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Prevalence of Inducible Clindamycin Resistant *Staphylococcus Species* in Clinical Isolates Recovered from Patients in Benin City, Nigeria

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Abstract: A total of 240 Staphylococci isolates were obtained from various clinical samples. These isolates were then identified using standard bacteriological techniques including colonial morphology, Gram staining, catalase and coagulase. The detection of lnducible clindamycin resistant (ICR) *Staphylococcus species* was done by D-Test method. Antimicrobial susceptibility test was performed using Kirby-Bauer diffusion method. The prevalence of ICR among *Staphylococcus species* obtained from this study was 22 (9.17%). Isolates that had inducible resistance to clindamycin was higher in methicillin resistant S. aureus isolates 16 (80.00%) than in methicillin sensitive S. aureus isolates 4 (20.00%). Resistance to cefotaxime recorded a significantly high prevalence in S. aureus 180 (96.78), CONS 54 (100.00%) and ICR 22 (100.00%) isolates. All isolates were more sensitive to imipenem/cilastin as compared to other antibiotics tested. The good oral absorption of clindamycin makes it an alternative option for use in treatment.

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I. INTRODUCTION

Macrolides, lincosamides, and streptogramin B are three separate antibiotics families with diverse chemical structures but related antibacterial spectrums and actions. They are a part of the macrolides, lincosamides, and streptogramin B (MLSB) subclass which comprises of a group bacteriostatic antibiotic. It has been suggested that these substances restrict the route by which nascent peptides leave the ribosome since their processes involve suppressing protein synthesis, with the target being the 23s rRNA of the 50S subunit of the bacterial ribosome (Tension et al., 2003). The most common method of resistance to macrolides and lincosamides is ribosomal methylation (Leclercq, 2002).

The resistance to antimicrobial agents among Staphylococci is an increasing problem. This has led to renewed interest in the usage of MLSB antibiotics to treat Staphylococcus aureus infections. The msrA gene, which codes for an efflux mechanism, or the erm gene, which codes for enzymes that confer inducible or constitutive resistance to MLSB antibiotics, can both mediate resistance to macrolides. Antimicrobials that share a method for accessing the cytoplasm, bind the same target, or are engaged in the same pathway that results in cell death or growth suppression are said to exhibit bacterial cross resistance (Colclough et al., 2019).

Clindamycin is an efficient and economic lincosamide drug employed in the treatment of staphylococci infection. It is regarded as an effective substitute medication for individuals who are allergic to penicillin for treating skin and soft tissue infections caused by S. aureus. It accumulates in abscesses, has high tissue penetration, and does not require dosage modifications in the context of renal impairment (Angel et al., 2008). According to Lim et al., (2006) *Staphylococcus species* can exhibit either constitutive or inducible clindamycin resistance. Target site modification by erm genes, which may express themselves either constitutively (cMLSB phenotype) or inducibly (iMLSB phenotype), is the most frequent route for such resistance, as they appear erythromycin resistant and clindamycin susceptible in vitro when not placed next to each other, strains with inducible resistance to clindamycin are challenging to identify in the typical laboratory setting. In these circumstances, in vivo treatment with clinda.

The D test can be employed as an easy-to-use adjunct technique to distinguish between constitutive and inducible clindamycin resistance in routine clinical laboratories. In iMLSB, an inducing agent is necessary for the creation of the enzyme, as opposed to cMLSB where it is constantly generated. A positive D test reveals the existence of an hidden resistance to clindamycin, which is brought about by an inducible methylase that modifies the shared ribosomal

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binding site for macrolides, clindamycin, and the group B streptogramins (Woods, 2009).

Methicillin is a narrow spectrum β-lactam antibiotics of the penicillin class. The rapid rise of Methicillin resistant Staphylococcus aureus (MRSA) has limited the therapeutic choices for the management of MRSA infections. This has led clinicians to choose the MLSB family of antibiotics, a reserved alternative, for the management of MRSA isolates (Thapa et al., 2021).

Inappropriate and excessive antibiotic use, prolonged hospital stays, the presence of chronic diseases, a lack of awareness, poor personal hygiene, a history of catheterization and a history of hospitalization are some of the factors that have been identified as potential risk factors for the development of MLSB and methicillin antimicrobial resistance in Staphylococcus aureus. By hospital, location, bacterial strain, and methicillin susceptibility, the percentage of staphylococci with in vitro inducible clindamycin resistance may differ. In particular, MRSA isolates of Staphylococcus aureus exhibit higher levels of iMLSB resistance.

II. MATERIALS AND METHODS

A total of 240 clinical isolates of *Staphylococcus species* were collected from various clinical samples submitted for routine culture and susceptibility testing at the Medical Microbiology Laboratory, University of Benin Teaching Hospital (UBTH), Benin City, Edo state. Conventional methods such as colonial morphology, Gram staining and biochemical testing (catalase and coagulase test) was used to identify all recovered isolates. Identified isolates were stored in nutrients agar slant at room temperature.

➢ *Ethical Approval*

Ethical Approval for this study was obtained from the Research and Ethics Committee, Ministry of Health, Edo state, Nigeria through their letter referenced HA/737/23/D/08210149.

- *Research Design*

A cross-sectional study design was used in conducting this research.

- *Inclusion Criteria*

Clinical isolates of *Staphylococcus species* were included in this research.

- *Exclusion Criteria*

Clinical isolates other than *Staphylococcus species* were excluded from this research.

➢ *Sample Size Determination*

The sample size for this study was calculated using the formula $N=\frac{Z^2P(1-p)}{d^2}$

(Naing et al., 2006).

Where:

Z= 1.96 (for confidence level of 95%)

d=0.05 (permissible error)

P=18% Prevalence rate of Inducible clindamycin resistance among *Staphylococcus species*, Karachi, Parkistan (Afridi et al.,2011).

Therefore; $N= 1.962 \times 0.18(1-0.18)$

0.052

$=226.8$

A total of 240 clinical isolates of *Staphylococcus species* were collected.

➢ *Antibacterial Agents*

The Antimicrobial susceptibility discs used for this research were purchased from a reputable pharmaceutical store [Celtech Diagnostic, Belgium Incorporated] and they include:

Amoxicillin clavulanate (30µg), Azithromycin (15µg), Cefuroxime (30µg), Cefotaxime (25µg), Cefoxitin (30µg), Cefexime (5µg), Ceftriaxone sulbactam (4µg), Ciprofloxacin (5µg), Clindamycin (2µg), Erythromycin (15µg), Gentamycin (10µg), Imipenem/ Cilastin (10/10µg), Levofloxacin (5µg), Ofloxacin (5µg).

➢ *Antibiotics Susceptibility Testing*

Antibiotics susceptibility testing was carried out on Muellar Hinton agar (MHA) medium (Oxoid Ltd., England). Modified Kirby-Bauer disc diffusion method for sensitivity reported by Sharma et al., (2014) was used for antibiotics susceptibility testing. Identified isolates from clinical samples were inoculated into nutrient agar slant and was stored at room temperature. Emergent colonies were then emulsified in sterile normal saline and the turbidity matched with 0.5 MacFarland standard. Once matched, a sterile cotton swab was dipped in the organism suspension and excess inoculum was allowed to drain off after which a lawn culture was made by streaking over the surface of the agar and applying bacteria inoculum of approximately 1-2 x 108CFU/mL to the surface of a large (150mm diameter) Mueller Hinton Agar plate.

These antibiotics disc were then placed aseptically on the agar surface. The plates were then incubated at 37°C overnight and observed for zones of inhibition. Sensitivity pattern of the isolates was determined by measurement in millimeters using a calibrated ruler. The degree of susceptibility of the isolates to each antibiotics was interpreted to be either sensitive (S) or resistant (R) according to the Clinical Laboratory Standard Institute (CLSI, 2012).

➢ *Detection of Methicillin Resistance*

The mecA gene, also known as the staphylococcal cassette chromosome mec (SCCmec), is responsible for mediating methicillin resistance (Ogefere and Ogunleye, 2019). Cefoxitin is a potent inducer of the mecA regulatory

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system. It has being recommended for detection of methicillin resistance in Staphylococcus aureus (MRSA) when using disc diffusion testing (Anand et al., 2009). A 0.5 McFarland equivalent suspension of organism was used streak the surface of the Muellar Hinton agar plate. Methicillin resistance was detected by placing Cefoxitin (30µg) disc on the agar plate, using modified Kirby-Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI, 2013). The plates were incubated at 37°C for 24 hours so as to allow for the growth of bacteria. Inhibition zone diameters ≤21mm indicates Cefoxitin resistance while diameters of ≥ 22mm indicates sensitivity.

- *Detection of Inducible Clindamycin Resistance Using the D-Test*

Detection of clindamycin resistance was carried out by performing D-test method as recommended by Clinical Laboratory Standard Institute (CLSI, 2013). A 0.5 McFarland equivalent suspension of organism was inoculated onto a Muellar Hinton agar (MHA) plate, the erythromycin (15 ug) disk was placed 20mm (edge-to-edge) apart from clindamycin (2 ug) disk on MHA plate. The plates were incubated for 24 hours at 37°C in an ambient air incubator. After incubation, isolates showing rounded zones of inhibition with diameter of ≤ 13 mm for erythromycin and ≥ 21 mm for clindamycin would be interpreted as negative for inducible resistance (D-test negative). Isolates with similar inhibitory zones of inhibition as above but with a D-shape zone around clindamycin would be interpreted as positive for inducible resistance (D-test positive).

- *Statistical Analysis*

Data obtained from this study was analyzed with Chi square (x2) using IBM SPSS version 24.0 for windows to determine the significance of the variables in the study. P-value of less than 0.05 was taken as statistically significant.

III. RESULTS

A total number of 240 isolates of *Staphylococcus species* were collected of which generally, the prevalence of Staphylococcus aureus was higher 186 (77.50%) than coagulase negative Staphylococcus 94 (22.50%). Staphylococcus aureus isolates recovered from females had a higher prevalence 111 (76.03%) than those from males 75 (79.79%) while coagulase negative Staphylococcus also had a higher prevalence for those from females 35 (23.97%) than from males 19(20.21).

The most prevalent phenotypes isolated were the cMLSB phenotype 166 (69.17%), S phenotype 27 (11.25%) and MSB phenotype 25 (10.42%) while the least prevalent phenotype was the iMLSB phenotype 22(9.17%). In all of the phenotypes recovered, each phenotype was more predominant among Staphylococcus aureus than in coagulase negative *Staphylococcus species*.

22 (9.17%) isolates recovered were positive for inducible clindamycin resistant *Staphylococcus species*. The prevalence was slightly higher in isolates recovered from males 10(10.64%) than those from females 12 (8.21%). The statistics was not significant (p=0.6856). This means that the gender of the patient is not a risk factor for the development of resistance.

A total of 153 (82.26%) were methicillin resistant while 33(17.74%) were methicillin sensitive. Amongst the methicillin resistant Staphylococcus aureus isolates tested, 16 (80.00%) were positive for inducible clindamycin resistant Staphylococcus aureus isolates while amongst the methicillin sensitive isolates 4 (20.00%) were positive for inducible clindamycin resistant staphylococcus. The prevalence of methicillin resistant Staphylococcus aureus and methicillin sensitive Staphylococcus aureus was statistically not significant (P = 0.7796). This means that whether the isolate is methicillin sensitive or methicillin resistant is not a risk factor for the development of resistance to clindamycin.

Age bracket 52-61 years had the highest prevalence of 5 (19.52%) while the lowest prevalence 0 (0.00%) was found in the age bracket 82-91. This was statistically not significant (P = 0.6949) and this means that there is no relationship between age of patients and inducible clindamycin resistance.

A total number of 22(9.17%) isolates were positive for inducible clindamycin resistant *Staphylococcus species* out of the 240 isolates tested. The highest prevalence for inducible clindamycin resistant *Staphylococcus species* was found amongst cerebrospinal fluid samples 1 (50.00%) while the lowest prevalence was found among Ear swab samples 1 (4.00%). This was statistically not significant (P = 0.4433).

Samples from Geriatric ward had the highest prevalence 1 (33.33%) when compared to samples recovered from other wards while samples from Special Care Baby Unit had the lowest prevalence of 1 (7.14%). This was however statististically not significant (P = 0.9642). This means that the ward the samples was recovered from was not a pre disposing factor for the development of inducible clindamycin resistance in *Staphylococcus species*.

A total number of 186 Staphylococcus aureus isolates were tested of which the highest resistance were found in Cefotaxime 180 (96.78%), Cefexime 154 (82.79%) and Ceftriazone Sulbactam 153 (82.26%). However, Staphylococcus aureus isolates recovered were highly susceptible to Imipenem/Cilastatin 82 (44.09%), Ciprofloxacin 68 (35.56%) and Ofloxacin 68 (36.56%).

A total number of 54 coagulase negative Staphylococcus isolates were tested of which the highest resistance were found in Cefotaxime 54 (100%), Cefexime 44 (81.49%) and Ceftriazone Sulbactam 43 (79.63%). However, coagulase negative Staphylococcus isolates recovered were highly susceptible to Imipenem/Cilastatin 22 (40.74%), Azithromycin 17 (31.48%) and Cefuroxime 17 (31.48).

The antibiotics susceptibility profile of clinical isolates of inducible clindamycin resistant *Staphylococcus species* tested showed that highest resistance were found in Cefotaxime 22 (100%), Cefexime 22 (100%) and Ceftriazone Sulbactam 18 (81.82%). However, inducible clindamycin

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resistant *Staphylococcus species* recovered were highly susceptible to Imipenem/Cilastatin 10 (45.45%), Ciprofloxacin 10 (45.45%) and Ofloxacin 8 (36.36%).

➢ *Detection of Inducible Clindamycin Resistant Staphylococcus Species*

The prevalence of inducible Clindamycin resistant *Staphylococcus species* in this study is 9.17%. A total of 22 (9.17) out of 240 isolates were identified as positive after performing the D – Test.

IV. DISCUSSION AND CONCLUSION

In this study, a total number of 240 non- duplicate isolates were collected from patients in Benin City of which 186 (77.50%) were Staphylococcus aureus and 54 (22.50%) were coagulase negative *Staphylococcus species*. The overall prevalence of inducible clindamycin resistant Staphylococcus isolates recovered was 22 (9.17%). Some other studies conducted in Nigeria has recorded varying prevalence. A study conducted in Ilorin recorded a prevalence of 5.4% (Ade et al., 2022). Nwokah and Abbey in 2016 recorded a prevalence of 11.2% in River state while a prevalence of 12.1% was recorded in Abia state (lfediora et al., 2019). Chika et al., (2018) reported that the prevalence of inducible clindamycin resistant *Staphylococcus species* isolated from a teaching hospital in Abakiliki was 15.4%, Medugu et al., (2021) recorded a prevalence of 29.1%. Meanwhile studies conducted outside Nigeria has also recorded varying prevalence. A study conducted in Pakistan by Rahbar and Hajia, (2007) reported a prevalence of 9.7% while

The prevalence of methicillin resistant Staphylococcus aureus (MRSA) isolates from this study was 153 (82.36%). This prevalence is significantly higher than the prevalence obtained from previous studies. Ifediora et al., (2019) and Abdullahi et al., (2022) have recorded a prevalence of 61.9%, 56.4% respectively. Among the MRSA isolates obtained, the prevalence of inducible clindamycin resistance was 16 (80.00%) in MRSA isolates. This prevalence is higher than prevalence obtained from previous studies 24.8% and 22.5% by Majhi et al., (2016) and Rabhar and Hajia, (2007) respectively. Such a variation might be due to differences in sample types, availability of drugs in the study areas, study participants involved, infection prevention practice, and unreasonable drug prescription. The prevalence of inducible clindamycin resistant isolates was significantly higher among MRSA isolates than in Methicillin sensitive Staphylococcus aureus (MSSA). This assertion is similar with the reports of Ifediora et al., (2019)

This study showed a high resistance to Amoxicillin Clavulanate 141 (75.81%) and Azithromycin 123 (66.13%) among Staphylococcus aureus isolates. This finding agrees with the study done by Ifediora et al., (2019) which reported a prevalence of 73.6% and 64.9% respectively. All Inducible clindamycin resistant isolates were susceptible to Cefotaxime and Cefexime with a prevalence of 22(100%). Meanwhile resistance to Gentamycin 16 (72.73%) in inducible Clindamycin resistant isolates was higher in this study as compared to 50.00% from the study conducted by Ade et al., (2022).

Among the 240 isolates of *Staphylococcus species* tested, this current study illustrated a high prevalence 166 (69.17%) of cMLSB phenotype indicating complete resistance to both erythromycin and clindamycin, the prevalence of the S phenotype was 27 (11.25%) indicating the strains that were susceptible to both clindamycin and erythromycin, prevalence of MSB phenotype was 25 (10.42%) this indicates the strains that were truly D- test negative and also susceptible to clindamycin in which case the use of clindamycin for treatment in these isolates would not result in treatment failure while those that had the iMLSB phenotype were 22 (9.17%) indicating those that were sensitive to clindamycin but had a D shaped zone of inhibition in the area around erythromycin. These isolates would have been taken as sensitive if a D test was not performed.

All of the variables analysed in relation to *Staphylococcus species* isolates tested including age, gender, ward and type of isolates recovered were all not statistically significant ($p>0.05$). This indicates that inducible resistance to clindamycin among *Staphylococcus species* was not dependent on any of those variables instead resistance was mainly based on genes of each strain. It is regrettable that the various genotypes of each isolate were not determined in this study. However, reports from previous studies indicates that ermA or ermC genes mediates resistance in cMLSB and iMLSB phenotypes while the msrA genes are seen in clindamycin susceptible isolates including the MSB phenotype (Li et al., 2015; Depardieu et al., 2007; Deotale et al., 2010).

The D-test is a simple and reliable method to detect inducible clindamycin resistance and has been recommended by the Clinical Laboratory Standard Institute (CLSI, 2013). Reporting susceptibility testing of Staphylococcal infections as susceptible to clindamycin without determining the inducible clindamycin resistance status can increase the risk of treatment failure during therapy. However, if in any case, clindamycin is administered for treatment of D- test positive isolates close follow up and monitoring is required to detect failure or relapse in treatment.

➢ *Conclusion*

The prevalence of inducible Clindamycin resistant *Staphylococcus species* reported in this study was 22 (9.17%). The aim of antimicrobial susceptibility testing is to improve proficiency in patient health care, this can be fostered by the timely administration of antimicrobial agents that are actually effective and sensitive against the infecting agents. Performing the D - test as a routine antimicrobial susceptibility testing technique on all identified isolate of Staphylococcus aureus and coagulase negative *Staphylococcus species* would help to achieve this aim.

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RECOMMENDATIONS

- The D - test should be included as a routine antimicrobial susceptibility testing technique on all identified isolate of Staphylococcus aureus and coagulase negative *Staphylococcus species* to reduce the risk of treatment failures due to unidentified D - test positive isolates.
- Public enlightenment on the consequence of antibiotics misuse should be done.
- There should be Public discouragement and strict regulation on over-the-counter sale of drugs to the public so as to combat the increasing prevalence of antibiotics resistance.
- The use of combination therapy is recommended so as to avoid the development of resistant strains among the limited range of antimicrobial agents currently available.

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APPENDIX

Table 1 Prevalence of Staphylococcus Aureus and Coagulase Negative *Staphylococcus Species* Among Clinical Isolates Recovered in Relation to Gender of Patients.

Gender	Number of isolates tested	*S. aureus* (%)	CONS (%)
Female	146	111 (76.09)	35 (23.97)
Male	94	75 (79.79)	19 (20.21)
Total	240	186 (77.50)	54 (22.50)

Table 2 Prevalence of MLSB Phenotypes Among Clinical Isolates of Staphylococcus Aureus and Coagulase Negative Staphylococcus Recovered from Patients in Benin City.

Phenotypes	Number of *S.aureus* isolates tested	Number of CONS isolates tested	Number of phenotypes obtained (%)
cMLSB (D-Test negative)	123	43	166 (69.17
MSB (True D- Test negative)	24	1	25 (10.42)
S (ERY-S; CD-S)	19	8	27 (11.25)
iMLSB (D-Test positive)	20	2	22 (9.17)
Total	186	54	240 (100.00)

Key:

CMLSB – Constitutive Macrolide Lincosamide Streptogramin B phenotype

MSB – Macrolide Streptogramin B phenotype

S – Susceptible phenotype

IMLSB – Inducible Macrolide Lincosamide Streptogramin B phenotype

Table 3 Prevalence of Inducible Clindamycin Resistant *Staphylococcus Species* Isolated from Clinical Isolates Based on Gender of Patient

Gender	Number of isolates tested	Positive (%)	P-Value
Female	146	12 (8.21)	0.6856
Male	94	10 (10.64)	
Total	240	22 (9.17)	

Table 4 Prevalence of Inducible Clindamycin Resistant *Staphylococcus Species* in Relation to Age of Patients.

Age (Years)	Number of isolates tested	Number of D- test positive isolates (%)	P – Value
<2	23	2(8.70)	0.6949
2-11	17	2 (11.76)	
12-21	21	2 (9.52)	
22-31	57	2 (3.51)	
32-41	38	3 (7.89)	
42-51	34	4 (11.76)	
52-61	26	5 (19.23)	
62-71	14	1 (7.14)	
72-81	7	1 (14.29)	
82-91	3	0 (0.00)	
Total	240	22 (9.17)	

Table 5 Prevalence of Inducible Clindamycin and Non-Inducible Staphylococcus Aureus Among Methicillin Resistant and Methicillin Sensitive Staphylococcus Aureus

D – Test	Number of isolates tested	MRSA (%)	MSSA (%)	P-value
Positive	20	16 (80.00)	4 (20.00)	0.7796
Negative	166	137 (82.53)	29 (17.47)	
Total	186	153 (82.26)	33 (17.74)	

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Table 6 Prevalence of Inducible Clindamycin Resistant *Staphylococcus Species* Among Clinical Isolates Recovered.

Samples	Number of isolates tested	Number of Positive isolates (%)	P - Value
Aspirate	6	1 (16.67)	0.4433
Breast Swab	2	0 (0.00) *	
Catheter Tip	7	0 (0.00) *	
Cerebrospinal fluid	2	1 (50.00)	
Ear Swab	25	1 (4.00)	
Endocervical swab	16	0 (0.00) *	
Eye Swab	15	0 (0.00) *	
High vaginal swab	19	0 (0.00) *	
Semen	1	0 (0.00) *	
Throat Swab	11	1 (9.09)	
Urethral Swab	11	2 (18.18)	
Urine	88	13 (14.77)	
Wound Swab	36	3 (8.33)	
Tonsil Swab	1	0 (0.00) *	
Total	240	22 (9.17)	

* Not Included in Statistical Analysis

Table 7 Prevalence of Inducible Clindamycin Resistant Isolates Recovered Based on Wards of Patients

Ward	Number of isolates tested	Number of positives (%)	P – value
Casualty	6	0 (0.00) *	0.9642
Children Emergency	14	2 (14.29)	
Consultant Outpatient Department	19	2 (10.53)	
Ear, Nose and Throat	21	0 (0.00) *	
General Practice Clinic	53	7 (13.21)	
Geriatric ward	3	1 (33.33)	
Gynaecological ward	12	0 (0.00) *	
Human Reproductive Research unit	1	0 (0.00) *	
Intensive Care Unit	4	0 (0.00) *	
Labour ward	12	1 (8.33)	
Maternity ward	5	0 (0.00) *	
Medical Emergency	10	1 (11.11)	
National Health Insurance Scheme	15	2 (13.33)	
Neurology ward	7	2 (28.57)	
Oncology ward	3	0 (0.00) *	
Orthopedic ward	1	0 (0.00) *	
Pediatric ward	10	0 (0.00) *	
Special Care Baby Unit	14	1(7.14)	
Surgical Out patient	3	0 (0.00) *	
Urology ward	2	0 (0.00)*	
Surgical ward	15	2 (13.33)	
Medical ward	10	1 (10.00)	
Total	240	22 (9.17)	

* Not Included in Statistical Analysis

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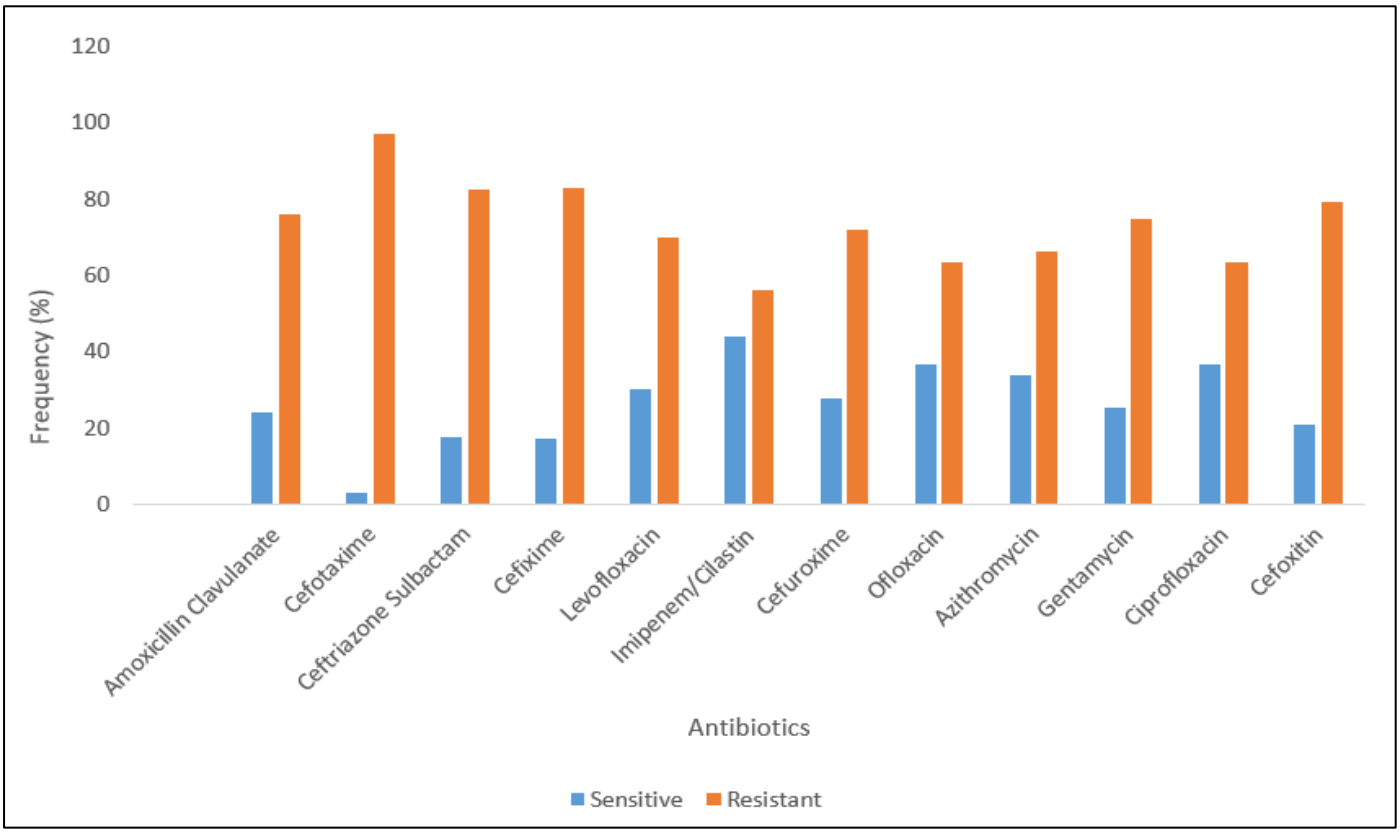

Fig 1 Antibacterial Susceptibility Profile of Staphylococcus Aureus Isolates Recovered Number of Isolates Tested = 186

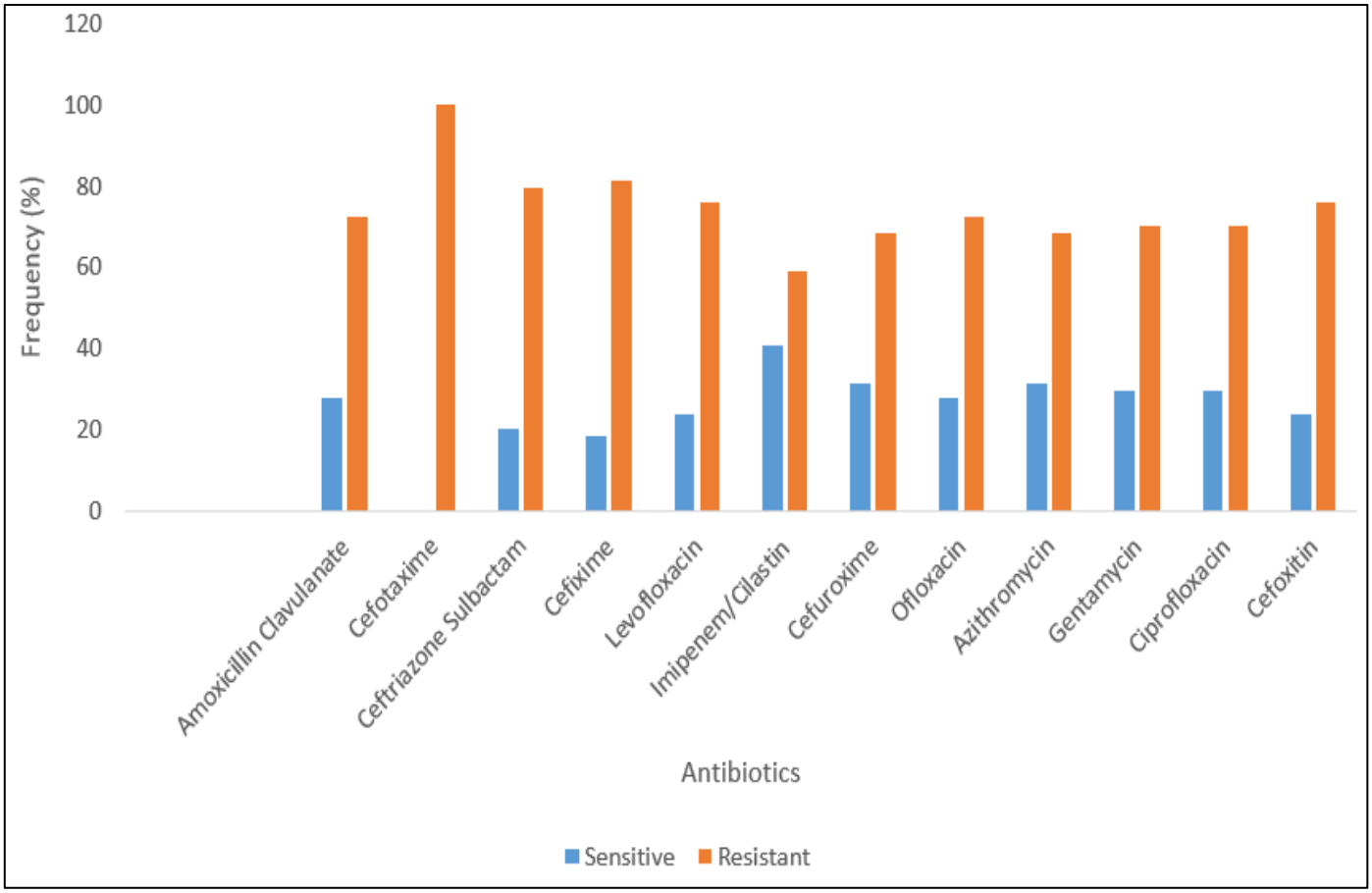

Fig 2 Antibacterial Susceptibility Profile of Coagulase Negative Staphylococcus Isolates Recovered Number of Isolates Tested = 54

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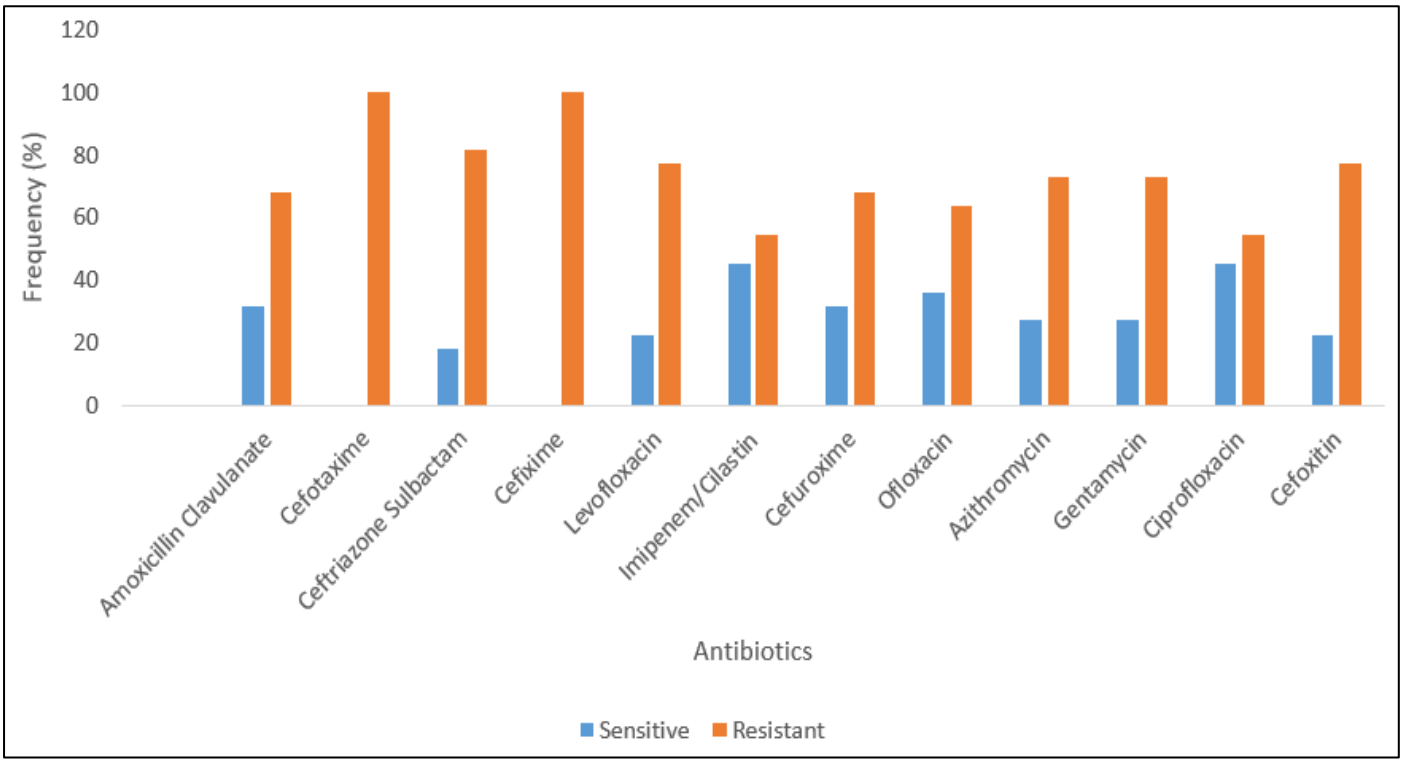

Fig 3 Antibacterial Susceptibility Profile of Inducible Clindamycin Resistant Isolates Recovered Number of Isolates Tested = 22

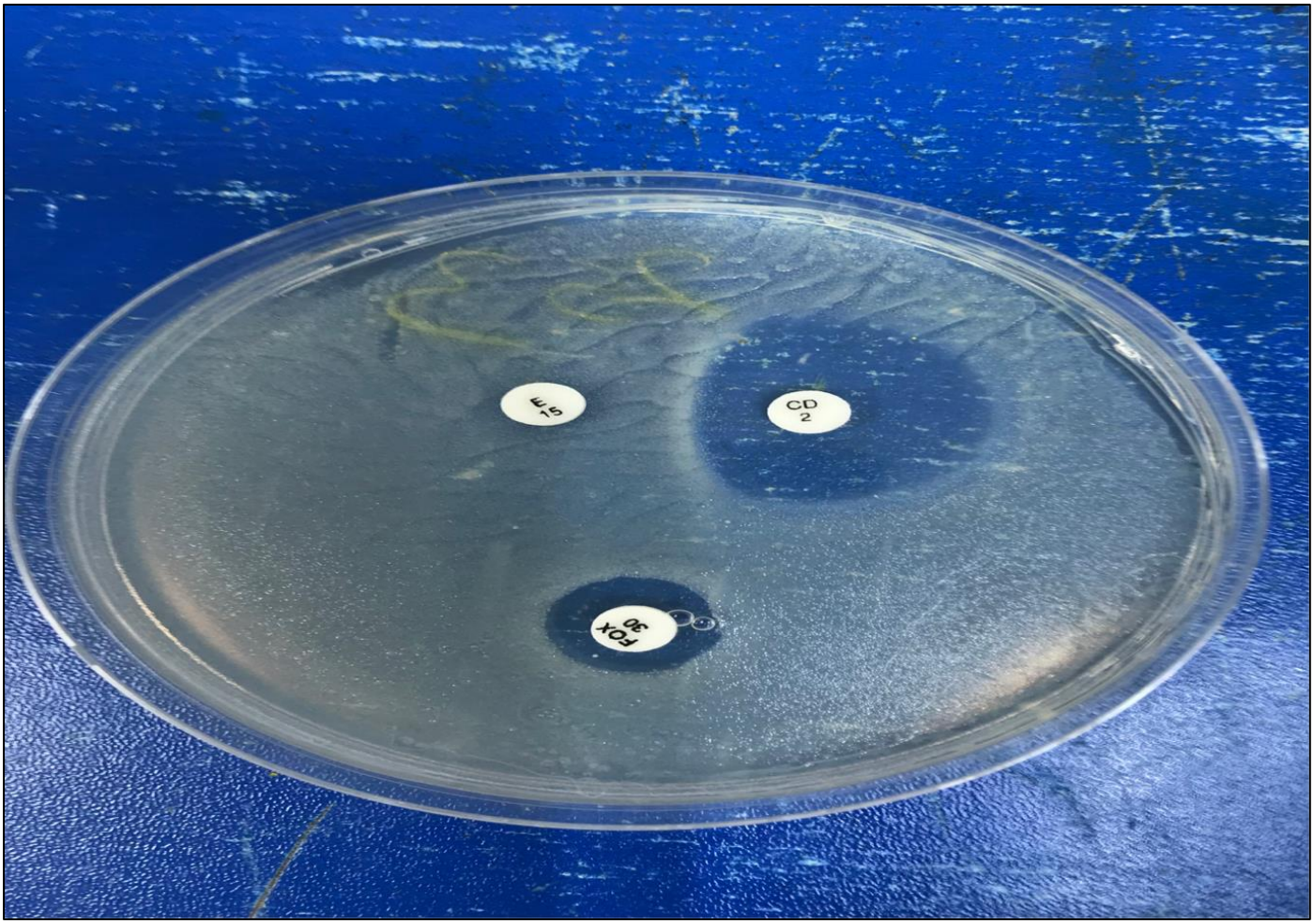

Fig 4 IMLSB Phenotype Determined by D – Test CD- Clindamycin E-Erythromycin FOX-Methicillin