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Final Abstract Number: 42.252
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wgMLST as a standardized tool for assessing the quality of genome assembly data

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Background: Continued advances in next-generation sequencing (NGS) technologies is accompanied with the development of many whole genome assembly approaches to convert the small sequences (reads) into larger regions (contigs/scaffolds). However, none of these is perfect. Up to now, genome assembly data is compared under standard statistics (N50, coverage, contig sizes, number bases etc.) and there is no commonly accepted and standardized method for comparison and assessing the assembly data

Methods & Materials: The raw data for S. aureus SA957 (paired end sequencing - SRR497751) produced by Illumina platform have been download from European Nucleotide Archive. Software such as Tadpole, Velvet, CLC genomic workbench, SeqMan NGen for de novo assembly and Bowtie2, BWA and CLC genomic workbench for mapping to reference have been used under default options to produce contigs/consensus. The assessing of the quality of genome assembly have been performed with using wgMLST implemented in SeqSphere software (Ridom). Concordace of genome assembly data was estimated with Rand index.

Results: Seven genomes have been assembled using de novo and reference mapping methods. The basic statistics of de novo assembled data showed that CLC genomic workbench tool gave the best assembly sequence. Statistic parameters of Tadpole assembly were less well in comparison with other (Table 1).

wgMLST analysis based on 2787 genes was performed on seven assembled genomes. As a result, CLC, Velvet and SeqMan Ngen assembly allowed to determine more than 2500 genes while Tadpole assembly with average contig length 1378 bp could identify 1560 out 2787 genes (table 2). Reference mapping assembly revielaed hign concordance (98-99%) between results. Minimum spanning tree clustered reference mapping results (picture1).

Conclusion: A standardized gene-by-gene wgMLST approach allows assessing not only the quality but also quantitative estimation of genome assembly data. Based on this approach the genomes assembled with different software can be compared and clustered

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Dependence of the genetic relatedness between isolates on the size of sequencing genes in MLST analysis

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Background: Multilocus-sequence typing (MLST) based on sequencing internal part of well-chosen housekeeping genes/loci has become the method of choice for typing of epidemiologically important strains.

Methods & Materials: 5 MLST schemes have been used for typing of 3496 of E.col genomes – whole gene MLST (645-2415bp), standard MLST(452-536bp) and 3 modified MLST: +100bp MLST (552-636bp), -100bp MLST (352-436bp), -200bp MLST (252-336 bp). Clonal complexes (CC) were defined as groups with six identical alleles and minimum 5 genotypes. Discriminatory power and concordance were estimated based on Simpson and adj. Wallace indices.

Results: In 3061 cases, genes/alleles have been determined successfully for all five MLST schemes. Discriminatory index for each genes (alleles) and combination of 7 genes (alleles) is presented in Table 1.

All MLST schemes concordance calculated with adj. Wallace index is presented in Table 2.

Based on CC parameters there have been 44 CC with 1951, 23CC with 1883, 17CC with 1720, 12 CC with samples and 4 with 1544 samples in gene MLST, +100bp MLST, standard MLST, -100bp MLST and -200bp MLST respectively.

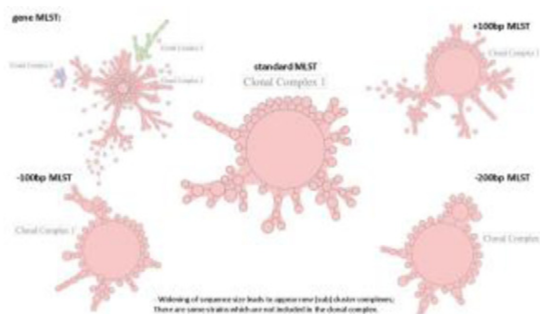
Table 1

MLST	adk	fumC	gyrB	icd	mdh	purA	recA	7genes
geneMLST	0.898	0.954	0.962	0.962	0.924	0.931	0.934	0.982
+100bpMLST	0.898	0.922	0.933	0.924	0.918	0.88	0.863	0.974
standardMLST	0.887	0.918	0.928	0.92	0.911	0.868	0.85	0.971
-100bpMLST	0.885	0.917	0.925	0.905	0.908	0.773	0.76	0.971
-200bpMLST	0.879	0.902	0.918	0.901	0.865	0.736	0.312	0.969

Table 2

	geneMLST	+100bpMLST	standardMLST	-100bpMLST	-200bpMLST
geneMLST		1.000	1.000	1.000	1.000
+100bpMLST	0.678		1.000	1.000	1.000
standardMLST	0.603	0.890		0.999	0.999
-100bpMLST	0.592	0.872	0.979		1.000
-200bpMLST	0.549	0.809	0.908	0.927	

As an example of transformation of Cluster Complex according to the sequencing size is shown in Figure 1



Conclusion: Artificial methods of subspecies typing gives a relative picture of the genetic relationship and clonal structure of microorganisms.

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In silico comparison of different PFGE and wgMLST

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Background: Pulsed field gel electrophoresis (PFGE) is acknowledged to be the 'gold standard' for the typing of strains of a number of bacterial species, including E. coli, and is used widely in clinical settings (van Belkum A., 2007).

Methods & Materials: In silico PFGE analysis of 138 complete E.coli genomes using classical XbaI and 5 other enzymes (Sse8647I, ApaI, AclN, SrfI and SdiI) have been performed by Geneious (Biomatter). Images with gel pattern have been analyzed by TotalLab 1D (Nonlinear Dynamics) to produce band matrix. wgMLST scheme with 2216 loci have created with SeqSpere (Ridom). Discriminatory power and concordance between different PFGE and wgMLST have been estimated based on Simpson and adj.Rand and Wallace indices.

Results: 138 genomes of E.coli have been used to produce different PFGE and wgMLST patterns. Sites of restriction, band (loci) numbers and discriminatory power are presented in Table 1.

Table 2

Enzymes for PFGE	adj.Rand	WallacewgMLST ->PFGE	WallacePFGE -> wgMLST
XbaI	0.809	1	0.737
Sse8647I	0.816	0.998	0.749
ApaI	0.818	0.998	0.75
AclN	0.821	1	0.751
SrfI	0.808	0.998	0.738
SdiI	0.805	1.0	0.741

The concordance between different PFGEs and wgMLST calculated on cluster complex is presented in Table 2.

Conclusion: PFGE using different restriction enzymes, which have different site restriction and produce different number (27-130) of band, have not shown the advance in discriminatory power and concordance with wgMLST.

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Hemophagocytic Lymhohistiocytosis(HLH) secondary to infections- Experiance at a tertiary care centre

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Background: **Background:** Hemophagocytic Lymphohistiocytosis (HLH) is a rare potentially life-threatening disorder characterized by immune dysregulation, overwhelming immune activation and inflammation. This condition can occur as primary or secondary to infections, autoimmune diseases and malignancies. HLH secondary to infections is an important clinical entity especially in tropical countries. We report our experience of HLH from our hospital.

Methods & Materials: **Materials and Methods:** This is a retrospective analysis of clinical information of patients presented to our hospital between March 2012 and November 2015. All fulfilled the revised criteria of HLH 2004. ackground:white'> This condition can occur as primary or secondary to infections, autoimmune diseases and malignancies. HLH secondary to infections is an important clinical entity especially in tropical countries. We report our experience of HLH from our hospital.

Results: **Results:** Total 5 cases were segregated with secondary HLH diagnosis. The mean age at diagnosis was 34 years (with a

Table 1

Enzyme	Recognition sequence	Median of band number	95%CI	#different types	Discriminatory index	Confidence interval (95% CI)
XbaI	TĈTAGA	39	27-51	131	0.999	[0.998 - 1.0]
Sse8647I	AGĜWCCT	73	57-104	131	0.999	[0.998 - 1.0]
ApaI	GGGCCĈ	77	62-130	132	0.999	[0.998 - 1.0]
AclN	AĈTAGT	79	53-95	129	0.999	[0.998 - 1.0]
SrfI	GCCCĜGGC	51	41-65	131	0.999	[0.998 - 1.0]
SdiI	GGCCNNNNN̂GGCC	38	31-66	129	0.999	[0.998 - 1.0]
wgMLST	2216 loci	-	-	129	0.999	[0.998 - 1.0]