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Molecular Detection of Macrolide-Induced Clindamycin Resistance Among Clinical Isolates of *Staphylococcus aureus* from Selected Hospitals in Katsina Metropolis

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Abstract

The emergence of inducible clindamycin resistance presents a significant challenge in treating Staphylococcus aureus (S. aureus) infections. This phenotype, evading routine susceptibility testing, compromises treatment efficacy and prolongs patient illness. Despite its clinical importance, limited data exist on its prevalence in Katsina Metropolis, Nigeria. This study aims to assess its prevalence and evaluate the phenotypic and genotypic characteristics among clinical isolates collected from selected hospitals in Katsina Metropolis. S. aureus isolates from various clinical specimens were obtained from three hospitals and identified using standard bacteriological methods. Antibiogram profiles were determined following CLSI guidelines, revealing varying efficacy among commonly prescribed antibiotics. Notably, chloramphenicol (87.8%), clindamycin (79.6%), tetracycline (69.4%), and azithromycin (67.3%) demonstrated high efficacy rates, while cefoxitin, ciprofloxacin, and trimethoprim-sulfamethoxazole exhibited the highest resistance level of (44.9%), (42.9%), and (40.8%) respectively. Prevalence of Macrolide Lincosamide Streptogramin B (MLS_B) phenotypes was assessed using the D-test, unveiling specific resistance phenotypes among the isolates. Polymerase chain reaction detected the ermC gene as predominant among D-test-positive isolates, all expressing the $iMLS_B$ phenotype. These findings shed light on the prevalence and mechanisms of inducible clindamycin resistance in S. aureus clinical isolates in Katsina Metropolis, emphasizing the importance of tailored treatment strategies and ongoing surveillance in combating antimicrobial resistance effectively.

Keywords: Antibiotic resistance, D-test, erm Genes, Katsina, MLSB and Staphylococcus aureus

INTRODUCTION

Macrolides, including erythromycin, clarithromycin, and azithromycin, lincosamides such as clindamycin, and streptogramin B antibiotics like quinupristin, belong to a distinct group of antibiotics that possess unique structures but share a similar mechanism of action. These antibiotics bind to the 23S ribosomal RNA within the 50S ribosomal subunit, thereby impeding the process of protein synthesis (Moosavian *et al.*, 2014; Heyar *et al.*, 2020). Clindamycin, in particular, offers several advantages. It is available in both parenteral and oral formulations, has high bioavailability and good permeability in the skin and soft tissues, inhibits toxin production, is cost-effective, and has fewer side effects (Baral and Khanal, 2017; Spížek and Řezanka, 2017; Heyar *et al.*, 2020). As a result, clindamycin is the most commonly prescribed antibiotic among the MLS_B group (Moosavian *et al.*, 2014; Khoshnood *et al.*, 2019). Unfortunately, the improper use and inappropriate utilization of MLS_B antibiotics have contributed to the proliferation of MLS_B-resistant strains of *S. aureus*.

This misuse has created selective pressure, allowing for the survival and spread of bacteria that possess mechanisms to evade the effects of these antibiotics. As a result, the effectiveness of MLS_B antibiotics in treating *S. aureus* infections has been compromised (Harkins *et al.*, 2017; Heyar *et al.*, 2020). Mechanisms of MLS_B

resistance include the activity of macrolide efflux pumps, modifications of the target site, and enzymatic inactivation of the antibiotics (Leclercq, 2002). The major resistance mechanism involves modifications or mutations in the ribosomal target, preventing the binding of the antibiotic. The erythromycin ribosome methylases (*erm*) gene, primarily *ermA* and *ermC*, in *S. aureus*, is responsible for mediating this mechanism (Leclercq, 2002; Cetin *et al.*, 2010).

Macrolides, lincosamides, and streptogramin B resistance can manifest in two primary forms: inducible and constitutive, which are not dependent on the type of *erm* gene (Leclercq, 2002; Moosavian *et al.*, 2014). Constitutive MLSB-resistant (cMLSB) phenotypes continuously produce functional methylase mRNA regardless of the presence of an inducer (Saderi *et al.*, 2011; Medugu *et al.*, 2021). In contrast, inducible MLSB-resistant (iMLSB) phenotypes produce non-functional mRNA that does not encode methylase under normal conditions but can become activated in the presence of an inducing agent.

These resistance phenotypes pose a challenge in clinical use, as they may not be detected by routine susceptibility testing methods, leading to treatment failure and prolonged illness in affected patients. Standard disk diffusion antibiotic susceptibility testing effectively detects constitutive MLSB-resistant phenotypes exhibiting resistance to erythromycin and clindamycin. However, to identify iMLSB-resistant phenotypes, D-test is employed. The D-test, also known as the Double-disk diffusion test, serves as a method for identifying macrolide-inducible clindamycin resistance. By performing this simple test, healthcare providers can determine the appropriate treatment options for infections caused by *S. aureus* (CLSI, 2023).

Understanding the prevalence and mechanisms of inducible clindamycin resistance in *S. aureus* infections is crucial for guiding treatment and infection control measures. Limited data exist on this resistance in Katsina Metropolis, Nigeria, emphasizing the need for local epidemiological insights.

This study assesses the prevalence and characterizes the phenotypic and genotypic aspects of inducible clindamycin resistance among clinical isolates of *S. aureus* obtained from Katsina Metropolis.

MATERIALS AND METHODS

The study was conducted in three hospitals in the Katsina metropolis: General Hospital Katsina, General Amadi Rimi Specialist Hospital, and Turai Umar Musa Yar'adua Women and Children Specialist Hospital. The study utilized a convenient sampling method, where *S. aureus* previously isolated from diverse clinical specimens like urogenital swabs, aspirates, urine, blood, and sputum were collected.

The sample size for this study was determined using the following formulae, considering a previous prevalence reported by (Medugu *et al.*, 2021) conducted in Nigeria:

$$n = \frac{Z^2 pq}{d^2}$$

Where;

n=Sample size

Z = Standard normal deviate at 95% $(1.96)^2 = 3.8416$

p = Prevalence, which is 7.0%, $\frac{7.0}{100} = 0.07$ (Medugu *et al.*, 2021).

q = Complement of p (1-p) = 0.07

d = Precision 5% $(0.05)^2 = 0.0025$

$n = \frac{3.8416 \times 0.07 \times 0.93}{0.0025} = \frac{0.25}{0.0025} = 100$ samples

In accordance with the dictates of ethics in medical research, approval for the research was sought from the Katsina State Ministry of Health (MOH/ADM/SUB/1152/1/530). Each isolate was inoculated onto quadrant plates of blood agar and mannitol salt agar and incubated at 37°C for 24 hours. *S. aureus* colonies on Mannitol salt agar showed a yellow halo, indicating mannitol fermentation, while on blood agar, colonies appeared greyish or golden yellow, ranged in size from medium to large (0.5-1.5 µm), and exhibited smooth texture, well-defined edges, and slightly raised elevation. Most colonies were pigmented creamy yellow and displayed beta-hemolysis. All predominant *S. aureus* isolates were further identified in accordance with Bannerman, 2003.

Gram staining was performed on recently cultured isolates to maintain staining capacity. Air-dried fixed smears were stained using the Gram staining technique. A catalase test was also conducted on colonies grown on Mannitol Salt Agar (MSA) by preparing a clean slide with 3% hydrogen peroxide. Subsequently, a coagulase production test was performed, starting with the rapid slide coagulase test. If clumping occurred, no further investigations

were required; however, if the result was negative, further testing with the tube coagulase test was conducted to confirm coagulase activity, considering the limitations of the slide coagulase test.

The susceptibility tests were conducted in accordance with CLSI (2023) guidelines, utilizing a turbidity standard of barium sulfate ($BaSO_4$) equivalent to a 0.5 McFarland standard to ensure consistent inoculum density. For isolates showing resistance to erythromycin and sensitivity to clindamycin, the D-test was performed to detect the iMLSB phenotype. This involved placing 15µg erythromycin and 2µg clindamycin disks on the agar surface, with a 15-26 mm distance between them. After incubation at 35°C for 18 hours, a flattened zone of inhibition adjacent to the erythromycin disk (D-zone) indicated inducible clindamycin resistance and a positive test.

Polymerase Chain Reaction

The genomic DNA of bacterial isolates was extracted using the Phenol-Chloroform DNA extraction method as described by Javadi *et al.* in 2014. Primers were adopted from Ghanbari *et al.* (2016), and Adeiza *et al.* (2019) were obtained from Inqaba Biotechnical Industries (Pty) Ltd. The primers' characteristics are shown in Table 1. For each isolate, three separate PCR reactions were performed using the BIO-RAD IQTM5 Multicolour Real-Time PCR Detection System, with 1µL of each forward and reverse primer, 15µL of master mix, 4µL of molecular-grade water, and 4µL of DNA template. Negative controls ('no template controls') and positive controls from known (*erm* positive) *S. aureus* isolates were included.

Table 1: Primers used in Polymerase Chain Reaction for Amplification of *erm* genes

Primer	Sequence (5'→3')	Product (bp)	Temp. (°C)	Reference
ermA F	GCCTGACTTTCAAAGGTAATTC	249	57.1	Ghanbari *et al.*, 2016; Adeiza *et al.*, 2019
R	TCGGATCAGGAAAAGGACAT	-	-	-
ermB F	GCCATGCGTCTGACATCTAT	192	58.7	-
R	CTGTGGTATGGCGGGTAAGT	-	-	-
ermC F	ATCTTTGAAATCGGCTCAGG	294	59.3	-
R	CAAACCCGTATTCCACGATT	-	-	-

Following the PCR amplifications, a 5µl aliquot of each PCR product was extracted and prepared for agarose gel electrophoresis. A 1% agarose gel, prepared in 1× Tris-borate-EDTA (TBE) buffer, was used for the electrophoresis. The gel was run at 100V for approximately 40 minutes. Midori Green Advance dye (Nippon Genetics) was added to the gel to facilitate the visualization of DNA bands during electrophoresis. To estimate the sizes of the PCR products, a 100-bp standard DNA ladder designed by Bioneer, which was ready-to-use, was employed as a size reference. The PCR products, along with the DNA ladder, were loaded onto the agarose gel for electrophoresis. Following electrophoresis, the gel was visualized using a gel documentation system, specifically the BIO-RAD Gel Doc 2000, allowing for the visualization and documentation of the DNA bands. The approximate sizes of the amplicons were determined by comparing the migration of the PCR products with the known sizes of the ladder bands.

RESULTS

During the 6 months study, 100 distinct clinical isolates of *S. aureus* were collected. Following standard bacteriological techniques, 49 (49%) were confirmed to be *S. aureus*. Table 2 illustrates the antibiogram profile of the utilized *S. aureus* isolates. Chloramphenicol, clindamycin, tetracycline, and gentamycin demonstrated the most effective outcomes among the tested antibiotics, with resistance recorded in only 8.2%, 6.1%, 30.6%, and 28.6% of cases, respectively (Figure 1). All bacterial isolates resistant to cefoxitin also exhibited resistance to erythromycin.

The findings from the PCR analysis, as presented in Table 3, indicate that only the ermC gene was identified among the three samples analyzed, as depicted in Figure 2. Conversely, the *ermA* and *ermB* genes were undetected in any isolates.

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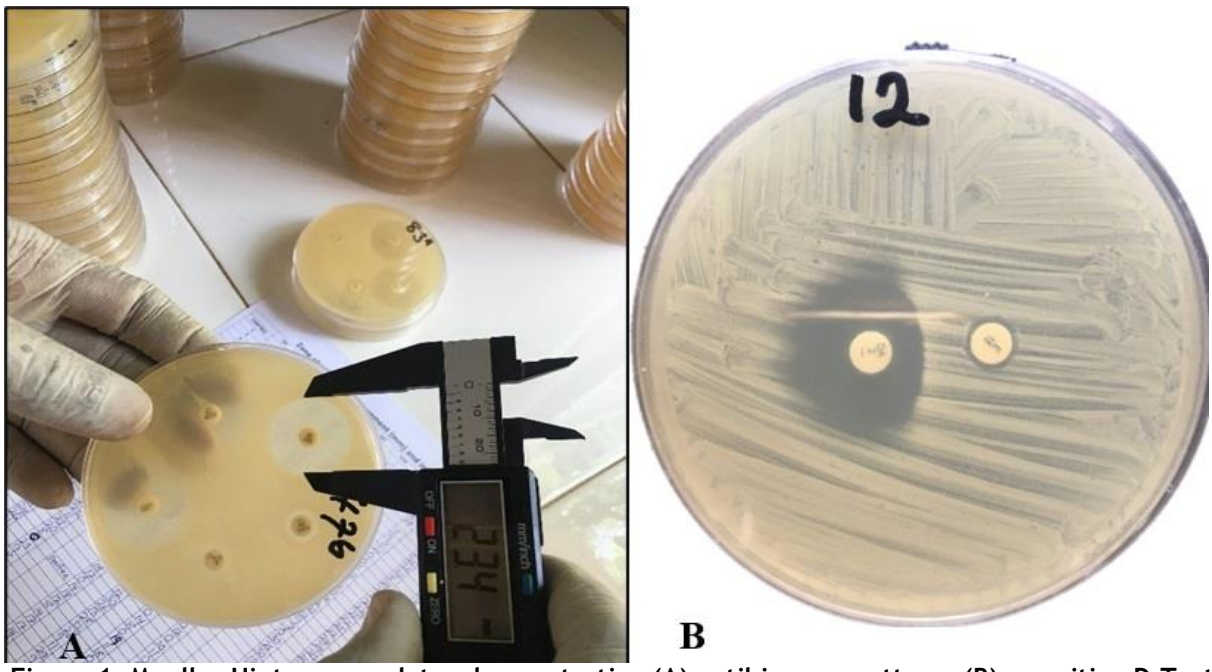

Figure 1: Mueller Hinton agar plates demonstrating (A) antibiogram pattern, (B) a positive D-Test

Table 2: Antibiogram Pattern of *S. aureus* Isolates

Antibiotics	Contents (µg)	No. (%) of isolates and Susceptibility pattern		
		Sensitive	Intermediate	Resistant
Clindamycin	2	39 (79.6)	07 (14.3)	3 (6.1)
Erythromycin	15	23 (46.9)	11 (22.4)	15 (30.6)
Gentamicin	10	29 (59.2)	06 (12.2)	14 (28.6)
Tetracycline	30	34 (69.4)	00 (0)	15 (30.6)
Trimethoprim-sulfamethoxazole	1.25/23.75	15 (30.6)	14 (28.6)	20 (40.8)
Ciprofloxacin	5	25 (51.0)	03 (6.1)	21 (42.9)
Chloramphenicol	30	43 (87.8)	02 (4.1)	04 (8.2)
Azithromycin	15	33 (67.3)	02 (4.1)	14 (28.6)
Cefoxitin	30	27 (55.1)	00 (0)	22 (44.9)

Table 3: Distribution of *erm* Genes Expression among the iMLS$_B$ Phenotypes

Isolates	D-Test	*ermA*	*ermB*	*ermC*
03	+	-	-	+
08	+	-	-	+
12	+	-	-	+

Key; (+) = detected, and (-) = not detected

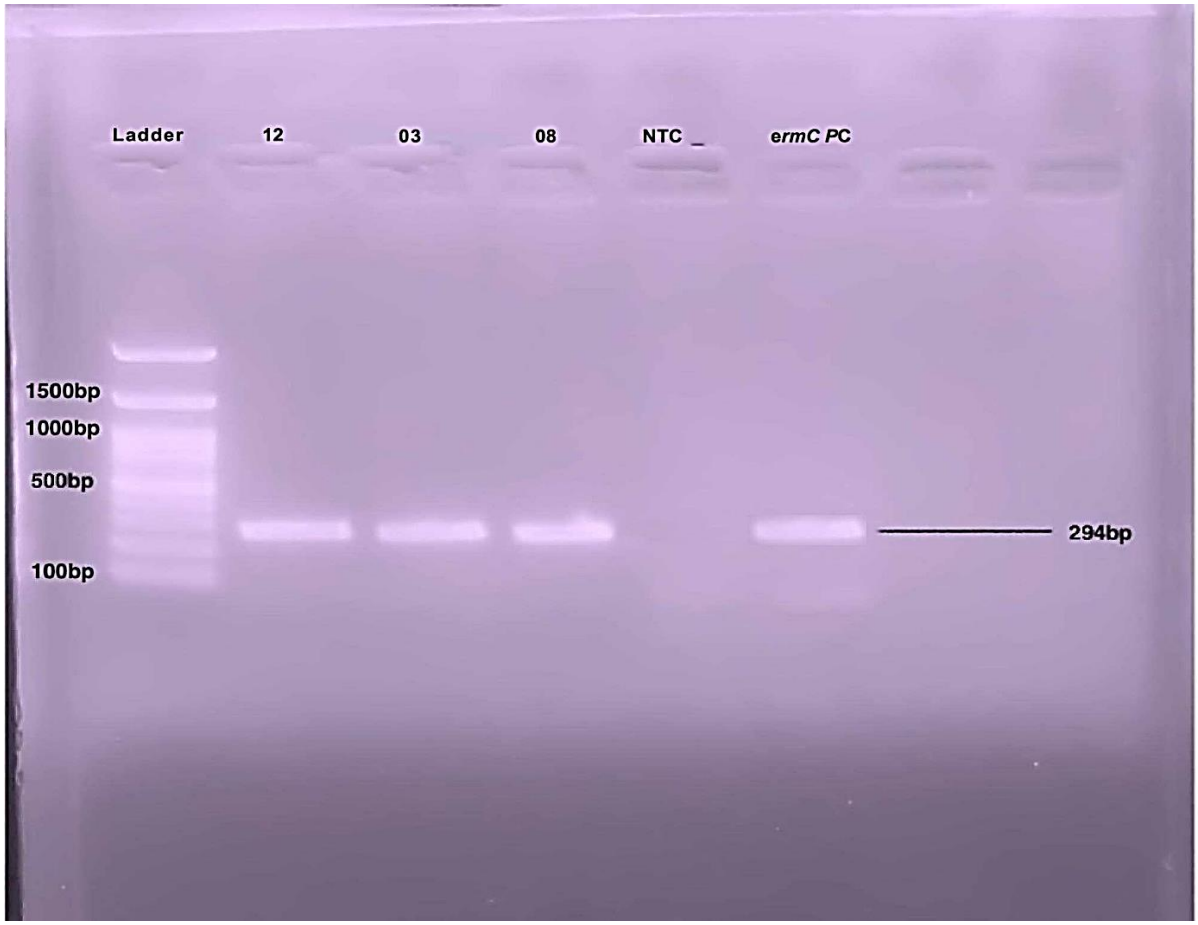

Figure 2: Electrophoretogram of polymerase chain reaction of *erm* genes for three D-Test positive isolates. In the figure: *ermC* positive 294bp (12, 03 and 08), Ladder is a 100 bp DNA ladder, NTC is a no template control, and *ermC* PC is a known positive control.

DISCUSSION

The antimicrobial susceptibility test revealed a moderate level of resistance among the isolates, notably to erythromycin (30.6%), cefoxitin (44.9%), ciprofloxacin (42.9%), trimethoprim-sulfamethoxazole (40.8%), and tetracycline (30.6%). These drugs, readily available and often misused empirically, are commonly used in Nigeria for treating various infections such as the common cold, typhoid fever, peptic ulcer, and sexually transmitted infections. The high resistance observed may indicate prior exposure of the isolates to these antibiotics, potentially contributing to the development of resistance mechanisms.

The isolates displayed the highest resistance to cefoxitin, commonly used as a phenotypic screening test for methicillin-resistant *S. aureus* (MRSA). This suggests that all isolates resistant to cefoxitin are likely MRSA, as cefoxitin resistance is a reliable indicator of methicillin resistance (CLSI, 2023). The presence of MRSA emphasizes the severity of antimicrobial resistance in the tested population, which is to be expected given that MRSA often carries additional drug-resistance genes, as documented in previous studies (Li *et al.*, 2018; Gan *et al.*, 2021).

Chloramphenicol has a reported resistance rate of 14.2% in Nigeria, according to a study by Medugu *et al.* (2021). However, compared to other antibiotics, chloramphenicol still maintains a relatively low resistance rate in this study, with only 8.2%. Despite its availability and cost-effectiveness, physicians in Nigeria do not commonly prescribe chloramphenicol due to concerns about potential side effects, such as the risk of inducing aplastic anaemia (Wolters *et al.*,2020). These concerns may contribute to the reduced use and abuse of chloramphenicol in clinical practice.

The findings indicate that the *ermC* gene was the predominant gene detected among all the inducible macrolide-lincosamide-streptogramin B ($iMLS_B$) isolates. This observation is consistent with a study conducted in Punjab (Heyar *et al.*, 2020), as well as other studies that have also reported *ermC* as the most prevalent gene (Ghanbari *et al.*, 2016; Mahfouz *et al.*, 2023). However, it is worth noting that some studies

have reported *ermA* as the most prevalent gene instead (Cetin *et al.*, 2010; Saderi *et al.*, 2011; Khoshnood *et al.*, 2019). The variation in the frequency of *erm* resistance genes observed in different studies can be attributed to two possible factors: the complex and diverse nature of erythromycin resistance and the possibility of small plasmids carrying *erm* genes being lost during bacterial replication or under the selective pressure of antibiotics. Even though the *erm* genes were amplified in our study, it's important to note that no additional molecular analysis or DNA sequencing of the amplicons was done.

CONCLUSION

Based on the available data, the study represents the first investigation of the frequency of erm genes among clinical isolates of S. aureus in Katsina. Detecting the *erm* gene further substantiates the sufficiency of the D-test in monitoring and assessing potential clindamycin treatment failures. However, chloramphenicol showed the highest efficacy against the *S. aureus* isolates.

RECOMMENDATIONS

The *erm* genes amplified should be further sequenced to determine their specific genetic sequences. Additionally, we suggest examining species other than *S. aureus* for the presence of the erm gene in future research studies.

REFERENCES

Adeiza, S. S., Onaolapo, J. A., and Olayinka, B. O. (2019). Exploration of erythromycin ribosomal methylase genotypes among D+ methicillin-resistant *S. aureus* strains in Sokoto, Nigeria. *Mediterranean Journal of Infection, Microbes and Antimicrobials*, **8**:39. **[Crossref]**

Bannerman, T. L. (2003) *Staphylococcus*, *Micrococcus*, and Other Catalase-Positive Cocci That Grow Aerobically. In: Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A. and Yolken, R. H., Eds., Manual of Clinical Microbiology, 8th Ed American Society for Microbiology, Washington DC, **4**(13), 384-404.

Baral, R. and Khanal, B. (2017). Inducible clindamycin resistance in *S. aureus* strains isolated from clinical samples. *International Journal of Biomedical Research*, **8**(02), 81-84. **[Crossref]**

Cetin, E. S., Gunes, H., Kaya, S., Aridogan, B. C. and Demirci, M. (2010). Distribution of Genes Encoding Resistance to Macrolides, Lincosamides and Streptogramins Among Clinical Staphylococcal Isolates in a Turkish University Hospital. *Journal of Microbiology, Immunology and Infection*, **43**(6), 524-529. **[Crossref]**

CLSI. (2023). Performance Standards for Antimicrobial Susceptibility Testing. *33rd ed. 2020 Supplement M100. Wayne, PA. Clinical and Laboratory Standards Institute*.

David, M. Z., and Daum, R. S. (2010). Community-Associated Methicillin-Resistant *S. aureus*: Epidemiology and Clinical Consequences of an Emerging Epidemic. *Clinical Microbiology Reviews*, **23**(3), 616-687. **[Crossref]**

Gan, T., Shu, G., Fu, H., Yan, Q., Zhang, W., Tang, H., . . .Lin, J. (2021). Antimicrobial resistance and genotyping of Staphylococcus aureus obtained from food animals in Sichuan Province, China. *BMC veterinary research*, **17**(1), 177. **[Crossref]**

Ghanbari, F., Ghajavand, H., Havaei, R., Jami, M. S., Khademi, F., Heydari, L., . . . Havaei, S. (2016). Distribution of *erm* genes among *S. aureus* isolates with inducible resistance to clindamycin in Isfahan, Iran. *Advanced Biomedical Research*, **5(1), 62. [Crossref]**

Harkins, C. P., Pichon, B., Doumith, M., Parkhill, J., Westh, H., Tomasz, A., . . . Holden, M. T. G. (2017). Methicillin-resistant *S. aureus* emerged long before the introduction of methicillin into clinical practice. *Genome Biology*, **18**(1), 130. **[Crossref]**

Heyar, A. K., Kaur, K., Gill, A. K. and Gill, P. K. (2020). Induction of clindamycin resistance in clinical isolates of *S. aureus* from a tertiary care hospital. *International Journal of Medical and Biomedical Studies*, **4**(12). **[Crossref]**

Javadi, A., Shamaei, M., Mohammadi Ziazi, L., Pourabdollah, M., Dorudinia, A., Seyedmehdi, S. M., and Karimi, S. (2014). Qualification study of two genomic DNA extraction methods in different clinical samples. *Tanaffos*, **13**(4), 41-47.

Kateete, D. P., Kimani, C. N., Katabazi, F. A., Okeng, A., Okee, M. S., Nanteza, A., . . . Najjuka, F. C. (2010). Identification of *S. aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Annals of Clinical*

Microbiology and Antimicrobials, **9**(1), 1-7. [Crossref]

Khoshnood, S., Shahi, F., Jomehzadeh, N., Abbasi Montazeri, E., Saki, M., Mortazavi, S. M., and Maghsoumi-Norouzabad, L. (2019). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among methicillin-resistant *S. aureus* strains isolated from burn patients. ***Acta Microbiologica et Immunologica Hungarica***, ***66***(3), 387-398. [Crossref]

Koneman, E. W. (2005). Koneman's color atlas and textbook of diagnostic microbiology (color atlas and textbook of diagnostic microbiology) 6th Edition. Lippincott Williams and Wilkins.

Leclercq, R. (2002). Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. *Clinical Infectious Diseases*, **34**(4), 482-492. [Crossref]

Li, S. M., Zhou, Y. F., Li, L., Fang, L. X., Duan, J. H., Liu, . . . Liu, Y. H. (2018). Characterization of the Multi-Drug Resistance Gene *cfr* in Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strains Isolated From Animals and Humans in China. *Frontiers in microbiology*, **9**, 2925. [Crossref]

Mahfouz, A. A., Said, H. S., Elfeky, S. M., and Shaaban, M. I. (2023). Inhibition of Erythromycin and Erythromycin-Induced Resistance among *S. aureus* Clinical Isolates. ***Antibiotics***, ***12***(3), 503. [Crossref]

Medugu, N., Nwajiobi-Princewill, P. I., Shettima, S. A., Mohammed, M. M., Mohammed, Y., Wariso, K., . . . Iregbu, K. C. (2021). A mini- national surveillance study of resistance profiles of *S. aureus* isolated from clinical specimens across hospitals in Nigeria. *Nigerian Journal of Clinical Practice*, **24**(2), 225-232. [Crossref]

Moosavian, M., Shoja, S., Rostami, S., Torabipour, M. and Farshadzadeh, Z. (2014). Inducible clindamycin resistance in clinical isolates of *S. aureus* due to *erm* genes, Iran. *Iranian Journal of Microbiology*, **6**(6), 421-427.

Saderi, H., Emadi, B., and Owlia, P. (2011). Phenotypic and genotypic study of macrolide, lincosamide and streptogramin B (MLSB) resistance in clinical isolates of *S. aureus* in Tehran, Iran. *Medical science monitor: international medical journal of experimental and clinical research*, **17**(2), 48-53. [Crossref]

Salgueiro, V. C., Iorio, N. L. P., Ferreira, M. C., Chamon, R. C., and Dos Santos, K. R. N. (2017). Methicillin resistance and virulence genes in invasive and nasal *Staphylococcus* epidermidis isolates from neonates. *BMC Microbiology*, **17**(1), 15. [Crossref]

Spížek, J., and Řezanka, T. (2017). Lincosamides: Chemical structure, biosynthesis, mechanism of action, resistance, and applications. *Biochemical pharmacology*, **133**, 20-28. [Crossref]

Wolters, M., Frickmann, H., Christner, M., Both, A., Rohde, H., Oppong, K., . . .Akenten, C. W. (2020). Molecular Characterization of *Staphylococcus aureus* Isolated from Chronic Infected Wounds in Rural Ghana. *Microorganisms*, **8**(12), 2052. [Crossref]