RESULTS

This study was carried out on 100 Pus samples collected from 100 patients attended Benha University Hospital and were suffering from different pyogenic infections.Those patients were classified into two groups as shown in table(1).

Group I: Thirty hospitalized patients (15 males and 15 females); their age ranged between 20 – 60 years. 15 samples (15.0%) were obtained from patients with diabetic foot and post operative sepsis in General Surgery Department, 10 samples (10.0%) from patients with post operative sepsis in Orthopedic Department and 5 samples (5.0%) from patients with the same clinical condition in Gynecological Department.

Group II: Seventy non hospitalized patients (46 males and 24 females) their age ranged between 1 – 70 years. 65 samples (65.0%) were obtained from patients with abscesses and infected wounds who attending General Surgery outpatient clinic and 5 samples (5.0%) from patients with otitis media who attending ENT outpatient clinics.

Table (1): Distribution of the collected samples according to the different departments:

Distribution / Source	No. of samples	Percent (%)
A) Inpatient Departments (30)		
- General surgery	15	15.0 %
- Orthopedic	10	10.0 %
- Gynacology	5	5.0 %
B) Outpatient Clinics (70)		
- General surgery clinic	65	65%
- ENT clinic	5	5.0 %
C) Total	100	100 %

Results of Gram (Gm) stain:

From a total of 100 (100%) pus samples stained by Gm stain: 52 (52.0%) showed staphylococci which appear as Gm+ve cocci arranged in clusters while 48 (48%) specimens were –ve for staphylococci (Table 2).

Table (2): Staphylococcal infection in the study group as diagnosed by Gram stain:

	Gm stain	No.	Percent (%)
Staph.	+ve	52	52.0 %
	- ve	48	48.0 %
Total		100	100 %

Results of culture on mannitol salt agar (MSA) medium:

A total of 52 (100%) Staphylococcal isolates were cultured on MSA medium; 39(75.0) of them were *S.aureus* (opaque yellow colonies surrounded by yellow zone due to acid fermentation) while 13(25.0) isolates were identified as other staphylococcal types (Table 3).

Table (3): Staphylococcal infection in the study group as diagnosed by culture on MSA medium:-

MSA medium	No.	Percent (%)
S.aureus	39	75.0 %
Other types of staph.	13	25.0 %
Total	52	100 %

Results of culture on blood agar:

Out of the 39 (100%) *S.aureus* strains cultured on MSA: 33 (84.6%) were haemolytic when cultured on blood agar while 6 strains (15.4%) were non haemolytic (Table 4).

Table (4):Haemolytic activity of *S. aureus* isolates cultured on blood agar.

Blood culture on agar medium	No. of *S. aureus* strains	Percent (%)
Haemolysis	33	84.6 %
No haemolysis	6	15.4 %
Total	39	100%

Results of gelatin liquefaction test:

From a total of 39 strains (100%) of *S. aureus* cultured on MSA: 37 (94.9%) liquified gelatin while 4 strains (5.1%) did not liquify it.

Table (5): Results of gelatin liquefaction test done on isolated *S.aureus* strains:

Gelatin liquefaction	No. of *S.aureus* strains	Percent (%)
Positive	37	94.9%
Negative	2	5.1 %
Total	39	100%

Results of coagulase test:

♦ **Slide coagulase test:** Out of 39 (100%) *S. aureus* strains isolated on MSA; only 10 (25.6%) showed positive slide coagulase test while 29 strains (74.4%) showed negative test (Table 6).

♦ **Tube coagulase test:** Out of 39 (100%) of *S. aureus* strains isolated from MSA: 35 strains (89.7%) showed positive tube coagulase test while 4 isolates (10.3%) showed negative test (Table 6).

Table (6): Results of Coagulase test done on isolated *S.aureus* strains.

Result / Type Of test	*S.aureus* positive strains		*S.aureus* negative strains		Total	
	No.	**Percent (%)**	**No.**	**Percent (%)**	**No.**	**Percent (%)**
Slide coagulase	10	25.6%	29	74.4%	39	100 %
Tube coagulase	35	89.7%	4	10.3%	39	100%
X^2	30.3					
p	<0.001 * H.S					

P <0.001 * H.S: high significance.

Phenotypic identification of MRSA by disc diffusion method:

♦Phenotypic identification of MRSA using oxacillin disc revealed that:

From 39 (100%) *S. aureus* strains isolated on MSA medium: 14 (35.9%) were oxacillin sensitive with zone diameter ≥ 13mm and 25 strains (64.1%) were oxacillin resistant with zone diameter ≤ 10mm (Table 7).

♦Phenotypic identification of MRSA using cefoxitin disc revealed that:

From 39 (100%) *S. aureus* strains isolated on MSA medium: 13 (33.3%) were cefoxitin sensitive with zone diameter ≥ 18mm and 26 strains (66.7%) were cefoxitin resistant with zone diameter ≤ 14mm (Table 7).

Table (7): Phenotypic identification of MRSA strains by disc diffusion method:

	Susceptibility to oxacillin			**Susceptibility to cefoxitin**			**X^2**	**P**
	Sensitive	Resistant		Sensitive	Resistant	Total	0.05	>0.05 * N.S
Zone diameter	≥ 13mm	≤ 10mm		≥ 18mm	≤ 14mm			
NO.	14	25	39	13	26	39		
Percent %	35.9%	64.1%	100%	33.3%	66.7%	100%		

P >0.05 * N.S: non significant

Phenotypic identification of MRSA by culture media:

♦By using ChromID™ MRSA (MRSAID) agar for MRSA isolation; culture of 39 *S.aureus* strains isolated previously on MSA, MSA showed that:

- After incubation for 24h; 25 (64.1%) strains showed growth (green colonies) while 14 (35.9%) strains showed no growth .
- After incubation for 48 h the number of strains showing growth was increased to 27 (69.2%) and only 12 (30.8%) strains showed no growth (Table 8).

♦Phenotypic identification of MRSA using Mannitol salt agar supplemented with cefoxitin (MSA-FOX) revealed that:

Out of 39 isolated *S. aureus* strains cultured and incubated on MSA-FOX for 24h ; 22 (56.4%) showed growth (yellow colonies) while 17 (43.6%) strains showed no growth.

After incubation for 48 h the number of strains showing growth was increased to 26 (66.7%) and only 13 (33.3%) strains showed no growth (Table 8).

Table (8): Phenotypic identification of MRSA by culture on MRSAID agar and MSA-FOX:

Results of growth	Culture media			
	MRSAID agar		MSA-FOX agar	
	Incubation time		Incubation time	
	24h	48h	24h	48h
Growth	25 (64.1%)	27 (69.2%)	22 (56.4%)	26 (66.7%)
No growth	14 (35.9%)	12 (30.8%)	17 (43.6%)	13 (33.3%)
Total	39	39	39	39
X^2	0.1		0.5	
P	>0.05		>0.05	

Results of PCR analysis:

PCR was applied to 39 (100%) *S. aureus* strains isolated on MSA medium for detection of mec A gene; 25 (64.1 %) isolates were positive for mec A gene (MRSA) while 14 (35.9%) isolates were negative for it (MSSA) (Table 9).

Table (9): Results of PCR used for mec A gene detection.

Distribution / **Results of PCR used for mec A gene detection**	*S.aureus* strains	Percent (%)
mec A +ve (MRSA)	25	64.1 %
mec A –ve (MSSA)	14	35.9%
Total	39	100%

Comparison between phenotypic identification of MRSA by oxacillin disc diffusion (ODD) and genotypic identification by PCR.

Table 10 shows that by the use of ODD method for phenotypic detection of MRSA: out of 39 isolated *S.aureus* strains, 25(64.1 %) resistant strains were detected (MRSA); 23(92.0%) of them were mec A positive and 2 (8.0%) were mec A negative. Fourteen strains were found to be sensitive to oxacillin (MSSA); 12 (85.7%) of them were mec A negative and 2 (14.3%) were mec A positive (i.e there are 2 false positive and 2 false negative cases).

Table (10): Sensitivity,specificity, positive predictive value (PPV) and negative predictive value(NPP) of oxacillin disc diffusion (ODD) method in relation to PCR.

ODD / PCR	Resistant (MRSA)		Sensitive (MSSA)		Total
	No.	Percent	No.	Percent	No.
Mec A positive	23	92.0 %	2**	14.3 %	25
Mec A negative	2*	8.0 %	12	85.7 %	14
Total	25	100 %	14	100 %	39

Sensitivity = 92.0 % PPV = 92.0 %
Specificity = 85.7 % NPV = 85.7 %

2*: Two false positive cases; they lacked mec A gene but showed resistance by ODD method (MRSA).

2**: Two false negative cases; they carried mec A gene but showed sensitivity by ODD method (MSSA).

Comparison between phenotypic identification of MRSA by cefoxitin disc diffusion (CDD) and genotypic identification by PCR.

Table 11 shows that by the use of CDD method for phenotypic identification of MRSA 26 resistant strains (MRSA) were detected out of 39 isolated *S.aureus* strains; 25(96.2 %) of them were mec A positive while 1 (3.8%) strain was mec A negative.Therteen (100 %) strains were found to be sensitive to cefoxitin (MSSA) ; all of them were mec A negative (i.e there is 1 false positive case and no false negative cases).

Table (11): Sensitivity,specificity, positive predictive value (PPV) and negative predictive value(NPP) of cefoxitin disc diffusion (CDD) method in relation to PCR

CDD / PCR	Resistant (MRSA)		Sensitive (MSSA)		Total
	No.	Percent	No.	Percent	No.
mec A positive	25	96.2 %	0**	0.0 %	25
mec A negative	1*	3.8 %	13	100 %	14
Total	26	100 %	13	100 %	39

Sensitivity = 100 % PPV = 96.2%

Specificity = 92.8 % NPP = 100 %

1*: One false positive case that lacked mec A gene and showed resistance to cefoxitin disc (MRSA).

0**: No false negative cases i.e all strains showed sensitivity to cefoxitin disc (13 cases) were negative for mec A gene (MSSA).

Comparison between phenotypic identification of MRSA by MRSAID and genotypic identification by PCR.

Table 12 shows that by culture of 39 isolated *S.aureus* strains on MRSAID medium and incubation for 24h: 25 strains showed growth (MRSA); 23 of them were mec A positive (True +ve) and 2were mec A negative (false +ve). Fourteen strains showed no growth (MSSA) 12 of them were mec A –ve (True –ve) and 2 were mec A positive (false –ve) (i.e there were 2 false positive and 2 false negative cases).

By culture of 39 isolated *S.aureus* strains on MRSAID medium and incubation for 48h: 27 strains showed growth (MRSA); 24 of them were mec A positive (True +ve) and 3 were mec A negative (false +ve) Twelve strains showed no growth (MSSA); 11of them were mec A –ve (True –ve) and one case was mec A positive (false –ve) (i.e there were 3 false positive and 1 false negative cases).

Table (12): Sensitivity, specificity, positive predictive value (PPV) and negative predictive value(NPP) of MRSAID medium in relation to PCR.

Cultureon MRSAID / PCR	Incubation time					
	24h			48h		
	+ve (MRSA)	-ve (MSSA)	Total	+ve (MRSA)	-ve (MSSA)	Total
mec A positive	23	2	25	24	1	25
mec A negative	2	12	14	3	11	14
Total	25	14	39	27	12	39

Sensitivity = 92.0 % Specificity = 85.7 % PPV = 92.0 % NPV = 85.7 %	Sensitivity = 96.0 % Specificity = 78.6 % PPV = 96.0 % NPV = 91.7 %

Comparison between phenotypic identification of MRSA by MSA-FOX and genotypic identification by PCR.

Table 13 shows that: by cuture of 39 isolated *S.aureus* strains on MSA-FOX medium and incubation for 24h: 22 strains showed growth (MRSA) ; 19 of them were mec A positive (True +ve) and 3 were mec A negative (false +ve). Seventeen strains showed no growth (MSSA); 11 were mec A –ve (True –ve) and 6 were mec A positive (false –ve) (i.e there were 3 false positive and 6 false negative cases).

By cuture of 39 isolated *S.aureus* strains on MSA-FOX medium and incubation for 48h: 26 strains showed growth (MRSA); 21 of them were mec A positive (True +ve) and 5 were mec A negative (false +ve). Therteen strains showed no growth (MSSA); 9 were mec A –ve (True –ve) and 4 were mec A positive (false –ve) (i.e there were 5 false positive and 4 false negative results).

Table (13): Sensitivity,specificity, positive predictive value (PPV) and negative predictive value(NPP) of MSA-FOX medium in relation to PCR.

MSA- FOX / PCR	Incubation time					
	24h			48h		
	+ve (MRSA)	-ve (MSSA)	Total	+ve (MRSA)	-ve (MSSA)	Total
mec A gene positive	19	6	25	21	4	25
mec A gene negative	3	11	14	5	9	14
Total	22	17	39	26	13	39
	Sensitivity = 76.0 % Specificity = 78.5 % PPV = 86.4 % NPV = 64.7 %			Sensitivity = 84.0 % Specificity = 64.3 % PPV = 80.8 % NPV = 69.2 %		

The source of MRSA isolates :

The source of the 25 isolated MRSA strains was as follow; 16 strains were isolated from 18 inpatient samples and 9 strains were isolated from 21 outpatient samples. Thus the rate of MRSA isolation from nosocomial infections is 88.8% (16 strains out of 18) while that from community acquired infections is 42.85% (9 strains out of 21).

Table (14): Source of MRSA isolates.

Source	Total No. of *S.aureus* isolates	No. of MRSA	Percentage (%)	z	P
- Hospital acquired infections (30)	(18)	(16)	88.8 %	5.2	> 0.05*
-Community acquired infections (70)	(21)	(9)	42.8 %	1.1	< 0.05**
Total (100)	39	25	64.1 %		

* P > 0.05: There is insignificant statistical difference between the number of the isolated MRSA and total number of *S.aureus* in hospital acquired infection cases.

** P < 0.05: There is a significant statistical relation between the number of the isolated MRSA and total number of *S.aureus* in community acquired infection cases.

The relation between MRSA isolation and patient's age:

The relation between MRSA isolation and patient 's age is demonstrated in (table 15). The age of patients from whom MRSA strains were isolated ranged between 4-70 years (mean 32±19.5). The age of patients from whom MSSA strains were isolated ranged between 2- 67 years (mean 31.1±17.1). (T = 0.2, P = >0.05).

Table (15): The relation between MRSA isolation and patient's age

Age/years	MRSA	MSSA	T	P
Age	(4 - 70)	(2 - 67)	0.2	>0.05 *N.S
Mean ± SD	32±19.5	31.1±17.1		

*N.S: There is insignificant statistical value as regards the relation between the age of patient's /years and MRSA isolation.

The relation between the duration of antibiotic intake and MRSA infection:

The relation between the duration of antibiotic intake / days and MRSA infection is demonstrated in (Table 16). The majority of MRSA were isolated from patients receiving antibiotic treatment for a mean period of (5.4±3.1) days, while MSSA were isolated from patients receiving antibiotic treatment for a mean period of (4.7±2.9) days.
(T = 1.1, P > 0.05)

Table (16): The relation between the duration of antibiotic intake / days and MRSA isolation.

Duration of antibiotic Intake / days	MRSA (25)	MSSA (14)	T	P
Mean ± SD	5.4±3.1	4.7±2.9	1.1	> 0.05*
Range	(0- 10)	(0- 10)		

*P > 0.05: There is insignificant statistical value as regards the relation between the duration of antibiotic treatment / days and the isolation of MRSA isolates.

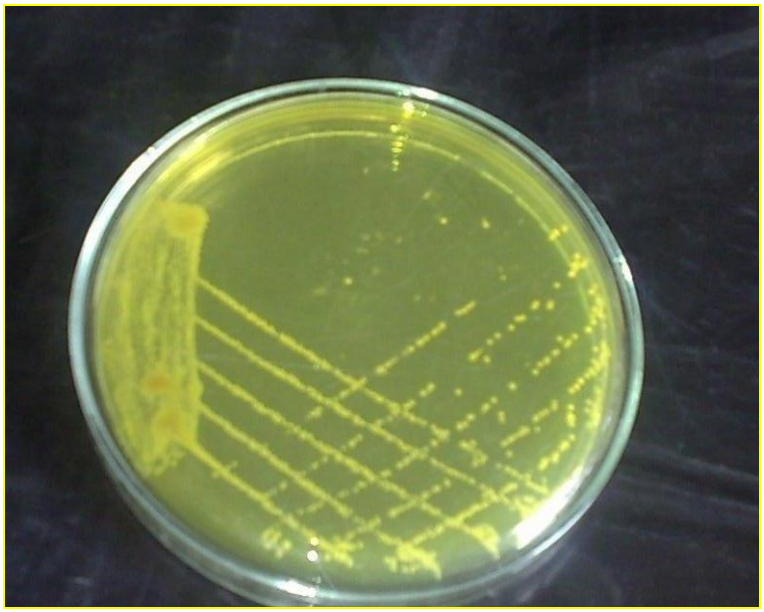

Figure (11): Cultured plate of MSA with *S.aureus* appear as opaque yellow colonies surrounded by yellow zone.

Figure (12): Cultured plate of MSA medium with staphylococcal species other than *S.aureus* which appear as opaque pink colonies.

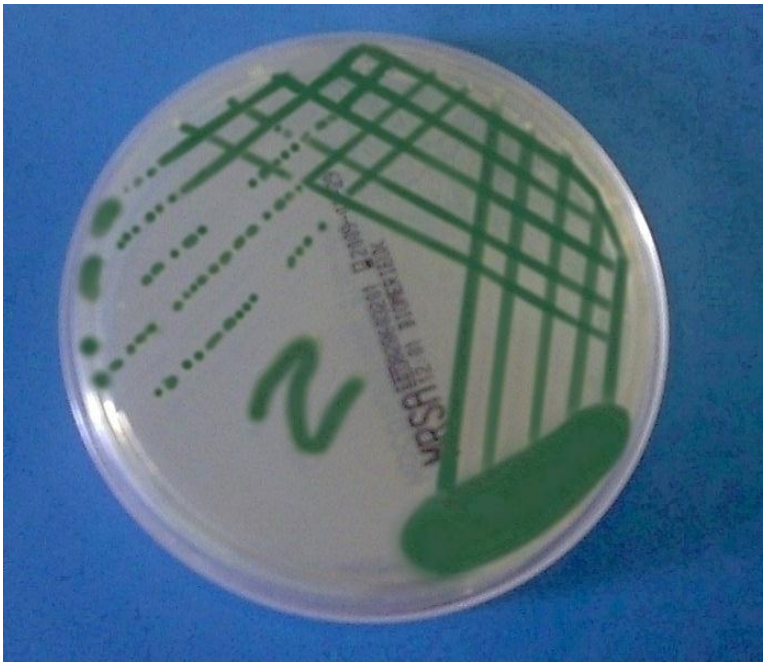

Figure (13): Cultured plate of MRSAID medium with MRSA strains appear as green colonies.

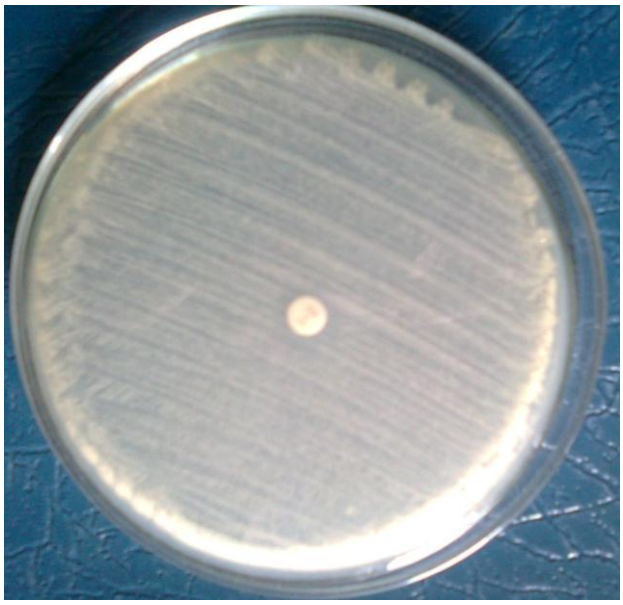

Figure (14): *S.aureus* strain cultured on Muller Hinton plate; it shows resistance to the tested antibiotic disc (inhibition zone diameter = zero)

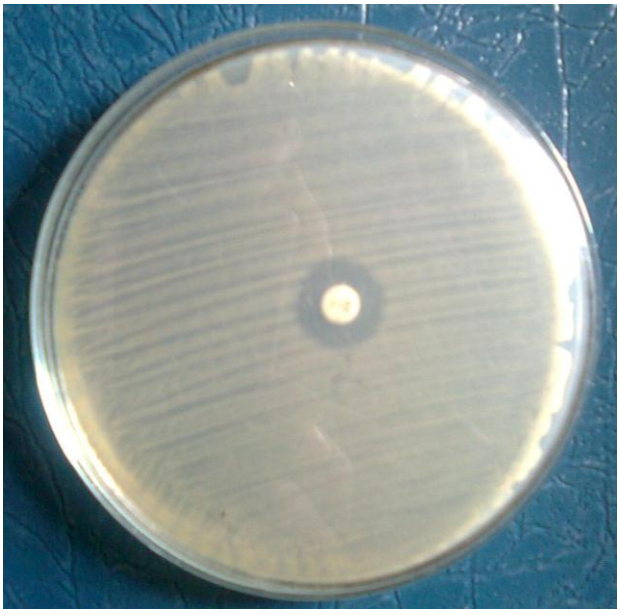

Figure (15): Oxacillin sensitive *S.aureus* strain cultured on Muller Hinton medium (inhibition zone diameter > 13mm)

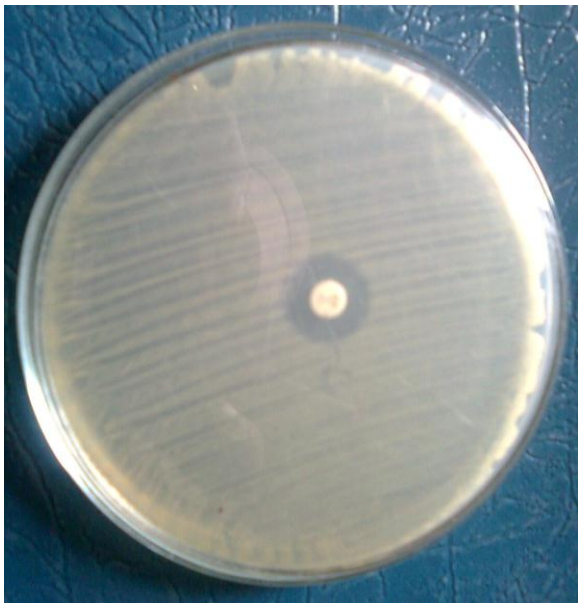

Figure (16): Cefoxitin sensitive *S.aureus* strain cultured on Muller Hinton medium (inhibition zone diameter > 18mm)

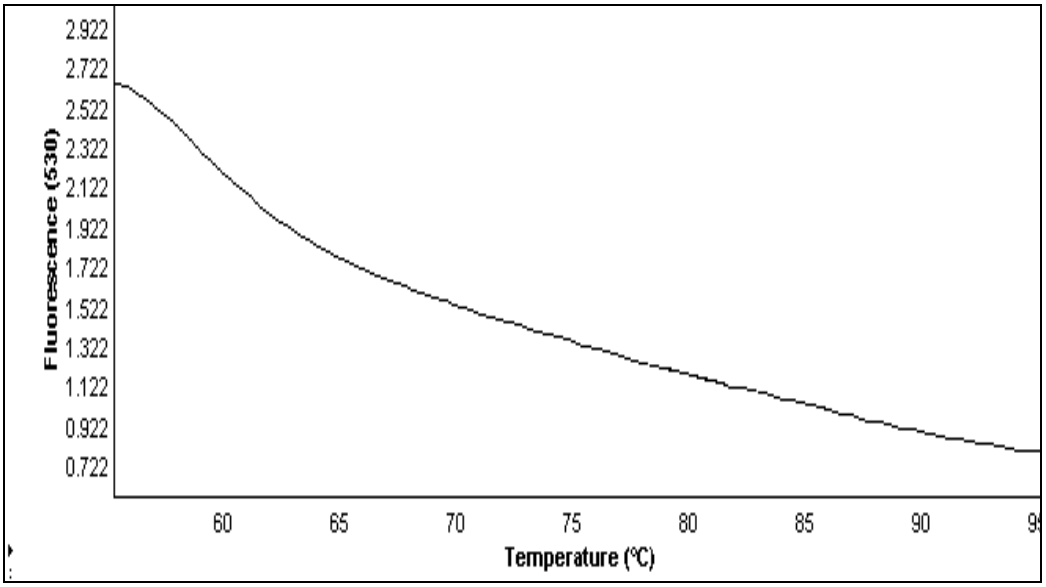

Figure (17): Melting curve of *S.aureus* mec A negative real time PCR.

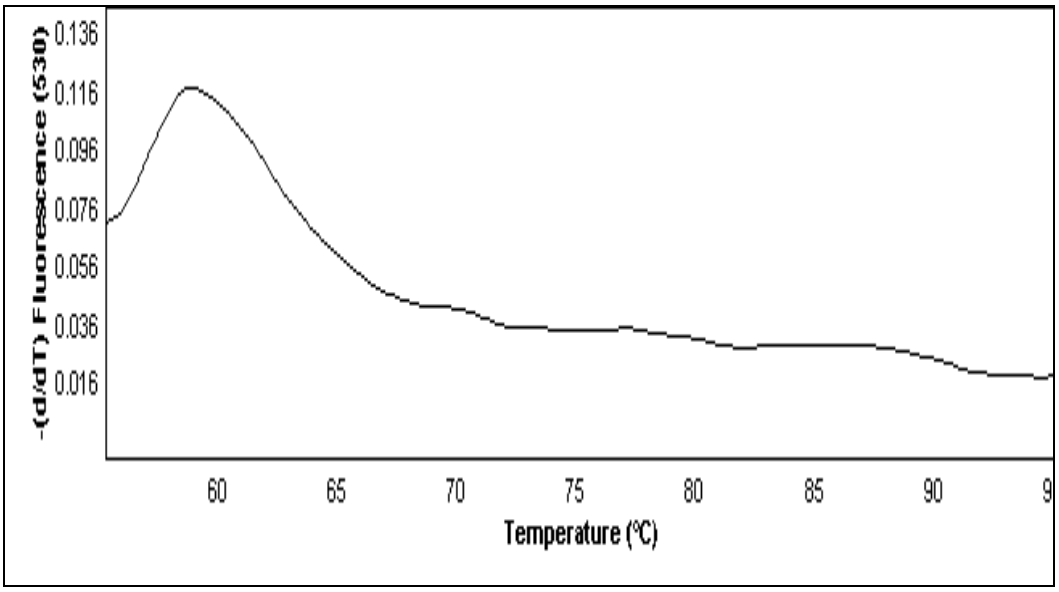

Figure (18): Melting peak of *S.aureus* mec A negative real time PCR.

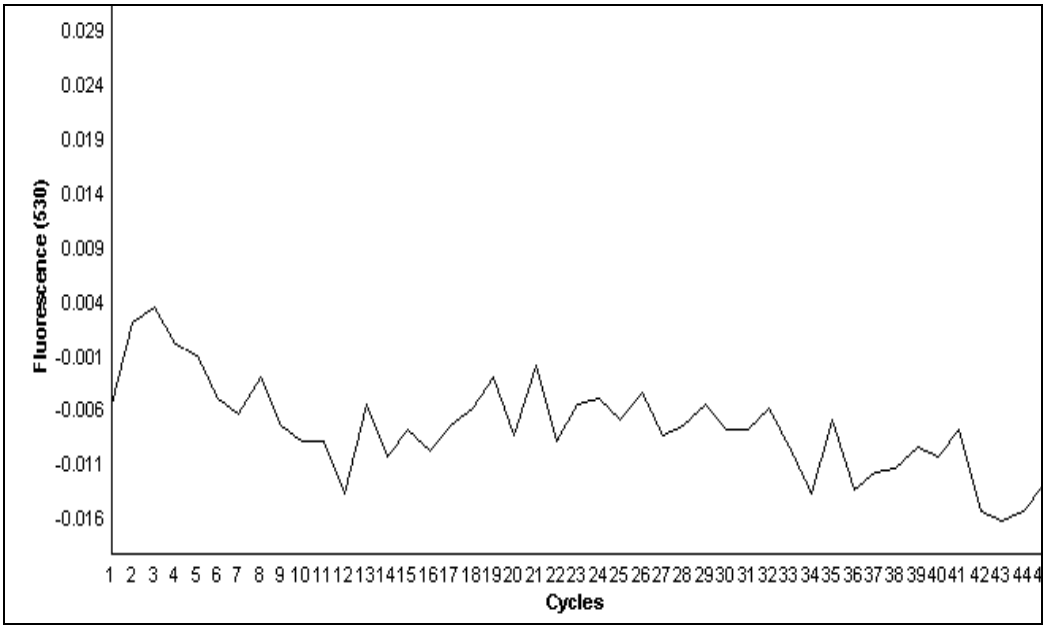

Figure (19): Amplification curve of *S. aureus* mec A negative real time PCR.

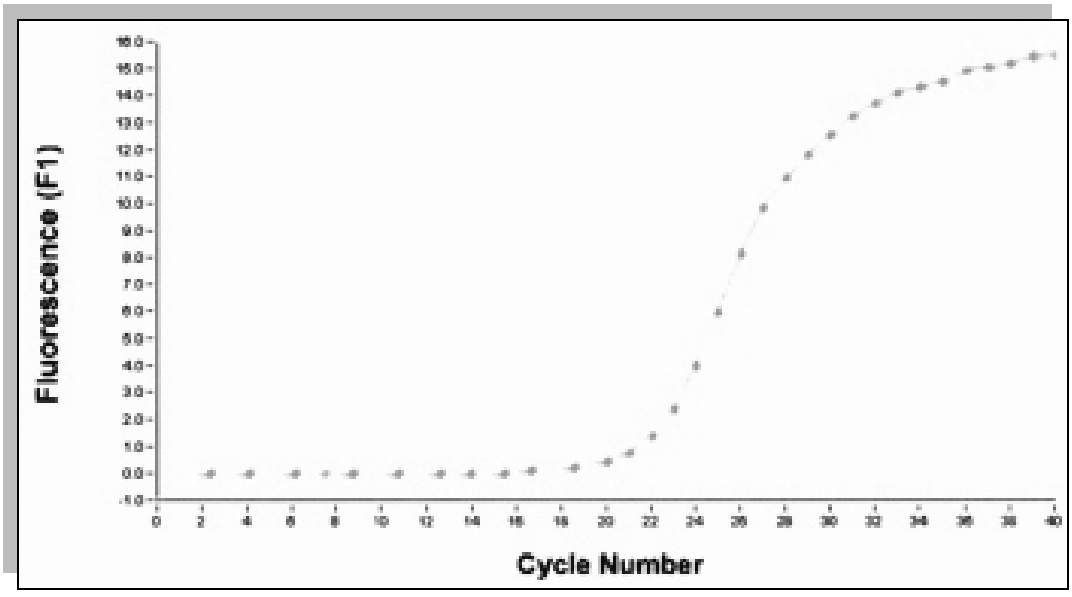

Figure (20): Amplification curve of *S.aureus* mec A positive real time PCR.

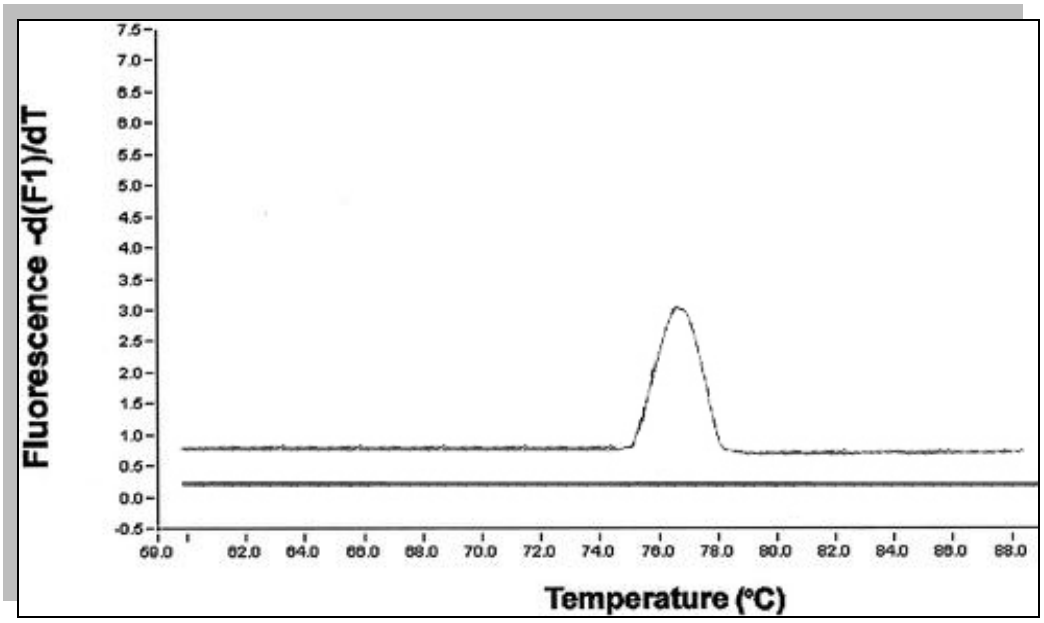

Figure (21): Melting peak of *S.aureus* mec A positive real time PCR.