



PHENOTYPIC DETECTION OF erm GENE ENCODING METHYLASE RESPONSIBLE FOR RESISTANCE TO MACROLIDE, LINCOSAMIDE-STREPTOGRAMIN-B (MSL-B) ANTIBIOTICS IN METHICILLIN SENSITIVE AND METHICILLIN RESISTANT STAPH AUREUS INFECTIONS.

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ABSTRACT... Objectives: Phenotypic Detection and sensitivity pattern of erm gene/D-Test positive Methicillin Sensitive (MSSA) and Methicillin Resistant Staphylococcus Aureus (MRSA). **Study Design:** Prospective Cohort study. **Setting:** Department of Pathology at Qazi Hussain Ahmed Medical Complex Nowshera. **Period:** From 1st March 2019 to 30th Sept 2019. **Material & Methods:** Relevant information's were entered in a SPSS version 16 for descriptive and inferential analysis of different variable. **Results:** Out of 186 patients Staph Aureus isolates, 52(27.95%) showed D-test Positive. Thirty (57.7%) cases were females and 22(42.3%) males. The Mean age with Standard Deviation was 28.36+3.8 years. In 45(86.5%) cases D-Test phenomenon was observed in MRSA while in 7(13.5%) cases in MSSA. Chi-square test showed a significant correlation in gender groups for acquiring resistance to Macrolide-Lincosamide-Streptogramin-B (MLS-B) antibiotic by expressing erm gene encoding methylase. The probability of resistance in female gender are 4.5 times more as compared to male gender ($p=0.09$, $OR=4.53$). The sensitivity pattern to D+ MRSA was; Vancomycine 100%, Lanezolid 100%, Rifampicin 84.23%, Fusidic acid 55.77%, Doxycyclin 32.59%, levofloxacin 21.15%, Gentamycine 13.46%, ciprofloxacin 13.46%. It is pertinent to mention that the sensitivity to cefoxetin (Beta lactam antibiotics) was seen only in cases with D+MSSA (13.5%) cases. **Conclusion:** D-Test phenomenon can be seen in both MRSA & MSSA infections. D+MSSA cases where sensitive to b-lactam antibiotics shall be treated with b-lactams only, and precious drugs like Vancomycine & Lanezolid should be kept reserved for D+ MRSA cases to reduce resistance.

Key word: Antibiotic Resistance, D-Test Phenomenon, erm Gene Encoding Methylase, Macrolide-Lincosamide-Streptogramin-B Antibiotics.

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INTRODUCTION

Staphylococcus Aureus is a major notorious bacterial strand causing nosocomial and community-acquired infections around the globe. Furthermore an enhancing prevalence of Methicillin Resistance among Staphylococci is alarming.¹

The resistance to antibiotic used for the treatment of infection caused by Staphylococci is increasing with passage of time and is challenging for the physician to treat.²

D test is very easy and cheaper test that involves disc diffusion (kerby Baur) methods and helps to

study the Macrolide Lincosamide Streptogramin-B (MLSB) resistance in Staphylococcus aureus. In this test, the disk of Macrolide i.e. Erythromycin and a Lincosamide extract i.e. Clindamycin are placed adjacent to each other on Mueller Hinton agar media and inoculated for interpretation.³

Clindamycin and streptogramin are golden drugs used for treating infections induced By Methicillin Resistant S. Aureus (MRSA). Therefore resistance to this precious antibiotic in the shape of D-test is annoying and challenging.³ It is pertinent to mention that unlawful and irrational use of MLS_B antibiotics (macrolide-lincosamide-streptograminB) is major contributing factor

to make the Staphylococcal strains vigilant to acquire resistance to MLS_B antibiotics.⁴

Common most and generally accepted mechanism of resistance to Clindamycine is due to mutation in the erm genes that can be expressed two ways by the Clindamycin that is constitutively or inducibly. In both case MLS-B Strains are involved and in vitro it appears erythromycin-resistant and clindamycin sensitive if the desks of both are not placed adjacently. But the therapy with clindamycine would result in failure.⁵

We conducted the study with aim to find out frequency of inducible Clindamycin resistance in *S. aureus* infections from a tertiary care hospital of District Nowshera using D-test phenomenon.

MATERIAL & METHODS

This Prospective cohort study was performed in the Pathology department of Qazi Hussain Ahmed Medical Complex Nowshera from 1stMar 2019 to 30th September 2019. A total of 186 pus and sputum samples were received for Culture and sensitivity out of which 52 cases reported to be phenotypically positive for erm-gene encoding methylase (27.95%).

The inclusion criteria were all cases irrespective of age and gender received in the laboratory. Exclusion criteria were samples received in the laboratory 24hour after collection, patient already on the antibiotic therapy and improperly collected sputum and pus samples.

The samples were received in the pathology section from the respective unit under observance of strict aseptic technique after education of patients on pus and sputum sample collection. Media were prepared as per CLSI (Clinical and laboratory standard institutes).

All samples were inoculated on selective medium MSA (Mannitol Salt Agar). Then the specimens were incubated under ambient air 35 +2 C for 18-20 hours. In case growth is obtained on MSA then further inoculated on Mueller Hinton agar for sensitivity to antibiotics as per CLSI recommendations. The antibiotic desks used

were; VA-Vancomycine, LZD-Lanezolid, RD-Rifampicin, Fd-Fusidic acid, Dox-Doxycyclin, Lev- Levofloxacin 21.15%, CN-Gentamycine, Cip-ciprofloxacin, SXZ (Cotrimaxazole) and Fox-cefoxetin.

Desk of Erythromycin and Clindamycine were placed at a distance of 20mm center to center. Phenotypically MSL-B resistance was confirmed as Inhibition of zone of Clindamycin towards erythromycin as a straight line, resembling the alphabet "D" and was considered to be positive for D-Test phenomenon (Figure-1).any haziness in the zone of inhibition of clindamycin is also phenotypically representative of resistance.

Plates were incubated for 18-20 hours and then zone of inhibition were calculated with caliper, including the size of the desk. Zones were compared with CLSI recommendations for sensitivity to be reported as sensitive, intermediate and resistant.

Finally the data obtained from the culture and sensitivity was entered in a SPSS version 16 for descriptive and correlation analysis of different parameters. Descriptive statistics were used for measuring central tendency values for the numerical variables like age and Gender values.

Chi square test was used to determine the relationship between gender and MLS-B resistant strains. Logistic regression analysis was used to show the probability of occurrence.

RESULTS

A total of 186 samples were studied, 52(27.9%) patients showed D-test phenomenon, that were positive for erm-gene encoding enzyme. Out of those 30(57.7%) were females and 22(42.3%) were males Table-I.

The mean age with standard deviation was 28.36±3.87. The age range was from 15 years to 45 years of age. Mode of age was 23years (Table-II).

In 45(86.5%) cases D-Test phenomenon was observed in MRSA while in 7(13.5%) cases it was

also noted in Methicillin Sensitive Staph Aureus.

When cross tabulation of gender group done with phenotypically positive cultures of erm (erythromycin resistant methylase) encoding gene, A significant relation though not at level of 95% confidence (p -value = 0.07) was recorded in gender groups for acquiring MSL-B resistant infections using chi-square test, females are more prone to develop these resistant infection. (Table-III).

Furthermore The regression analysis was attempted to show the probability of MLS-B positive culture (D-Test Positivity) in gender group and it was observed that the probability of resistance in female gender are 4.5 times more as compared to male gender (p -value 0.09, OR=4.53). Table IV.

Thirty six (69.23%) patients with D-Test Positive were referred from medical unit of QHAMC. The

rest of the patients were referred for Culture from surgical, pediatric and emergency units.

The sensitivity pattern to D+MRSA was; Vancomycine 100%, Linezolid 100%, Rifampicin 84.23%, Fusidic acid 55.77%, Doxycyclin 32.59%, levofloxacin 21.15%, Gentamycine 13.46%, ciprofloxacin 13.46%..

It is pertinent to mention that the sensitivity to betalactam antibiotics was seen only in cases with D+MSSA (11.5%) cases. while no sensitivity recorded in both the groups for co-trimazole. (Table V).

The sensitivity to Vancomycine & Linezolid was 100% in both D+MSSA and D+MRSA but was not reported in cases of D+MSSA to the clinicians to avoid its misuse of these precious drugs for simple D+MSSA cases that can easily be treated with betalactam antibiotics.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Female	30	57.7	57.7	57.7
	Male	22	42.3	42.3	100.0
	Total	52	100.0	100.0	

Table-I. Gender statistics

Total Number of Patients		52
Mean		28.36
Median		25.00
Mode		23.00
Std. Deviation		3.87
Range		30.00
Minimum		15.00
Maximum		45.00

Table-II. Age statistics

		Culture Report		Total
		MSSA D-Positive	MRSA D-Positive	
Sex	Male	5	16	21
	Female	2	29	31
Total		7	45	52
Percentages		13.5	86.5	100
Chi-Square Tests				
		Value	Df	Asymptotic Significance (2-sided)
Pearson Chi-Square		3.238 ^a	1	0.072
Continuity Correction ^b		1.919	1	0.166

Table-III. Correlation of gender groups for acquiring MLS-B resistance in MRSA & MSSA.

Table 4/a. Classification Table

	Observed	Predicted		Percentage Correct	
		Report			
		MSSA D-Positive	MRSA D-Positive		
Step 1	Culture and sensitivity Report	MSSA Positive	0	7	0
		MRSA D-Positive	0	45	100
	Overall Percentage				86.5

Table 4/b. Regression table

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	Sex	1.511	0.893	2.865	1	0.091	4.531
	Constant	-0.348	1.259	0.076	1	0.782	0.706

a. Variable(s) entered on step 1: sex.

Table-IV. Regression analysis of gender with D-Test positive isolate

Antibiotics	Sensitivity to D+MSSA Positive Cultures	Sensitivity to D+MRSA Positive Cultures	Total Sensitivity	Total Cases	Percentage %
Vancomycine	7	45	52	52	100
Lanezolid	7	45	49	52	100
Rifampicin	6	38	44	52	84.62
Fusidic acid	1	28	29	52	55.77
Doxycycline	3	14	17	52	32.69
Levofloxacin	2	9	11	52	21.15
Gentamycin	1	6	7	52	13.46
Ciprofloxacin	1	6	7	52	13.46
Cefoxetin(b-lactam antibiotics)	4	2	6	52	11.54
Cotriamaxazole	0	0	0	52	100.00

Table-V. Sensitivity pattern to D-Test Positive MSSA and MRSA isolates.

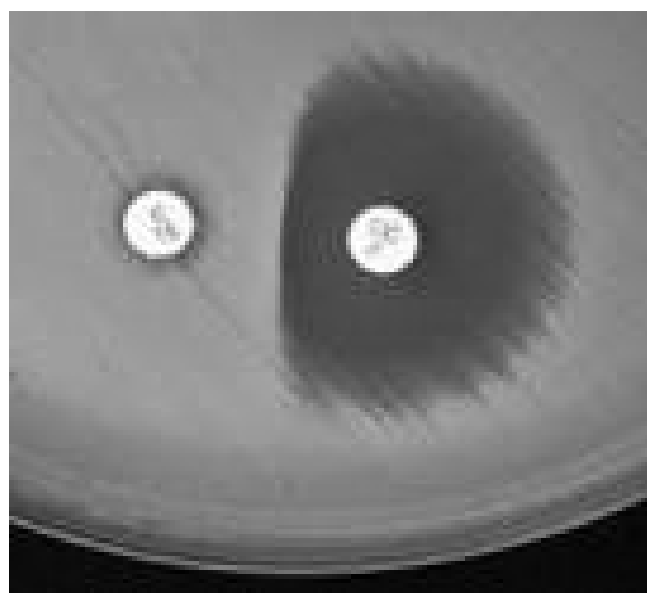


Figure-1. D-Test phenomenon

DISCUSSION

The resistant strains of *Staphylococcus Aureus* in the form of MRSA emerged soon after the initiation of antibiotics in early 1940.⁶ Resistance to Macrolides, Lincosamides, and Streptogramins (MLS) antibiotics by expression of *erm* genes detected by D-Test, can occur in both MSSA and MRSA infections.⁷ Resistance to Clindamycin induced by erythromycin mediated through a methylase enzyme that alters the common ribosomal binding site for Macrolides, Clindamycin and the group B Streptogramins (quinupristin) makes *Staph Aureus* more resistant to another strand of antibiotic after beta-lactam antibiotics.⁸

This emerging resistance of *Staph Aureus* to Macrolides, Lincosamides, and Streptogramin-B

(MLS-B) limits the use of these drugs in infections caused by Staph Aureus.

In present study frequency of D-Test positivity in Staph Aureus infections was 52/186(27.9%). D-test phenomenon is resistance to MLS-B antibiotics due to erm gene caused by a methylase enzyme. Out of those 30(57.7%) were females and 22(42.3%) were males. In 45(86.5%) cases D-Test phenomenon was observed in MRSA while in 7(13.5%) cases it was also noted in Methicillin Sensitive Staph Aureus infections. It is important learning point that D-test phenomenon can be observed in both the strands that is MSSA & MRSA.

Macrolide, Lincosamide and Streptogramin type B (MLSB) antibiotics are clinically important easily available and cost effective antibiotics used for treatment of Staph aureus infection, and an existence of isolates with integral ability due to genetic mutation in MLSB antibiotics is worrisome. They reported a higher prevalence of 58% of their isolates of Staph infections to be positive for erm gene, representing resistant isolates to MLS-B antibiotics.⁹

A prospective cohort reported from Poland show that the prevalence and frequency of phenotype of erm gene encoding enzyme in Staph Infections for the years 2010, 2011 was 59%, 69.7%, respectively. But after taking remedial action and lawful use of antibiotic the frequency of D+ isolates in 2012 decreased to 21.7%.¹⁰

Erythromycin is a macrolide and clindamycin is a lincosamide extract, usually treated by clinician as macrolides but chemically and biochemically Clindamycin is different from other macrolides as it belongs to Lincosamide group. Hence these molecules represent two distinct classes of antibacterial drugs, that inhibit synthesis of protein by inhibiting 50S ribosomal subunits in ribosomes of SA. In staphylococci, resistance to both of these agents is caused by a methylase enzyme that causes mutation in erm genes encoding methylase responsible for resistance to MLS-B antibiotics.^{1,10}

It is to emphasize that in this study we determined both the frequency of D-Test positive isolates along with sensitivity pattern to those isolates. The sensitivity pattern to D+MRSA in our study was; Vancomycine 100%, Lanezolid 100%, Rifampicin 84.23%, Fusidic acid 55.77%, Doxycyclin 32.59%, levofloxacin 21.15%, Gentamycine 13.46%, ciprofloxacin 13.46%.

Another study reported that all isolates were found to be sensitive to vancomycin 100%, they also suggested that Gentamycin to be used an alternative for treatment of S. aureus infections with d-test positive, however as per CLSI aminoglycosides alone are not indicated as monotherapy and should be prescribed in combination with another antibiotics. Their results further showed that vancomycin was the only antimicrobial agent to be prescribed clinically in infection caused by D+ MRSA.¹¹

The sensitivity to Vancomycine & Lanezolid was 100% in both D+MSSA and D+ MRSA but we did not reported its sensitivity in cases of D+MSSA to the clinicians to avoid the misuse of these precious drugs for simple D+MSSA infections that can easily be treated with beta-lactam antibiotics. Developed countries have developed strategies for the use of vancomycin in clinical practice. An example is USA where they have designed a computerized structured system in hospital to bound the clinicians starting vancomycin for treating resistant infections like D+MRSA, where the clinicians were supposed to follow a protocol with clear mention of proper indication of vancomycin and updating the treatment record in the a computerized interconnected system that was strictly observed by the decision makers under Management information system to avoid its misuse.¹²

Irrational, unlawful and inappropriate use of precious antimicrobial agents like vancomycin and lanizolid is responsible for emerging resistance in bacteria and also increase the budget/hospital stay on general public.¹³

It is concluded that resistance to MLS-B antibiotics must be kept in mind while dealing

with infections caused by gram positive bacteria. A comprehensive strategy using advocacy, communication social mobilization and CME events can help in understanding healthcare provider in proper selection of antibiotics for treatment of various bacterial infections.



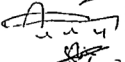

There is need for multidisciplinary approached at national, international and authorities (Medical Teaching institutions MTIs) level to develop consensus on lawful and evidence based use of antibiotic to safeguard the future clinical challenges.

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2	Fazli Bari	Data collection, Critical review.	
3	Tayyaba Basharat	Data collection, Critical review.	
4	Saadullah	Data collection, Manuscript writing.	
5	Shabbir Hussain	Data collection, Critical review.	