation of Metagenomic Next-

Generation sequencing and identification of risk factors in patients with severe community-acquired pneumonia. Inf. D. Resis., 5135-5147. doi: https://doi.org/10.2147/IDR.S421721

12. Lamy, B., Sundqvist, M., & Idelevich, E. A. (2020). Bloodstream infections–Standard and progress in pathogen diagnostics. Clin. Microb. Inf., 26(2), 142-150.doi: https://doi.org/10.1016/j.cmi.2019.11.017
13. Ma, L. N., Zhang, J., Chen, H. T., Zhou, J. H., Ding, Y. Z., & Liu, Y. S. (2011). An overview on ELISA techniques for FMD. Vir jou, 8, 1-9.doi: https://doi.org/10.1186/1743-422X-8-419
14. Moghnia, O. H., & Al-Sweih, N. A. (2022). Whole genome sequence analysis of multidrug resistant Escherichia coli and Klebsiella pneumoniae strains in Kuwait. Micr, 10(3), 507.doi: https://doi.org/10.3390/microorganisms10030507
15. Moragues-Solanas, L., Le-Viet, T., McSorley, E., Halford, C., Lockhart, D. S., Aydin, A., & Gilmour, M. W. (2024). Development and proof-of-concept demonstration of a clinical metagenomics method for the rapid detection of bloodstream infection. BMC Med. Geno., 17(1), 71.doi: https://doi.org/10.1186/s12920-024-01835-5
16. Nie, S., Zhang, Q., Chen, R., Lin, L., Li, Z., Sun, Y., & Chen, X. (2023). Rapid detection of pathogens of peritoneal dialysis-related peritonitis, especially in patients who have taken antibiotics, using metagenomic next-generation sequencing: a pilot study. Ren. Fail., 45(2), 2284229.doi: https://doi.org/10.1080/0886022X.2023.2284229
17. Qian, M., Li, C., Zhang, M., Zhan, Y., Zhu, B., Wang, L., & Cheng, Y. (2023). Blood metagenomics next-generation sequencing has advantages in detecting difficult-to-cultivate pathogens and mixed infections: results from a real-world cohort. Front. Cell. Inf. Microb., 13, 1268281.doi: https://doi.org/10.3389/fcimb.2023.1268281
18. Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R., ... & Naghavi, M. (2020). Global, regional and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. T Lan, 395(10219), 200-211.doi: https://doi.org/10.1016/S0140-6736(19)32989-7
19. Somasekar, S., Lee, D., Rule, J., Naccache, S. N., Stone, M., Busch, M. P., ... & Chiu, C. Y. (2017). Viral surveillance in serum samples from patients with acute liver failure by metagenomic next-generation sequencing. Cli. Inf. Dis., 65(9), 1477-1485.doi: https://doi.org/10.1093/cid/cix596
20. Vijayvargiya, P., Jeraldo, P. R., Thoendel, M. J., Greenwood-Quaintance, K. E., Esquer Garrigos, Z., Sohail, M. R., ... & Patel, R. (2019). Application of metagenomic shotgun sequencing to detect vector-borne pathogens in clinical blood samples. PLo. On., 14(10), e0222915.doi: https://doi.org/10.1371/journal.pone.0222915
21. Wang, L., Li, S., Qin, J., Tang, T., Hong, J., Tung, T. H., ... & Qian, J. (2023). Clinical diagnosis application of metagenomic next-generation sequencing of plasma in suspected sepsis. Inf. Dru. Res., 891-901.doi: https://doi.org/10.2147/IDR.S395700
22. Wilson, M. R., Naccache, S. N., Samayoa, E., Biagtan, M., Bashir, H., Yu, G., & Chiu, C. Y. (2014). Actionable diagnosis of neuroleptospirosis by next-generation sequencing. New Eng. J. Med., 370(25), 2408-2417. DOI: https://doi.org/10.1056/NEJMoa1401268
23. Yu, J., Zhang, L., Gao, D., Wang, J., Li, Y., & Sun, N. (2024). Comparison of metagenomic next-generation sequencing and blood culture for diagnosis of bloodstream infections. Front. Cell. Inf. Microb., 14, 1338861.doi: https://doi.org/10.3389/fcimb.2024.1338861
24. Zuo, Y. H., Wu, Y. X., Hu, W. P., Chen, Y., Li, Y. P., Song, Z. J., ... & Zhang, J. (2023). The clinical impact of metagenomic next-generation sequencing (mNGS) test in hospitalized patients with suspected sepsis: a multicenter prospective study. Diag., 13(2), 323.doi: https://doi.org/10.3390/diagnostics13020323