Pathogenic Detection by Metagenomic Next-generation Sequencing in Skin and Soft Tissue Infection

TING-KUANG YEH[1,2], YAO-TING HUANG[3], PO-YU LIU[1,2,4,5,6], YAN-CHIAO MAO[7],
CHIH-SHENG LAI[8], KUO-LUNG LAI[9], CHIEN-HAO TSENG[1], CHIA-WEI LIU[1],
WEI-HSUAN HUANG[1], HSIEN-PO HUANG[1] and KUAN-PEI LIN[1]

[1]Division of Infectious Diseases, Department of Internal Medicine,
Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.;
[2]Genomic Center for Infectious Diseases, Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.;
[3]Department of Computer Science and Information Engineering,
National Chung Cheng University, Chiayi, Taiwan, R.O.C.;
[4]Ph.D. Program in Translational Medicine, National Chung Hsing University, Taichung, Taiwan, R.O.C.;
[5]Rong Hsing Research Center for Translational Medicine,
National Chung Hsing University, Taichung, Taiwan, R.O.C.;
[6]Department of Post-Baccalaureate Medicine, College of Medicine,
National Chung Hsing University, Taichung, Taiwan, R.O.C.;
[7]Division of Clinical Toxicology, Department of Emergency Medicine,
Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.;
[8]Division of Plastic and Reconstructive Surgery, Department of Surgery,
Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.;
[9]Division of Allergy, Immunology and Rheumatology, Department of Medicine,
Taichung Veterans General Hospital, Taichung, Taiwan, R.O.C.

Abstract. *Background/Aim: Skin and soft tissue infections (SSTIs) can be life-threatening, but the conventional bacterial cultures have low sensitivity and are time-consuming. Metagenomic next-generation sequencing (mNGS) is widely used as a diagnostic tool for detecting pathogens from infection sites. However, the use of mNGS for pathogen detection in SSTIs and related research is still relatively limited. Patients and Methods: From January 2020 to October 2021, 19 SSTI samples from 16 patients were collected in a single center (Taichung Veterans General Hospital, Taichung, Taiwan). The clinical samples were simultaneously subjected to mNGS and conventional bacterial culture methods to detect pathogens.*

Correspondence to: Kuan-Pei Lin, Postal address: 1650, Sec.4, Taiwan Boulevard, Taichung City, Taiwan, R.O.C. E-mail: taylor0711@vghtc.gov.tw

Key Words: Clinical metagenomics, next-generation sequencing, skin and soft tissue infection.

Clinical characteristics were prospectively collected through electronic chart review. The microbiological findings from conventional bacterial culture and mNGS were analyzed and compared. Results: The mNGS method detected a higher proportion of multiple pathogens in SSTIs compared to conventional bacterial culture methods. Pseudomonas spp. was among the most commonly identified Gram-negative bacilli using mNGS. Additionally, the mNGS method identified several rare pathogens in patients with SSTIs, including Granulicatella adiacens, Bacillus thuringiensis, and Bacteroides fragilis. Antimicrobial resistance genes were detected in 10 samples (52.6%) using the mNGS method, including genes for extended-spectrum beta-lactamase, Ambler class C β-lactamases, and carbapenemase. Conclusion: mNGS not only plays an important role in the detection of pathogens in soft tissue infections, but also informs clinical professionals about the presence of additional microbes that may be important for treatment decisions. Further studies comparing conventional pathogen culture with the mNGS method in SSTIs are required.

Skin and soft tissue infections (SSTIs) range from mild to serious life-threatening infections, such as necrotizing skin and soft-tissue infections (nSSTIs), which are not common, but often require intensive unit care (1). SSTIs are one of the most

common infectious diseases and their incidence is 2-fold that of urinary tract infection and 10-fold that of pneumonia (2). Additionally, due to the diversity of pathogens, clinical doctors prefer to choose broad-spectrum antibiotics as empirical treatment. However, this will increase the prevalence rate of resistant bacteria (3). Currently, we rely on pathogen culture report to offer appropriate antibiotic treatment, but the conventional bacterial cultures have low sensitivity and are time consuming, which has great impact on physician's decisions when treatment is not sufficient.

There are several advanced methods for pathogen detection in SSTIs. Broad-range PCR of the 16S rRNA/rDNA gene is a widely used method for pathogen detection. However, due to the inability of identifying polymicrobial infections, the sensitivity was not high, approximately 70% (4). In addition, multiplex PCR can detect several specific pathogens in one assay, but the number of targets is limited (5).

Metagenomic next-generation sequencing (mNGS) is an approach for nearly all infectious agents with DNA or RNA genomes (6). It is a powerful method for identifying and sequencing nucleic acids from a mixed population of microorganisms (7). Compared to conventional methods such as culture, mNGS has high precision and efficacy, and it is thought that it will become an important clinical diagnostic tool in the current era (8). However, etiological diagnosis of SSTIs by clinical application of mNGS is relatively less studied. There have been 96 SSTIs cases studies showing that the sensitivity of mNGS to detect pathogens was superior to traditional culture methods, and that the use of mNGS testing could guide antibiotic treatment strategies and improve clinical outcomes (9). In our study, we evaluated the performance of mNGS in universally detecting pathogens from various types of soft tissue infection samples in a tertiary hospital in Taiwan. We compared the positive rate and pathogen identification between conventional and mNGS methods. Furthermore, we focused on the resistance genes detected using mNGS and compared the relationship between antibiotic susceptibility test and genotype.

Patients and Methods

Study population. The present study initially included 19 soft tissue infection samples from 16 patients who underwent conservative or surgical therapy for SSTIs. The institutional review board of the Taichung Veterans General Hospital granted ethical approval for this study (CE24012B). Data were prospectively collected and analyzed at a single institution (Taichung Veterans General Hospital, Taichung, Taiwan, ROC), over a 22-month period, from January 2020 to October 2021. The patients included met one of the diagnoses below: cutaneous abscess, cellulitis, acute lymphangitis, necrotizing fasciitis, superficial or deep surgical site infection or pressure sore. The physicians and surgeons diagnosed patients with infectious diseases according to clinical manifestation, laboratory tests, imaging, and culture examinations. The tissues of the wounds were debrided from mainly pressure sore, surgical wound and abscess. Tissue swabs were

collected in deep wounds. The samples were delivered to the microbiology laboratory for simultaneous bacterial culture and mNGS.

Initial species identification was conducted using Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (bioMérieux, Lyon, France) according to the manufacturer's instructions. The minimum inhibitory concentration (MIC) values were determined by VITEK®2 (bioMérieux) and interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines. If the results of mNGS encompassed all pathogens identified by bacterial culture at the species level or genus level, we considered them to be concordant. If the results of mNGS included only a portion of the pathogens identified by bacterial culture, we considered the results to be partially concordant. If the pathogens identified by mNGS were entirely different from those identified by bacterial culture at the genus level, we considered the results to be discordant.

Clinical sample preparation and sequencing. The tissue samples were firstly grounded and mixed with 1 g of 0.5-mm diameter glass beads and then placed on a vortex mixer for 30 min at 3,000 rpm. DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) was used for DNA extraction from 300 μl of the sample according to manufacturer's instructions. We used an enzymatic method to fragment the DNA to 150-200 bp long. The DNA library was built using end-repaired adapter and polymerase chain reaction amplification. The DNA Qubit Assay (Thermo Fisher, Waltham, MA, USA) was used to determine DNA concentrations. DNA quality was assessed electrophoretically using the Agilent 2100 system (Agilent Technologies, Santa Clara, CA, USA). The DNA library was built using end-repaired adapters and polymerase chain reaction amplification using the MGIEasy FS DNA Library Prep Kit (MGI, ShenZhen, PR China). We then transformed the single-strand circularized DNA library into DNA nanoballs (DNB) and sequenced using DNBSeq-G50 (MGI) with an average read length of 50 bp.

Bioinformatics analysis. The sequencing reads were preprocessed by removing low-quality (*i.e.*, reads <80% phred score Q30), duplicated reads, and reads shorter than 35 bp in length. The remaining high-quality reads were aligned to the human genome (hg38) using the Burrows-Wheeler Aligner (BWA) to remove reads derived from human sequences (10). The non-human reads were BWA-aligned to the NCBI microbial reference genomes (RefSeq) for taxonomic classification. The species of lower read counts were considered as reagent/environmental contamination or alignment errors due to short-read mapping ambiguity.

Results

Characteristics of the study participants. In total, 16 patients diagnosed with SSTIs were included in this study; 11 were male and 5 were female. A total of 19 SSTI samples were collected from 16 patients, two patients subjected to surgery at least 2 times and therefore offered more than one sample. Samples were classified into three types: pressure sore (four cases, 21.0%), surgical wound (six cases, 31.5.0%), and wound infection (nine cases, 47.3%) (Figure 1). Details of the demographic characteristics of the patients enrolled in this study are provided in Table I. Sample 11 and Sample 14 were collected from the same patient with diagnosis of left
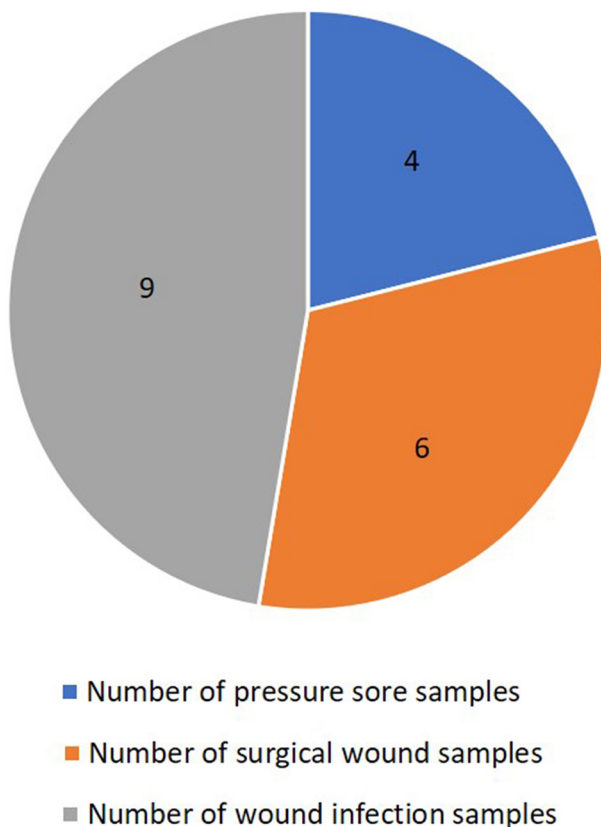
Different Type of SSTIs

Figure 1. *Among the 19 samples from 16 patients with soft tissue infection, four cases were pressure sore, six cases were surgical wounds, and nine cases were wound infection tissues.*

tonsil squamous cell carcinoma, who was subjected to surgery twice. Samples 12, 13, and 16 were collected separately from the same patient with left dorsal foot open wound who was subjected to debridement thrice.

Pathogen detection using mNGS and traditional bacterial culture methods. All pathogens detected in different specimens using traditional bacterial cultures and mNGS method are listed in Table II. The microbiological cultures were positive in 19 out of 19 samples and mNGS showed positive results in 18 out of 19 samples.

The numbers of different pathogens detected by mNGS and traditional bacterial cultures are shown in Figure 2. Among the 47 pathogens detected using mNGS or traditional bacterial cultures, gram-negative bacillus was predominant, especially *Pseudomonas* spp. (n=10), followed by *Staphylococcus aureus* (n=6), and *Streptococci* (n=3). The mNGS method showed

higher positive detection rates than the culture in *Staphylococcus aureus*, *Streptococci* spp., *Enterococcus* spp., *Enterobacter* spp. and *Bacteroids* spp.

The results in seven of 19 samples (36.8%) obtained using mNGS and conventional bacterial culture were concordant at the species level; seven of 18 (36.8%) were concordant at the genus level. Among 19 samples, three (15.8%) were regarded partially concordant, and two (10.5%) were discordant (Figure 3). Sample T17 obtained from a wound of a snake bite (Table II) was negative in mNGS but positive for *Staphylococcus epidermis* in conventional culture.

The mNGS also showed a higher proportion of polymicrobial pathogen detection in soft tissue infections (Figure 4). Multiple pathogens were detected in 73% of the 19 samples by the traditional bacterial culture method, while mNGS detected multiple pathogens in 84% of the 19 samples.

Antimicrobial resistance gene detection using mNGS. In this study, several types of antimicrobial resistance genes were detected (Table III and Table IV), including carbapenemase gene, broad spectrum and extended spectrum beta-lactamase gene (bla_{TEM}, bla_{SHV}, bla_{OXA}), Ambler class C (AmpC) beta-lactamase, *Bacteroides* spp. related beta-lactamase gene (bla_{CfXA} and bla_{CepA}), *Bacillus* spp. related resistant gene (bla_{BCI} and bla_{Bla}), SCC*mec* and quinolone resistance gene.

Discussion

Metagenomic sequencing is a promising and novel tool for the etiological diagnosis of SSTIs, particularly for identifying viruses, anaerobes, fastidious, and multi-pathogen infections (9). In this study, the clinical performance of mNGS was assessed in patients with soft tissue infection, in comparison with conventional microbiology testing. This study demonstrated the capability of mNGS as the first-line pathogen test. The mNGS method could detect more pathogens compared to conventional culture. Furthermore, mNGS was shown to overcome limitations of current diagnostic tests, such as 16S rRNA PCR, allowing universal pathogen detection without prior knowledge of specific microorganisms.

In our study, most of microbiological findings in each sample were concordant and partially concordant between traditional bacterial cultures and mNGS (17 of 19). For discordant and partially concordant samples, the discrepancy was primary on anaerobes, fastidious pathogens, and viral pathogens.

The detection of anaerobes, especially obligate anaerobes, requires a strict anaerobic environment during specimen collection and transport. In our study, mNGS revealed the presence of *Bacteroides fragilis* in three samples (T2, T10, T19), but traditional bacterial culture showed its presence in only one sample (T19). *Bacteroides* spp. are small, pleomorphic gram-negative bacilli. Among the more than 50 known *Bacteroides* spp. (11), *Bacteroides fragilis* is the most

Table I. *Characteristics of patients and sample sources.*

Sample	Sex	Age	Sample source	Diagnosis
T1	M	25	Primary wound	Left foot fracture with bone and tendon exposure
T2	M	55	Primary wound	Right plantar foot abscess
T3	M	64	Pressure sore	Necrotizing fasciitis
T4	F	69	Surgical wound	Left tibia and fibula shaft open fracture
T5	M	66	Primary wound	Left temporal bone necrosis
T6	F	81	Surgical wound	Malignant tenosynovial giant cell tumor
T7	M	80	Pressure sore	Pressure sore
T8	F	73	Pressure sore	Pressure sore
T9	M	96	Primary wound	Scalp abscess
T10	F	92	Pressure sore	Pressure sore
T11	M	73	Surgical wound	Left hard palate necrosis
T12	M	20	Primary wound	Left dorsal foot wound
T13	M	20	Primary wound	Left dorsal foot wound
T14	M	73	Surgical wound	Left hard palate necrosis
T15	M	70	Surgical wound	Right lower leg chronic ulcer
T16	M	20	Primary wound	Left dorsal foot dirty wound
T17	M	51	Primary wound	Snake bite wound
T18	F	50	Surgical wound	Right lower leg ulcerative wound
T19	M	53	Primary wound	Peripheral artery occlusive disease with gangrene change

important in human disease. It is the most common anaerobe isolated from intra-abdominal infections, and the most frequent anaerobic isolate in patients with bacteremia (12). This highlights the importance of quick detection of anaerobes using mNGS, which can provide novel and fast turnaround time in the detection of *Bacteroides fragilis*.

In addition, mNGS identified several fastidious pathogens in patients with SSTI, such as *Granulicatella* spp. (formerly known as nutritionally variant streptococci). It is an important etiologic species in blood culture negative endocarditis (BCNE) (13). Besides BCNE, *Granulicatella adiacens* are also involved in prosthetic joint infection, and are often being missed due to difficult culture, especially in the cases of polymicrobial infection. In our study, *G. adiacens* was found in three samples (T11, T13, T14) using mNGS, but was not detected using conventional bacterial culture. A study showed that prolonged antimicrobial treatment (≥8 weeks) should be considered for prosthetic joint infection involving *G. adiacens* (14). The ability of the mNGS method to detect fastidious pathogens in SSTI helps guiding antimicrobial agent selection and determining duration of treatment.

Additionally, due to viral pathogens usually not being taken seriously in SSTI condition, mNGS provides an effective way to detect viral pathogens. In the T9 sample, left occipital carbuncle, the bacterial culture detected *Staphylococcus aureus, Pseudomonas aeruginosa* and mNGS *Staphylococcus aureus, Pseudomonas aeruginosa* and *Human betaherpesvirus* 5. Although *Human betaherpesvirus* 5 (also known as *Human cytomegalovirus*) infection is typically asymptomatic in the immunocompetent population, this infection can still result in

conditions, such as mononucleosis and certain cancers (15). In nowadays, mNGS is also used in central nervous system infections due to the inefficiency of the current diagnostic workflows. mNGS offers the chance to circumvent these challenges by using unbiased laboratory and computational methods (16).

The major advantage of mNGS in soft tissue infection compared to conventional bacterial culture is not only the much shorter turnaround time but also the acquisition of drug resistance data that can guide optimal antibiotic therapy at an early stage of infection. In this study, several types of antimicrobial resistance genes were detected (Table III and Table IV), including carbapenemase, broad- and extended spectrum beta-lactamase (bla_{TEM}, bla_{SHV}, bla_{OXA}), AmpC beta-lactamase, *Bacteroides* spp.-related beta-lactamase (bla_{CfXA} and bla_{CepA}), and *Bacillus* spp. related resistant gene (bla_{BCI} and bla_{Bla}), SCC*mec*.

One of the problems with the detected antimicrobial resistance genes is that it is difficult to attribute a resistant gene to a specific species. However, because certain resistant genes tend to be present in certain bacterial species, there are still hints that can be used for identification. For example, in the T8 sample, *Escherichia coli* and *Enterococcus faecium* were detected using mNGS. The resistant genes bla_{CMY}, bla_{OXA}, and bla_{KPC-17} were more likely to belong to *E. coli*. In the T19 sample, SCC*mecA*, SCC*mecR,* and SCC*mecI* were more recognized as belonging to *S. aureus*.

Specific resistance genes mainly appear in specific bacterial species. When using mNGS to simultaneously detect multiple pathogens and multiple resistance genes in a sample,

Table II. *Pathogen detection by traditional culture vs. mNGS.*

Sample	Sample source	Traditional culture	mNGS
T1	Primary wound	*Aeromonas hydrophila*	*Pseudomonas aeruginosa*
		Pseudomonas aeruginosa	*Staphylococcus epidermidis*
			Aeromonas hydrophila
T2	Primary wound	*Proteus penneri*	*Bacteroides fragilis*
		Citrobacter koseri	*Porphyromonas asaccharolytica*
			Anaerococcus vaginalis
			Proteus penneri
			Citrobacter koseri
T3	Pressure sore	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
		Enterobacter cloacae complex	*Streptococcus gallolyticus*
		Clostridium cadaveris	*Clostridium cadaveris*
			Enterobacter hormaechei
T4	Surgical wound	*Stenotrophomonas maltophilia*	*Staphylococcus aureus*
			Stenotrophomonas geniculata
T5	Primary wound	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
		Corynebacterium spp.	*Corynebacterium resistens*
T6	Surgical wound	*Serratia marcescens*	*Serratia nevei*
T7	Pressure sore	*Pseudomonas aeruginosa*	*Prevotella bivia*
		Acinetobacter pittii	*Pseudomonas aeruginosa*
			Bilophila wadsworthia
			Pyramidobacter piscolens
			Staphylococcus aureus
			Klebsiella pneumoniae
			Fusobacterium nucleatum
			Citrobacter sedlakii
			Dialister pneumosintes
T8	Pressure sore	*Escherichia coli*	*Escherichia coli*
			Enterococcus faecium
T9	Primary wound	*Staphylococcus aureus*	*Staphylococcus aureus*
		Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
			Human betaherpesvirus 5
T10	Pressure sore	*Staphylococcus aureus*	*Bacteroides fragilis*
		Corynebacterium spp.	*Staphylococcus aureus*
			Corynebacterium striatum
T11	Surgical wound	*Pseudomonas aeruginosa*	*Granulicatella adiacens*
		Enterococcus faecalis	*Streptococcus constellatus*
		Klebsiella aerogenes	*Klebsiella pneumoniae*
			Pseudomonas aeruginosa
			Schaalia odontolytica
			Enterococcus faecalis
T12	Primary wound	*Serratia marcescens*	*Bacillus thuringiensis*
		Pseudomonas mosselii	*Enterobacter hormaechei*
		Pseudomonas aeruginosa	*Paraclostridium dentum*
			Serratia ureilytica
			Enterobacter cancerogenus
			Citrobacter amalonaticus
T13	Primary wound	*Serratia marcescens*	*Pseudomonas aeruginosa*
		Pseudomonas aeruginosa	*Enterobacter hormaechei*
			Bacillus thuringiensis
			Granulicatella adiacens
			Enterococcus faecalis
			Serratia ureilytica
T14	Surgical wound	*Klebsiella aerogenes*	*Prevotella oris*
		Klebsiella pneumoniae	*Finegoldia magna*
		Enterobacter cloacae complex	*Granulicatella adiacens*
			Streptococcus constellatus
			Enterococcus faecalis
			Klebsiella pneumoniae
			Schaalia odontolytica

Table II. *Continued*

Table II. *Continued*

Sample	Sample source	Traditional culture	mNGS
T15	Surgical wound	*Pseudomonas aeruginosa* *Escherichia coli*	*Pseudomonas aeruginosa* *Escherichia coli*
T16	Primary wound	*Pseudomonas aeruginosa*	*Staphylococcus epidermidis* *Pseudomonas aeruginosa*
T17	Primary wound	*Staphylococcus epidermidis*	None
T18	Surgical wound	*Enterococcus faecium* *Burkholderia cepacia* complex	*Corynebacterium tuberculostearicum*
T19	Primary wound	*Staphylococcus aureus* *Bacteroides fragilis* *Klebsiella pneumoniae*	*Bacteroides fragilis* *Staphylococcus aureus* *Klebsiella pneumoniae*
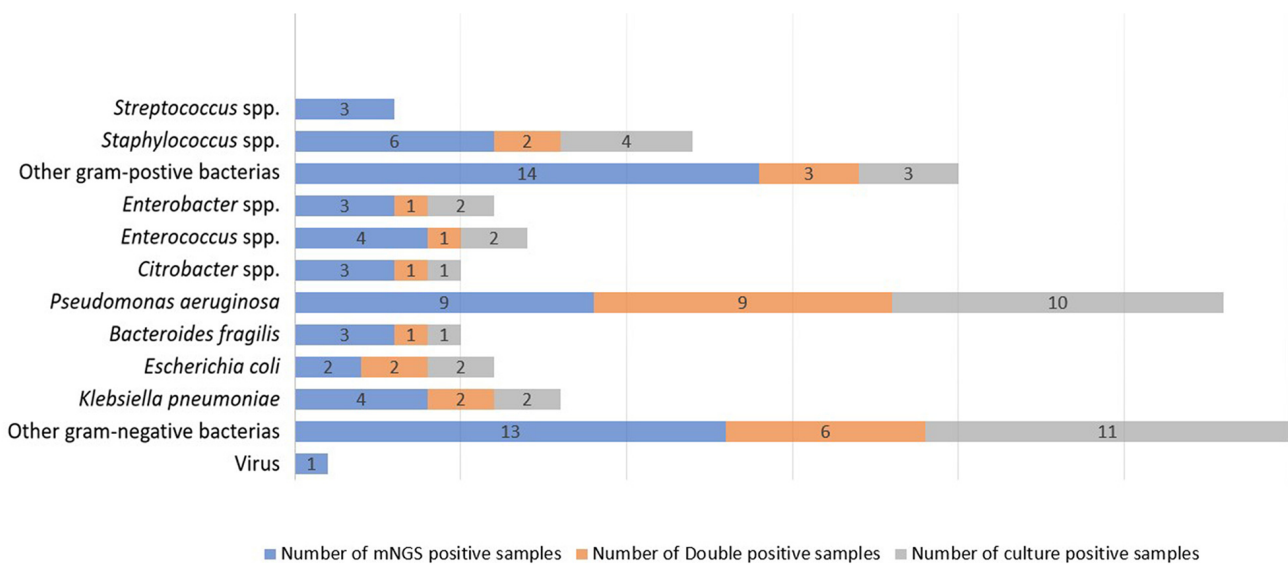
Figure 2. *Comparison the pathogen detection between mNGS and culture in SSTI cases. Higher positive rate for Streptococcus, Staphylococcus, other gram-positive bacteria and other gram negative bacterial was demonstrated by mNGS when compared to culture.*

if a specific resistance gene is identified, it can be linked to a specific bacterial species. The bla_{CfxA} is known to be present in *Bacteroides* spp. and oral anaerobic bacteria, such as *Prevotella* spp. and *Capnocytophaga* spp. (17). Insertional activation of bla_{cepA} leads to high-level beta-lactamase expression in clinical isolates of *B. fragilis* (18). In our study, mNGS showed the presence of *B. fragilis* reads in the T2, T10, T19 samples. Beta-lactamase genes, such as bla_{CfxA}, bla_{cepA}, and bla_{CfiA}, which are often found in *Bacteroides* spp., were also detected in T2, T10, and T19 samples. Although other pathogens were also detected in the T2, T10, and T19 samples, based on the characteristics of bla_{CfxA}, bla_{cepA}, and bla_{CfiA}, these genes are likely to belong to *Bacteroides* spp. in these samples. In addition, bla_{BCI} and bla_{Bla1} were detected in *Bacillus* spp. Expression of the *bla1*

and *bla2* genes in certain strains of *Bacillus anthracis* is associated with penicillin resistance (19). In sample T12, *Bacillus thuringiensis* and bla_{BCI} and bla_{Bla} resistant genes was detected using mNGS.

In *Pseudomonas* spp., the main resistance mechanism against β-lactams is the expression of the class C bla_{PDC} (Pseudomonas-derived cephalosporinase) resistant gene (20). In our study, *P. aeruginosa* was detected using mNGS and conventional bacterial culture in T3, T6, T15. The bla_{PDC} gene was also detected in those samples using mNGS.

There are still some limitations to the use of mNGS for pathogen and resistant gene detection. First, in our study, mNGS could detect 24 more potential pathogens from samples positive based on mNGS and culture. However, culturable pathogens were also missed by mNGS because of
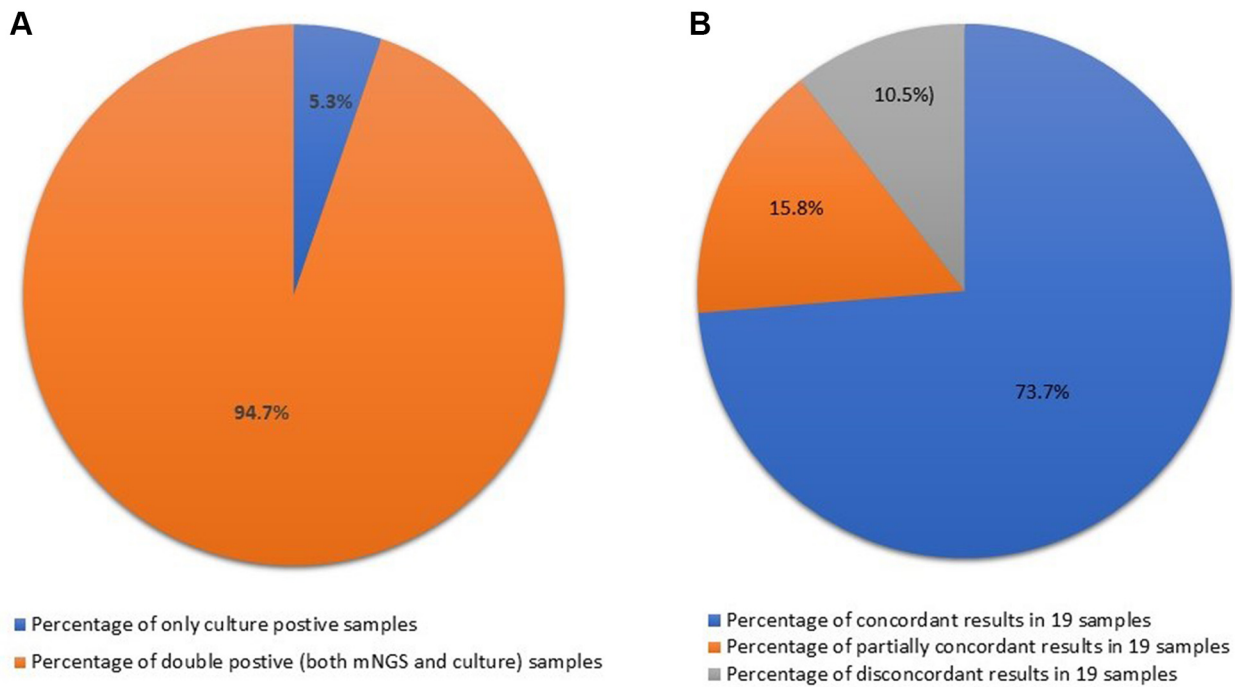
A

B

Figure 3. *Composite pie chart showing consistency between mNGS and conventional bacterial culture results. There was a 95% rate of double-positive results. Most results were concordant between the two methods (14 cases, 73.7%), with only two cases (10.5%) being discordant.*
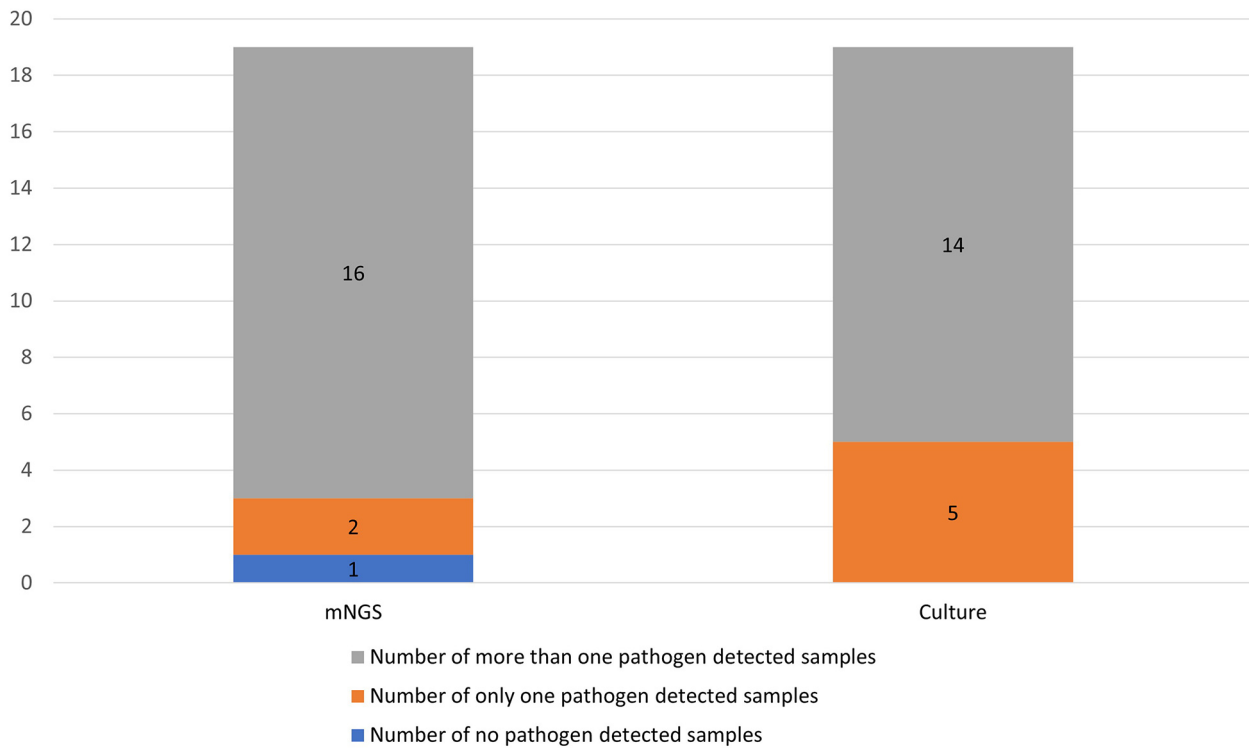
Figure 4. *Bar graph showing the proportions of multiple pathogens in SSTI cases as detected using mNGS and culture. mNGS identified multiple pathogen infections in 16 cases, which was more than those detected using conventional bacterial culture (14 cases).*

Table III. *Comparison of genotype and phenotype in antimicrobial resistance gene detection.*

ID	mNGS	AMR	Bacterial culture	Resistance to drug						
				SAM	CZ	TZP	CAZ	CRO	FEP	IPM
T2	*Proteus penneri*	bla_{CfxA}	*Proteus penneri*	I	R	S	S	S	S	R
	Citrobacter koseri	bla_{CepA}	*Citrobacter koseri*	S	S	S	S	S	S	S
	Bacteroides fragilis									
	Porphyromonas asaccharolytica									
	Anaerococcus vaginalis									
T3	*Pseudomonas aeruginosa*	bla_{OXA-50}	*Pseudomonas aeruginosa*	-	-	S	S	-	S	S
	Streptococcus gallolyticus	bla_{PDC}	*Enterobacter cloacae* complex	R	R	S	R	R	S	S
	Enterobacter hormaechei		*Clostridium cadaveris*	-	-	-	-	-	-	-
	Clostridium cadaveris									
T6	*Pseudomonas aeruginosa*	$bla_{OXA-903}$	*Pseudomonas aeruginosa*	-	-	R	R	-	I	S
	Corynebacterium resistens	bla_{PDC}	*Corynebacterium* spp.	-	-	-	-	-	-	-
T8	*Escherichia coli*	bla_{CMY}								
	Enterococcus faecium	bla_{OXA}	*Escherichia coli*	R	R	R	R	R	R	R
		bla_{KPC-17}								
T10	*Bacteroides fragilis*	$bla_{CepA-44}$	*Corynebacterium*	-	-	-	-	-	-	-
	Corynebacterium striatum	bla_{CfxA}	*Staphylococcus aureus*	-	-	-	-	-	-	-
	Staphylococcus aureus	$bla_{OXA-347}$								
T12	*Bacillus thuringiensis*	$bla_{OXA-851}$	*Serratia marcescens*	R	R	S	S	S	S	S
	Enterobacter hormaechei	bla_{Bla}	*Pseudomonas mosselii*	-	-	S	S	-	S	S
	Paraclostridium dentum	bla_{BCI}	*Pseudomonas aeruginosa*	-	-	S	S	-	S	S
	Serratia ureilytica									
	Enterobacter cancerogenus									
	Citrobacter amalonaticus									
T14	*Prevotella oris*	bla_{CfxA3}	*Klebsiella aerogenes*	R	R	R	R	R	S	S
	Finegoldia magna	bla_{LAP-1}								
	Granulicatella adiacens	bla_{ACT}								
	Streptococcus constellatus	bla_{TEM}								
	Enterococcus faecalis									
	Klebsiella pneumoniae									
	Schaalia odontolytica									
T15	*Pseudomonas aeruginosa*	bla_{PDC-64}	*Pseudomonas aeruginosa*	-	-	S	S	-	S	S
	Escherichia coli	$bla_{OXA-395}$	*Escherichia coli*	R	R	S	R	R	R	S
		bla_{CTX-M}								
		bla_{EC-5}								
T19	*Bacteroides fragilis*	bla_{CfiA18}	*Bacteroides fragilis*	R	-	R	-	-	-	-
	Klebsiella pneumoniae	bla_{TEM-20}	*Klebsiella pneumonia*	R	R	S	R	R	R	S
	Staphylococcus aureus	bla_{DHA}	*Staphylococcus aureus*							

mNGS: Metagenomic next-generation sequencing; AMR: antimicrobial resistance; SAM: Ampicillin/Sulbactam; CZ: Cefazolin; TZP: Piperacillin/Tazobactam; CAZ: Ceftazidime; CRO: Ceftriaxone; FEP: Cefepime; IPM: Imipenem; R: resistant; S: susceptible; I: intermediate.

Table IV. *Comparison of genotype and phenotype in SCCmec antimicrobial resistance gene detection.*

ID	mNGS	AMR	Bacterial culture	Resistance to drug						
				MET	OXA	TMP/SMX	CLI	VAN	TEC	LZD
T10	*Bacteroides fragilis*		*Corynebacterium* spp.		R	R	R	S	S	S
	Corynebacterium striatum		*Staphylococcus aureus*							
	Staphylococcus aureus									
T19	*Bacteroides fragilis*	SCC_{mecA}	*Bacteroides fragilis*	S	-	-	R	-	-	-
	Klebsiella pneumoniae	SCC_{mecR}	*Klebsiella pneumonia*	-	-	R	-	-	-	-
	Staphylococcus aureus	SCC_{mecI}	*Staphylococcus aureus*	R	R	R	R	S	S	S

mNGS: Metagenomic next-generation sequencing; AMR: antimicrobial resistance; MET: Metronidazole; OXA: Oxacillin; TMP/SMX: trimethoprim-sulfamethoxazole; CLI: Clindamycin; VAN: Vancomycin; TEC: Teicoplanin; LZD: Linezolid; R: resistant: S: susceptible.

the low read numbers or low relative abundance. For example, T3 culture revealed *Enterobacter cloacae* complex, but mNGS did not detect *Enterobacter hormaechei* due to low reads. T7 conventional bacterial culture (a bedsore wound) yielded *Acinetobacter pittii* but not mNGS. Second, resistant gene detection can't always correlate to specific pathogens precisely. For example, there are several samples in our study where there was simultaneous detection of *P. aeruginosa* and other non-*Pseudomonas* Enterobacterales using mNGS. However, if OXA beta-lactamase was detected, we cannot determine which pathogen carried the resistant gene until a phenotype report is issued. However, there are also some specific resistance genes, such as bla_{PDC}, which can be more confidently interpreted as originating from *P. aeruginosa*. This implies that interpretation plays a more and more significant role when mNGS is implemented in clinical conditions.

Conclusion

The mNGS plays a significant role in pathogen detection in soft tissue infection. It identifies more pathogens and can allow clinical practitioners to make precise patient treatment decisions. However, careful interpretation of the mNGS report is needed. We need further studies comparing conventional bacterial cultures with mNGS in SSTI.

Conflicts of Interest

The Authors declare no competing interests in relation to this study.

Authors' Contributions

Funding

References

1 Peetermans M, de Prost N, Eckmann C, Norrby-Teglund A, Skrede S, De Waele JJ: Necrotizing skin and soft-tissue infections in the intensive care unit. Clin Microbiol Infect 26(1): 8-17, 2020. DOI: 10.1016/j.cmi.2019.06.031

2 Miller LG, Eisenberg DF, Liu H, Chang CL, Wang Y, Luthra R, Wallace A, Fang C, Singer J, Suaya JA: Incidence of skin and soft tissue infections in ambulatory and inpatient settings, 2005-2010. BMC Infect Dis 15: 362, 2015. DOI: 10.1186/s12879-015-1071-0

3 Moffarah AS, Al Mohajer M, Hurwitz BL, Armstrong DG: Skin and soft tissue infections. Microbiol Spectr 4(4), 2016. DOI: 10.1128/microbiolspec.DMIH2-0014-2015

4 Huang Z, Wu Q, Fang X, Li W, Zhang C, Zeng H, Wang Q, Lin J, Zhang W: Comparison of culture and broad-range polymerase chain reaction methods for diagnosing periprosthetic joint infection: analysis of joint fluid, periprosthetic tissue, and sonicated fluid. Int Orthop 42(9): 2035-2040, 2018. DOI: 10.1007/s00264-018-3827-9

5 Malandain D, Bémer P, Leroy AG, Léger J, Plouzeau C, Valentin AS, Jolivet-Gougeon A, Tandé D, Héry-Arnaud G, Lemarié C, Kempf M, Bret L, Burucoa C, Corvec S, Centre de Référence des Infections Ostéo-articulaires du Grand Ouest (CRIOGO) Study Team: Assessment of the automated multiplex-PCR Unyvero i60 ITI® cartridge system to diagnose prosthetic joint infection: a multicentre study. Clin Microbiol Infect 24(1): 83.e1-83.e6, 2018. DOI: 10.1016/j.cmi.2017.05.017

6 Gu W, Miller S, Chiu CY: Clinical metagenomic next-generation sequencing for pathogen detection. Annu Rev Pathol 14: 319-338, 2019. DOI: 10.1146/annurev-pathmechdis-012418-012751

7 Shi Y, Wang G, Lau HC, Yu J: Metagenomic sequencing for microbial DNA in human samples: emerging technological advances. Int J Mol Sci 23(4): 2181, 2022. DOI: 10.3390/ijms 23042181

8 Zeng X, Wu J, Li X, Xiong W, Tang L, Li X, Zhuang J, Yu R, Chen J, Jian X, Lei L: Application of metagenomic next-generation sequencing in the etiological diagnosis of infective endocarditis during the perioperative period of cardiac surgery: a prospective cohort study. Front Cardiovasc Med 9: 811492, 2022. DOI: 10.3389/fcvm.2022.811492

9 Wang Q, Miao Q, Pan J, Jin W, Ma Y, Zhang Y, Yao Y, Su Y, Huang Y, Li B, Wang M, Li N, Cai S, Luo Y, Zhou C, Wu H, Hu B: The clinical value of metagenomic next-generation sequencing in the microbiological diagnosis of skin and soft tissue infections. Int J Infect Dis 100: 414-420, 2020. DOI: 10.1016/j.ijid.2020.09.007

10 Li H: Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv: 1303.3997, 2013. DOI: 10.48550/arXiv.1303.3997

11 Wexler HM: Bacteroides: the good, the bad, and the nitty-gritty. Clin Microbiol Rev 20(4): 593-621, 2007. DOI: 10.1128/CMR.00008-07

12 Polk BF, Kasper DL: Bacteroides fragilis subspecies in clinical isolates. Ann Intern Med 86(5): 569, 1977. DOI: 10.7326/0003-4819-86-5-569

13 Reyes C, Barthel ME: [Granulicatella spp]. Rev Chilena Infectol 32: 359-360, 2015. DOI: 10.4067/S0716-10182015000400017

14 Quénard F, Seng P, Lagier JC, Fenollar F, Stein A: Prosthetic joint infection caused by Granulicatella adiacens: a case series

and review of literature. BMC Musculoskelet Disord 18(1): 276, 2017. DOI: 10.1186/s12891-017-1630-1

15 Fulkerson HL, Nogalski MT, Collins-McMillen D, Yurochko AD: Overview of human cytomegalovirus pathogenesis. Methods Mol Biol 2244: 1-18, 2021. DOI: 10.1007/978-1-0716-1111-1_1

16 Piantadosi A, Mukerji SS, Ye S, Leone MJ, Freimark LM, Park D, Adams G, Lemieux J, Kanjilal S, Solomon IH, Ahmed AA, Goldstein R, Ganesh V, Ostrem B, Cummins KC, Thon JM, Kinsella CM, Rosenberg E, Frosch MP, Goldberg MB, Cho TA, Sabeti P: Enhanced virus detection and metagenomic sequencing in patients with meningitis and encephalitis. mBio 12(4): e0114321, 2021. DOI: 10.1128/mBio.01143-21

17 Binta B, Patel M: Detection of cfxA2, cfxA3, and cfxA6 genes in beta-lactamase producing oral anaerobes. J Appl Oral Sci 24(2): 142-147, 2016. DOI: 10.1590/1678-775720150469

18 Rogers MB, Bennett TK, Payne CM, Smith CJ: Insertional activation of cepA leads to high-level beta-lactamase expression in Bacteroides fragilis clinical isolates. J Bacteriol 176(14): 4376-4384, 1994. DOI: 10.1128/jb.176.14.4376-4384.1994

19 Chen Y, Tenover FC, Koehler TM: Beta-lactamase gene expression in a penicillin-resistant Bacillus anthracis strain. Antimicrob Agents Chemother 48(12): 4873-4877, 2004. DOI: 10.1128/AAC.48.12.4873-4877.2004

20 Colque CA, Albarracín Orio AG, Tomatis PE, Dotta G, Moreno DM, Hedemann LG, Hickman RA, Sommer LM, Feliziani S, Moyano AJ, Bonomo RA, K Johansen H, Molin S, Vila AJ, Smania AM: Longitudinal evolution of the pseudomonas-derived cephalosporinase (PDC) structure and activity in a cystic fibrosis patient treated with β-lactams. mBio 13(5): e0166322, 2022. DOI: 10.1128/mbio.01663-22