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Original Research Article

Inducible Clindamycin Resistance among Staphylococcus Aureus from Clinical Isolates Samples in Tertiary Care Hospital Center, Indore (M.P)

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Abstract:

Introduction: Antimicrobial resistance agents of staphylococci species are raising problems all around the epidemic area. Staphylococcus aureus infections to treatment by antibiotics were renewed be attractive to age of macrolide-lincosamide-streptogramin B (MLSB). Medical disturbance have to describe due to all of kind mechanisms that confer resistance to MLSB antibiotics. In this present study were aimed to detect the iCR and sentivity to erythromycin in staphylococcus aureus and where these study the was correspondence between clindamycin and Methicillin resistance other than methicillin sensitivity.

Materials and Methods: From July 2020 to June 2021in this period, out of 155 (46.3%) staphylococcus aureus were isolated from different clinically specimens in the study. According to CLSI-2019-20,21 guidelines detection of antimicrobial susceptibility test (AST) was done by Kirby-Bauer's disc diffusion method. For using perception inducible clindamycin resistance and erythromycin résistance was perform to detection by d test according to CLSI guideline and where deferent phenotypes method were interpreted as methicillin-sensitive (MS) phenotype negative test, constitutive MLSB phenotype and inducible (iMLSB) phenotype as positive test.

Results: Among 155 Staphylococcus aureus were isolated predominate from pus 48 (30.9%) followed by 36 (23.7%) were urine and where 27 (17.4%) were blood. In this present study were isolated to sensitivity such as linezolid and vancomycin. Out of which 155 (51.7%) were isolated in staphylococcus aureus resistant to erythromycin and among in this present study 91 (58.7%) were MRSA followed by 64 (42.3%) were MSSA. Among the 155 isolates resistant to erythromycin, where 50 (32.3%) inducible iMLSB D were test positive followed by 69 (44.5%) were negative test among MS phenotype and where 36 (23.2%) were isolated among cMLSB phenotype. Compare to more than one method using were detection inducible percentage %, constitutive cMLSB and MS phenotype resistance were equal in the MRSA and MSSA in staphylococcus aureus.

Conclusion: d-testing might help to decide whether to use Clindamycin sensitivity in Staphylococci species infections when erythromycin resistance as confirmation by Kirby Bauer disc diffusion method according CLSI 2020-21 guideline.

Keywords: iMLSB Phenotype, MS phenotype, MRSA, constitutive MLSB, MSSA, staphylococcus aureus and Clindamycin resistance.

Introduction

Among multidrug résistance, there is high raising in MRSA with macrolides resistant to clarithromycin, erythromycin and lincosamides resistant to lincomycin clindamycin**.** Recently newer drugs using like a, quinupristin-dalfopristin, and linezolid have been prescribe to the management for isolates, but the latest upgrade AST reports of resistance using raising real concerns then these sensitivity will hold good[1] . Major problem of public health Infection with MRSA has emerged importance. MRSA has usually conferred by altered PBP-2a which that causes resistance to all ß-lactam AST agents [2]. Inducible clindamycin resistant to isolate is not recommended caused for any infection. Infections of MRSA isolation with are sensitive to clindamycin on routine using for tests and resistant to erythromycin isolates. Staphylococcus aureus's isolates samples have to using detection by d-test routinely in all microbiology laboratory but not recommended clindamycin to patients because patients infections caused by iCR. So suggested negatile avoiding switch to treatment clindamycin [5].

Isolates samples MRSA are increasingly being reported as multidrug resistant with high resistance to macrolides (erythromycin, clarithromycin) and lincosamides (clindamycin, lincomycin), leaving very few therapeutic options[6]. Newer antibiotics like vancomycin, linezolid, and quinupristin-dalfopristin have been advocated in the management of such isolates, but recent reports of resistance to these agents raise real concerns over how long these uniform susceptibilities will hold good[7]. This suspicion has led clinicians to choose the macrolides lincosamide-streptogramin B (MLSB) family of antibiotics which is used, in place of MRSA resistance antibiotic. Clindamycin is comely used ideal antibiotic among MLSB family which has outlasting pharmacokinetic [8] MRSA gene initiate mechanism of constitutively resistant in which for the conditions i.e, erythromycin resistance and clindamycin sensitivity in staphylococcus aureus in both in vivo and in vitro.

During treatment clindamycin resistance does not develop in constitutively resistant staphylococcus aureus. Among staphylococcus aureus methicillin avidity and imurical resistance of clindamycin cefferd according to the condition and area. A difference study has been done which reveal 20 to 58% prevalence of MRSA worldwide. In a study we aimed explore research the burreden of MR and iCR in diffraction types of staphylococcus species in considerably as per settings and regions. Different types of study have reported the highly increasable rate in world ranging of MRSA [9].

Aims and Objectives

The present study was undertaken with the following aim and objectives:

- By conventional methods to detection species of MRSA from clinically significant samples.
- By using Kirby Bauer disc diffusion method was detection erythromycin resistance among MRSA.
- Methicillin resistance among the isolated species of staphylococcus aureus MRSA
- All in some staphylococcus aureus species were gives to inducible or constitutive clindamycin resistance MRSA.
- Detection accurately inducible, constitutive clindamycin resistance with methicillin resistance (MR)

Materials and Methods

Ethical and research clearance was obtained from the Ethical Committee of Microbiology department at Index Medical College, Hospital & Research Centre. Permission to conduct the study was sought.

Study Setting – In this study were isolates samples from clinically in department of microbiology at Index Medical College, Hospital & Research Centre (IMCHRC) Indore (M.P).

Study duration and sample size - From June 2020 to July 2021 and deferent types of species of staphylococcus aureus isolated from various clinical samples were included in the study.

Study subjects - Patients visiting IPD, OPD and ICU's of Hospital in Index medical college, hospital & research center fulfill criteria.

Inclusion criteria - All consecutive, non-duplicate isolates of Staphylococcus aureus collected from various specimens of patients attending various outpatient departments as well as admitted in wards like OPD, IPD and ICUs in hospital at Index Medical College, Hospital & Research Centre. All kind of specimens like urine, blood, and pus/wound were included in the study.

Exclusion criteria - Clinical such as coagulase negative Staphylococcus, gram negative bacteria, and fungi were excluded in this study.

Sample storage - After isolated growth of staphylococcus species were sub-cultured on to mannitol agar then stored at 2˚C to 8˚C.

Identification of Staphylococcus aureus with AST testing

Standard microbiological procedure was followed to culture the specimen's protocols. Rotten culture media like blood agar , mannitol salt agar for the inoculation and then place in the incubator for overnight at at 37 °C in aerobic condition. For clarification of staphylococcus aureus was done by the morphology of broth on culture gram staining s and confirmed with biochemical reaction CLSI guideline were followed for the antibiotic susceptibly test such as catalase- positive, coagulase-positive[9]

Disk diffusion was the used for detection of antibiotic susceptibility. The suspension of staphylococcus aureus was prepaired according to the 0.5 McFarland standerization dilution. A strile cotton swab then was dip in solution sepesion then stecked on mullore hinton agar. Erythro mycin disk 15 µg was placed in proximity to a clindamycin disc on MHA. The MHA plate watch incubated over night at 37°C[11]. All sentivity testes were detection by Kirby-baure method then results were interpretation according to CLSI 2020-21 and other tests were routinely using to detection of AST such like chloramphenicol (30 µg, amikacin (30 µg), gentamicin (30 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg) followed by linezolid (30 µg) and vancomycin (30 µg).

Isolates by d-test: Described according to CLSI 2020-21 guidelines were isolates erythromycin resistant and inducible resistance. To detection by Kirby baur method makes a flattening zone on muller hinton agar to detection, after overnight showing inhibition that like D letter and where inducible clindamycin resistance that gives were d test positive zone[11].

MS phenotype: Among erythromycin have to ≤13 mm diameter and where clindamycin ≥21 mm giving showing to d-test negative.

iMLSB phenotype: Among erythromycin ≤13 mm diameter where to gives the sensitive to clindamycin ≥21 mm diameter were showing d-test positive.

cMLSB phenotype: Among both erythromycin and clindamycin with circular shape zone of inhibition around clindamycin like as constitutive MLSB.

Result

The study entitled was carried out in the department of Microbiology, Index medical college; Malwanchal University & Research centre Indore (M.P).

Table 1: Gender wise distribution of MRSA and MSSA isolated (n=155)

S.N	Gender	No of S. aureus	Percentage %
1	Male	86	55.5
2	Female	69	44.5
	Total	155	100

Table 2: Frequency of staphylococcus aureus was isolated from various clinical samples (n=155)

S.N	Specimens	Number of samples	Percentage %
1	Pus	48	30.9
2	Blood	27	17.4
3	Sputum	12	7.7
4	Urine	36	23.3
5	Synovial fluid	14	9.0
6	Ascitic fluid	10	6.5
7	High virginal swab	8	5.2

Table 3: Frequency of age group of staphylococcus aureus (n=155)

S.No	Age group	Staphylococcus aureus	
		No of patients	Percentage %
1	0-10	21	13.5
2	10-20	17	10.9
3	20-30	35	22.6
4	30-40	15	9.7
5	40-50	11	7.0
6	50-60	45	29.0
7	60-70	7	4.5
8	>70	4	2.6
	Total	**155**	**100**

Table 4: Frequency association of Clindamycin resistance with Methicillin resistance and sensitive (n=155)

S.N	Parameter	Methicillin-resistant 91 (58.7%)	Methicillin-sensitive 64 (42.3%)
1	**MS phenotype**	40 (43.9%)	29 (45.3%)
2	**iMLSB phenotype**	28 (30.8%)	22 (34.4%)
3	**Constitutive MLSB phenotype (%)**	23 (25.3%)	13 (20.3%)
	Total	91	64

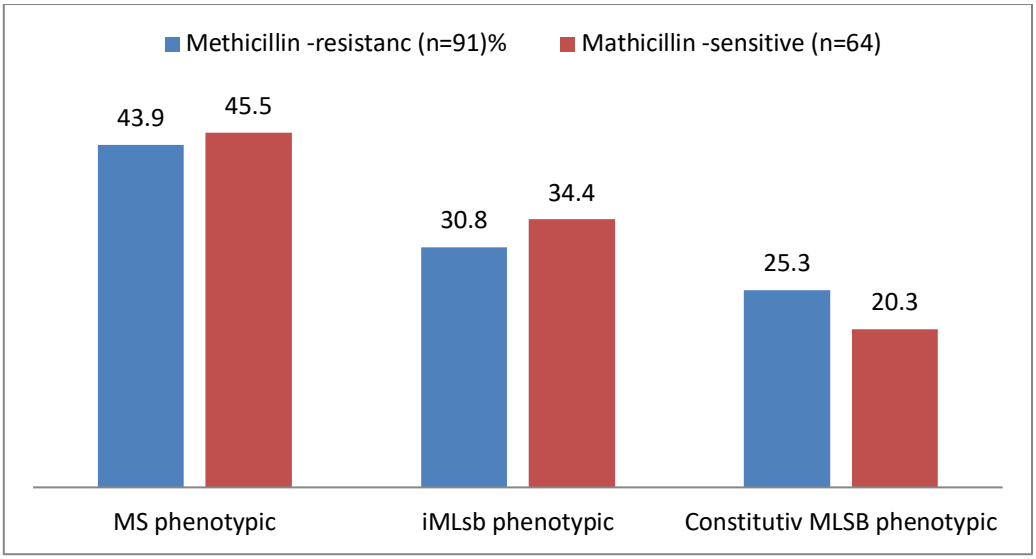

Figure 1: Showing Clindamycin resistance with Methicillin resistance and sensitive (n=155)

Table 5: Frequency of antimicrobial resistance pattern in d-test positive and negative

S.N	Antibiotics	d test negative	d- test positive
1	Penicillin	92.2	97.2
2	Amikacin	24.67	37.25
3	Gentamicin	78.5	48.87
4	Clindamycin	34.5	64.4
5	Erythromycin	89.67	15.01
6	Ciprofloxacin	74.6	95.45
7	Cotrimoxazole	72.5	81.47
8	Rifampicin	67.15	69.24
9	Nitrofurantion	19.7	24.5
10	Vancomycin	0	0
11	Linezolid	0	0

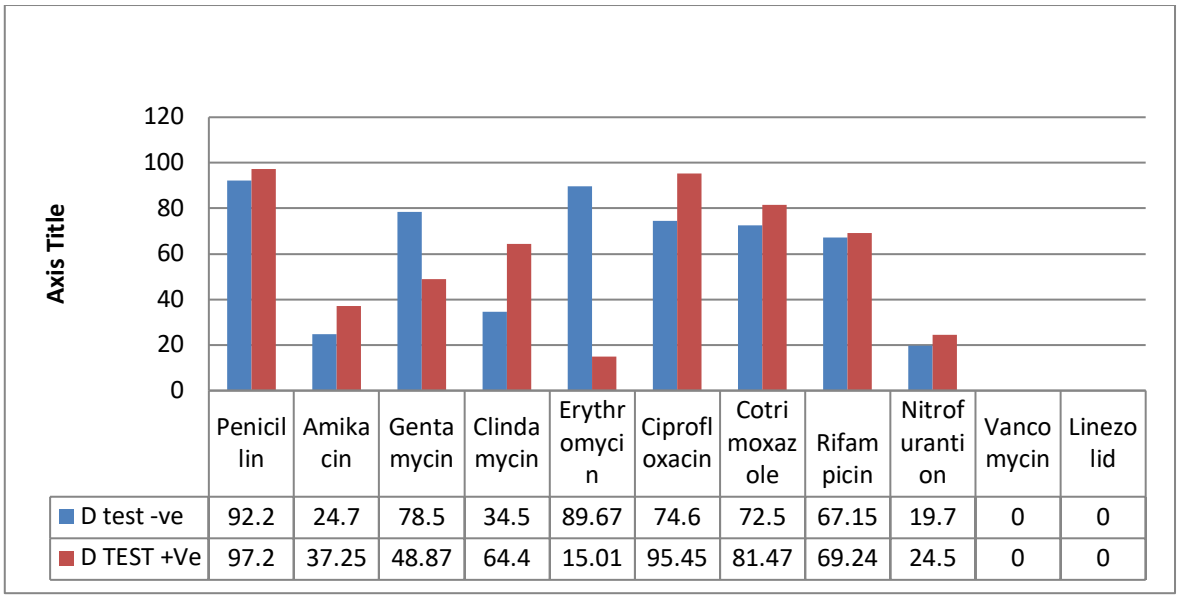

Figure 2: Frequency of antimicrobial resistance pattern use to d- test positive and negative isolates

Out present study 342 were isolates of staphylococci species from various clinical samples. like Pus, urine, Blood, sputum, pleural fluid, Asiatic fluid and high vaginal swab were received in clinical microbiology laboratory. Out of 342 isolated samples of staphylococcus species, 187 (54.7%) isolates samples were Coagulase negative staphylococcus (CONS) and 155 (46.3%) isolates samples were staphylococcus aureus. Out of 155 Staphylococcus aureus 91 (58.7%) were MRSA and 64 (42.3%) were MSSA.

Out of 155 Staphylococcus aureus isolates 48 (30.9%) were obtained from pus while 36 (23.3%) from obtained urine followed by 27 (17.4%) were blood, 14 (9.0%) were synovial fluid, 12 (7.7%) were sputum, 10 (6.5%) were ascetic fluid and also were obtained from high vaginal swab for culture sensitivity 8(5.2%). Most common predominantly infection samples were among in pus 28 (32.2%) followed by urine 25 (28.7%) and Blood 15 (17.2%). Most common predominantly infection were surgery word 36 (41.4%) followed by Medicine 24 (27.6%) and gynecology 11 (12.6%).Among, these present studies were highly infected patients in the age group of MRSA 50–60 45were isolated (29.0%) followed by 30 – 40 years in age group 35 (22.6%) were isolated.

Out of present this study 155 (51.7%) staphylococcus aureus isolates were resistant to erythromycin, out of which 91 (58.7%) were MRSA and 64(42.3%) were MSSA. Among the 155 isolates resistant to erythromycin, D test was positive in 50 (32.3%) (Inducible MLSB Phenotype) followed by 69 (44.5%) were negative in isolates (MS phenotype) and where 36 (23.2%) were isolated in constitutive MLSB phenotype . Acccording to result was comaperble , constitutive, inducible and MS phenotype resistance was almost equal and where using the MS A and MR in staphylococcus aureus.

Discussion

In this present study 342 isolates of staphylococcus species from various clinical samples like pus, urine, blood, sputum, pleural fluid, synovial fluid, and ascitic fluid received in clinical microbiology laboratory. Out of 322 isolates, 187 were coagulase negative staphylococcus (CoNS) and 155 isolates were staphylococcus aureus. However out of 155 Staphylococcus aureus, 58.7% were MRSA and 42.3% were MSSA while similar study was conducted by G. Liliana, M.Ligozzi, et al[22]In the present study, MRSA 86 (55.5%) of isolates were from male and 69 (45.5%) were from females. In this similar study conducted by Christian et[22]al in 2019.

Among, 91 (58.7%) staphylococcus aureus MRSA were isolates most common predominantly infection 48 (30.9%) were obtained from pus while 36 (23.3%) were obtained from urine followed by 27 (17.4%) were blood etc. Similar study were find out from clinical isolated samples the increasing of MRSA isolation (61.7%) was obtain in pus conducted by Mallick and Basak in Maharashtra (61.4%) followed by (42%) were by Tiwari et al.

Among, these present studies were highly infected patients in the age group of MRSA 50–60 45were isolated (29.0%) followed by 30 – 40 years in age group 35 (22.6%) were isolated. This similar study was conducted by Anna Bertoncelli et al[81] in 2019 where more affected age group was of elderly patients 34.6% were >60 years in study followed by 0-15 years age group patients (22.2%).

Geographical region were significantly resistance deffer to incidence of iMLSB. In this present study,which was erythromycin-resistant strainsof inducible clindamycin resistance were more in MSSA (34.4%) staphylococcus aureus then MRSA (30.8%) this similar study was conducted by Sasirekha B et al[13] in Bangalore, showing staphylococcus aureus were given 9.15%

isolates using iclindamycin resistance and erythromycin-resistant were given 22.4% staphylococcus aureus[14] this similar study conduct by by Schrecken berger et al[l6] and Levin et al15the giving showing which that higly infective inducible resistance in MSSA (20%) then MRSA (12.5%).

Clindamycin susceptibility were specific and provide incredible therapeutic option staphylococcus aureus. Where clindamycin susceptible was without checking inducible resistance for appropriate clindamycin therapy may result in institution. Other than negative result for inducible clindamycin resistance confirms [20]. This test were using for routine test in laboratory to identification suitable drug of choice for specific treatment staphylococcus aureus which that test were depending upon d- test positive enables to guiding and where it can giving to drug of choice d test negative isolates.

Conclusions:

Our study find out clindamycin sensitivity and erythromycin resistance might help clinicians to decide whether to using that clindamycin for s*taphylococcal species infections* detection by d-testing. The deferent types of frequency of constitutive and iCR in staphylococcus aureus MRSA isolates need to using for routine AST to detect by d-test the susceptibility to clindamycin as the inducible resistance and phenotype can inhibit the action of clindamycin were most common affect the treatment.

Among 155 isolates find out resistant to erythromycin where d- test was positive in 50 (32.3%) (inducible MLSB Phenotype) followed by 69 (44.5%) were negative in isolates (MS phenotype) and where 36 (23.2%) were isolated in constitutive MLSB phenotype. Compare to more than one method using were detection inducible percentage %, constitutive cMLSB and MS phenotype resistance were equal in the MRSA and MSSA in staphylococcus aureus

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Ethical Clearance

Ethical approval from Malwanchal university, Index medical college & Research Board and Institutional Ethical Committee (IEC Ref ID: MU/Research/EC/Ph.D/2020/54(a) was obtained before study commencement.

Data availability

In this present study collection the data using to findings of this study are available from the corresponding author to request.

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