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Bacterial whole genome sequencing as powerful tool for hospital molecular epidemiology: *Acinetobacter baumannii* as a model

Abdalla Ahmed[1,2*], Bashir Sirag[1], Fahad Raees[1], El-Shiekh Kidir[3], Tayseer Ali[2], Mohammad Atiqur Rahman[1], Sami Ashgar[1], Abeer Barhameen[1], Abdelrahman Elsawy[4], Asmaa Mostafa[4] and Sheerin Shalam[2]

[1]Department of Microbiology, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia

[2]Microbiology Unit, Department of Laboratory and Blood Bank, King Abdullah Medical City, Makkah, Saudi Arabia

[3]Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Saudi Arabia

[4]Medical Microbiology Department, Al-Noor Specialist Hospital, Makkah, Saudi Arabia

Abstract

In this study, the power of whole genome sequence data in the characterization of 40 clinical isolates of Acinetobacter baumannii was explored. The aim is this study is to demonstrate how bacterial genomic data can be analyzed using easy and semi-automated bioinformatics tools to find answers to clinical microbiology diagnostic problems. These bioinformatics tools can use assembled or un-assembled genome data for species identification, prediction of antibiotics resistant mechanisms and genotyping. In the studied sample, genomics data was successfully used to correct species identification and confirm resistant phenotypes. In addition, multi locus sequence types with three novel sequence types were determined. In conclusion, next generation whole genome sequence data with minor improvement and customization of currently available bioinformatics tools will shortly change the shape of clinical microbiology laboratory services.

Introduction

Recently, the use of Next Generation Sequencing technologies provided unprecedented amount of microbial genomics data. The availability of these genome data is rapidly changing our understanding of microbial behavior, interactions, virulence, antibiotic resistance and genotyping. With the availability of this sequencing technology, it become so popular and many studies have been published in the last few years reporting the whole genome sequences of clinically important bacterial species such as *Klebsiella pneumoniae* and *Acinetobacter baumannii* [1-4]. Whole genome sequence data has been used to study antibiotics resistance [1-3, 5], molecular epidemiology [4,6] and comparative genomics [7,8]. With few exceptions most of these reports were research articles describing bacterial whole genome sequencing with complex data presentation, complex bioinformatics workflow and many bioinformatics terms. These type of research articles are difficult to understand by general readers, such as clinical microbiology practitioners, without special training in bioinformatics. This knowledge barrier gives wrong impression about the unlimited applications and endless possibilities of using whole genome sequence data in routine clinical microbiology laboratory, which can be directly applied to routine microbiology laboratory. However, a good number of research articles has also been recently published describing a powerful, user-friendly and publicly accessible web-tools with direct applications in clinical microbiology laboratory [9-12]. Only basic knowledge of bioinformatics is needed for the run of these tools and for the interpretation of the generated reports. These tools are extremely useful in microbial characterization and genotyping, and with more minor customization it will become part of the routine microbiology workflow [13].

In this study, we describe original whole genome data used for the study of the molecular epidemiology of *Acinetobacter baumannii* in tertiary referral hospital in Saudi Arabia. DNA sequencing and data analysis were all done in clinical microbiology departments with no special bioinformatics trained staff. The sequencing results and data analysis will be presented as simple as possible and no complex terms will be mentioned. The aim of this study is to encourage microbiologist to start using these rapidly evolving tools for uncovering the fascinating world of microbial genomics.

Materials and methods

Acinetobacter baumannii isolates

During an apparent outbreak of multidrug resistant *A. baumannii* during 2013, 40 clinical isolates from two hospitals in Makkah, Saudi Arabia, were studied using next generation whole genome sequencing. *A. baumannii* clinical isolates were obtained from both medical and surgical wards including different intensive care units at King Abdullah Medical City and Al-Noor Specialized Hospital in Makkah, Saudi Arabia. Identification and susceptibility testing in the two hospitals were done routinely using Siemens MicroScan® WalkAway®-96 Plus

Correspondence to: Abdalla Ahmed, Department of Microbiology, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia, Tel: +966543031577; **E-mail:** aoaahmed@uqu.edu.sa

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System (Siemens, Germany). Clinical isolates were stored at -20°C in 10% glycerol peptone water. DNA sequencing and data analysis were done in the Department of Microbiology, College of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.

DNA extraction and genome sequencing

Bacterial cells from fresh cultures were used for DNA extraction. Cells were harvested from overnight cultures and washed using sterile Tris EDTA buffer (TE) pH 8.0 in 2 mL screw cap tubes and then re-suspended in 500 µl TE buffer. The cell wall was disrupted using 0.1 mm glass beads in BioSpec Mini-Beadbeater-16 (BioSpec Inc., USA) for 5 minutes then cooled in ice for additional 5 minutes. Aqueous layer containing DNA was separated from proteins and cell debris using two Phenol/Chloroform (1:24 pH 8.0) extractions. DNA was then precipitated by iso-propanol, washed by 70% ethanol, dried at room temperature and re-suspended into 35 µl TE buffer pH 8.0. The quantity and quality of the isolated DNA was determined using Qubit® (Invitrogen, Applied Bio systems, USA), and Agilent Bio analyzer 2100 using 1000 DNA Chip (Agilent Inc., USA).

Library preparation for DNA sequencing

A. baumannii DNA libraries for whole genome sequencing were prepared using Illumina NexteraXT Library Preparation Kit and samples were barcoded using NexteraXT Index Kit (Illumina Inc., USA). DNA sequencing libraries were prepared using 1 ng input genomic DNA, and validated and quantified directly without normalization using Agilent Bio analyzer 2100 High Sensitivity DNA Chip (Agilent Inc., USA). *A. baumannii* genomes were sequenced in Illumina MiSeq using pair ends protocol and version-3 600 cycles kit. The quality of the pair ends sequence reads were checked by FastQC before sequence assembly (BaseSpace Labs, Illumine Inc., USA).

Genome assembly

De novo assembly of *A. baumannii* genomes were done using DNASTAR SeqMan NGen 12.3.1 (DNASTAR, Madison, USA) using default settings, which include terming of low quality sequences ends.

Species identification and sequence-based typing

Assembled genomes were used for 16s rRNA based species identification and Multi Locus Sequencing Typing (MLST). 16s species identification and MLST were done using SpeciesFinder 1.0 Server and MLST 1.8 Server from the Center for Genomics Epidemiology [10,14].

Predication of antibiotics resistance mechanisms

In this study, antibiotics resistance mechanisms were predicted using multiple available tools. The antibiotics resistant genes were predicted using ResFinder Server from the Center for Genomics Epidemiology and SRST2 (BaseSpace Labs, Illumine Inc., USA) [11,15]. Antibiotics resistant genes, in selected strains of *A. baumannii,* were also predicted using Resistance Gene Identifier (RGI), which is designed and developed by the laboratories of Drs. Gerry Wright and Andrew G. McArthur of McMaster University [16,17]. The RGI provides a preliminary annotation of DNA or protein sequence(s), based upon the data available in the Comprehensive Antibiotic Resistance Database (CARD). With all above mentioned tools, only genes with >80% template coverage and minimum match percentage of 90% were reported.

Results

MicroScan® WalkAway®-96 Plus System was able to identify all isolates as *A. baumannii*. The antimicrobial susceptibility testing showed resistance of all isolates to meropenem and imipenem. Half of the isolates were resistant to colistin with (minimum inhibitory concentration of equal to or higher than 4 µg/ml.

Thirty-nine isolates were successfully sequenced and only one isolate failed sequencing. The summary of the *de novo* assembly is shown in table 1. Based on the 16s rRNA sequences, 36 isolates were correctly identified as *A. baumannii* using SpeciesFinder tool [14]. One isolate was found to be *Stenotrophomonas maltophilia* and another isolate was identified as *Acinetobacter* species. One of the isolates was identified with low confidence as *Serratia marcescens* (only 33% of reads were aligned to species level). The three non-*A. baumannii* isolates were excluded from analysis in this study.

Six known sequence types were identified (ST195, ST218, ST208, ST281, ST557 and ST884). ST195 was the most common sequence type

Table 1. Summary of assembly statistics of whole genome sequencing of 36 clinical isolates of *A. baumannii.* All isolates were sequenced in the same MiSeq sequencing run using pair-end library with 600 sequencing cycles. Sequencing coverage were variable, but sequence data were enough for isolates characterization. Genome assembly was done using DNASTAR SeqMan NGen 12.3.1 (DNASTAR, Madison, USA).

Strain ID	Assembled Sequences	Contigs number	Contigs >2K	Contig N50	Average Coverage	Average Quality
AB254	539471	123	114	61000	35	35
AB250	1506011	124	65	135000	93	36
AB393	404991	136	117	48000	24	35
AB263	2866840	142	62	122000	180	35
AB578	393169	146	133	52000	23	36
AB466	793272	148	116	64000	52	35
AB252	836036	169	135	47000	49	36
AB487	673304	175	127	60000	42	35
AB601	431553	181	142	57000	27	35
AB559	543683	184	152	44000	32	35
AB469	359745	198	155	39000	21	36
AB596	566539	216	164	41000	35	36
AB354	767629	228	190	37000	51	35
AB309	1400536	244	109	69000	74	36
AB357	373552	249	204	34000	24	35
AB388	676161	286	228	26000	43	36
AB552	349431	293	253	27000	20	36
AB492	539559	319	272	22000	29	36
AB321	273488	321	293	20000	17	35
ABNH8	254274	333	319	19000	16	35
ABNH6	462054	452	411	16000	23	35
AB363	303315	466	427	12000	19	36
AB314	168044	509	484	7000	10	36
AB595	157553	513	500	8000	9	35
AB543	537413	523	362	14000	23	36
AB576	222018	541	489	8000	14	35
AB217	552147	551	435	12000	29	36
AB558	269568	575	501	9000	17	35
AB432	404823	693	496	9000	24	36
ABNH1	261285	695	602	6000	16	35
ABNH2	294471	766	627	6000	18	35
ABNH7	292329	770	636	6000	18	35
AB417	267777	775	634	6000	16	36
AB462	863503	782	494	8000	43	36
ABNH4	292141	809	661	5000	18	35
ABNH3	218238	816	718	4000	13	35
Average	558776	401	329	32222	33	35
Maximum	2866840	816	718	135000	180	36
Minimum	157553	123	62	4000	9	35

accounting for 47% of all *A. baumannii* isolates (17/36). Three novel sequence types were found among seven isolates collected from both hospitals (Table 2). These novel sequence types were submitted to the *A. baumannii* MLST database (http://pubmlst.org/ abaumannii/) [18]. The new novel sequence types were designated as ST1286, ST1287 and ST1288 (Table 2). ST218 was found in more than half (4/7) of *A. baumannii* isolates from AL-Noor Hospital, in which two of the remaining isolates had two different novel sequence types and the third one was found to be ST195, which was the major prevalent sequence types as described above. No clear correlations were noticed between sequence types and certain wards, specimens, or infection date. No correlation was identified between sequence types and biotypes as determined by the MicroScan® WalkAway®-96 Plus System (Table 2).

Bioinformatics tools predicted the presence of large number of resistant genes known to confer resistant to a wide range of major antibiotics classes. Resistant genes to Aminoglycoside, Beta-lactam, Fluoroquinolone, Macrolide, Lincosamide, Phenicol, Sulphonamide and Tetracycline were detected in most of the isolates (Table 3 and 4). OXA-51-like carbapenemases (OXA-66) was found in all isolates, followed by OAX-23, which was found in more than 90% of the isolates. OXA-40-like (OXA-72) was found in only 5 strains. No other Carbapenem-hydrolyzing OXA-type, NDM-type, VIM-type, or IMP-type carbapenemases were detected in our study population (Table 3 and 4).

Discussion

Good results are always obtained when comparing genomic data with phenotypic results generated by commercially available microbiology automated identification and antimicrobial susceptibility testing systems such as Microscan or Vitek2. These systems provide acceptable and reliable data of species identification and antibiotics

Table 2. Clinical data, antibiotics susceptibility results, biotypes and multi locus sequences types of 36 clinical isolates of *A. baumannii*. Antibiotics susceptibility and biotypes were determined using MicroScan® WalkAway®-96 Plus System. Sequence types were predicted using whole genome assembled contigs using the MLST server from the Center for Genomics Epidemiology.

Isolate Number	Site	Ward	Date	Colistin	Imipenem Susceptibility	Meropenem Susceptibility	Biotype	Sequence type genes alleles							Sequence Type
								cpn60	gdhb	glta	gpi	gyrb	reca	rpod	
AB217	Wound swab	ER	Oct 2012	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_97	gyrb_3	reca_2	rpod_3	ST-208
AB250	Sputum	ICU	Oct 2012	Resistant	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_97	gyrb_3	reca_2	rpod_3	ST-208
AB252	sputum	ICU	Oct 2012	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB254	Wound swab	Surgical	Nov 2012	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB263	sputum	ICU	Nov 2012	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB309	Blood	ER	Jan 2013	Resistant	Resistant	Resistant	60770	cpn60_2	gdhb_3	glta_1	gpi_100	gyrb_35	reca_2	rpod_3	Novel 1 (ST1286)
AB314	Blood	ICU	Jan 2013	Susceptible	Resistant	Resistant	62720	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB321	Wound Swab	ICU	Jan 2013	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB354	Sputum	ICU	Feb 2013	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB357	Rectal Swab	ICU	Feb 2013	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_99	gyrb_17	reca_2	rpod_3	ST-281
AB363	Rectal Swab	ICU	Feb 2013	Susceptible	Resistant	Resistant	62730	cpn60_1	gdhb_2	glta_18	gpi_83	gyrb_87	reca_28	rpod_71	ST-884
AB388	Rectal Swab	CCU	Feb 2013	Resistant	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB393	Sputum	CCU	Feb 2013	Susceptible	Resistant	Resistant	66730	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB417	Blood	HEAM	Feb 2013	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_100	gyrb_35	reca_2	rpod_3	Novel 1 (ST1286)
AB432	Rectal Swab	CCU	Mar 2013	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_66	gyrb_38	reca_2	rpod_3	ST-557
AB462	Blood	SICU	Mar 2013	Resistant	Resistant	Resistant	62743	cpn60_2	gdhb_3	glta_1	gpi_100	gyrb_35	reca_2	rpod_3	Novel 1 (ST1286)
AB466	Rectal Swab	SICU	Mar 2013	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_66	gyrb_38	reca_2	rpod_3	ST-557
AB469	Urine	LTCU	Apr 2013	Susceptible	Resistant	Resistant	62720	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB487	Urine	CCU	Apr 2013	Resistant	Resistant	Resistant	2062770	cpn60_2	gdhb_3	glta_1	gpi_97	gyrb_3	reca_2	rpod_3	ST-208
AB492	Blood	CCU	Apr 2013	Susceptible	Resistant	Resistant	24620	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB508	Blood	ICU	May 2013	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB543	Blood	ICU	Jul 2013	Susceptible	Resistant	Resistant	62720	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB552	Blood	LTCU	Jul 2013	Susceptible	Resistant	Resistant	2062620	cpn60_2	gdhb_3	glta_1	gpi_100	gyrb_35	reca_2	rpod_3	Novel 1 (ST1286)
AB558	Sputum	ICU	Jul 2013	Susceptible	Resistant	Resistant	62720	cpn60_2	gdhb_3	glta_1	gpi_100	gyrb_35	reca_2	rpod_3	Novel 1 (ST1286)
AB559	Rectal Swab	ICU	Jul 2013	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_99	gyrb_17	reca_2	rpod_3	ST-281
AB576	Rectal Swab	ICU	Aug 2013	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB578	Blood	ER	Aug 2013	Susceptible	Resistant	Resistant	66624	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB595	Rectal Swab	ICU	Sep 2013	Resistant	Resistant	Resistant	62760	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB596	Urine	ER	Sep 2013	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
AB601	Rectal Swab	ICU	Sep 2013	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195
ABNH1	Blood	ICU	Apr 2014	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_102	gyrb_3	reca_2	rpod_3	ST-218
ABNH2	Tissue	Surgical	Apr 2014	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_102	gyrb_3	reca_2	rpod_3	ST-218
ABNH3	Tissue	Surgical	Apr 2014	Resistant	Resistant	Resistant	62730	cpn60_2	gdhb_115	glta_1	gpi_102	gyrb_3	reca_2	rpod_3	Novel 1 (ST1287)
ABNH4	Wound swab	Surgical	Apr 2014	Resistant	Resistant	Resistant	62770	cpn60_2	gdhb_3	glta_1	gpi_102	gyrb_3	reca_2	rpod_3	ST-218
ABNH6	Wound swab	Surgical	Apr 2014	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_102	gyrb_3	reca_2	rpod_3	ST-218
ABNH7	Wound swab	Surgical	Apr 2014	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_117	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	Novel 1 (ST1288)
ABNH8	Wound swab	Surgical	Apr 2014	Susceptible	Resistant	Resistant	62730	cpn60_2	gdhb_3	glta_1	gpi_96	gyrb_3	reca_2	rpod_3	ST-195

Table 3. Antibiotics resistant genes in *Acinetobacter baumanniias*. SRST2 version 1.0.0 (Illumina BaseSpace) was used to predict the resistant genes using ARG-ANNOT database. Only genes detected with >90% coverage are reported.

Antibiotics Resistant Genes																									
Strain ID	**aac6-Ib**	**Aac3**	**AacA4**	**aada1**	**AadA2**	**aph3**	**AphA6**	**armA**	**BlaA1**	**BlaA2**	**CatB8**	**Mb1**	**MphE**	**MsrE**	**OXA-23**	**OXA-66**	**OXA-72**	**StrA**	**StrB**	**Sul1**	**Sul2**	**TEM-ID**	**TetB**	**Zn dependent hydrolase**	**bla class C**
AB217	ND	ND	ND	ND	ND	D	D	ND	ND	D	ND	D	ND	ND	D	D	ND	D	D	ND	ND	D	D	D	D
AB252	D	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB254	D	ND	ND	D	ND	D	D	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB263	D	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB309	D	D	ND	ND	ND	ND	ND	ND	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB314	D	D	ND	D	ND	D	D	D	ND	D	D	D	D	D	ND	D	ND	D	D	D	ND	ND	D	D	D
AB321	ND	ND	D	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB354	ND	ND	D	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB363	ND	ND	ND	ND	ND	ND	D	ND	ND	ND	ND	D	ND	ND	D	D	ND	D	D	D	D	ND	ND	D	D
AB388	D	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB393	D	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB417	D	D	ND	ND	ND	ND	ND	ND	ND	D	ND	D	D	D	ND	D	D	D	D	D	D	ND	D	D	D
AB432	ND	ND	ND	ND	ND	ND	D	ND	ND	D	ND	D	D	D	D	D	ND	D	D	D	D	ND	D	D	D
AB462	D	D	ND	ND	ND	ND	ND	ND	D	D	ND	D	D	D	ND	D	D	D	D	D	D	ND	D	D	D
AB466	ND	D	ND	ND	ND	ND	D	ND	ND	D	ND	D	D	D	D	D	D	D	D	D	D	ND	D	D	D
AB469	D	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	ND	ND	D	D	D	D
AB487	ND	ND	ND	ND	ND	ND	D	ND	ND	D	ND	D	D	D	D	D	ND	D	D	ND	ND	ND	D	D	D
AB492	D	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB543	ND	ND	ND	ND	ND	D	ND	ND	ND	D	ND	D	ND	ND	D	D	ND	D	D	ND	ND	D	D	D	D
AB552	D	D	ND	ND	ND	ND	ND	ND	ND	D	ND	D	D	D	D	D	D	D	D	ND	D	ND	D	D	D
AB558	D	D	ND	ND	ND	ND	ND	ND	ND	D	ND	D	D	D	D	D	D	D	D	D	D	ND	D	D	D
AB559	ND	D	ND	D	ND	D	ND	ND	ND	D	ND	D	ND	ND	D	D	ND	D	D	D	D	D	D	D	D
AB576	D	ND	ND	D	ND	D	D	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB578	D	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB595	D	ND	ND	ND	ND	D	ND	D	ND	ND	ND	D	D	D	D	D	ND	D	D	ND	ND	D	D	D	D
AB596	D	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
AB601	ND	ND	D	D	ND	D	ND	D	ND	D	D	D	D	D	D	D	ND	D	D	D	ND	D	D	D	D
ABNH1	ND	ND	ND	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	ND	D	D	D	D	D	D	D	ND
ABNH2	ND	ND	ND	ND	ND	D	D	D	ND	ND	ND	D	D	D	D	D	ND	D	D	D	D	D	D	D	ND
ABNH3	ND	ND	ND	ND	ND	D	D	D	ND	D	ND	D	D	D	D	D	ND	D	D	ND	ND	D	D	D	ND
ABNH4	ND	ND	ND	ND	ND	D	ND	D	ND	D	ND	D	D	D	D	D	ND	D	D	ND	ND	D	D	D	D
ABNH6	ND	ND	ND	ND	ND	D	D	D	ND	ND	ND	D	D	D	D	D	ND	D	D	ND	ND	D	D	D	D
ABNH7	ND	ND	ND	ND	ND	ND	ND	D	ND	ND	ND	D	D	D	D	D	ND	D	D	ND	ND	ND	D	D	ND
ABNH8	ND	ND	ND	ND	ND	ND	ND	D	ND	D	ND	D	D	D	D	D	ND	D	D	ND	ND	ND	D	D	D
				D	Resistant Gene Detected									ND	Resistant Gene not detected										

susceptibility profiling for routine clinical setting and offer excellent assistance in patient management. However, in case of atypical strains or in case of hospital outbreaks, these phenotypic data usually has limited resolution required for understanding complex resistant phenotypes, outbreak clones, colonization dynamics and species identity of poorly differentiated organisms. In routine hospital outbreak investigation, many molecular biology techniques are used to understand the genetic basis of resistant to a single antibiotic and/or to determine the genotype(s) responsible for the outbreak. These molecular tools include DNA sequencing of tens and hundreds of target genes to search for resistant and typing markers to resolve the resistant mechanisms and to determine the genotype of each outbreak isolate.

In the presence of currently available next generation technology, huge sequence data become available for each clinical isolates, which can provide immediate microbiology diagnostic solutions. In the current study, we demonstrate power of currently available bioinformatics tools that are capable of analyzing whole genome sequence data and provide total clinically relevant data within acceptable short time frame that can influence patient care. Species identification and antimicrobial susceptibility testing, which are the most important and routine duty of the clinical microbiology laboratory, can be determined directly as soon as the bacterial genomic data become available from the DNA sequencing platform [11,15]. However, there is still need for better bioinformatics tools that directly handle DNA sequence data and perform a sequence of automated bioinformatics workflow followed by automated data interpretation tools to generate easily understandable clinical reports.

Whole genome sequence data can also be used to answer questions beyond the routine clinical microbiology daily needs. This is typically useful in case of outbreak investigations, when genetics relation between different clinical isolates from the same species need to be determined. In this study, some tools have been used to study the clonal relationships between relatively large number of clinical isolates

Table 4. Prediction of antibiotics resistant genes in *A. baumannii* strains AB250 and AB508 using Resistance Gene Identifier, which is designed and developed by the laboratories of Drs. Gerry Wright and Andrew G. McArthur of McMaster University.

Resistant Gene	*A. baumanniias* strains		Antibiotic Resistant Ontology Category
	AB250	AB508	
aac(6')-ib9	D	D	antibiotic inactivation enzyme; aminoglycoside resistance gene
aada	D	D	antibiotic inactivation enzyme; aminoglycoside resistance gene
abem	D	D	efflux pump conferring antibiotic resistance
abes	D	D	efflux pump conferring antibiotic resistance
acrf	D	D	efflux pump conferring antibiotic resistance; beta-lactam resistance gene; fluoroquinolone resistance gene
adea	D	D	efflux pump conferring antibiotic resistance; tetracycline resistance gene
adef	D	D	efflux pump conferring antibiotic resistance; tetracycline resistance gene; fluoroquinolone resistance gene
adeh	D	D	efflux pump conferring antibiotic resistance; tetracycline resistance gene; fluoroquinolone resistance gene
adei	D	D	chloramphenicol resistance gene; lincosamide resistance gene; macrolide resistance gene; fluoroquinolone resistance gene; efflux pump conferring antibiotic resistance; aminocoumarin resistance gene; tetracycline resistance gene; rifampin resistance gene; beta-lactam resistance gene; trimethoprim resistance gene
adek	D	D	chloramphenicol resistance gene; lincosamide resistance gene; macrolide resistance gene; fluoroquinolone resistance gene; efflux pump conferring antibiotic resistance; aminocoumarin resistance gene; tetracycline resistance gene; rifampin resistance gene; beta-lactam resistance gene; trimethoprim resistance gene
aden	D	D	chloramphenicol resistance gene; gene modulating antibiotic efflux; lincosamide resistance gene; macrolide resistance gene; fluoroquinolone resistance gene; efflux pump conferring antibiotic resistance; aminocoumarin resistance gene; tetracycline resistance gene; rifampin resistance gene; beta-lactam resistance gene; trimethoprim resistance gene
ader	D	D	efflux pump conferring antibiotic resistance; tetracycline resistance gene; gene modulating antibiotic efflux
ades	D	D	efflux pump conferring antibiotic resistance; tetracycline resistance gene; gene modulating antibiotic efflux
aph(3')-ia	D	D	antibiotic inactivation enzyme; aminoglycoside resistance gene
aph(3")-ib	D	D	antibiotic inactivation enzyme; aminoglycoside resistance gene
aph(6)-id	D	D	antibiotic inactivation enzyme; aminoglycoside resistance gene
arma	D	D	antibiotic target modifying enzyme; aminoglycoside resistance gene
catb8	D	D	chloramphenicol resistance gene; antibiotic inactivation enzyme
mext	D	D	efflux pump conferring antibiotic resistance; chloramphenicol resistance gene; trimethoprim resistance gene; gene modulating antibiotic efflux; fluoroquinolone resistance gene
msre	D	D	efflux pump conferring antibiotic resistance; streptogramin resistance gene; macrolide resistance gene
oxa-23	D	D	antibiotic inactivation enzyme; beta-lactam resistance gene
oxa-66	D	D	antibiotic inactivation enzyme; beta-lactam resistance gene
sul1	D	D	antibiotic target replacement protein; sulfonamide resistance gene
tem-1	D	ND	antibiotic inactivation enzyme; beta-lactam resistance gene
teta	D	D	efflux pump conferring antibiotic resistance; tetracycline resistance gene
	D	Resistant Gene Detected	ND Resistant Gene not detected

Table 5. Prediction of antibiotics resistant genes in *A. baumannii* strains AB250 and AB508 using ResFinder form Center for Genomics Epidemiology. Only gene with minimum of 90% similarity and 80% template coverage are reported.

Resistance gene	AB508		AB250		Phenotype
	Identity %	Accession no.	Identity %	Accession no.	
aac(6')Ib-cr	99.23	EF636461	99.23	EF636461	Fluoroquinolone and aminoglycoside resistance
aacA4	99.64	KM278199	99.64	KM278199	Aminoglycoside resistance
aadA1	100	JQ414041	100	JQ414041	Aminoglycoside resistance
aph(3')-Ic	100	X62115	99.88	X62115	Aminoglycoside resistance
armA	100	AY220558	100	AY220558	Aminoglycoside resistance
blaADC-25	99.8	EF016355	99.91	EF016355	Beta-lactam resistance
blaOXA-23	100	HQ700358	100	HQ700358	Beta-lactam resistance
blaOXA-66	100	FJ360530	100	FJ360530	Beta-lactam resistance
blaTEM-1D	Not detected		100	AF188200	Beta-lactam resistance
catB8	100	AF227506	100	AF227506	Phenicol resistance
mph(E)	100	EU294228	100	EU294228	Macrolide resistance
msr(E)	100	EU294228	100	EU294228	Macrolide, Lincosamide and Streptogramin B resistance
strA	100	M96392	100	M96392	Aminoglycoside resistance
strB	100	M96392	100	M96392	Aminoglycoside resistance
sul1	100	AY224185	100	CP002151	Sulphonamide resistance
tet(B)	100	AP000342	100	AP000342	Tetracycline resistance

of *A. baumannii* from two closely related hospitals. Using draft genome sequence, therefore assembled contigs, multi locus sequence types were determined using web based free tool from the Center for Genomics Epidemiology [10,14]. Similar tool, SRST2, is also available from the Illumina BaseSpace, which is an application that reports the presence of sequences types and/or reference genes from a database of sequences for virulence genes, resistance genes, and plasmid replicons.

In this study, 40 clinical isolates were multiplexed in one MiSeq

sequencing run using version 3 pair-end library with 600 sequencing cycles. Only one samples failed sequencing, but the remaining samples produced sequence data enough for full isolates characterization (Table 1). Larger number of bacterial genomes can be studied in one batch using sequencing platforms with higher data output such as NextSeq and HiSeq. Therefore, for big clinical microbiology larger sample size can still be sequences and genomics reports can be generated within the same time-frame. In routine microbiology, no single test can be used to produce comparable data with similar power. In this study, only species, antibiotics resistant genes and sequence types were determined. However, using the same genome sequence data many other features can be studied using many freely available and user-friendly tools. The advancement of sequencing technology foster the development of several tools for immediate virulence genes detections, plasmids profiling, serotypes predictions and much more [19-21].

The presence of multiple sequence types in our *A. baumannii* isolates indicate that apparent resistant outbreak was not caused by a single clone. The most prevalent sequence types was ST195 accounting for 47% of all *A. baumannii* isolates. Similar results were recently reported from the same region [22,23]. ST195 and ST557 were reported by Alyamani and his group from isolates collected form the same city [22], while ST195 and ST208 were reported by study done by Zowawi *et al.* [23] in isolates representing the Arabian Gulf region [23]. ST195, ST208 and ST218 were found to be closely related to each other with only difference in one allele. ST195 in *A. baumannii* was also reported from many other different regions such as India, China and Malaysia [24-26]. In addition to the known sequence types, three novel sequence types were found among seven isolates collected from both hospitals (Table 2). Zowawi *et al.* [23] also reported three novel sequence types in the Arabian Gulf region study [23]. In this study, these novels sequence types were curated and assigned to new sequence types (ST1286, ST1287 and ST1288) Oxford scheme at the *A. baumannii* MLST database.

Using genome sequence data, a wide range of antimicrobial resistance genes to major antibiotics classes were predicted, which were in consistent with the phenotypic data obtained by Microscan. Different carbapenem resistant genes were reported in different studies in our region [22,23,27-31]. However, most of these studies used PCR based detection, which need careful design to insure coverage of all carbapenems resistant genes. In addition, by using PCR-based detection of resistant markers it is difficult to use the term "molecular characterization" for even a single class of antibiotics. Therefore, the power of whole genome sequence remains unbeatable in the screening of all acquired and naturally occurring resistant mechanisms not only for carbapenems, but also for all resistant mechanisms to all known antibiotics classes. In our study, large number of resistant mechanisms were identified (Tables 3,4 and 5). Only whole genome sequence data was used for the prediction of these antibiotics resistant mechanisms. Many user friendly bioinformatics tools were tested for prediction of antibiotics resistant mechanisms, which were nicely consistent with each other (Tables 4 and 5). One of these tools is the Illumia BaseSapce SRST2, which can provide clinically relevant antibiotics resistant data within acceptable time frame that can influence patient care.

In classical molecular hospital epidemiology, antibiogram data with genotyping results are usually combined to trace infections source and to understand colonization patterns in patients, healthcare workers and hospital environment. However, when whole genome sequence data become available from clinical isolates, better hospital molecular epidemiology data with high resolution will help in identifying complex outbreak dynamics and evolution. With genome data, unlimited features can be studied and proper microbial molecular characterization can be achieved.

In conclusion, next generation sequencing data is transforming clinical microbiology routine services. In near future, genomics based characterization will replace number of currently used microbiology techniques such as routine bacterial identification, susceptibility testing and serotyping. Microbial genotyping, which normally carried out in case of hospital outbreaks investigation or as part of research projects, will be part of routine the clinical microbiology reports. In near future, new hospital patients admission will routinely be screened for all known antibiotics resistant mechanisms instead off only being screened for carbapenems or methicillin resistance.

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