Results

This study was conducted on 80 patients admitted to Benha University Hospital during the period from October 2008 to the end of September 2009. The age of the patients ranged from days to 74 years. They were 39 males (48.8%) and 41 females (51.2%).

Stool samples were collected from the 80 patients in different hospital departments. Enterococci were isolated from 77 cases out of 80 patients under study.

Antimicrobial susceptibility pattern by disc diffusion method were done for all enterococcal isolates.

Also vancomycin susceptibility tests were done for enterococcal isolates by agar screening method and confirmed by broth dilution method that determined the minimum inhibitory concentration (MIC) which was ≥ 64μg/ml.

Genotyping of the VRE strains by real time PCR revealed that 4 strains had *van*A gene and 1 strain had *van*B gene.

The results of this study are represented in tables 1- 20.

Tables from 1 to 6 Show the clinical data of all the studied cases.

Table 1: Distribution of the study group according to sex:

Sex	No. of cases	Percent (%)
Female	41	51.2 %
Male	39	48.8 %
Total	80	100 %

Table 2: Distribution of the study group according to age:

Age	No. of cases	Percent (%)
Neonate → till 1 month	1	1.25 %
One month → till 1 year	5	6.25 %
One year → 20 years	4	5 %
Twenty years → 40 years	13	16.25 %
Forty years → 60 years	32	40 %
Sixty years → 80 years	25	31.25 %
Total	80	100 %

Table 3: Number of cases collected from different hospital departments:

Department	No. of cases under study	Percent (%)
ICU	23	28.8 %
CCU	7	8.8 %
Hemodialysis	17	21.2 %
Internal medicine	21	26.2 %
Surgery	5	6.2 %
Gastroenterology/ Hepatology	1	1.2 %
NICU	6	7.5 %
Total	80	100 %

ICU= Intensive care unit. CCU= Cardiac care unit. NICU= Neonatal ICU.

Table 4: Classification of the study group according to different diseases:

Disease	No. of cases	Percent (%)
Neonatal diseases	6	7.5 %
Renal	17	21.25 %
Cardiac	8	10 %
Diabetes mellitus	11	13.75 %
Oncological	13	16.25 %
Trauma	7	8.75 %
Hepatic	13	16.25 %
stroke	2	2.5 %
anemia	3	3.75 %
Total	80	100 %

Table 5: Distribution of the study group according to the history of previous hospitalization:

Previous hospitalization	No. of cases	Percent (%)
No	38	47.5 %
Yes	42	52.5 %
Total	80	100 %

Table 6: Distribution of the study group according to the current antimicrobial intake:

Antimicrobial drug	No. of cases	Percent (%)
Vancomycin + other antimicrobial	5	6.25 %
Cephalosporins	33	41.25 %
Others	21	26.25 %
none	21	26.25 %
Total	80	100 %

Table 7 shows the number of enterococcal strains isolated from the study group. There were 77 (96.25%) strains isolated from 80 cases.

Table 7: Number of enterococcal strains isolated from the study group:

Total number of cases	No. of enterococcal isolates
80	77(96.25%)*

*Seventy seven stool samples out of 80 samples from the studied cases showed growth of enterococci on bile aeusculin agar while 3 samples showed no growth.

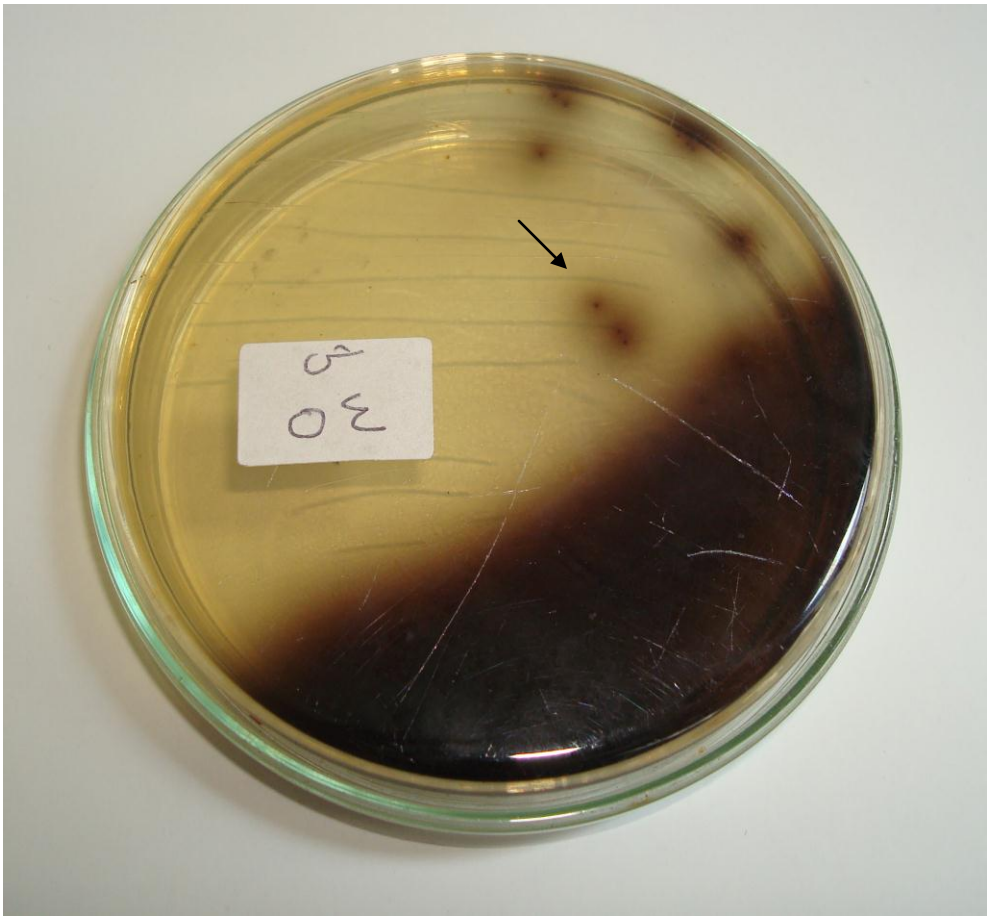

Figure 1: A plate of bile aesculin azide agar showing colonies of enterococci with blackening of the medium around them due to hydrolysis of aesculin.

Figure 2: Growth of enterococci on MacConkey agar: producing small dark red lactose fermenting colonies.

Table 8 shows the antibiotic sensitivity pattern for the isolated enterococci by disc diffusion method. It reveals that:

Out of 77 enterococcal isolates, 74 strains (96.1%) were sensitive to vancomycin and 3 strains (3.9%) were resistant with no intermediately resistant strains.

Thirty five strains (45.5%) out of 77 strains were sensitive to ciprofloxacin, 11 (14.2%) were intermediately resistant and 31 (40.3%) were resistant.

Forty two strains (54.5%) out of 77 strains were sensitive to amoxicillin, 2 (2.6%) were intermediately resistant and 33 (42.9%) were resistant.

Thirteen strains (16.8%) out of 77 strains were sensitive to chloramphenicol, 21 (27.2%) were intermediately resistant and 43 (55.8%) were resistant.

Seventy four strains (96.1%) out of 77 strains were sensitive to gentamycin, 3 (3.9%) were intermediately resistant with no resistant strains.

Fifteen strains (19.5%) out of 77 strains were sensitive to cephradine and 62 strains (80.5%) were resistant with no intermediately resistant strains.

Table 8: Antibiotic sensitivity pattern for the isolated enterococci by disc diffusion method:

	Sensitive	Intermediate	Resistant	Total
Vancomycin	74 (96.1 %)	—	3 (3.9 %)	77(100%)
Ciprofloxacin	35 (45.5 %)	11 (14.2 %)	31 (40.3 %)	
Amoxicillin	42 (54.5 %)	2 (2.6 %)	33 (42.9 %)	
Chloramphenicol	13 (16.8 %)	21 (27.2 %)	43 (55.8 %)	
Gentamycin	74 (96.1 %)	3 (3.9 %)	—	
Cephradine	15 (19.5 %)	—	62 (80.5 %)	

Out of 77 enterococcal isolates, 3(4%) cases were diagnosed as VRE by disc diffusion method. By agar screen and broth dilution methods, 2 additional cases were diagnosed as VRE. So in this study 5 (6.5%) cases out of 77 were diagnosed as VRE. (table 9, 10)

Table 9: Vancomycin susceptibility test for the isolated enterococci by agar screening method:

No. of isolated enterococci	No. of VSE*	No. of VRE**
77	72 (93.5%)	5** * (6.5%)

*VSE= vancomycin sensitive enterococci.

**VRE = vancomycin resistant enterococci.

*** Five cases include 3 resistant strains diagnosed by disc diffusion method.

Table 10: Number of VRE as diagnosed by disc diffusion and agar screening methods:

Method	No. of VSE	No. of VRE	Total
Disc diffusion	74 (96%)	3 (4%)	77 (100%)
Agar screening	72 (93.5%)	5 (6.5%)	

Table 11 shows the Antibiotic sensitivity pattern for the five isolated VRE using the disc diffusion method for vancomycin. The first three strains were diagnosed by disc diffusion method. The fourth and fifth strains in addition to the first three strains were diagnosed by agar screen and confirmed by broth dilution methods.

Table 11: Antibiotic sensitivity pattern for the five isolated vancomycin resistant enterococci (VRE) using the disc diffusion method:

VRE strains / Antimicrobials	1	2	3	4	5
Vancomycin	R	R	R	S	S
Ciprofloxacin	R	R	R	S	S
Amoxicillin	R	R	R	S	S
Chloramphenicol	R	R	R	R	I
Gentamycin	S	S	S	S	S
Cephradine	R	R	R	S	S

R = resistant , S = sensitive , I = intermediately resistant

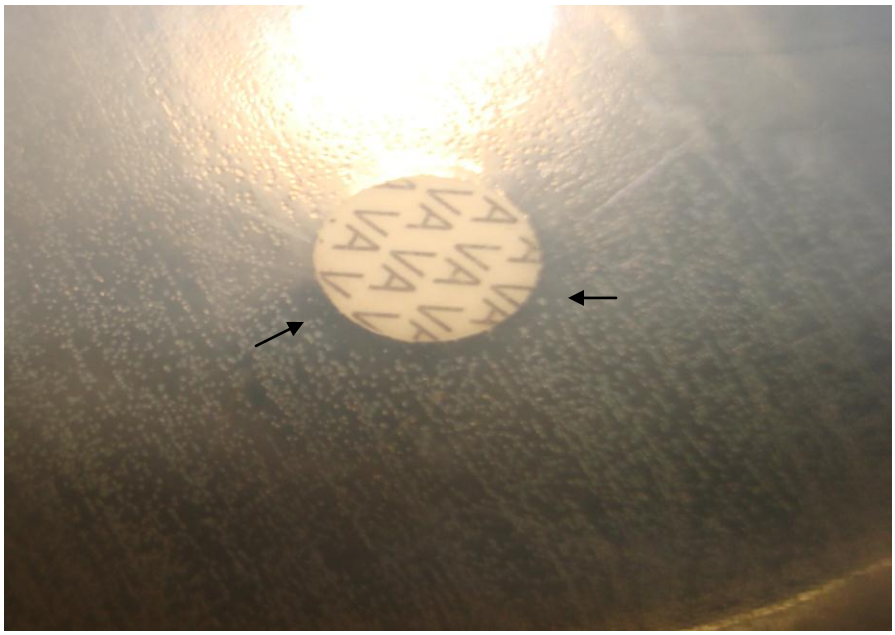

Figure 3: Disc diffusion method on Muller Hinton agar showing VRE as diagnosed by vancomycin disc. Diameter of the inhibition zone < 14 mm.

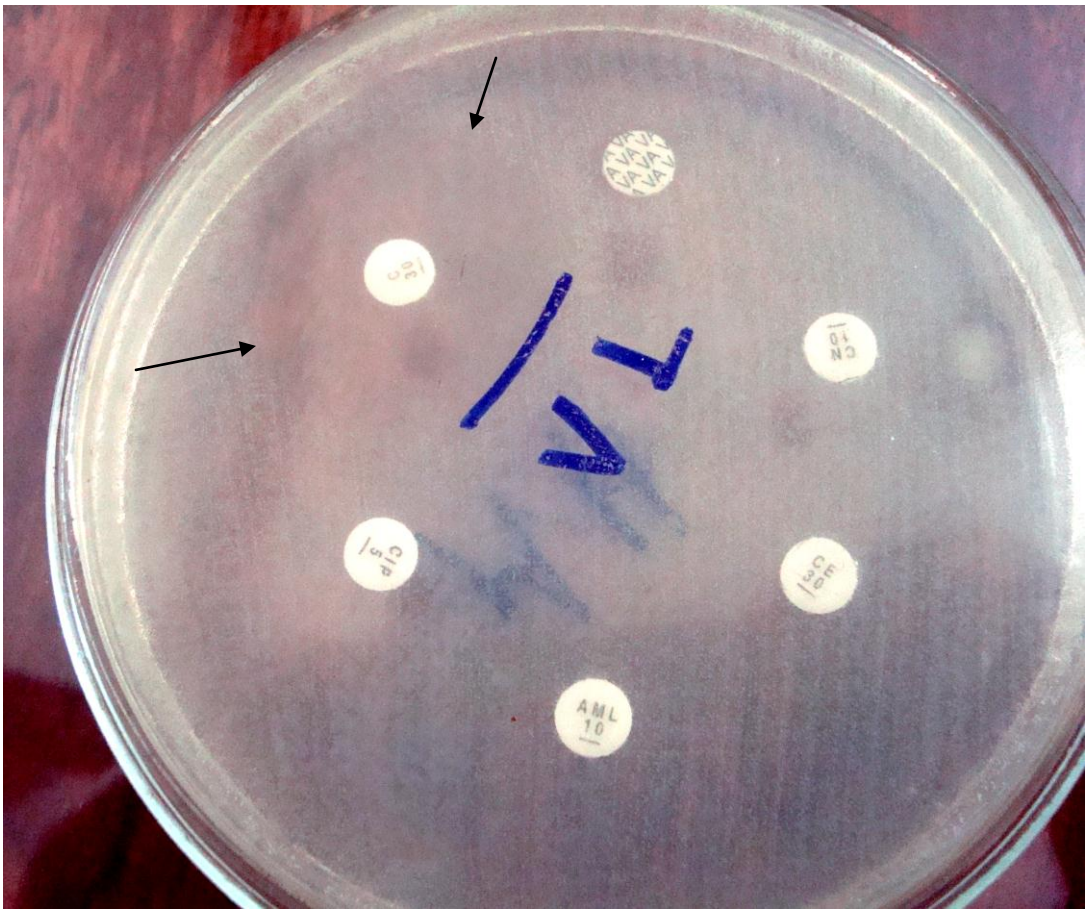

Figure 4: Disc diffusion method on Muller Hinton agar showing the sensitivity pattern of one VRE isolate. The figure showes that the strain is resistant to vancomycin (VA), chloramphenicol (CN), cephradine (CE), amoxicillin (AML) and ciprofloxacin (CIP) but sensitive to gentamycin (G). (The black arrows point to the sensitive zone to (G) disc)

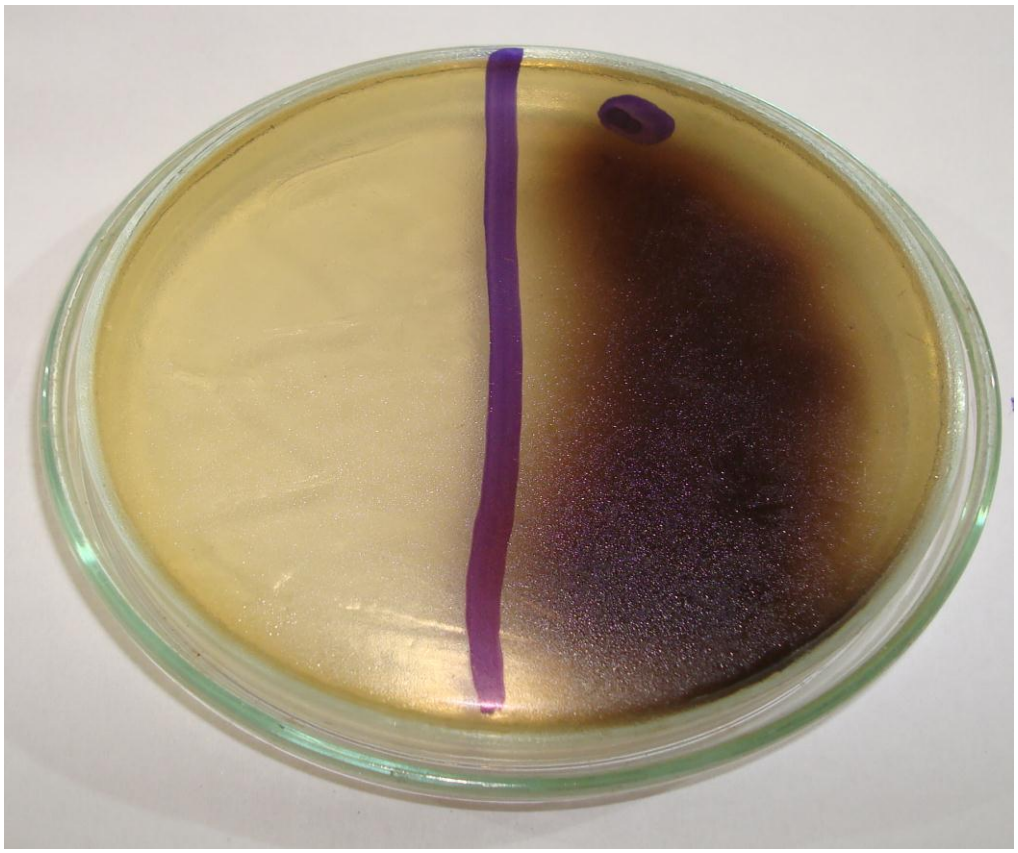

Figure 5: Vancomycin agar screening plate (bile aesculin azide agar medium containing 6 µg /ml vancomycin) Showing VRE on the right side and VSE on the left.

Table 12 shows the Relation between the patients' sex and their enterococcal vancomycin sensitivity. There is insignificant statistical value in the relation between the patients' sex and vancomycin sensitivity of their enterococcal isolates.

Table 12: Relation between the patients' sex and their enterococcal vancomycin sensitivity:

Vancomycin sensitivity	sex			X^2	p
	female	male	Total		
Sensitive strains	37 (51.4%)	35 (48.6%)	72(100%)	0.6	>0.05*
Resistant strains	4 (80%)	1 (20%)	5(100%)		
Total	41	36	77		

* None significant.

Table 13 shows the Relation between the patients' age groups and their enterococcal vancomycin sensitivity. There is insignificant statistical value between the age groups of the studied patients and the sensitivity of the isolated enterococci to vancomycin.

Table 13: Relation between the patients' age groups and their enterococcal vancomycin sensitivity:

Age groups	No.	VSE	VRE	z	p
Neonate → till 1 month	1	1(1.3%)	0	0.3	>0.05*
One month → till 1 year	2	2(3%)	0	0.4	>0.05*
One year → 20 years	4	4(5%)	0	0.5	>0.05*
Twenty years → 40 years	13	12(15.5%)	1(1.3%)	0.2	>0.05*
Forty years → 60 years	32	29(37.6%)	3(3.9%)	0.9	>0.05*
Sixty years → 80 years	25	24(31%)	1(1.3%)	0.6	>0.05*
Total	77	72	5		

The percentages in this table are calculated according to the total 77 cases.

*None significant.

Table 14 shows the Relation between the patient's age and their enterococcal vancomycin sensitivity. There is insignificant statistical value between the age of the studied patients and the sensitivity of the isolated enterococci to vancomycin.

Table 14: Relation between the patients' age and their enterococcal vancomycin sensitivity:

	VSE (neonate →80 years)	**VRE** (30 → 74 years)	**p***
Age (neonate →80 years) (X±SD)	43.7±20.7	47.4±16.5	>0.05**

*t- test is used.

**None significant.

Table 15 shows the Relation between the duration of hospitalization / days and enterococcal vancomycin sensitivity. There is insignificant statistical value between the duration of hospitalization of the studied cases and the sensitivity of their isolated enterococci to vancomycin.

Table 15: Relation between the duration of hospitalization and enterococcal vancomycin sensitivity:

	VSE	**VRE**	**P***
Duration of hospitalization/ days (X±SD)	13.2±14.1	15.4±8.8	>0.05**

***** Mann-Whitney Test is used

** None significant.

Table 16 shows the Relation between the history of previous hospitalization and enterococcal vancomycin sensitivity. There is insignificant statistical value between the history of previous hospitalization of the studied cases and the sensitivity of their isolated enterococci to vancomycin.

Table 16: Relation between the history of previous hospitalization and enterococcal vancomycin sensitivity:

Vancomycin sensitivity / previous hospitalization	VSE	VRE	Total	X^2	p
Yes	38 (90.4%)	4 (9.5%)	42	0.5	>0.05*
No	34 (97%)	1(3%)	35		
Total	72 (93.5%)	5 (6.5%)	77		

N.B: Percentage is calculated according to the total No. of each group.

* None significant.

Table 17 shows that in neonate, patients with renal failure, leukemia, trauma and hepatic diseases there is a significant statistical difference between the no. of VSE and VRE.

Table 17: Number of vancomycin sensitive enterococci (VSE) and vancomycin resistant enterococci (VRE) isolated in relation to different diseases under study:

Diagnosis		No. of cases	No. of isolated enterococci (77)		P
			VSE	VRE	
Neonatal diseases		6*	3 (3.9%)	0	<0.05**
Renal failure		17	17 (22%)	0	<0.05**
Cardiac diseases		8	7 (9 %)	1 (1.3%)	>0.05***
Diabetes		11	11 (14.2%)	0	>0.05***
Tumors (13)	**Leukemia**	9	6 (7.8%)	3 (3.9%)	<0.05**
	Lymphoma	2	2 (2.6%)	0	>0.05***
	Cancer ovary	1	1 (1.3%)	0	>0.05***
	Brain tumor	1	1 (1.3%)	0	>0.05***
Trauma		7	7 (9%)	0	<0.05**
Hepatic diseases		13	13 (16.8%)	0	<0.05**
Stroke		2	2 (2.6%)	0	>0.05***
Anemia		3	2 (2.6%)	1 (1.3%)	>0.05***
Total		80	72	5	

-The percentage in this table is calculated according to the total no. of isolated enterococci.

- Four cases out of five VRE carriers were isolated from hematology ward of internal medicine department.

* From stool samples of 6 neonatal cases, 3 samples only showed growth on bile aeusculine agar.

**Significant

***None significant.

Table 18 shows Comparison between VSE and VRE as regards current antimicrobial intake. There is a significant statistical value between the use of vancomycin and colonization with VRE. There is insignificant statistical value between the use of cephalosporins and VRE.

Table 18: Comparison between vancomycin sensitive enterococci (VSE) and vancomycin resistant enterococci (VRE) as regards current antimicrobial intake:

Antimicrobial	No. of cases	VSE	VRE	Z	P
None	21	20 (26%)	1 (1.3%)	4.5	<0.05***
Vancomycin + other antimicrobials	5*	3 (3.9%)	1 (1.3%)	2.1	<0.05***
Cephalosporins	33	30 (39%)	3 (3.8%)	1.4	>0.05*
Others	21**	19 (24.7%)	—		
Total	77	72	5		

* One case out of 5 stool sample showed no growth on Bile aeusculine agar.

**Two cases of them; their stool samples showed no growth on bile aeusculine agar.

***Significant

*None significant

Table 19 shows the history of the VRE cases as regards age, sex, hospital department, underlying disease, duration of hospitalization, antimicrobials used and previous hospitalization.

Table 19: Data of cases of vancomycin resistant enterococci carriers:

History of VRE cases	Case no.				
	1	**2**	**3**	**4**	**5**
Age	74	30	43	50	40
Sex	female	female	female	male	female
Hospital department	CCU	Hematology	Hematology	Hematology	Hematology
Underlying disease	Cardiac disease (atrial fibrillation, hypertension)	Acute lymphocytic leukemia	Acute myeloid leukemia	Acute myeloid leukemia	Anemia for investigations
Duration of hospitalizati-on	5 days	20 days	25 days	20 days	7 days
Antimicrob-ials used	No	Cephalosporins	Vancomycin in combination with other antimicrobials	Cephalosporins	Cephalospori-ns
Previous hospitalizat-ion	Yes	Yes	Yes	Yes	No

Table 20 shows the genotypic analysis of vancomycin resistant enterococcal isolates. Four cases (80%) out of five VRE cases had *van*A gene while one case (20%) had *vanB* gene.

Table 20: Genotypic analysis of vancomycin resistant enterococcal isolates:

Genotype	No. of VRE isolates	Percent (%)
vanA	4	80%
vanB	1	20%
Total	5	100%

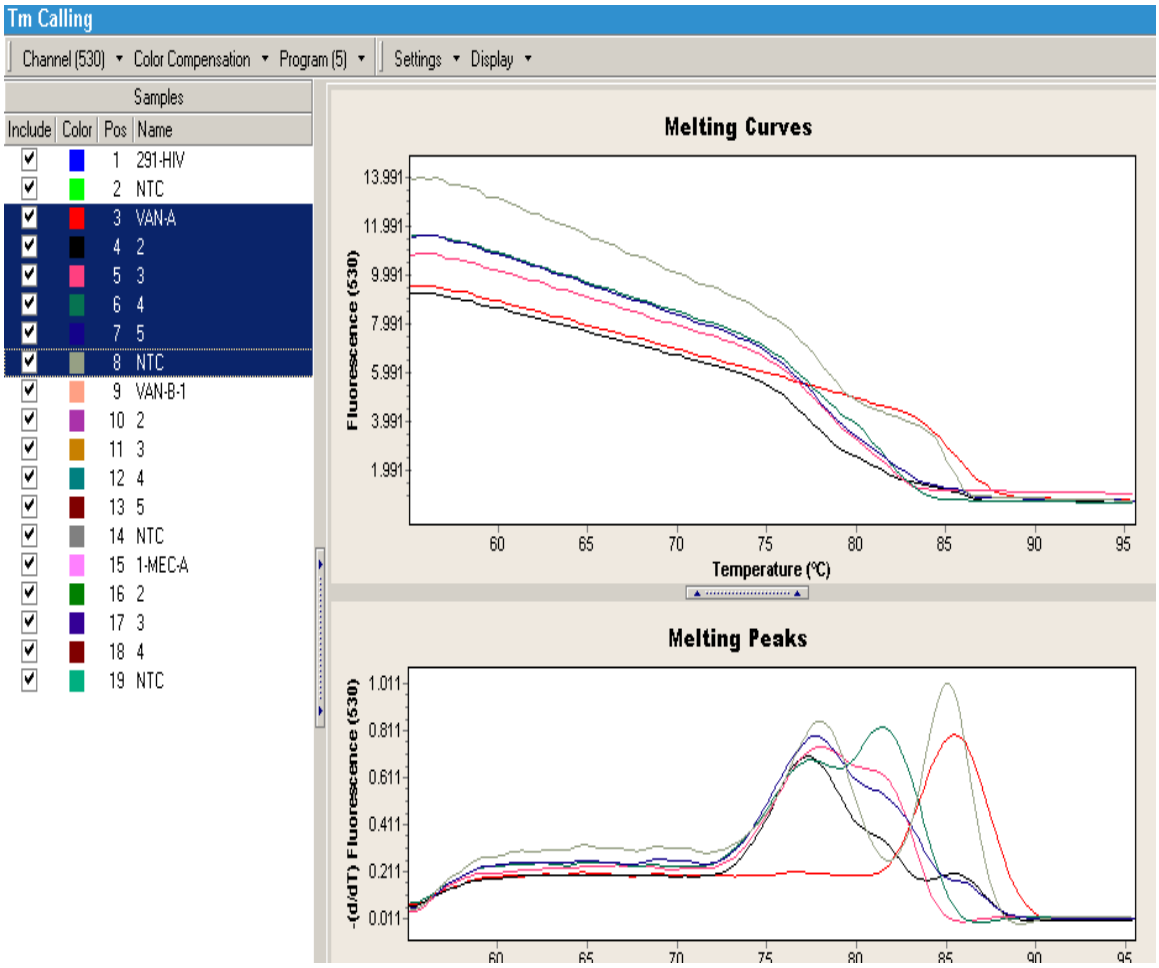

Figure 6: Melting curve analysis for LightCycler *van*A detection assay. The melting temperatures were determined by melting curve analysis. They were as follows: *van*A 80º±5.

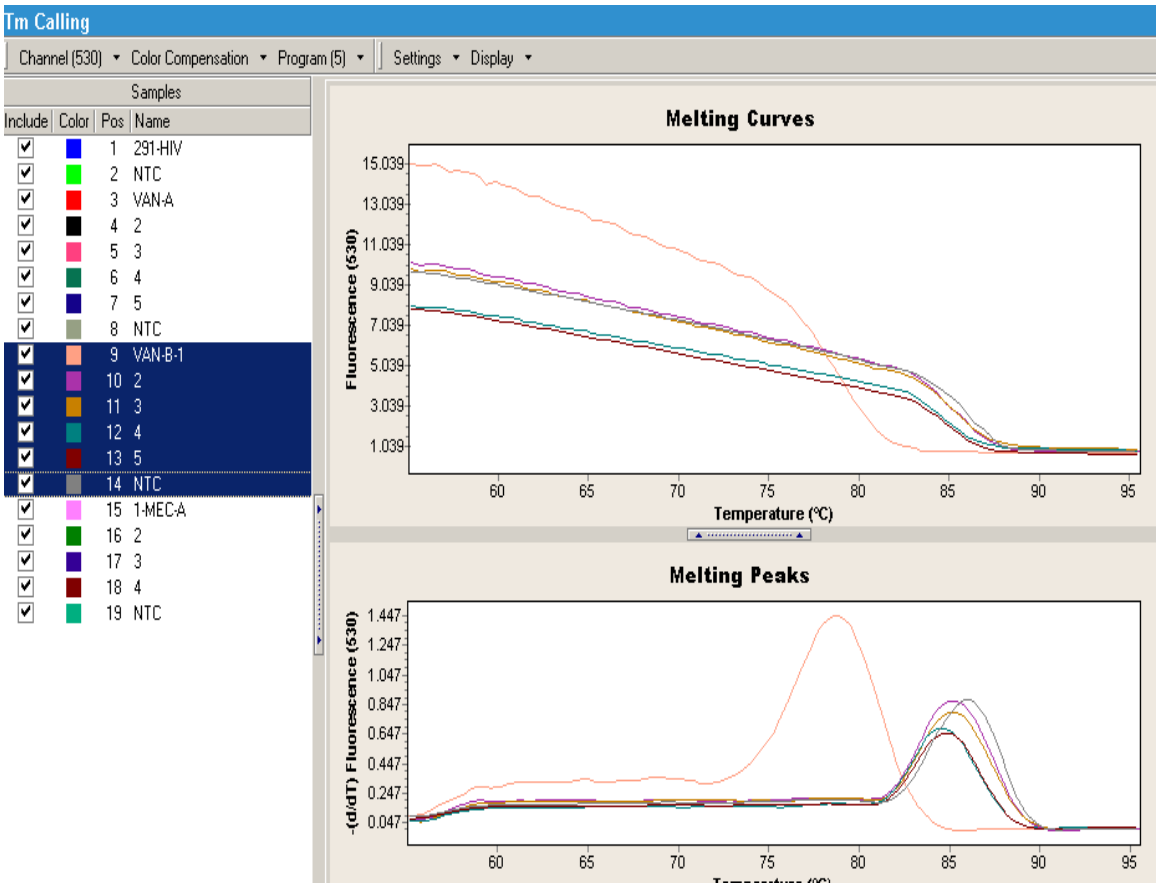

Figure 7: Melting curve analysis for LightCycler *van*B detection assay. The melting temperatures were determined by melting curve analysis .they were as follows: *van*B 78º±3.