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Phenotypic Detection of erm Gene Encoding Methylase for Resistance to Macrolide, Lincosamide-Streptogramin-B (MSL-B) in Methicillin Sensitive and Methicillin Resistant *Staph aureus* Infections in a Tertiary Care Hospital of Nowshera

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ABSTRACT

Objectives: Phenotypic detection of erm gene encoding enzyme in methicillin sensitive and methicillin resistant *Staphylococcus aureus* isolates (sputum and pus samples) in a tertiary care hospital of Nowshera and to find the sensitivity pattern to D+MSSA and D+MRSA.

Methods: This prospective cohort study was performed in the pathology laboratory of Qazi Hussain Ahmed Medical Complex Nowshera from 1st March 2019 to 30th Sept 2019. A total of 186 *S. aureus* isolates were selected for routine antibiotic susceptibility testing. 52 samples showed D-test positive *i.e.,* (induced clindamycine resistance). Relevant information were recorded on a predesigned proforma prepared as per CLSI recommendation for data collection.

Results: Out of 186 patients *Staph aureus* isolates were selected for antibiotic susceptibility, 52 (27.95%) isolates showed inducible clindamycin resistance using D-test as per CLSI recommendations. Out of total, 30 (57.7%) were females and 22 (42.3%) were males. The mean age with standard deviation was 28.36 ± 3.8. The age range was from 15 years to 45 years of age. Mode of age was 23 years. 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* (MSSA). 36 (69.23%) patients with phenotypically

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positive erm gene encoding methylase were referred from medical unit of QHAMC. The sensitivity pattern to D+MRSA was; vancomycine 94.23%, lanezolid 94.23%, 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 amoxicillin, cefradine, ceftriaxone was seen only in cases with D+MSSA cases only (13.5%) cases. While no isolate sensitivity to co-trimaxazole.

The sensitivity to vancomycine and lanezolid 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 alternative b-lactam antibiotics.

Conclusion: D+MSSA cases where sensitive to b-lactam antibiotics and shall be treated with these conventional antibiotics and precious drug like vancomycine and lanezolid should be kept reserved for D+MRSA cases to avoid misuse of antibiotics and to reduce resistance. D+type of resistant infection are now a challenge to the clinician to treat and is a public health threat that needs accumulative response through advocacy, communication and social mobilization.

Keywords: Antibiotic resistance; MLS-antibiotics; *Erm gene encoding methylase*

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. 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. D-test is very easy and cheaper test that involves disc diffusion (Kerby Baur) methods and helps to study the Macrolide Lincosamide Streptogramin resistance (MLSB) in *Staphylococcus aureus.* In this test, the desk 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.

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. It is pertinent to mention that unlawful and irrational use of MLSB antibiotics (macrolide-lincosamide-streptogramin B) is major contributing factor to make the staphylococcal strains vigilant to acquire resistance to MLSB antibiotics. 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 klindamycin 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. We conducted the study with aim to find out frequency of inducible clindamycin resistance in *S. aureus* infections from our geographical location using D-test phenomenon.

MATERIALS AND METHODS

This prospective cohort study was performed in the pathology department of Qazi Hussain Ahmed Medical Complex Nowshera from 1st Mar 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 24 hour after collection, patient already on the antibiotic therapy and improperly collected sputum and pus samples [1].

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 (Manitol 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 recommendatios. 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 klindamycine were placed at a distance of 20 mm center to center. Phenotypically MSL resistance was confirmed as Inhibition of zone of clindamycin towards erythromycin as a strainght 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 [2]. 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.

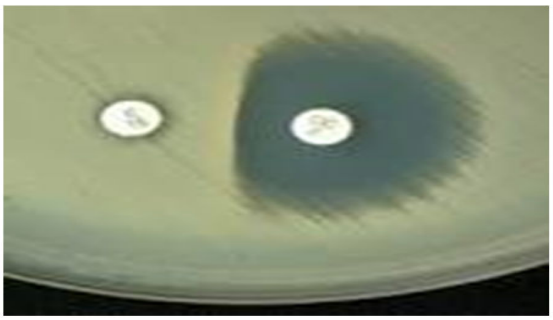

Figure 1: D-test phenomenon.

RESULTS

A total of 186 samples were studied, 52 (27.9%) patients showed D-test phenomenon, that were positive for erm-gene encoding enzyme [3]. Out of those 30 (57.7%) were females and 22 (42.3%) were males (**Table 1**).

Table 1: Gender statistics.

Variables		Frequency	Percent	Valid percent	Cumulative percent
Valid	Female	30	57.7	57.7	57.7
	Male	22	42.3	42.3	100
	Total	52	100	100	

The mean age with standard deviation was 28.36 ± 3.87. The age range was from 15 years to 45 years of age [4]. Mode of age was 23 years (**Table 2**).

Table 2: Age statistics.

N	**Valid**	52
	Missing	0
	Mean	28.3654
	Median	25
	Mode	23
	Std. deviation	3.87145
	Range	30
	Minimum	15
	Maximum	45

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* (**Table 3**).

Table 3: C/s report of phenotypic detection of erm gene encoding methylas in MSSA and MRSA.

Variables		Frequency	Percent	Valid percent
Valid	MSSA D-positve	7	13.5	13.5
	MRSA D-positve	45	86.5	86.5
	Total	52	100	100

36 (69.23%) patients with D-test positive were referred from medical unit of QHAMC [5].

The rest of the patients were referred for culture from surgical, pediatric and emergency units (Table 4).

Table 4: Culture and sensitivity report sex unit cross tabulation.

Unit			Gender		Total
			Female	Male	
FMW	CS report	SA D-P positve	2		2
		MRSA D-positve	14		14
		Total	16		16
FSW	CS report	MRSA D-positve	11		11
		Total	11		11
GYNE	CS report	MRSA D-positve	2		2
		Total	2		2
MMW	CS report	SA D-positve	0	4	4
		MRSA D-positve	0	16	16
		Total	0	20	20
MSW	CS report	SA D-positve		1	1
		MRSA D-positve		2	2
		Total		3	3
Total	CS report	SA D-positve	2	5	7
		MRSA D-positve	28	17	45
		Total	30	22	52

The sensitivity pattern to D+MRSA was; vancomycine 94.23%, lanezolid 94.23%, rifampicin 84.23%, fusidic acid 55.77%, doxycyclin 32.59%, levofloxacin 21.15%, gentamycine 13.46%, ciprofloxacin 13.46% [6].

It is pertinent to mention that the sensitivity to betalactam antibiotics was seen only in cases with D +MSSA (11.5%) cases [7]. while no sensitivity in any both the groups seen for co-trimaxazole (Table 5).

Table 5: Sensitivity pattern of D-test positive MSSA and MRSA.

Antibiotics	Sensitivity to D +MSSA positive cultures	Sensitivity to D +MRSA Positive Cultures	Total sensitivity	Total cases	Percentage
Vancomycine	6	43	49	52	94.23
Lanezolid	5	44	49	52	94.23
Rifampicin	6	38	44	52	84.62
Fusidic acid	1	28	29	52	55.77
Doxycycline	3	14	17	52	32.69
Levofloxacine	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	0

The sensitivity to vancomycine and lanezolid 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 [8].

DISCUSSION

The resistant strains of *Staphylococcus aureus* of emerged soon after the initiation of antibiotics in early 1940s. Macrolides, Lincosamides and Streptogramins (MLS) genes are notorious for development of resistance to these antibiotics in MSSA and MRSA infections. Resistance to clindamycin induced by microclide mediated through a methylase enzyme that alters the common ribosomal binding site for macrolides, clindamycin and the group B streptogrammins (quinupristin) makes *Staph aureus* more resistant to another strand of antibiotic.

This emerging combined resistance of *Staph aureus* to Macrolides, Lincosamides and Streptogramin-B (MLS-B) limits the use of these drugs in infections caused by these bacteria.

In present study frequency of MLS-B phenotype in *staph* infections was 52/186 (27.9%). D-test phenomenon that is resistance due to MLS-B gene caused by a methylase enzyme [9]. 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*.

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 1991/2010-2011 and 2012 was 59%, 76.9%, 69.7%, respectively [10]. But after taking remedial action and lawful use of antibiotic the frequency of D+isolates in 2012, derived from the culture dramatically decreased to 21.7%.

Erythromycin is a macrolide and clindamycin is a lincosamide usually treated by clinician as macrolides but chemically and biochemically clindamycin is different from other macrolides as it is belongs to lincosamide group. Hence represent two distinct classes of antibiotic/antibacterial drugs, agents 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 cause mutation in MLS-B antibiotics mediated by erm genes encoding enzyme.

The sensitivity pattern to D+MRSA in our study was; vancomycine 94.23%, lanezolid 94.23%, rifampicin 84.23%, fusidic acid 55.77%, doxycyclin 32.59%, levofloxacin 21.15%, gentamycine 13.46%, ciprofloxacin 13.46% [11].

Another study reported that all isolates were found to be sensitive to vancomycin 100%, the D-test was found to be positive in 82.4% of the isolates they also suggested that gentamycin to be used an alternative for treatment of *S. aureus* infections with D-test positive, however as per CLSI aminglycosides alone are not indicated as montherphy and should be prescribed in combination with another antibiotics [12]. Their results further showed that vancomycin was the only antimicrobial agent to be prescribed clinically in infection caused by D+MRSA.

CONCLUSION

The sensitivity to vancomycine and 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 stratagies 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, 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 to be strictly observed by the decision makers under management information system to avoid its misuse.

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.

It is concluded that 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 challanges.

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