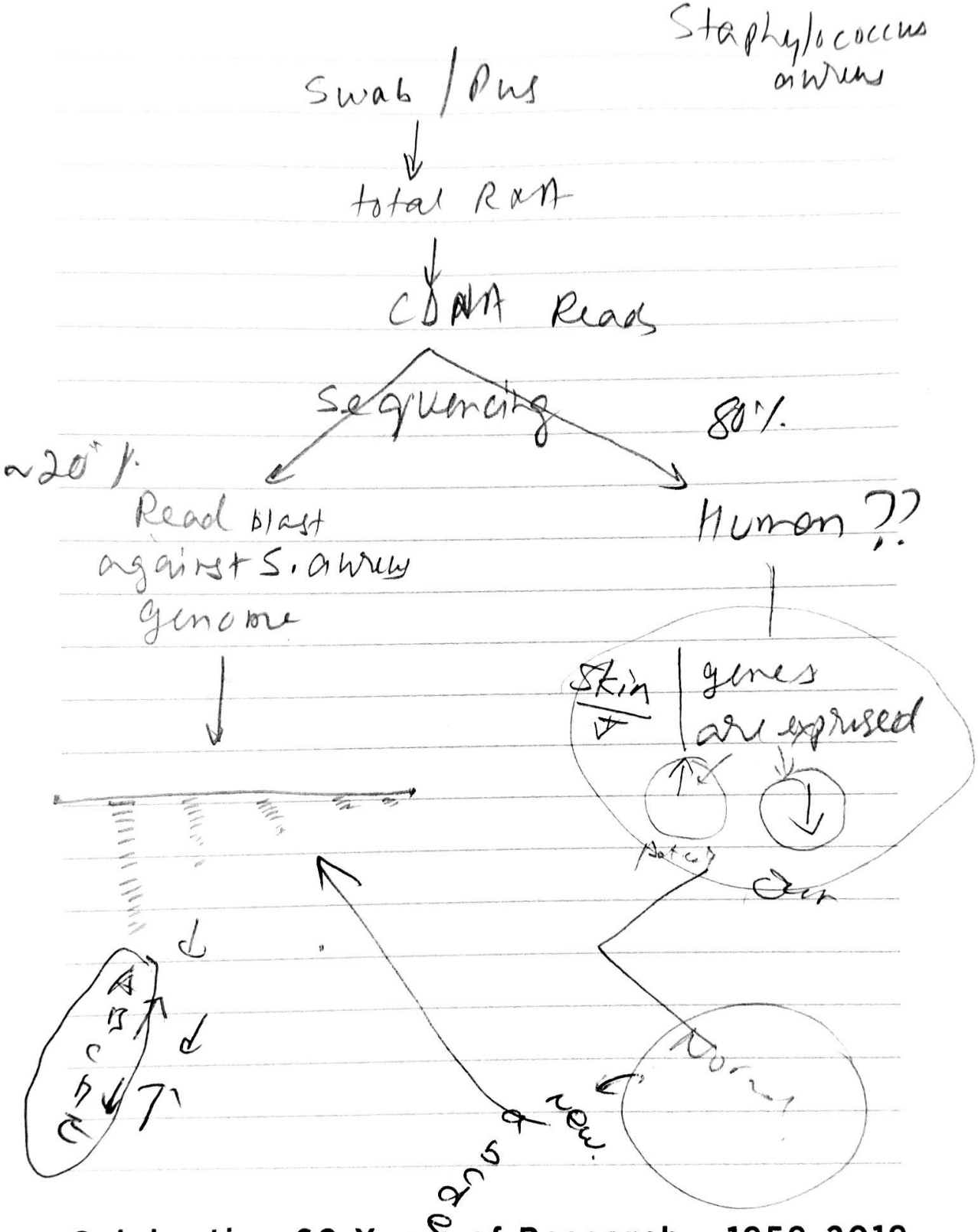
Staphylococcus aureus
Swab / Pus
total RNA
cDNA Reads
sequencing
~20%
80%
Read Blast against S. aureus genome
Human ??
Skin
genes are expressed

ECCMID 2018
P1255

In vivo expression of *Staphylococcus aureus* virulence genes in human skin and soft tissue infections

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ABSTRACT

Background: *Staphylococcus aureus*, the most common pathogen in skin and soft tissue infections (SSTI), harbors many well-characterized and putative toxin genes. However, the role of many of these genes in acute SSTIs is unknown. Our goals were to identify and quantify *in vivo* expression of virulence genes during SSTI. We hypothesized that virulence genes with a role in SSTIs are expressed during the disease process.

Materials/Methods: Fifty-three subjects presenting to urgent care and emergency departments with SSTI at two medical centers in Wisconsin, United States, were enrolled in the study. Purulent material was collected following surgical drainage of the infections using either a sterile cotton swab or syringe depending upon the volume of material present. Samples were immediately placed on dry ice and stored at -80ºC until processed. Total mRNAs were extracted from the collected materials, made into cDNA and then sequenced on a MiSeq platform (Illumina, Inc, San Diego, CA). BWA-MEM v. 0.7.13 was used to align fastq files to reference genomes MW2 (GenBank # BA000033.2) and USA300 (GenBank # CP000255.1). The cDNA counts and coverage were generated with SAMTOOLS v.1.3.

The study was approved by the Institutional Review Boards of both Marshfield Clinic and UW-Madison, WI; each subject had given informed written consent for the study.

Results: Out of 53 clinical samples, 30 grew *S. aureus* of which 20 were MRSA. cDNAs could be made from 24 clinical samples (16 MRSA, 7 MSSA, and 1 *S. capitis* positive samples). The average number of cDNA reads per sample was 5,432,957 of which 43,660 on average matched with a locus on a MW2 or USA300 genome.

Expression of as many as 858 *S. aureus* genes was observed of which 273 genes had at least 5 cDNA on average. Panton-Valentine leukocidin genes, *lukF* and *lukS*, were among the highly expressed genes with an average of 97.5 and 80.82 reads respectively followed by *rpoC* (DNA-directed RNA polymerase subunit beta), *fusA* (elongation factor G), *atl* (bifunctional autolysin), *rpoB* (DNA-directed RNA polymerase subunit beta), *ahpF* (alkyl hydroperoxide reductase subunit F) and *isaA* (immunodominant staphylococcal antigen A). Transcripts of additional genes included but not limited to, *lukSF*, *lukD*, *lukE*, (leukocidin genes); *hla*, *hlb*, *hld*, *hlgA*, *hlgB*, *hlgC* (hemolysins); fibronectin binding protein genes; and adhesins. Transcripts for genes not detected were *seb*, *sec*, *sed*, *see*, *seh*, *sej*, *eta*, *etb*, and *seg2*. Transcripts of seven housekeeping genes of the multilocus sequence typing scheme were identified although their averages (1.2 to 7) were low.

Conclusions: Interrogation of *in vivo S. aureus* gene expression in SSTI identified a number of known and unsuspected virulence genes, suggesting that *S. aureus* SSTI pathogenesis is likely due to the a variety of virulence proteins acting simultaneously.

STUDY AIMS

Our goals were to identify and quantify *in vivo* expression of *S. aureus* (Figure 1) virulence genes during SSTI.

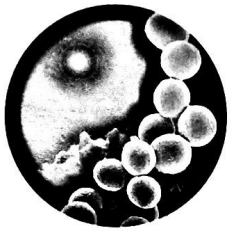

Figure 1. Scanning electron micrograph of *S. aureus*. Photo Courtesy: National Institute of Allergy and Infectious Diseases

MATERIAL AND METHODS

Processing of the samples: Pus or swab samples (Figure 2) from the SSTI were immediately transferred in a dry ice bucket and then stored in -80ºC until processed. Total RNA was extracted from the clinical samples by Trizol-chloroform method. RNA purity was measured by OD_{260}/OD_{280} ratio in a Rad SmartSpec spectrophotometer. mRNAs were converted to cDNA by the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). cDNAs were purified and concentrated using the QIAquick PCR Purification kit (QIAGEN). cDNA libraries were prepared for sequencing using the KAPA HyperPlus Library Preparation Kit (KAPA Biosystems). The libraries were pooled in equimolar ratios, diluted to 10 pM, and sequenced on a MiSeq using the V3 150 cycle reagent kit (Illumina®). Fastq files from read 1 and 2 were assembled.

Figure 2. An example of a skin and soft tissue infection from which clinical sample with a cotton swab was collected.

RESULTS

Table 1. # of cDNA reads at different steps of data processing

# of sequencing reads per sample	3,867,530	7,552,309	5,432,957
# of cDNA reads that matched to *S. aureus* USA300	255	247,463	43,660
# of sequences that matched to an annotated loci	0	77,765	11,345
# of loci that have at least one sequence coverage	0	2,372	668

Table 2. Total number and average cDNA counts of the eight most highly expressed genes.

lukF	Panton-Valentine leukocidin	1658	97.53
rpoC	DNA-directed RNA polymerase subunit beta	1507	88.65
fusA	Elongation factor G	1415	83.24
lukS	Panton-Valentine leukocidin	1374	80.82
atl	Autolysin	1317	77.47
rpoB	DNA-directed RNA polymerase subunit beta	1248	73.41
ahpF	Alkyl hydroperoxide reductase subunit F	1016	59.76
isaA	Immunodominant staphylococcal antigen A	1015	59.71

Table 3. Total number and average counts of additional known virulence genes.

Gene	Gene name	Total cDNA count	Average coun
clfB	Clumping factor B	809	47.59
clfA	Clumping factor A	288	16.94
fnbpA	Fibronectin-binding protein A	433	25.47
fnbpB	Fibronectin-binding protein B	278	16.94
hlgB	Gamma hemolysin B	186	10.94
hlgA	Gamma hemolysin A	32	1.88
hlgC	Gamma hemolysin C	110	6.47
sdrC	Serine-aspartate repeat-containing protein C	71	4.18
sdrE	Serine-aspartate repeat-containing protein E	46	2.71
sdrD	Serine-aspartate repeat-containing protein D	44	2.59
lukD	Leucotoxin D	23	1.35
lukE	Leucotoxin E	21	1.24

RESULTS (continued)

Fifty three subjects with SSTI who presented as outpatients/inpatients at Marshfield Clinic and to the emergency department at UW Madison were enrolled.

Demographics and microbiological data: 30 subjects with SSTI yielded *S. aureus* on a blood agar plate from the wound sample. Only eight SSTI were large enough to yield pus samples while wounds of the remaining 22 subjects were small and collected by a swab. The average age of the *S. aureus*-positive subjects was 42.33 years and 17 of the 30 (56.67%) were male. Twenty *S. aureus* was *mecA* positive. The 23 *S. aureus* were represented by 11 *spa* types (t008=17, t024=2, and t002, t068, t078, t159, t216, t723, t1578, t4045, t7712 one each). cDNA could be made only from 23 *S. aureus*-positive samples and one *S. capitis* positive-sample (Figure 3).

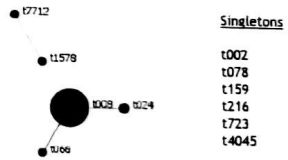

Figure 3. Based Upon Repeat Pattern (BURP) analysis of the 11 *spa* types. Spa-CC t008 was the major clonal complex and there were six singletons.

DISCUSSION

Our preliminary analysis of the *S. aureus* genes expressed *in vivo* in SSTI showed that both Panton-Valentine leucocidin is perhaps the most significant toxin in SSTI.

Identification of immunodominant staphylococcal antigen A (*isaA*) and autolysin (*atl*) genes needs to further investigated in SSTI.

Staphylococcal superantigen like (*ssl*) and putative virulence genes such as lipoprotein lipases (*lpl*) genes were identified infrequently.

Our approach to directly measure the *S. aureus* gene expression in vivo will help identify the combination of virulence genes at interplay in SSTI.

FUNDING

The study was supported by funding from Marshfield Clinic Research Institute and University of Wisconsin, Madison.