LIFE: International Journal of Health and Life-Sciences
ISSN 2454-5872

Ahmed et al, 2017

Volume 3 Issue 3, pp.69-87

Date of Publication: 21th December, 2017

DOI-https://dx.doi.org/10.20319/lijhls.2017.33.6987

This paper can be cited as: K.N, N; Daniel, B.B & Varghese, J. (2017). The Influence of Plasmids and Other MGE's Over Antibiotic Resistance in Multidrug Resistant Staphylococcus Aureus Isolated from HIV+ Patients. LIFE: International Journal of Health and Life-Sciences, 3(3), 69-87.

THE INFLUENCE OF PLASMIDS AND OTHER MGE'S OVER ANTIBIOTIC RESISTANCE IN MULTIDRUG RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM HIV+ PATIENTS

Nihal Ahmed K N
Research student at St. Joseph's college, Bangalore University, Bangalore, India
nihal95ahmed@gmail.com

Betty Bagyam Daniel
Professor of Botany, St Joseph's college, Bangalore University, Bangalore, India
joyrachel321@yahoo.co.in

Jancy Varghese
Research scholar, St Joseph's college, Bangalore University, Bangalore, India
jansjc@gmail.com

Abstract

Bacterial opportunistic infections are common among immunocompromised patients. Antibiotic resistance hinders efforts to treat such infections. Our objectives are to detect the prevalence of Staphylococcus aureus in immunocompromised HIV patients, to analyze their antibiogram, to correlate antibiotic resistance with the presence of plasmids and to identify crucial antibiotic resistance genes and understand their connectivity with MGEs. Bacterial strains were collected from HIV+ve patients. Pure cultures of Staphylococcus aureus were isolated and its clones were selected for further studies. Their Antibiogram and plasmid profiles were analysed. Vancomycin resistant strains were selected and PCR was conducted to trace the presence of Van A in both the gDNA and plasmids. There wasn't much correlation between antibiotic resistance and

plasmid profile. The most resistant strains didn't have high copy number plasmids than the less resistant ones. Thus Plasmids weren't the sole determinants of antibiotic resistance. This led us to explore other MGEs that could aid in conferring antibiotic resistance -an MGE that could be stationed on both gDNA & Plasmids. Literature shows that b-lactam resistance is due to transposons. A Tn3 family that's non-constitutive, replicative DNA transposon. This transposon stays on the gDNA further lowering the possibilities of losing it via vertical gene transfer. VanA is a part of similar transposon Tn 1546 of the Tn3 family. PCR for Van A on Vancomycin resistant strains showed positive results both on gDNA and plasmids. The outcomes open up other possible pathways to tackle MDR strains than antibiotics.

Keywords

MGE's(Mobile Genetic elements), Vancomycin(A glycopeptide antibiotic), Plasmid profile, Transposons

1. Introduction

Staphylococcus aureus is an opportunistic pathogen that causes a wide variety of self-limiting to life-threatening diseases in humans (Murray, P. R., Baron E J, Jorgenson J H, Landry M L, Pfaller M A, and Yolken R)

20% of the human population are long-term carriers of *S. aureus* that can be found as part of the normal skin flora, in the nostrils, and the lower reproductive tract of women (Ehiwario N.J* and Oshilim) Staphylococcus aureus has emerged as a potential opportunistic pathogen among HIV and AIDS patients in both hospital and community settings. Recent studies have shown greater frequency and morbidity of this organism among HIV positive individuals (J Chacko et al)(Alicia I Hidron, Russell Kempker, Abeer Moanna, and David Rimland,)

1.1 Antibiotic resistance

The unique characteristic of S. aureus is the diverse mechanism of antibiotic resistance and variety of virulence factors responsible for establishment of staphylococcal infections (F. D. Lowy). Resistance is very often acquired by horizontal transfer of genes from outside sources, although mutation and antibiotic selection are important too.

The emergence of antibiotic-resistant strains such as methicillin-resistant S. aureus (MRSA) is a global problem in clinical medicine. There is no approved vaccine for S. aureus despite much research and development (Dennis de Carvalho Ferreira et al)

Recently, *S. aureus* acquired vancomycin resistance genetic elements from enterococci, resulting in the emergence of vancomycin-resistant *S. aureus* (VRSA) (Weigel LM, et al).

Vancomycin has the most reliable antimicrobial activity against MRSA. The emergence of resistance to vancomycin in S. aureus has been predicted based on high levels of Vancomycin resistance in enterococci and transfer of the vanA-containing plasmid from enterococci into S. aureus (Anita Bhalla David C Aron, and Curtis J Donskey).

S. aureus isolates with reduced susceptibility to vancomycin appears to have developed from pre-existing methicillin or related drug resistant S. aureus infection(Tenover FC[1], Biddle JW)

The reduced susceptibility of S. aureus strains to vancomycin leaves clinicians with but little therapeutic options to treat these infections. Thus it highlights the significance of prudent use of antibiotics (Alicia I Hidron, Russell Kempker, Abeer Moanna, and David Rimland).

Misuse of vancomycin could lead to the emergence of resistant strains in S. aureus.

1.2 Plasmid profile

Many species of bacteria possess plasmids, thus plasmid profile typing has been used to investigate outbreaks of many bacterial diseases and trace inter- and intra-specific spread of antibiotic resistance (L W Mayer).

The evolution and spread of resistance plasmids has been promoted by the rampant use of antibiotics in medicines and animal husbandry. In the presence of antibiotics, the acquisition of resistance plasmids by the host cells is definitely beneficial. Cells with Plasmids multiply and survive, whilst non-plasmid carrying cells will be eliminated. Plasmids are passed on vertically to the cell's progeny during cell division. Nonetheless, many resistance plasmids are capable of mediating their transfer to other organisms, and indeed to the organisms of other species, via conjugation. This greatly increases the potential for the spread of resistance genes situated on plasmids. The plasmid, which is separate from the cell in evolutionary terms, ensures its maintenance in the cell whether or not antibiotic selection pressure is present. Resistance plasmids seem to be stable in bacterial populations, even in the absence of selective pressure; indeed their stability will benefit the progeny of the cell in future encounters with the antibiotic, which may be likely in the case of commonly used drugs (Bennett PM, Linton AH)

Staphylococci usually carry one or more plasmids per cell and these plasmids have varied gene content. Staphylococcal plasmids are classified into one of the three following groups: 1) small multi-copy plasmids that are cryptic or carry a single resistance determinant; 2) larger (15–30 kb) low copy (4–6/cell) plasmids, which usually carry several resistance determinants; and 3) conjugative multi-resistance plasmids (Berg T, Firth N, Apisiridej S, Hettiaratchi A, Leelaporn A, Skurray RA). Larger plasmids undergo theta replication, whereas small plasmids usually replicate by the rolling-circle mechanism (Lindsay JA). As a consequence of the limited ability of *S. aureus* to acquire DNA from the environment compared to bacteria such as *Escherichia coli* or *Bacillus subtilis*, intercellular transfer of staphylococcal plasmids most often occurs by transduction or conjugation. Upon entering the bacterial host, staphylococcal plasmids remain as free circularized DNA or linearize and integrate into the chromosome (Natalia Malachowa and Frank R. DeLeo)

1.3 Transposons

Transposons are a class of mobile genetic elements that can “jump” to different locations within a genome. Although these genetic elements are frequently called “jumping genes,” they are always maintained at an integrated site in the genome. These include class II transposons, miniature inverted-repeat transposable elements (MITEs, or class III transposons), and retrotransposons (class I transposons).

Class I transposons are a heterogeneous group of mobile genetic elements that carry a variety of resistance genes that share similar structural and functional properties, but share little

DNA homology. The class II transposons are more homogeneous, comprising three different but related families, the Tn3, Tn21 and Tn2.501 group (Lafond M, Couture F, Vezina G, Levesque RC).

The Tn3 class transposons are replicative, non-composite transposons. They are large transposons of about 5000 nucleotide base pairs that are flanked by simple inverted repeats (38 to 40 nucleotide pairs). T*n*3 has three genes namely *tnpA*, *tnpR* and *bla* that encodes for transposase, repressor or resolvase and beta lactamases respectively. Transposase and Resolvase help in regulating transposition while beta lactamases provide resistance against the Ampicillin and other beta lactamases (Nicolas E, Lambin M, Dandoy D, Galloy C, Nguyen N, Oger CA, Hallet B).

Unlike cut and paste mechanism of IS elements these move by replicative mechanism. Transposition occurs by fusing two molecules of plasmids one carrying T*n*3 and other not. This fusion is aided by transposase and the fused structure is called as cointegrate. During the fusion process T*n*3 is replicated and two copies of T*n*3 are present in the cointegrate at each junction. Both the T*n*3 elements are arranged in the same orientation. In the next step, resolvase resolves the cointegrate into two plasmids again each having one copy of T*n*3. The enzyme resolvase also facilitates the recombination between the two copies of T*n*3. This event occurs at the *res* site which is located between the genes *tnp*A and *tnp*R. This region is also called as internal resolution site (IRS) (Nicolas E, Lambin M, Dandoy D, Galloy C, Nguyen N, Oger CA, Hallet B)

Tn 1546 that belongs to the same family of Tn 3 transposon is responsible for resistance against Vancomycin. It is flanked by two inverted repeats with a region for Transposase, Resolvase and Van operon. Van operon contains a cluster of seven genes (*vanS*, *vanR*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ*) (Nicolas E, Lambin M, Dandoy D, Galloy C, Nguyen N, Oger CA, Hallet B).

2. Material and methods

Many isolates were taken from HIV patients from across Bangalore. Pure cultures were isolated from Staph specific agar and grown in Brain Heart infusion broth. 10 random pure culture isolates were chosen for the project, 5 from skin, and 5 from throat. 10 strains were labelled accordingly and used for further studies.

2.1 Antibiotic sensitivity test

This test was conducted for all the strains of the cultures. Petri plates and the media containing Mueller Hinton Agar were autoclaved and transferred to the LAF. After the plates cooled, the agar was poured into 10 petri plates, each for one strain. About 0.5mm of agar was poured in each of the 10 plates. It was let to cool and solidify. After the agar solidified, the 18 hour cultures with Mc Farland standard value of 0.5 were swabbed on each plate using a sterile cotton bud. The cultures were uniformly swabbed and the antibiotic discs were gently lowered onto the plate using a sterile forceps. The plates were immediately covered with the lid. The sides of the Petri plate locked tight with cling film. They were then transferred into the incubator, and let for 18 hours. After 18 hours, they were taken out and checked for sensitivity.

2.2 Plasmid Isolation

Plasmid isolation was done by Alkaline lysis method. 18 hour-cultures of the strains in LB Broth were used.1.5ml of each strain was transferred into sterilized Eppendorf tubes. These were now labelled with the strain names and ready for processing. They were centrifuged at 20,000 rpm for 10 minutes. The supernatant was discarded. Into the pellet that remained in the tubes, 100µl of suspension (Phosphate buffer) was added. The pellet was re-suspended with a vortex and 5µl of alkaline proteinase – Proteinase K was added. The tubes were then placed in a water bath at 37^{o}C for 10 minutes.100µl of NaOH/SDS Detergent solution was added. The tubes were shaken for 2 minutes and let to stand at room temperature for 5 minutes.100 µl of 3M CH_3COONa was added. The tubes were shaken for 2 minutes and placed in ice for 5 minutes. They were then spun in a microfuge for 5 minutes, and the resultant supernatant was transferred into fresh tubes.500 µl of ice cold, 100% ethanol was added and mixed well. The tubes were placed in ice for 5 minutes. They were then centrifuged for 2 minutes at 20000 rpm. The resultant supernatant was discarded. The pellets were dried. 10µl of TE Buffer was added to the tubes and stored in the refrigerator for 24 hours.

2.3 Gel electrophoresis for the plasmids

After the plasmids were obtained , they were subjected to gel electrophoresis to analyse the plasmid profile.0.7% Agarose was prepared by dissolving 0.7g of agarose in 100ml of 1X TAE Buffer. The solution was heated to completely dissolve the agarose. When it was about 60^{O}C, 3µl of Ethidium Bromide was added and mixed well. The gel was then transferred to the electrophoresis chamber and a suitable comb was placed. The set up was left undisturbed till the gel solidified. After the gel solidified, the comb was removed and the chamber transferred to the tank. 1X TAE Buffer was added till the whole gel submerged. Simultaneously, 5µl of gel loading

dye and buffer were added to the plasmid isolates of all the strains and shaken well. 10µl of the plasmid was then introduced into the well, without air bubbles. After loading all the 10 strains into the gel, the electrical connection was made. The plasmids were made to run on the gel for 3 hours at 50V.After the run time, the gel was carefully transferred onto the UV Trans-illuminator to observe the bands.

2.4 Genomic DNA isolation

100ug/ml lysozyme was added to the pellet and incubated at RT for 30 minutes. 600 µl of cell lysis buffer (Guanidium-iso-thiocyanate, SDS, Tris-EDTA), was added and mixed by inverting the vial for 5 minutes. The cells which did not get lysed were further treated with additional detergent and incubated for 10 minutes with gentle mixing till the suspension looked almost transparent. 700 µl of Isopropanol was layered on top of this solution. The two layers were mixed gently till white strands of DNA were seen and until the solution is homogenous. The strands of DNA were spooled with the help of a pipette tip and transferred into a fresh vial. 500 µl of 70% ethanol was added to the spooled DNA. The spooled DNA was spun to precipitate DNA at 10,000 rpm for 10 minutes. The supernatant was discarded. The pellet air dried (without allowing it to dry completely). 50 µl of 1X TE was added and the pellet suspended. (Incubated for 5 min at 55–60 degree to increase the solubility of genomic DNA). 5 µl of the freshly extracted DNA along with 3 µl of gel loading dye was loaded onto the 1% agarose gel and subjected to electrophoresis.

2.5 Purification of both gDNA and Plasmid DNA for PCR

The column was placed with a collection tube in vial stand. 400 µl of equilibration buffer was added and centrifuged at high speed for 1 minute (10000 rpm). The collected buffer was discarded. The DNA sample was added into the column, 400 µl of equilibration buffer was added, mixed gently and centrifuged at high speed for 1 minute. The flow through was collected and discarded, and the step was repeated until the entire DNA sample was added. 500 µl of wash buffer 1 was added and centrifuged at high speed for 1 minute. Collected wash buffer 1 was discarded. 500 µl of wash buffer 2 was added and centrifuged at high speed for 1 minute. Collected wash buffer 2 two was discarded. The column was then centrifuged with empty collection tube for 2 minutes to completely remove the wash buffer. The column was placed in a new collection tube and 30 µl of prewar med Elution buffer was added at the center of the filter membrane. Incubated for 2 minutes and centrifuged at high speed for 1minute. 2 µl of the eluted DNA was collected, made up to 100 µl and quantified using UV spectrophotometer.

2.6 PCR

PCR for Van A gene was done on five Vancomycin resistant strains. Primers for VanA were selected using Bio informatics software. The oligonucleotide sequence for forward primer was 5' ATGAATAGAATAAAAGTTGCAATAC 3' and for reverse primer was 5'CCCCTTTAACGCTAATACGAT 3'. 1 µl containing 100pmol of each primer was used in the vial. 5 µl of 1x Taq assay buffer, 1.3 units of Taq polymerase enzyme, 20 µl of dntp mix containing 200 µM of each dntp and 2 µl of template DNA containing 50ng of plasmid was taken in a microfuge and the total volume was made up to 50 µl. 30 cycles were carried out at denaturation temperature 94 degree for 2 minutes initially, followed by denaturation for 30 seconds, annealing temperature of 50 degrees based on melting temperatures of both the primers for 45 seconds and elongation temperature of 72 degrees for 1 minute. Same protocol was followed for 2 µl of genomic DNA.

3. Results and Discussion

3.1 Antibiotic sensitivity

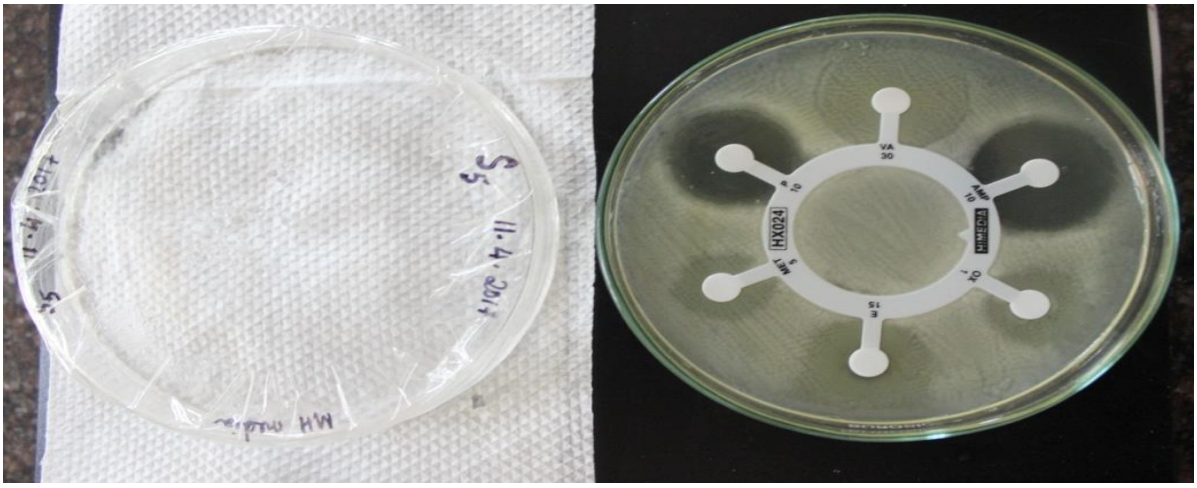

Figure 3.1a: *Antibiotic sensitivity of strain S5*

Table 3.1a

S5

ANTIBIOTICS	DIAMETER (CM)
AMPHICILIN	2.4
VANCOMYCIN	2.6
PENCILLIN	2.2
ERY	2.3
OX	1.9
METHICILLIN	1.7

Figure 3.1b: *Antibiotic sensitivity of strain S7*

Table 3.1b

S7

ANTIBIOTICS	DIAMETER (CM)
AMPHICILIN	2.5
VANCOMYCIN	-
PENCILLIN	1.5
ERY	-
OX	-
METHICILLIN	-

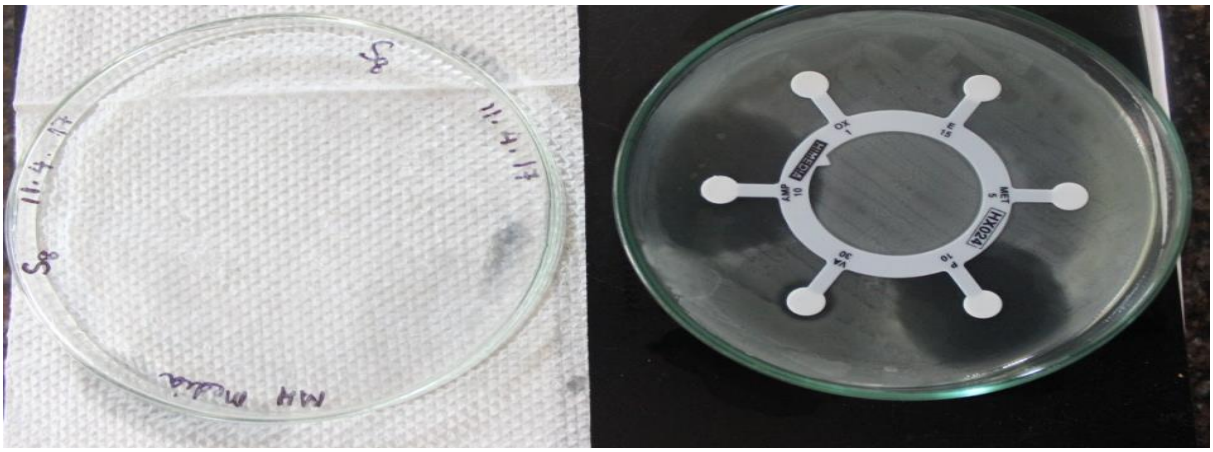

Figure 3.1c: *Antibiotic sensitivity of strain S8*

Table 3.1c

S8

ANTIBIOTICS	DIAMETER(CM)
AMPHICILIN	3.4
VANCOMYCIN	0.7
PENCILLIN	2.7
ERY	-

OX	**-**
METHICILLIN	**-**

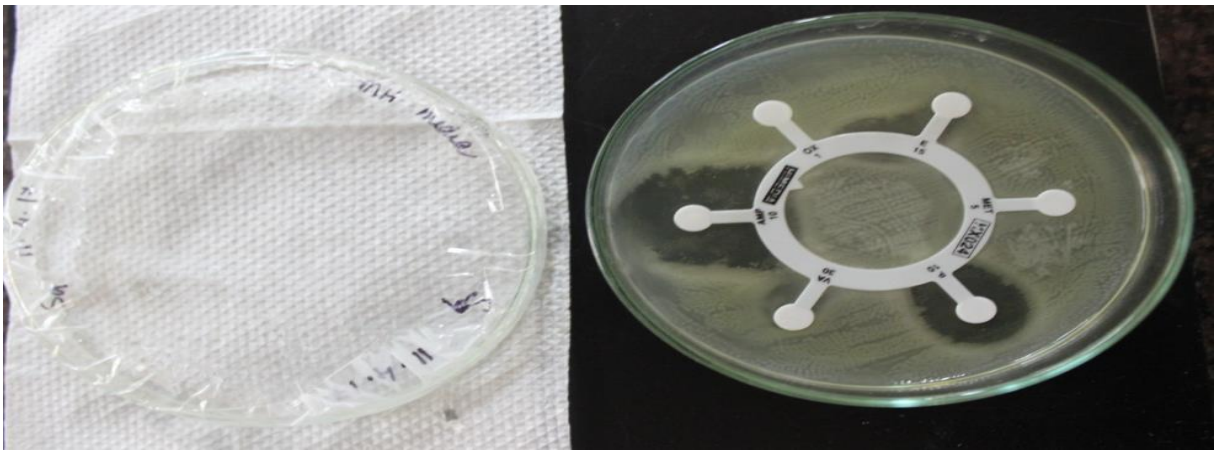

Figure 3.1d: *Antibiotic sensitivity of strain S9*

Table 3.1d

S9

ANTIBIOTICS	**DIAMETER(CM)**
AMPHICILIN	**2.7**
VANCOMYCIN	**-**
PENCILLIN	**1.7**
ERY	**1.0**
OX	**-**
METHICILLIN	**-**

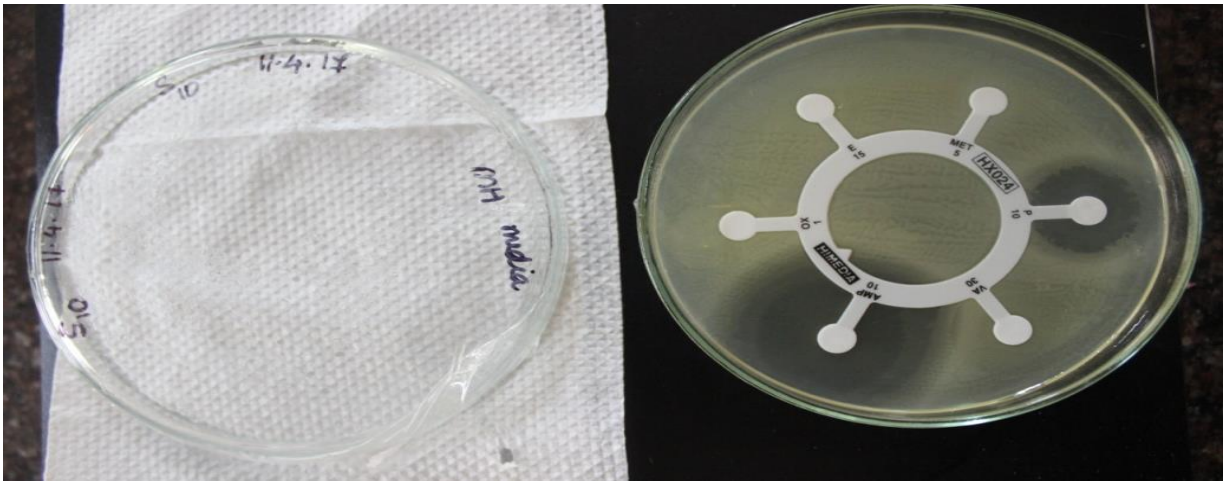

Figure 3.1e: *Antibiotic sensitivity of strain S10*

Table 3.1e

S10

ANTIBIOTICS	DIAMETER (CM)
AMPHICILIN	3.5
VANCOMYCIN	
PENCILLIN	1.6
ERY	
OX	
METHICILLIN	

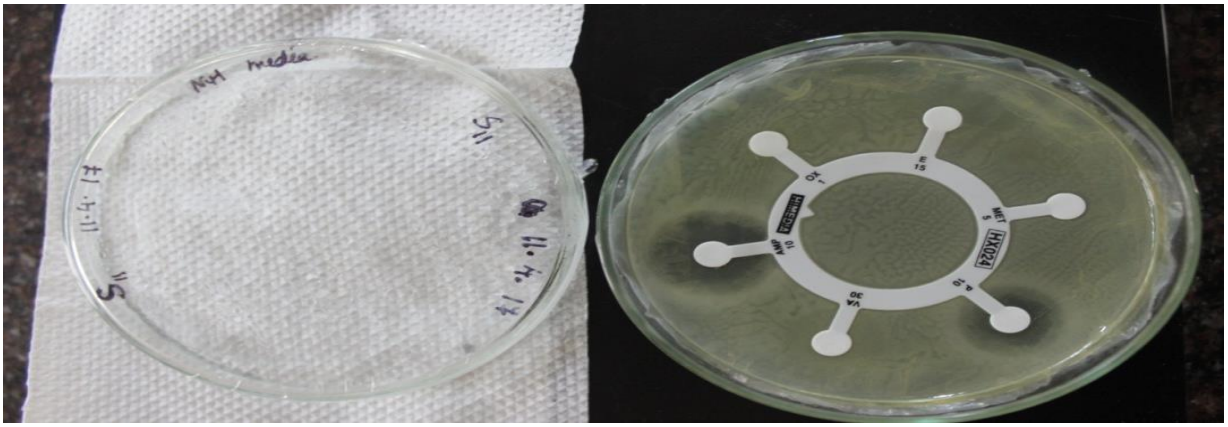

Figure 3.1f: *Antibiotic sensitivity of strain S11*

Table 3.1f

S11

ANTIBIOTICS	DIAMETER (CM)
AMPHICILIN	2.0
VANCOMYCIN	-
PENCILLIN	1.2
ERY	-
OX	-
METHICILLIN	-

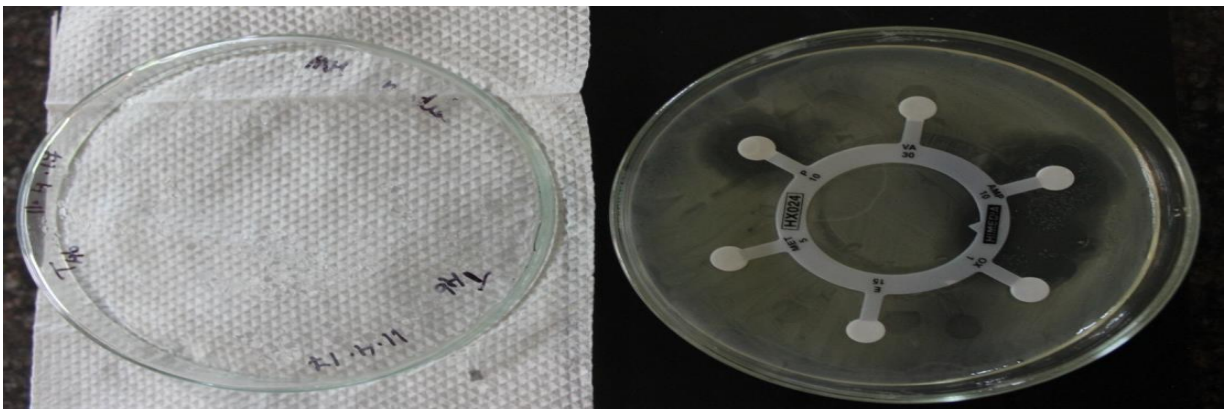

Figure 3.1g: *Antibiotic sensitivity of strain T46*

Table 3.1g

T46

ANTIBIOTICS	DIAMETER(CM)
AMPHICILIN	2.0
VANCOMYCIN	0.5
PENCILLIN	1.3
ERY	-
OX	3.4
METHICILLIN	-

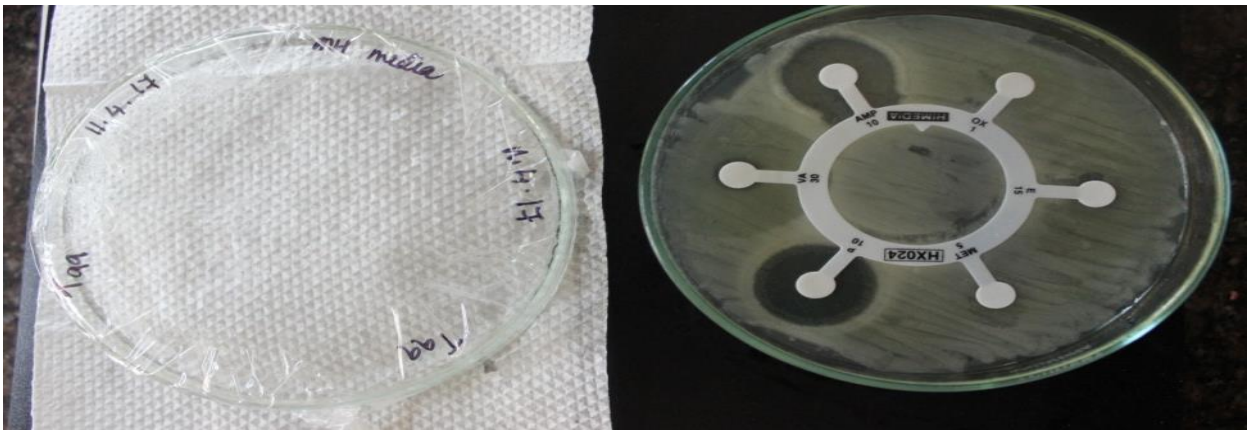

Figure 3.1h: *Antibiotic sensitivity of strain T99*

Table 3.1h

T99

ANTIBIOTICS	DIAMETER (CM)
AMPHICILIN	1.7
VANCOMYCIN	3.0
PENCILLIN	2.0
ERY	-
OX	-
METHICILLIN	1.0

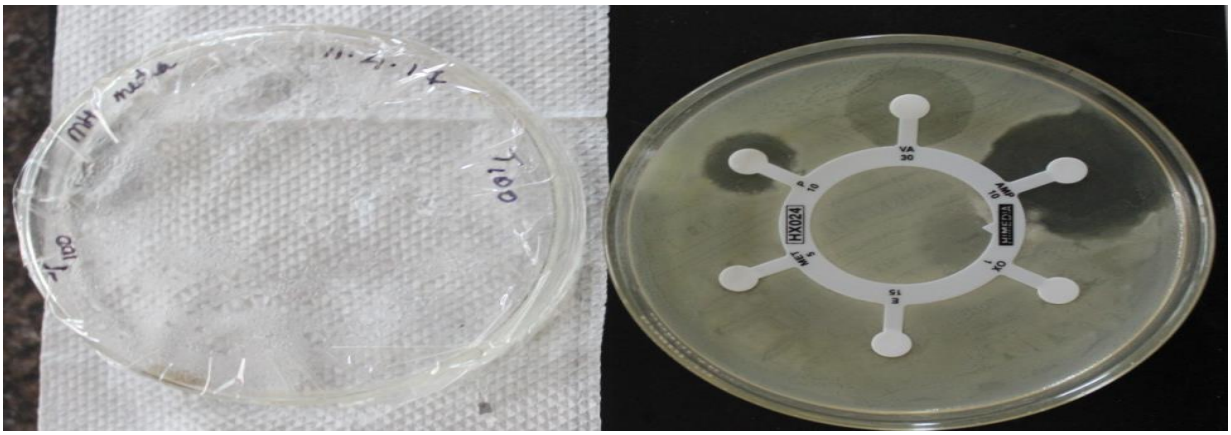

Figure 3.1i: *Antibiotic sensitivity of strain T100*

Table 3.1i

T100

ANTIBIOTICS	DIAMETER (CM)
AMPHICILIN	3.2
VANCOMYCIN	2.0
PENCILLIN	1.5
ERY	-
OX	-
METHICILLIN	-

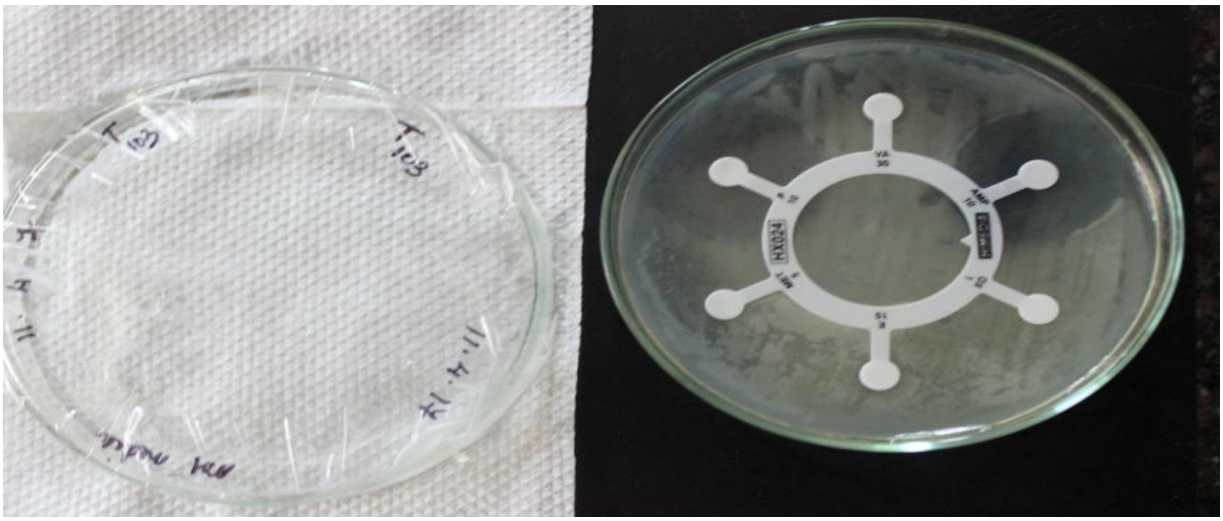

Figure 3.1j: *Antibiotic sensitivity of strain T103*

Table 3.1j

T103

ANTIBIOTICS	Diameter(CM)
AMPHICILIN	2.7
VANCOMYCIN	-
PENCILLIN	3.0
ERY	-
OX	-
METHICILLIN	-

The Antibiotic sensitivity tests concluded that S5 is the weakest of all strains as it shows susceptibility to all the antibiotics used in the experiment. S7, S10, S11, and T103 strains showed resistance to 4 antibiotics, Methicillin, Vancomycin, Oxacillin and Erythromycin respectively. S8, S9 and T100 showed resistance to 3 antibiotics each of which Methicillin and Oxacillin were common. T46 and T99 showed resistance to 2 antibiotics Methicillin and Erythromycin. S7 S9

S10 S11 and T103 strains showed resistance to Vancomycin. Two strains S11 and T103 showed maximum resistance to 4 antibiotics (Vancomycin, Methicillin, Oxacillin and Erythromycin).

3.2 Plasmid profile

Three plasmid runs of all strains were conducted for concordant results

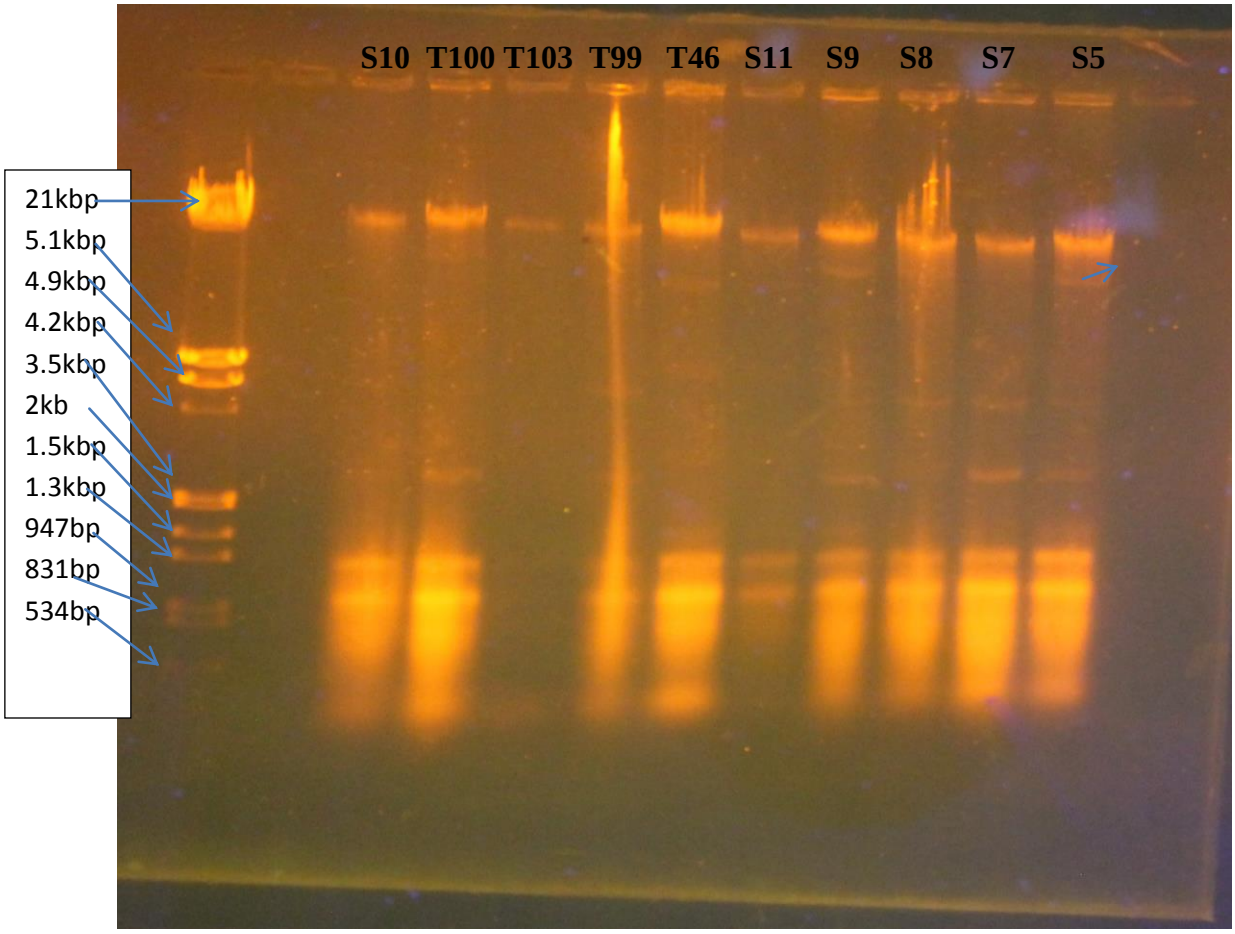

Figure 3.2a: *Plasmid profile*

All strains showed a linear 23kbp linear plasmid and two supercoiled plasmids, S11 and T103 did not show high plasmid diversity despite being most resistant to most number of antibiotics used. Strain T 103 showed the presence of only one band at 23kbp

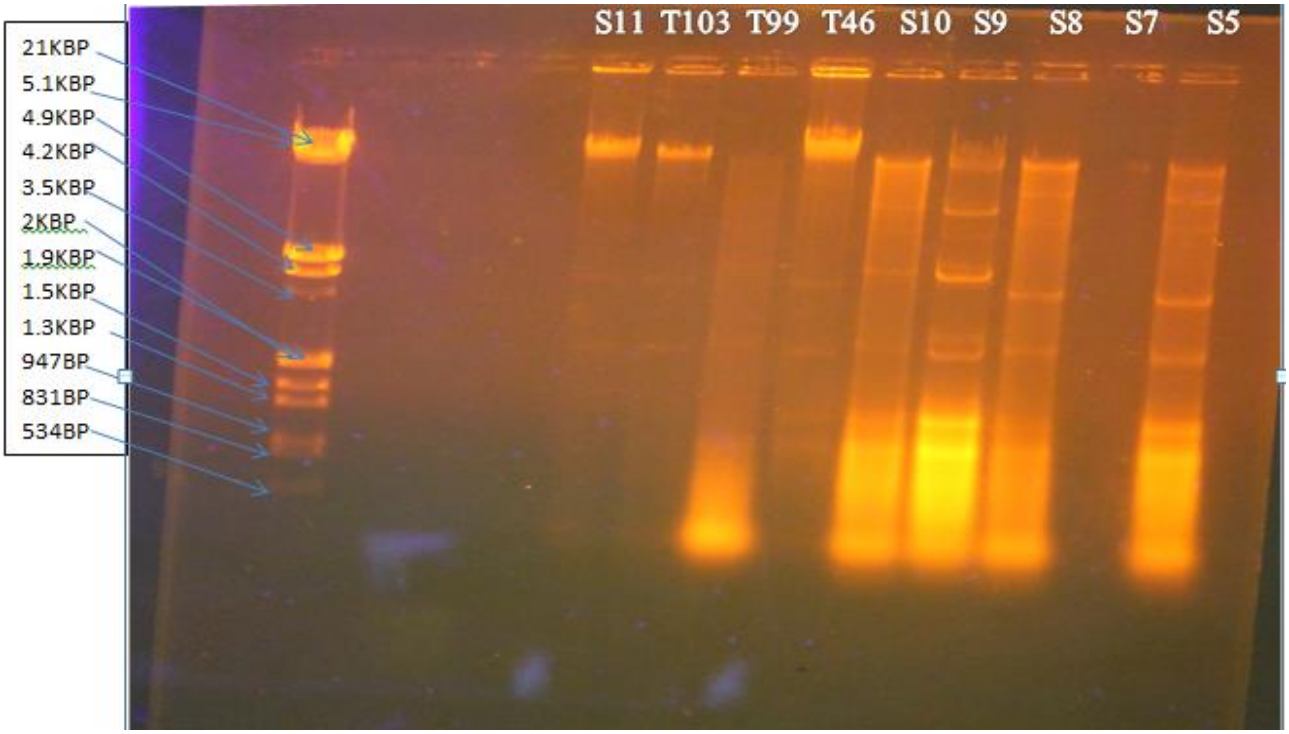

Figure 3.2b: *Plasmid profile*

S11 and T103 show a linear plasmid a 21kbp linear plasmid two coiled plasmids as opposed all the other less resistant strains which continue to show high plasmid diversity. It is thus no hard and fast rule that high resistance to antibiotics is equated to high plasmid diversity and number.

This apparent paradox led us to explore the role of other MGEs that are independent of Plasmids. MGEs that could be permanently stationed on gDNA and possibly shuttle between Plasmids and gDNA. Resistance to beta lactamases was largely because of a Tn 3 class of transposons, which is non composite replicative transposon, Methicillin resistance is due to a staphylococcal caste chromosome. Vancomycin resistance was acquired in Staphylococcus via Horizantal gene transfer from Enterococcus faecalis. Tn 1546 that belongs to the same family of Tn 3 transposon is responsible for resistance against Vancomycin. It is flanked by two inverted repeats with a region for Transposase, Resolvase and Van operon containing the *vanA* resistance locus comprising a cluster of seven genes (*vanS*, *vanR*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ*) [18].

With Tn 1546 transposon's replication mechanism, a replica of it on the genome is highly likely thus paving the way for permanent acquisition of Vancomycin. Thus a PCR of a key gene from Van operon becomes vital to conclusively prove it.

3.3 PCR gDNA

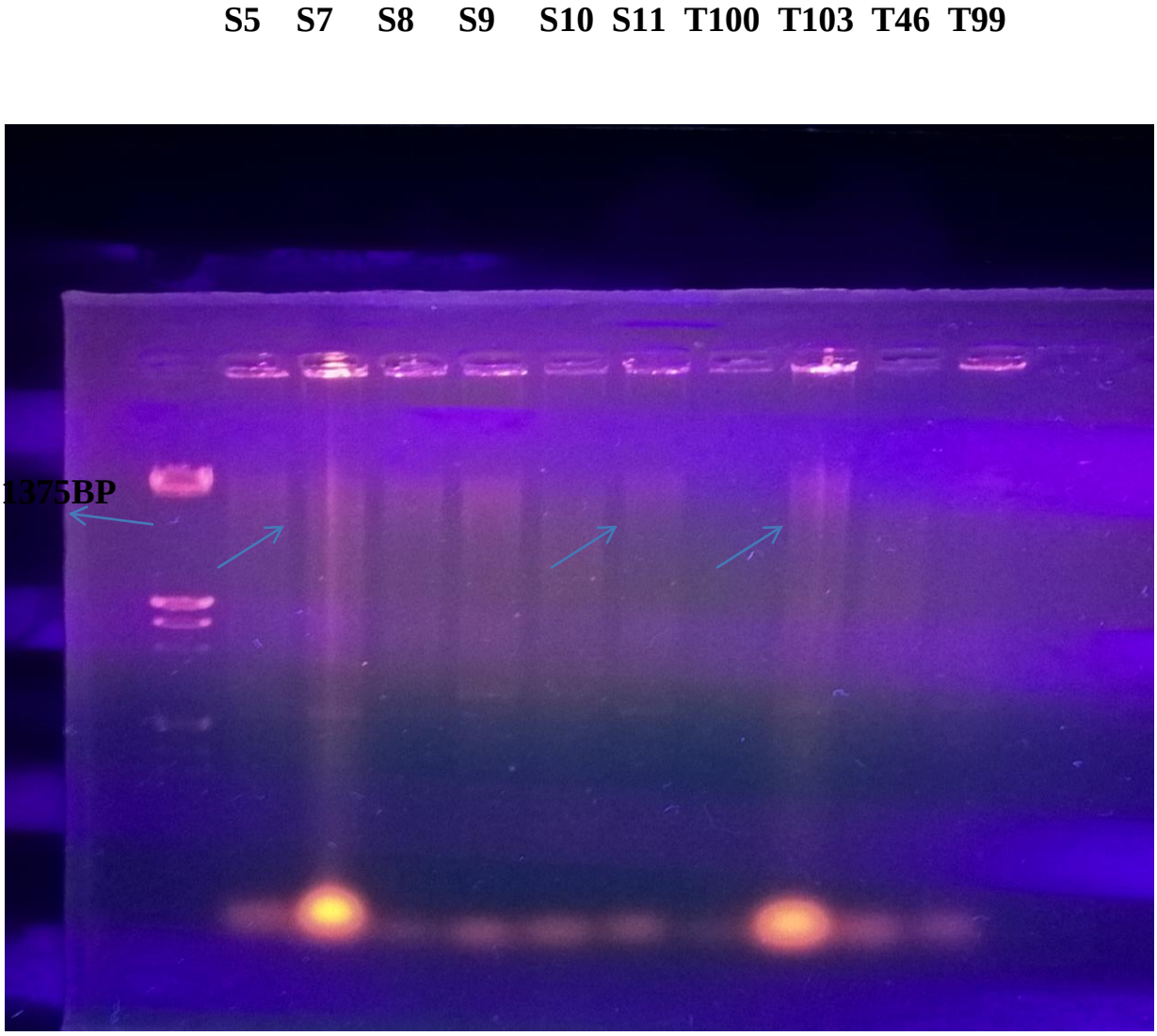

Figure 3.3a: *PCR for VanA on gDNA*

Bands at around 1375 bp were seen in S7, S11 and T103 when PCR was done on genomic DNA indicating the presence of a transposon containing VanA gene.

3.4 PCR on plasmid DNA

T103 S11 S10 S9 S8 S7

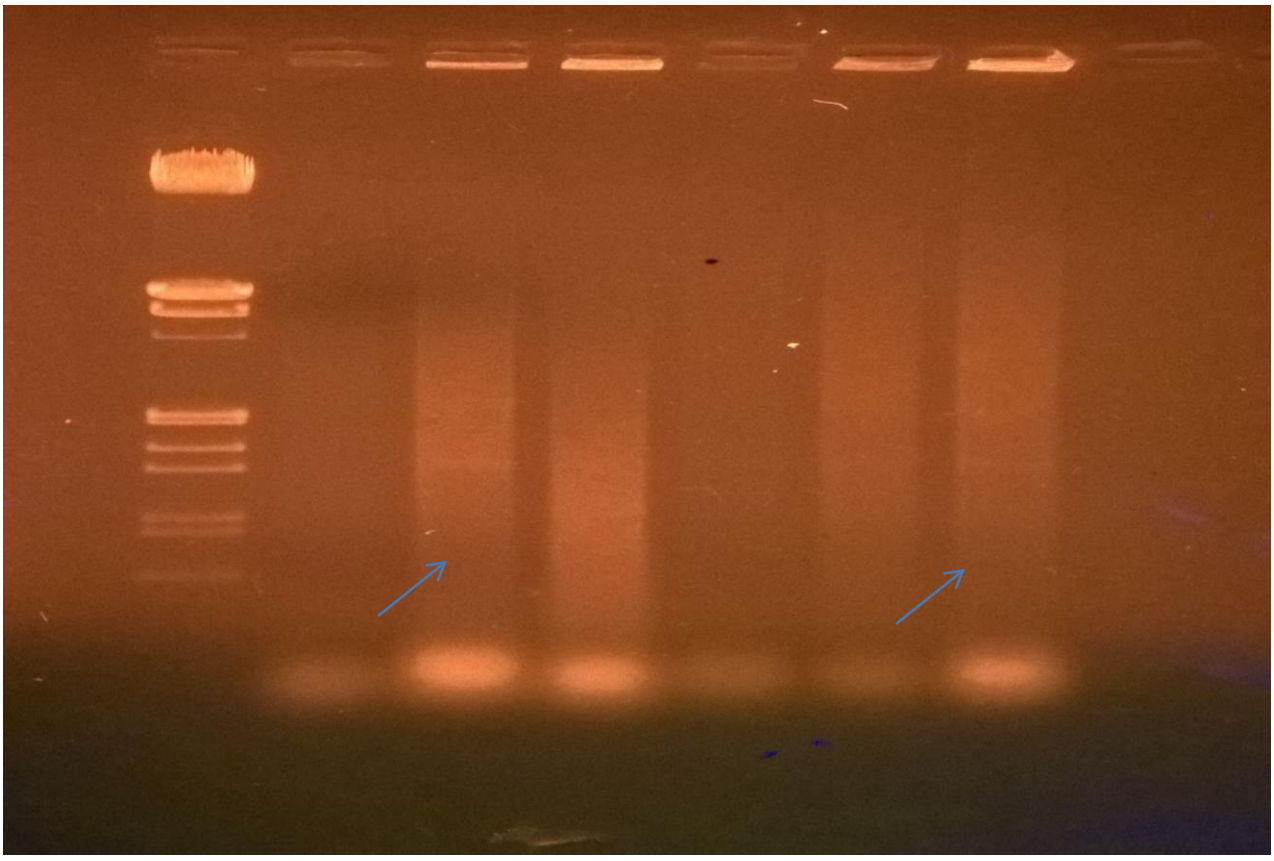

Figure 3.4a: *PCR of VanA on plasmids*

S11 and S7 showed the presence of VanA at around 1375bp when PCR was conducted on plasmids.

4. Concluding Remarks

There doesn't seem to be a proportional relationship between Antibiotic resistance and Plasmids. The most resistant strains showed much less bands compared to the relatively less resistant strains thus compelling us to believe otherwise, that Plasmids aren't the sole determinants of Antibiotic resistance. Vancomycin resistance in Staphylococcus is acquired via Horizontal gene transfer from Enterococcus faecalis (Susana Gardete and Alexander Tomasz). The incoming plasmid is highly likely to be lost in the succeeding generations in the absence of antibiotics (Colin Miller). The strains were sub-cultured several times in the absence of Vancomycin in an attempt to have cultures devoid of the plasmid containing the VanA gene.

PCR results however showed positive for Genomic DNA in 3 out of 5 Vancomycin resistant strains thus proving that the VanA gene showed it presence on the gDNA. S11 and S7 Strains however were VanA positive for both the gDNA and Plasmid. We conclude through our results that VanA being a part of Vancomycin operon of the non-composite replicative transposon Tn 1546 (Dennis de Carvalho Ferreira et al) showed its presence on both genomic DNA and Plasmid in three strains and remained on the Plasmid in two strains. Thus we propose that, if the incoming plasmid from a foreign source contains the resistance gene in a non-composite replicative transposon, it is highly likely for the transposon's replica to be seen on the gDNA. Once on the gDNA, it is least likely for the resistant gene to be lost during vertical transfer thus attributing for the strain's long lasting virulence. Antimicrobials targeting the transposition machinery could help prevent permanent acquisition of Virulence.

References

Alicia I Hidron, Russell Kempker, Abeer Moanna, and David Rimland,: Methicillin-resistant *Staphylococcus aureus* in HIV patients:

Anita Bhalla David C Aron, and Curtis J Donskey: Factors affecting the nasal carriage of methicillin-resistant Staphylococcus aureus in human immunodeficiency virus-infected patients

Bennett PM, Linton AH.: Do plasmids influence the survival of bacteria? J Antimicrobial Chernotlwr 1986

Berg T, Firth N, Apisiridej S, Hettiaratchi A, Leelaporn A, Skurray RA

Colin Miller: Loss of antibiotic resistance in e coli

Dennis de Carvalho Ferreira et al :A study on Risk factors associated with colonization and/or infection and methods for characterization of isolates - A systematic review

Ehiwario N.J* and Oshilim. Effect of Gentamicin and Amoxicillin on methicillin resistant Staphylococcus aureus (MRSA) against different time and concentrations

F. D. Lowy,:"Staphylococcus aureus infections," The New England Journal of Medicine, vol. 339, pp. 520–532, 1998. J Bacteriol. 1998 Sep; 180(17):4350-9.: Complete nucleotide sequence of pSK41: evolution of staphylococcal conjugative multi-resistance plasmids.

J Chacko et al from the Department of Skin and VD, Kasturba Medical College, Mangalore-575 001, India

L W Mayer: Use of plasmid profiles in epidemiologic surveillance of disease outbreaks and in tracing the transmission of antibiotic resistance.

Lafond M, Couture F, Vezina G, Levesque RC.: Evolutionary perspectives in multiresistance #Mactamase transposons. J Bacteriol 1989; 171: 6423-6429. https://doi.org/10.1128/jb.171.12.6423-6429.1989

Lindsay JA : Int J Med Microbiol. 2010 Feb; 300(2-3):98-103.:Genomic variation and evolution of Staphylococcus aureus. Review

Murray, P. R., Baron E J, Jorgenson J H, Landry M L, Pfaller M A, and Yolken R (2003). Manual of clinical Microbiology(8th edition). Hedron V A United States of America: Americal Society for Microbiology.

Natalia Malachowa and Frank R. DeLeo: Mobile genetic elements of *Staphylococcus aureus*

Nicolas E, Lambin M, Dandoy D, Galloy C, Nguyen N, Oger CA, Hallet B.: The Tn3-family of Replicative Transposons.

S. Simjee,[1,*] D. G. White,[1] P. F. McDermott,[1] D. D. Wagner,[1] M. J. Zervos,[2] S. MDonabedian,[2] L. L. English,[1] J. R. Hayes,[1,3] and R. D. Walker[1]: Characterization of Tn*1546* in Vancomycin-Resistant *Enterococcus faecium* Isolated from Canine Urinary Tract Infections: Evidence of Gene Exchange between Human and Animal Enterococci

Susana Gardete and Alexander Tomasz: Mechanisms of vancomycin resistance in *Staphylococcus aureus*

Tenover FC[1], Biddle JW, Lancaster : Increasing resistance to vancomycin and other glycopeptides in Staphylococcus aureus.

Weigel LM, et al.: Genetic analysis of a high-level vancomycin-resistant isolate of Staphylococcus aureus. Science.

Wiedemann B, Meyer JF, Ziihlsdorf MT.: Insertions of resistance genes into Tn21-like transposons. J Antimiiroh Chemother 1986; 18 Suppl C: 85-9