Results

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200 isolates were collected from 220 clinical samples from Benha university hospital.

The age of the patients ranged from 3 days to 55 years with the mean age 27.5years.

The sex of the patients under the study was 65 female and 155 male.

The 200 isolates were collected from; pus (128), ear discharge (2), urine (43) and blood (27) as shown in table 1.

- It was shown that the prevalence of gram positive cocci was 41.5% (83 Gram+ve cocci out of 200 isolates).
- The prevalence of Staphylococcus aureus isolates was 28% (56 S.aureus isolates out of 200 isolates).
- The prevalence of other gram positive cocci was 13.5% (27 out of 200 isolates), 25 of them were coagulase negative staphylococci and 2 were streptococci.
- The prevalence of gram negative bacilli was 58.5% (117 out of 200).

Table 1: Distribution of the 200 isolates in relation to clinical samples.

Organism	Clinical samples*											
	Total no of isolates		Wound pus		Abscess aspirate		Ear pus		Blood		Urine	
	No	%	No	%	No	%	No	%	No	%	No	%
Staphylococcus aureus	56	28	36	18	4	2	0	0	8	4	8	4
Coagulase – ve staphylococci	25	12.5	10	5	2	1	0	0	0	0	13	6.5
Streptococci	2	1	0	0	0	0	2	1	0	0	0	0
Gram –ve bacilli	117	58.5	74	37	2	1	0	0	19	.5	22	11
Total	200	100	120	60	8	4	2	1	27	3.5	43	21.5

It is noted from the table that the frequency of S.aureus among all isolates was 28% (56 out of 200) and they were mainly from wound pus (36 out of 56).

*No growth detected from the following samples in aerobic culture:-

- 5 in wound infection.
- 2 in abscess.
- 3 in ear pus.
- 5 in blood.
- 5 in urine .

Table 2: Value of mannitol salt agar for detection of Staph.aureus.

Isolates	Mannitol salt agar			
	Growth without color change		Growth with lemon yellow color	
	No	%	No	%
Staphylococcus. aureus (56)	0	0	56	100
Coagulase –ve Staphylococci (25)	22	88	3	12
Other organisms (119)	0	0	0	0

As shown in table 2 mannitol salt agar was successful in detecting all 56 S.aureus isolates but some (3) coagulase negative staphylococcal isolates showed positive growth .On the other hand, mannitol salt agar inhibited the growth of the other organisms.

Table 3: The results of disk diffusion method with different antibiotics applied to 56 isolated S.aureus.

Disk diffusion method	S .aureus (56)			
	MRSA		MSSA	
	No	%	No	%
Oxacillin 1 ug*	22	39.3	34	60.7
Oxacillin 5 ug*	28	50	28	50
Cefoxitin 30 ug*	24	42.9	32	57.1

*incubation was carried out for 24-48 h at 37 ^{0}C

Table 4: Frequency of MRSA isolates in relation to clinical samples

Samples	Staph. aureus isolates (56)				Total	
	MRSA		MSSA			
	No	%	No	%	No	%
Wound pus	12	21.4	24	42.9	36	64.3
Abscess aspirate	0	0	4	7.1	4	7.1
Blood	6	10.8	2	3.6	8	14.3
Urine	4	7.1	4	7.1	8	14.3
Total	22	39.3	34	60.7	56	100

This table shows that the frequency of MRSA among S.aureus isolates was 39.3% (22out of 56) and they were mainly from wound pus (12out of 22) and the MRSA isolation from blood was 6 out of 22 and it was higher than MSSA isolation from blood (2 out of 32).

Table 5: The results of oxacillin E test strips applied on 56 S.aureus isolates.

Method	S .aureus isolates (56)					
	MRSA		BORSA*		MSSA	
	MIC(>4ug/ml)		MIC(4-8 ug/ml)		MIC(<2 ug /ml)	
	No	%	No	%	No	%
Oxacillin E-test**	20	35.7	2	3.6	34	60.7

** Incubation was carried out for 24-48 h at 37 ^{0}C

*BORSA (borderline resistant S.aureus).

This table shows the presence of 2 BORSA strains among isolates (MIC4-8 ug/ml) this strains produce resistance most probably due to hyperproduction of B lactamases.

Table 6: Agreement between oxacillin 1 ug and 5 ug disk diffusion methods for detection of MRSA

Organism	Test				Agreement
	Oxacillin 1ug		Oxacillin 5ug		
	No	%	No	%	
MRSA	22	39.3	28	50	78.5%
MSSA	34	60.7	28	50	82.4%

Table 7: Agreement between oxacillin 1 ug and cefoxitin 30ug disk diffusion methods for detection of MRSA

Organism	Test				Agreement
	Oxacillin 1ug		Cefoxitin 30ug		
	No	%	No	%	
MRSA	22	39.3	24	42.9	91.7 %
MSSA	34	60.7	32	57.1	94.1%

It is noted from the table that agreement between oxacillin 1 ug and cefoxitin 30 ug disk diffusion methods was 91.7%in detecting MRSA isolates and 94.1% in detecting MSSA isolates.

Table 8: Agreement between oxacillin 1 ug disk diffusion method and oxacillin E test method for detection of MRSA

Organism	Test				Agreement
	Oxacillin 1ug		Oxacillin E-test		
	No	%	No	%	
MRSA	22	39.3	22	39.3	100%
MSSA	34	60.7	34	60.7	100%

It is noted from the table that agreement between oxacillin 1 ug disk diffusion method and oxacillin E-test was 100%in detecting MRSA isolates and 100% in detecting MSSA isolates

Table 9: The results of latex agglutination test applied on 56 S.aureus isolates

Method	S.aureus isolates (56)			
	MRSA No	%	MSSA No	%
Latex agglutination test	22	39.3	34	60.7

Table 10: Agreement between oxacillin 1ug disk diffusion method and latex agglutination test for detection of MRSA

Organism	Test				Agreement
	Oxacillin 1ug		Latex agglutination		
	No	%	No	%	
MRSA	22	39.3	22	39.3	100%*
MSSA	34	60.7	34	60.7	100%*

* It is noted from the table that agreement between oxacillin 1 ug disk diffusion method and latex agglutination test was 100% in detecting MRSA isolates and 100% in detecting MSSA isolates. But it is not a true agreement it is only numerical agreement as 2 cases detected positive with latex agglutination were susceptible by oxacillin 1 ug disk diffusion method, and other 2 cases detected negative with latex agglutination were resistant by oxacillin 1 ug disk diffusion method.

Table 11: The results of real time PCR for mec A gene detection applied on 56 S.aureus isolates.

Method	S.aureus isolates (56)			
	mec A positive No	%	mec A negative No	%
Real time PCR	22	39.3	34	60.7

Table 12: Agreement between oxacillin 1 ug disk diffusion method and real time PCR detection of MRSA

Organism	Test				Agreement
	Oxacillin 1ug No	%	Real time PCR No	%	
MRSA	22	39.3	22	39.3	100%*
MSSA	34	60.7	34	60.7	100%*

* It is noted from the table that agreement between oxacillin 1 ug disk diffusion method and real time PCR was 100% in detecting MRSA isolates and 100% in detecting MSSA isolates. But it is not a true agreement it is only numerical agreement as 2 cases detected positive with real time PCR were susceptible by oxacillin 1 ug disk diffusion method, and 2 cases detected negative with real time PCR were resistant by oxacillin 1 ug disk diffusion method.

Table 13: The results of different methods for detection of methicillin resistance in S.aureus isolates.

Method	S .aureus isolates (56)			
	MRSA		MSSA	
	No	%	No	%
Oxacillin 1ug disk diffusion	22	39.3	34	60.7
Oxacillin 5ug disk diffusion	28	50	28	50
Cefoxitin 30ug disk diffusion	24	42.9	32	57.1
Oxacillin E-test MIC	22	39.3	34	60.7
Latex agglutination test	22	39.3	34	60.7
Real time PCR	22	39.3	34	60.7

Table 14: MRSA strains detected by different methods in different samples.

Sample type	Total	Disk diffusion methods						E –test		Latex agglutination test		Real time PCR	
		Ox 1ug		Ox 5ug		Fox 30ug							
		R	S	R	S	R	S	R	S	R	S	R	S
Wound	36	12	24	18	18	14	22	12	24	12	24	12	24
Abscess	4	0	4	0	4	0	4	0	4	0	4	0	4
Urine	8	4	4	4	4	4	4	4	4	4	4	4	4
Blood	8	6	2	6	2	6	2	6	2	6	2	6	2
Total	56	22	34	28	28	24	32	22	34	22	34	22	34

Ox:oxacillin
Fox: cefoxitin
R: Resistant
S: Sensitive

Table 15: Time taken by each method to detect MRSA strains.

	Methods					
	Disk diffusion			E –test MIC	Latex agglutination test	Real time PCR*
	OX 1ug	OX 5ug	FOX 30ug			
Time taken to detect MRSA from isolated colonies	24-48 hours	24-48 hours	24-48 hours	24-48 hours	15 minutes	<60 minutes

*The 22 MRSA isolates detected by real time PCR 16 of them were detected from isolated colonies and 6 of them were detected directly from the samples. Both took the same time.

Table 16: Phenotypic expression of oxacillin resistance in S.aureus by different methods versus genotypic expression of mec A gene by real time PCR.

Phenotypic expression	Genotypic expression		Chi square	P value
	Real time PCR			
	mec A positive	mec A negative		
Oxacillin 1 ug disk diffusion			40.485	<0.001
MRSA 22	20	2		
MSSA 34	2	32		
Oxacillin 5 ug disk diffusion			24.257	<0.05
MRSA 28	20	8		
MSSA 28	2	26		
Cefoxitin 30ug disk diffusion			48.314	<0.001
MRSA 24	22	2		
MSSA 32	0	32		
Oxacillin E-test trips			40.485	<0.001
MRSA 22	20	2		
MSSA 34	2	32		
Latex agglutination test			56	<0.001
MRSA 22	22	0		
MSSA 34	0	34		
Total for each method	22	34		

It is noted from the table that; By oxacillin 1 ug disk diffusion method,20 isolates from the resistant 22 S.aureus isolates were mec A positive and 2 were mec A negative .From the 34 sensitive S.aureus

isolates 32 were mec A negative and 2 were mec A positive .Chi square for the relation between phenotypic expression of oxacillin resistance in S.aureus by ox 1ug disk and the presence of mec A gene by real time PCR revealed that square was (40.485)and P value was <0.001 which means that there a strong association between the presence of mec A gene and phenotypic expression of oxacillin resistance in S.aureus by ox 1ug disk .

By Oxacillin 5 ug disk diffusion method 20 isolates from the resistant 28 S.aureus isolates were mec A positive and 8 were mec A negative and from the 28 sensitive S.aureus isolates 26 were mec A negative and 2 were mec A positive. Chi square for the relation between phenotypic expression of oxacillin resistance in S.aureus by ox 5ug disk and the presence of mec A gene by real time PCR revealed that square was (24.257) and P value was <0.05 which means that there is association between the presence of mec A gene and phenotypic expression of oxacillin resistance in S.aureus by ox 5ug disk.

By Cefoxitin 30ug disk diffusion method 22 isolates from the resistant 24 S.aureus isolates were mec A positive and 2 were mec A negative and all of the 32 sensitive S.aureus isolates were mec A negative. Chi square for the relation between phenotypic expression of oxacillin resistance in S.aureus by fox 30ug disk and the presence of mec A gene by real time PCR revealed that square was (48.314) and P value was <0.001 which means that there is strong association between the presence of mec A gene and phenotypic expression of oxacillin resistance in S.aureus by fox 30ug disk.

By Oxacillin E-test strips 20 isolates from the resistant 22 S.aureus isolates were mec A positive and 2 were mec A negative .From the 34

sensitive S.aureus isolates 32 were mec A negative and 2 were mec A positive. Chi square for the relation between phenotypic expression of oxacillin resistance in S.aureus by oxacillin E- test and the presence of mec A gene by real time PCR revealed that square was (40.485) and P value was <0.001 which means that there is strong association between the presence of mec A gene and phenotypic expression of oxacillin resistance in S.aureus by oxacillin E-test .

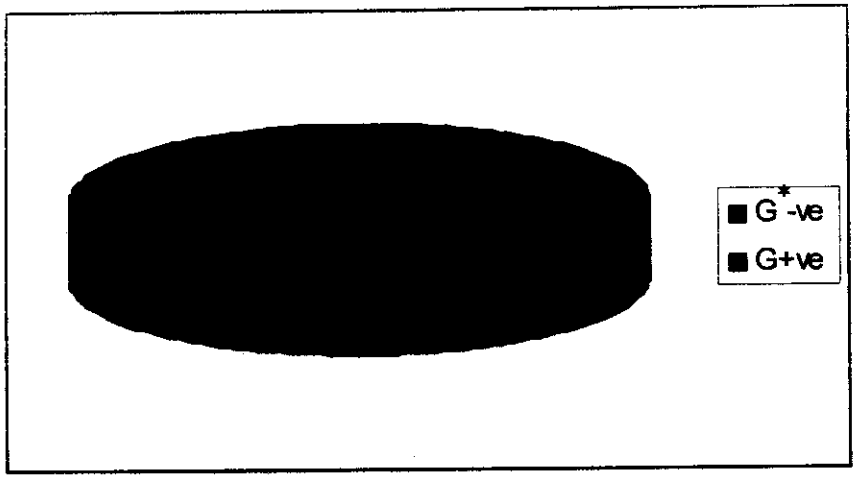
By latex agglutination test 22 of the 56 S.aureus isolates were resistant (have PBP2a) .All these 22 isolates were mec A positive and all 34 sensitive S.aureus were mec A negative. Chi square for the relation between the presence of PBP2a in S.aureus by Latex agglutination test and the presence of mec A gene by real time PCR revealed that square was (56) and P value was <0.001 which means that there is strong association between the presence of PBP2a in S.aureus by Latex agglutination test and the presence of mec A gene.

Table 17:

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the different methods used in relation to mec A presence by real time PCR

Method	False positive	False negative	Sensitivity	Specificity	PPV	NPV
Ox 1ug disk diffusion	2	2	90.9%	94.1%	90.9%	94.1%
Ox 5ug disk diffusion	8	2	90.1%	76.5%	71.4%	94.1%
Fox 30ug disk diffusion	2	0	100%	94.1 %	100%	94.1%
Oxacillin E-test	2	2	90.1%	94.1%	90.9%	94.1%
Latex agglutination test	0	0	100%	100%	100%	100%

It is noted from the table that cefoxitin 30ug disk diffusion method is the only disk diffusion method that gave no false negative results and latex agglutination test gave neither false positive nor false negative results and both of them gave the best sensitivity (100%) but specificity of cefoxitin was 94.1% and that of latex was 100%.



G-ve:Gram negative
G+ve:Gram positive

Figure 1:

This figure shows the distribution of Gram positive cocci among 200 isolates

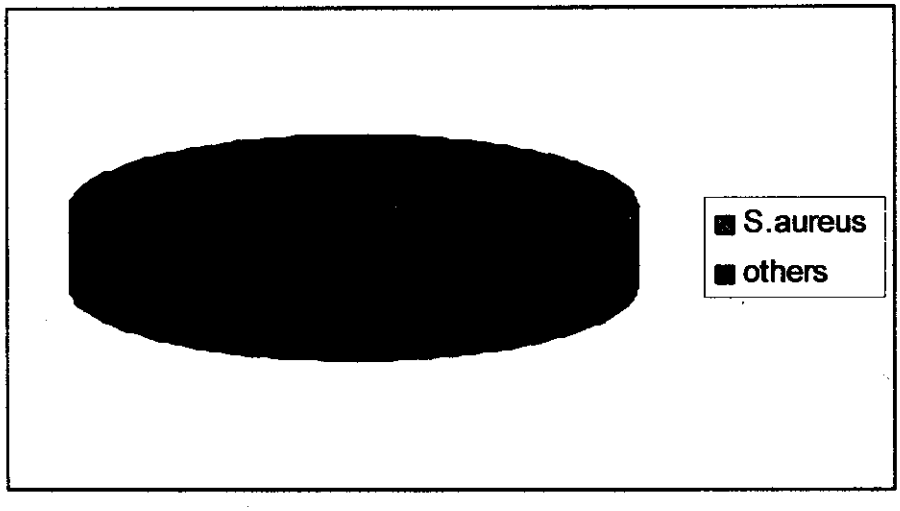


Figure 2:

This figure shows the distribution of S.aureus among 200 isolates

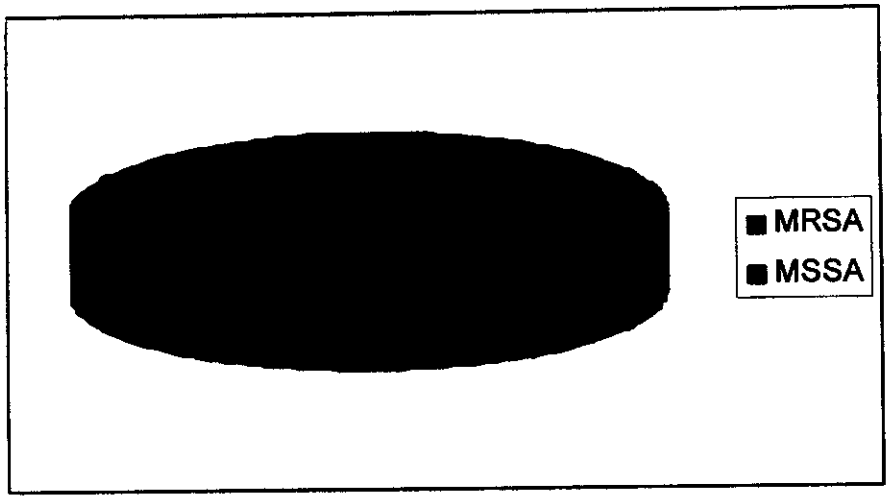


Figure 3:

This figure shows the distribution of MRSA among 56 S.aureus isolates.

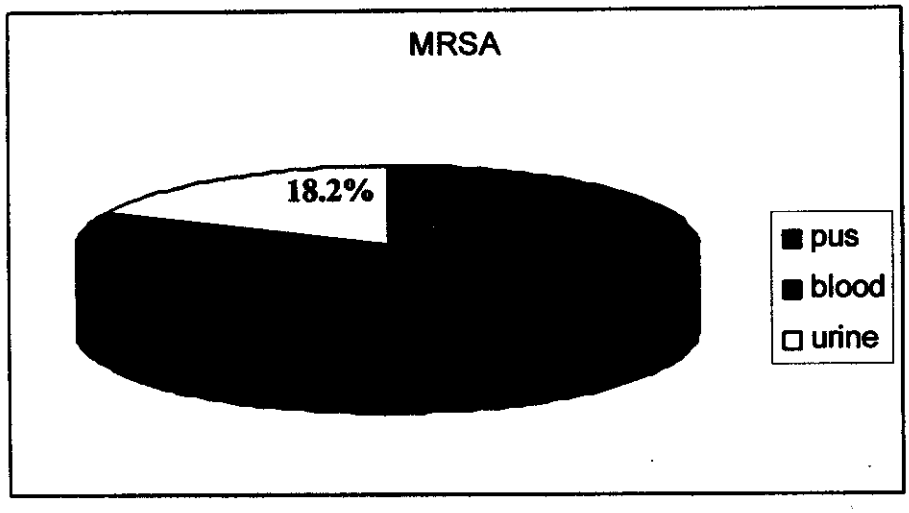


Figure 4:

This figure shows the distribution of MRSA isolates in relation to clinical samples.

Figure 5:

Growth of opaque yellow colonies of S.aureus surrounded by yellow zone on mannitol salt agar

Figure 6:

Resistance of S.aureus to ox 1ug, ox 5ug and fox 30ug disks (no inhibition zone).

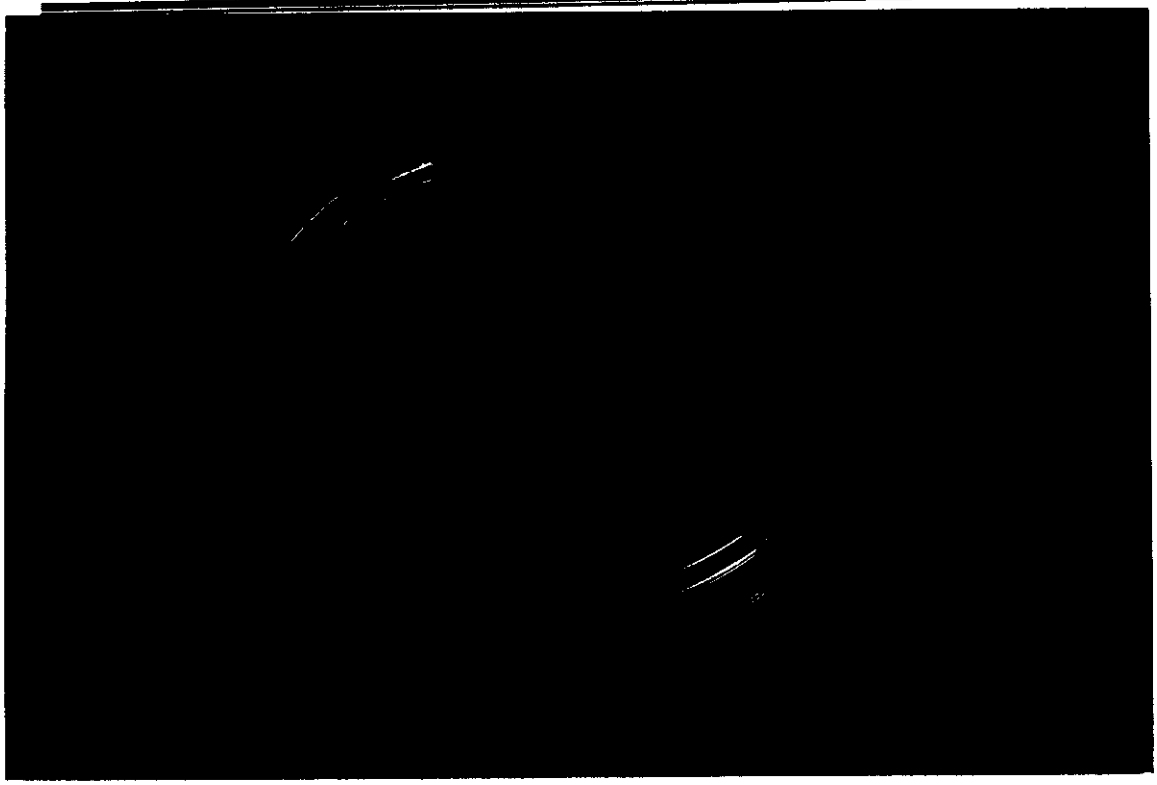


Figure 7:
Resistant subpopulation of S.aureus inside the zone of inhibition of ox 1ug, ox 5ug and fox 30ug disks.

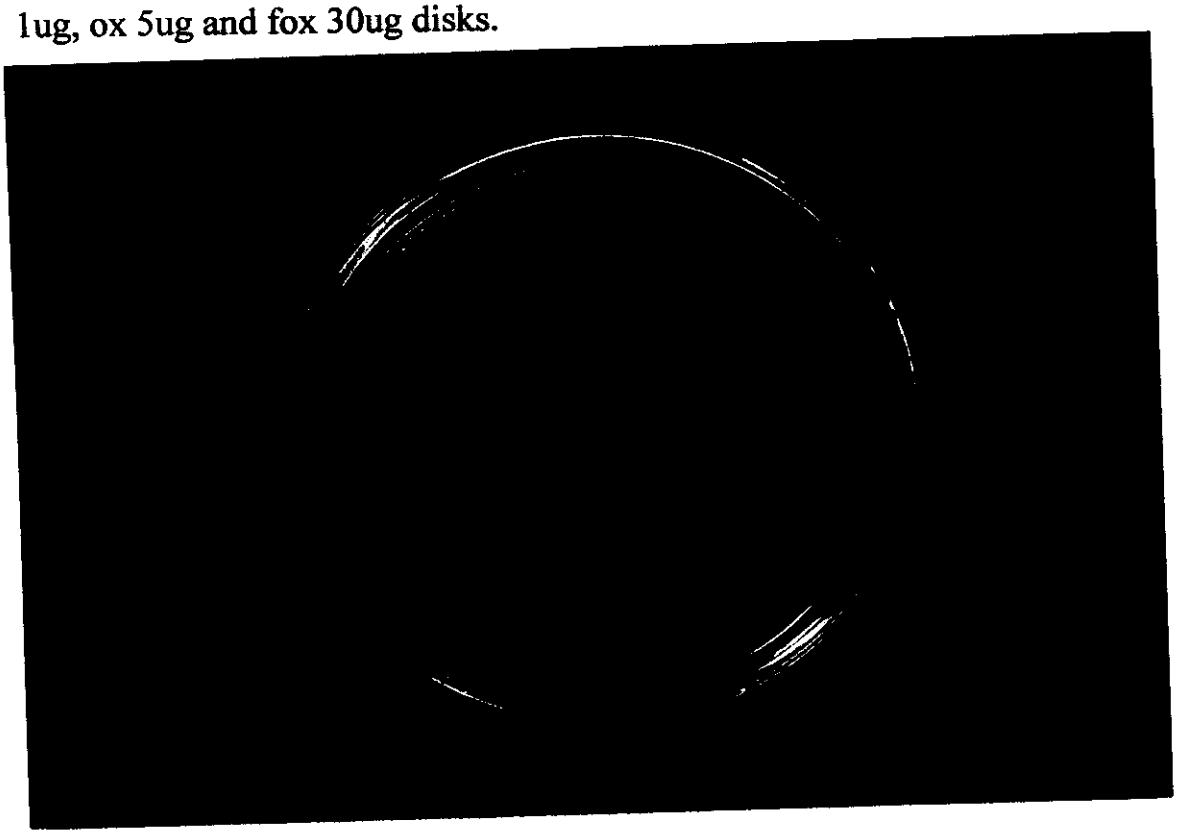


Figure 8:
Oxacillin sensitive strain of S.aureus by oxacillin E-test strip (MIC 0.38ug/l)

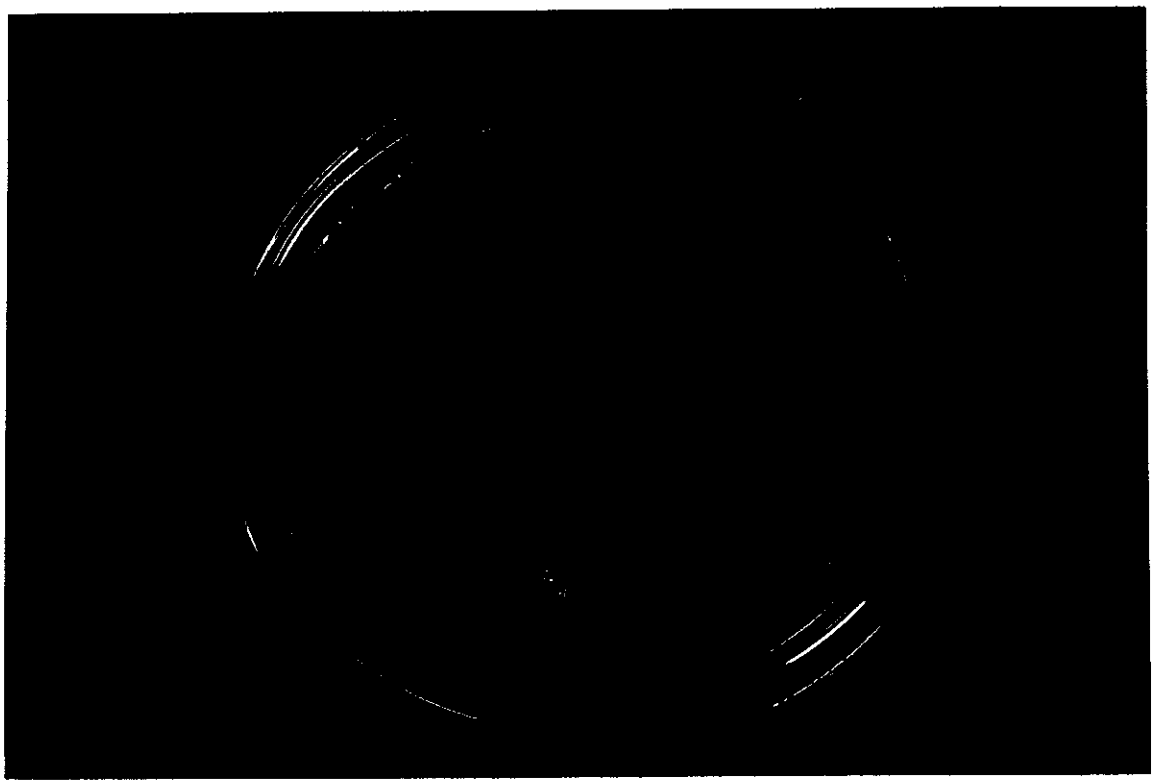


Figure 9:
Resistant strain of S.aureus by oxacillin E-test strip (MIC 256ug/l)

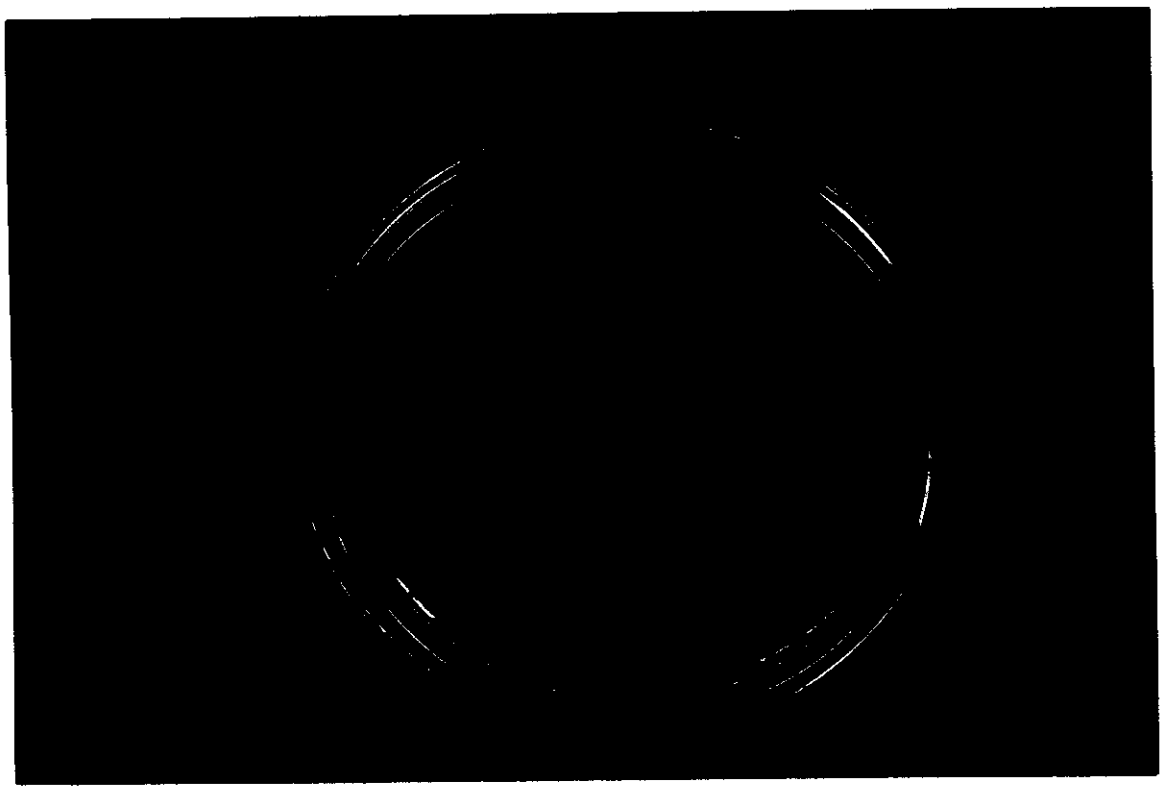


Figure 10:
Resistant strain of S.aureus by oxacillin E-test strip (MIC 24ug/l)

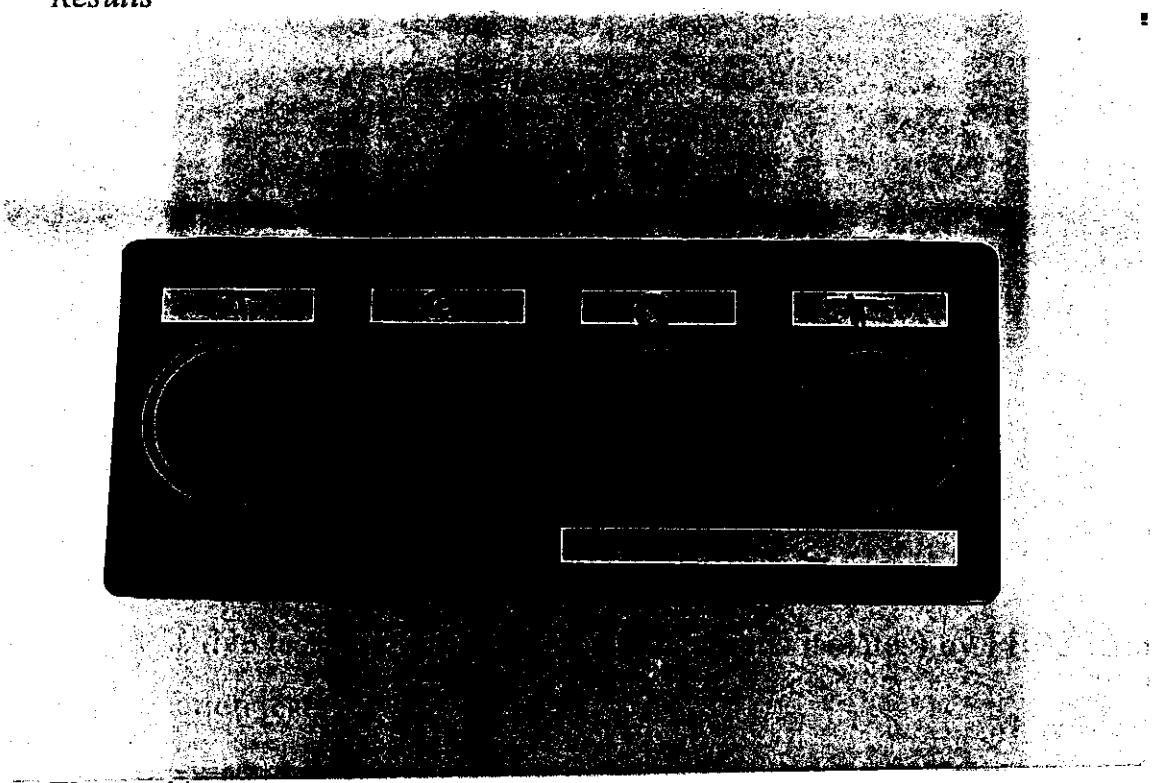


Figure 11:

Positive test (agglutination of one S.aureus isolates) and negative test (no agglutination of another S.aureus isolates) by PBP2' latex

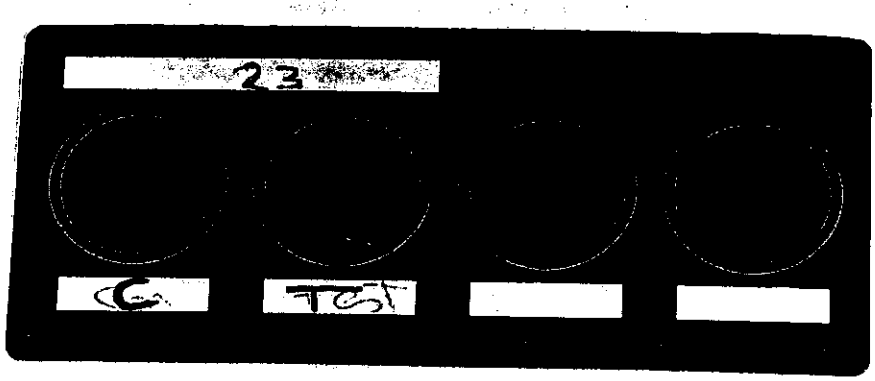


Figure 12:

Positive test (agglutination of one S.aureus isolates) by PBP2' latex agglutination test.

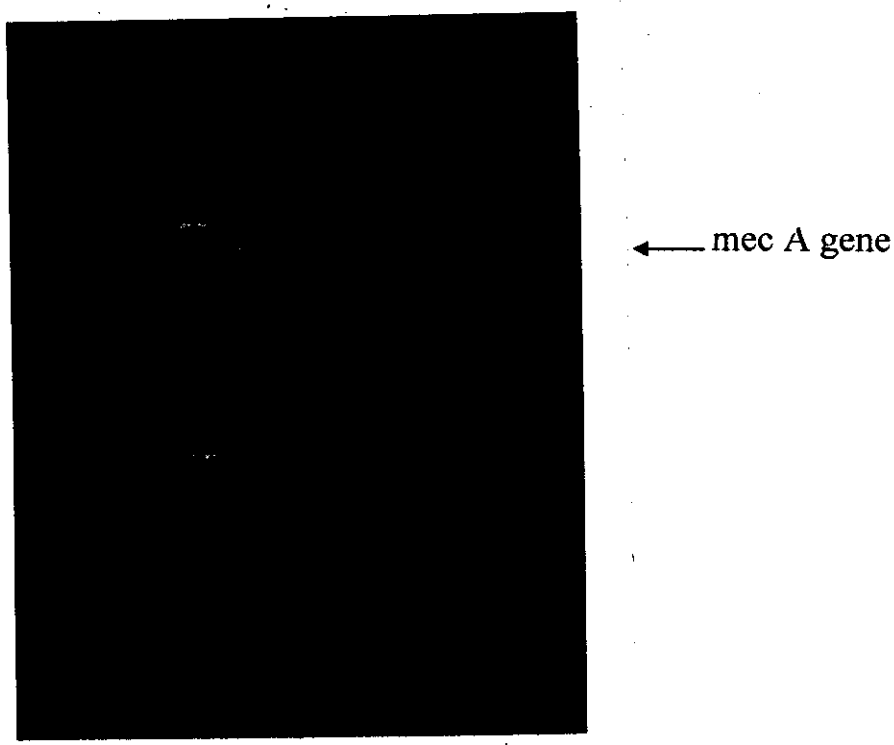


Figure 13:

Agarose gel electrophoresis shows bands of PCR amplified DNA of MRSA.

Note that:

The product (mec A gene) at: (878 bp).

Control at: (100 bp).

Marker :(100-200-300-400-500-600-700-800-2000)

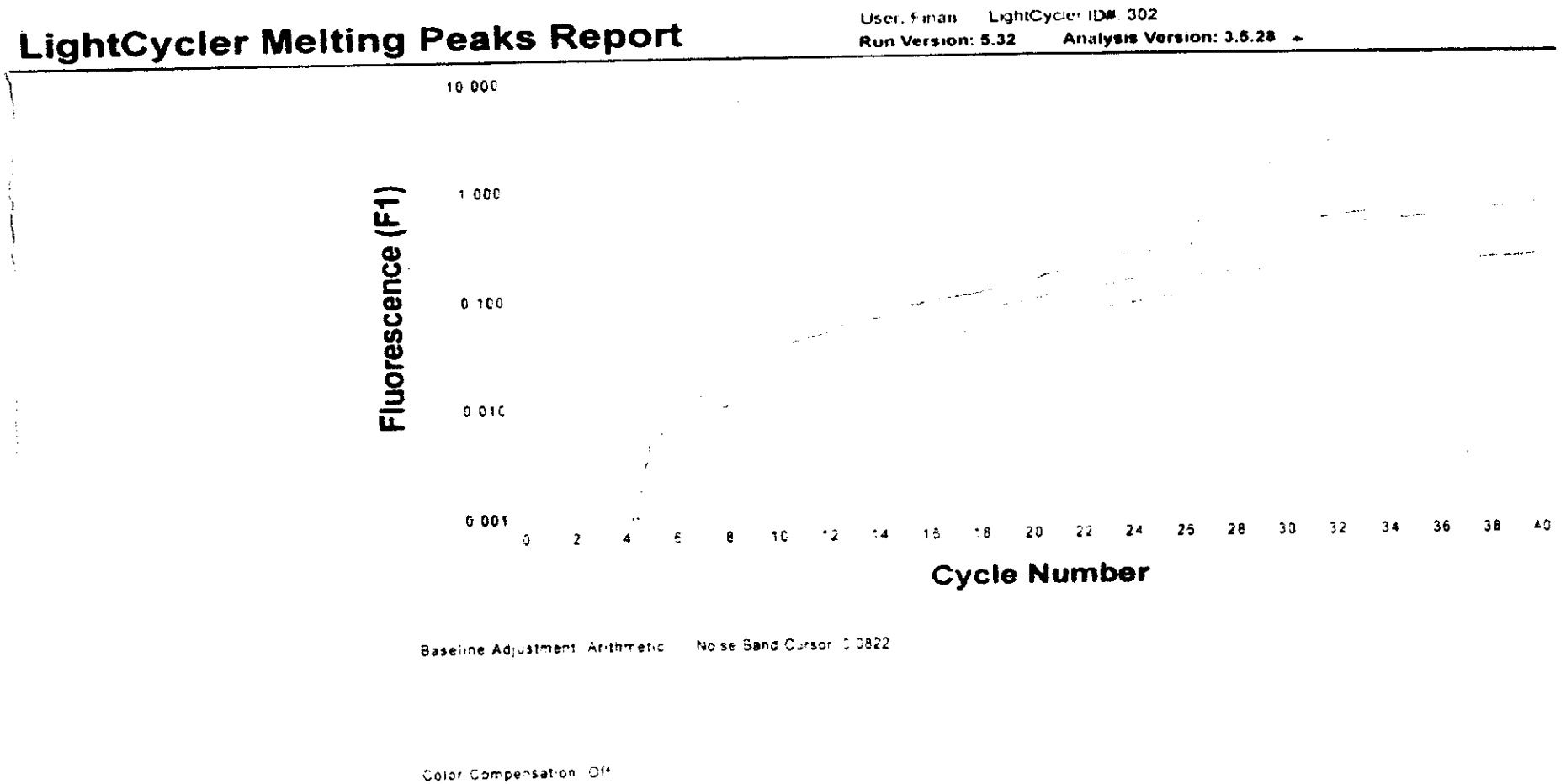


Figure 14: Sensitivity of real time PCR assay for detection of mec A gene

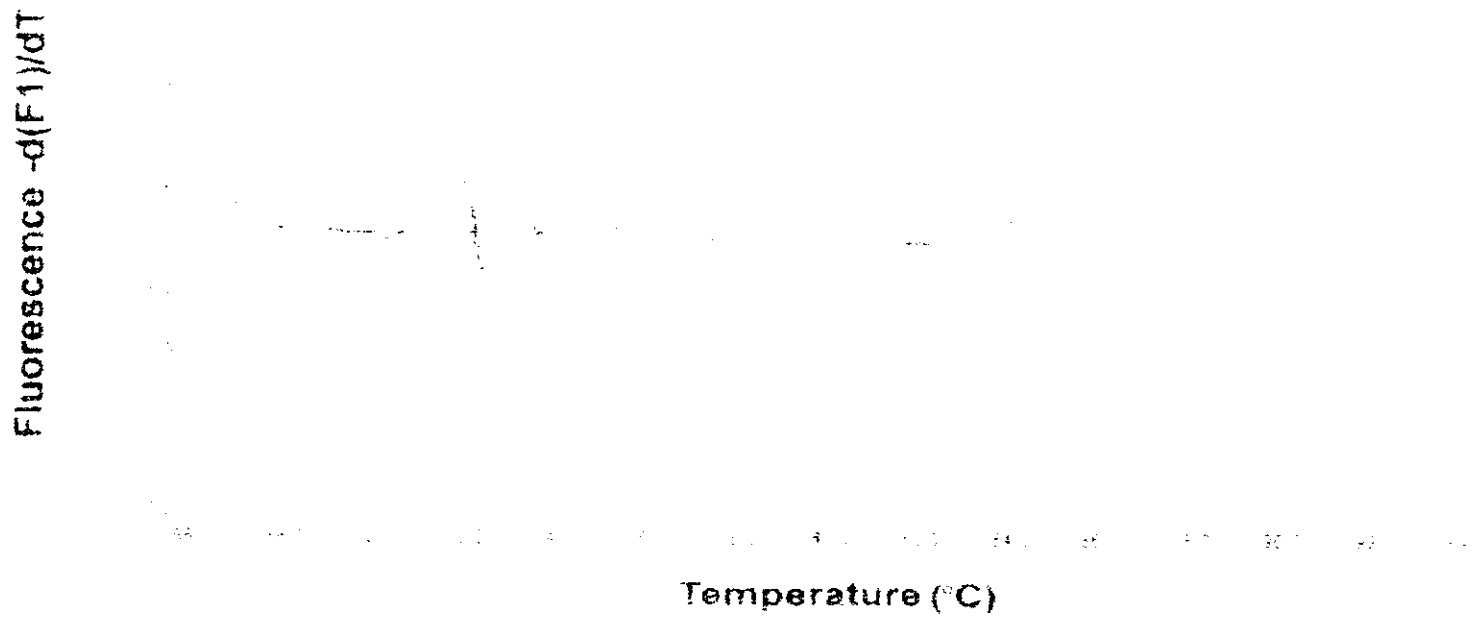


Figure 15: Melting temperature curve (Tm curve).
Tm specific for MRSA 80 °C

Table 18: Grouping of the studied 56 S.aureus isolates by results of all typing methods.

Isolate designation	E –test MIC	Disk diffusion			Latex agglutination test	mec A status
		OX 1ug	OX5ug	FOX30ug		
1	0.38	S	S	S	S	-ve
2	0.38	S	S	S	S	-ve
3	0.38	S	S	S	S	-ve
4	0.5	S	S	S	S	-ve
5	1	S	S	S	S	-ve
6	256	R	R	R	R	+ve
7	8	R	R	R	S	-ve
8	0.5	S	I	S	S	-ve
9	16	R	R	R	R	+ve
10	1	S	I	S	S	-ve
11	0.5	S	R	S	S	-ve
12	0.38	S	I	S	S	-ve
13	0.5	S	I	S	S	-ve
14	0.38	S	I	S	S	-ve
15	0.5	S	R	S	S	-ve
16	0.5	S	S	S	S	-ve
17	256	R	R	R	R	+ve
18	1	S	I	S	S	-ve
19	0.5	S	R	S	S	-ve
20	64	R	R	R	R	+ve
21	0.5	S	S	S	S	-ve
22	24	R	R	R	R	+ve
23	64	R	R	R	R	+ve
24	256	R	R	R	R	+ve
25	24	R	R	R	R	+ve
26	2	S	S	R	R	+ve
27	96	R	R	R	R	+ve

28	256	R	R	R	R	+ve
29	0.38	S	S	S	S	-ve
30	1	S	S	S	S	-ve
31	0.38	S	S	S	S	-ve
32	0.5	S	S	S	S	-ve
33	1	S	S	S	S	-ve
34	256	R	R	R	R	+ve
35	8	R	R	R	S	-ve
36	1	S	I	S	S	-ve
37	16	R	R	R	R	+ve
38	1	S	I	S	S	-ve
39	0.5	S	R	S	S	-ve
40	0.38	S	I	S	S	-ve
41	0.5	S	I	S	S	-ve
42	0.38	S	I	S	S	-ve
43	0.5	S	R	S	S	-ve
44	0.5	S	S	S	S	-ve
45	256	R	R	R	R	+ve
46	1	S	I	S	S	-ve
47	0.5	S	R	S	S	-ve
48	256	R	R	R	R	+ve
49	0.5	S	S	S	S	-ve
50	24	R	R	R	R	+ve
51	64	R	R	R	R	+ve
52	256	R	R	R	R	+ve
53	24	R	R	R	R	+ve
54	2	S	S	R	R	+ve
55	16	R	R	R	R	+ve
56	96	R	R	R	R	+ve