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Clindamycin Resistance among Methicillin Resistant *Staphylococcus aureus* Isolated from Human and Respective Household Swine in Greater Kabale Region—South Western Uganda

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Abstract

Introduction: *S. aureus* is recognized as the common cause of nosocomial and community-acquired infections. Macrolide-Lincosamide-Streptogramin B (MLS_B) is thought to be alternative therapies against MRSA infections. Clindamycin is the most favored agent because of exceptional pharmacokinetic characteristics. However, increasing resistance to clindamycin among MRSA strains is a serious challenge. The current study investigated the profile of clindamycin resistance among MRSA isolates from Humans, and their respective livestock (in particular swine) using D-test in greater Kabale region. **Materials and Methods:** Three hundred phenotypic MRSA isolates previously isolated from Humans and swine were confirmed by mecA PCR. We performed D-test using erythromycin (15 μg) and clindamycin (2 μg) discs in accordance to Clinical and Laboratory Standards Institute (CLSI) protocol. **Results:** Of all 300 MRSA isolates, 6% (n = 18) were sensitive to Erythromycin and Clindamycin (S). The rate of inducible clindamycin resistance (iMLSB) was 42% (n = 125) and 38% (n = 115) was resistance to both Erythromycin and clindamycin (cMLSB). However, 14% (n = 42) were resistant to erythromycin but sensitive to clindamycin (MS) without "D" zone negative. **Conclusion:** Clindamycin resistance (both cMLSB and iMLSB) among MRSA was high and "D" test should be adopted routinely during antimicrobial susceptibility testing by disc diffusion testing to rapidly detect iMLSB and cMLSB.

1. Introduction

S. aureus is recognized as one of the most common organisms causing nosocomial and community-acquired infections worldwide. The emergence of multidrug resistant *S. aureus* strains, especially methicillin resistant *S. aureus* (MRSA), is of a particular concern. In Uganda, this has been largely attributed by empirical antibiotic prescriptions [1]. The cumulative MRSA problem is an indicator for urgent need for new antibiotics.

Macrolide-Lincosamide-Streptogramin B (MLS$_B$) antibiotics have been thought about as alternative solution to treat MRSA infections [2]. The most commonly used antibiotic in the MLSB group are the macrolides (e.g. erythromycin and azithromycin) and clindamycin which is a Lincosamide [3].

Macrolides act through inhibition of protein synthesis by binding irreversibly to the 23S ribosomal RNA (rRNA) on the bacterial 50S ribosomal subunit and subsequent disruption of the growing peptide chain by blocking translocation [4]. Lincosamide [e.g. clindamycin and lincomycin] bind to the 50S ribosomal subunit and prevent peptide elongation by interfering with the peptidyl transfer during protein synthesis [5]. Clindamycin is the most preferred agent because of exceptional pharmacokinetic characteristics [6] and is regularly used in the management of severe infections, caused by macrolide resistant *S. aureus* infections including MRSA [7]. However, increasing resistance to clindamycin among MRSA strains and other S*taphylococcus* is a serious challenge [8]. The expression of clindamycin resistance in *Staphylococcus* species can be constitutive or inducible [9] [10] through *erm* genes which codes for ribosomal methylases [11]. In addition, the resistance to the lincosamides (clindamycin), macrolides (erythromycin), and streptogramins (quinupristin/dalfopristin) is facilitated by three related genes, *erm*A, *erm*B, and *erm*C, that encode for erythromycin resistance methylases [12]. Methylase enzymes binds on to the ribosome resulting in a conformational change or modification in the ribosomal target and consequently, decreasing the ability of these drugs to bind to the ribosome [5] [13].

Inducible Clindamycin resistance (iMLSB) cannot be identified by standard methods of antibiotic susceptibility testing and failure to detection may result into treatment failure with Clindamycin [6]. Erythromycin-resistant *staphylococci* are routinely considered to be resistant to Clindamycin by clinicians [14], a phenomenon that is wrong that shuns Clindamycin prescription to patients infected with macrolide-resistant isolates that may be sensitive to Clindamycin. It is rational to routinely test for presence of iMLSB strains and this can be achieved cheaply by use of Erythromycin and clindamycin discs placed adjacent

to each other during routine antibiotic sensitivity testing by Kirby Bauer technique for *S. aureus* [2] [15] [16]. Data on profiles of clindamycin resistance among MRSA in Kabale region—South western Uganda is not available. Therefore, the current study investigated the profile of clindamycin resistance among MRSA isolates from Humans, and their respective livestock (in particular swine) using D-test.

2. Materials and Methods

This was cross-sectional study, where 300 phenotypic MRSA isolates previously isolated from humans (n = 200) and swine (n = 100) during the period of January 2015 to June 2016 and stored in glycerol (20%v/v) at −80°C.

2.1. DNA Extraction

These isolates, were subjected to DNA extraction following Queipo *et al.* and Teeraputon *et al.*, techniques [8] [17] by boiling using 100 µl of the bacterial suspension in 1.5 ml cryogenic vials (Eppendorf, Germany) followed by centrifugation at 3000 rpm for 15 minutes. The supernatant was removed, and the pellet suspended using molecular biology-grade water (Eppendorf, Germany) and re centrifuged at 3000 rpm for 10 min. The supernatant was discarded, and the pellet suspended in 100 µl of molecular biology-grade water. The suspension was subjected to boiling at 100°C for 10 min, cooled on ice, and centrifuged at 15,000 rpm for 10 seconds before it was stored at −20°C.

2.2. PCR Amplification

Aliquots of 2 µl of template DNA was used for PCR to detect and amplify mecA gene with origonucleotide primer mecA F;
5-TCCAATTACAACTTCACCAGG-3 and mecA R
5-CCACTTCATATCTTGTAACG-3 synthesized by GenxBio to confirm their MRSA status. The reaction mixture (25 µl) consisting of 100 pmol of each primer, Taq polymerase (2.5 U), Mg^{2+} (2.5 mM), 2.5 µl PCR buffer and 3 µl template DNA. The PCR program was as follows: 3 min at 94°C; followed by 40 cycles of a 30 seconds denaturation step at 94°C, a 30 second annealing step at 45°C and a 30 second extension at 72°C; and a final 10 minutes extension step at 72°C. The amplified product was a 533 bp sequence, which was detected by 1% agarose gel electrophoresis with (0.5 mg/L) ethidium bromide stain and observation under UV light. All strains positive for the mecA gene and designated MRSA. MecA positive (ATCC 43300) and MecA negative (ATCC 29213) were used as positive and negative controls.

2.3. Detection of Phenotypic Clindamycin Resistance

To detect clindamycin resistance, a suspension of 0.5 McFarland standard equivalent was prepared from all genotypically confirmed MRSA isolates and a lawn culture of bacterial suspension was seeded on to sterile Muller Hinton Agar

(MHA) plates. Onto the seeded plates, disk of Clindamycin (2 µg) and Erythromycin (15 µg) was place in approximately 15 mm apart (measured edge to edge). The inoculated plates were further incubated at 37˚C for 16 to 18 hours. The zone of clearance with flattening characteristics (D-shaped) around clindamycin in the area between the two adjacent discs, indicated iMLSB [16].

2.4. Media Quality Control

Before use, Mueller Hinton agar (MHA) was quality controlled (QC) by checking its physical appearance, sterility after preparation and capacity to support growth. Sterility testing was performed on 5% of each batch of new medium prepared in house media selected randomly and incubated for 48 hours at 35˚C to 37˚C under aerobic conditions to check for contamination. Fertility test was performed using QC control strains (*Staphylococcus aureus ATCC* 25923, *Escherichia coli ATCC* 25922, *Pseudomonas aeruginosa ATCC* 27853) and subjected to Kirby-Bauer Susceptibility Testing protocol [2] using three MHA plates from each batch prepared and incubated for 18 - 24 hours at 35˚C to 37˚C under aerobic atmosphere. All the media used passed quality control measures.

3. Results

Among the MRSA isolates (n = 300), 6% (n = 18) of the strains were sensitive to both erythromycin and clindamycin, designated as **S** phenotype. However, 38% (n = 115) MRSA strains showed resistance to both erythromycin (zone size ≤ 13 mm) and clindamycin (zone size ≤ 14 mm) and these strains were constitutively resistant to clindamycin and designated as "**cMLSB**" phenotype. In addition, 42% (n = 125) showed resistance to erythromycin (zone size ≤ 13 mm) and sensitive to clindamycin (zone size ≥ 21 mm) with a "D-shaped" zone of inhibition around. These were identified as inducible clindamycin resistant strains and they were designated as **iMLSB**. Of note, 14% (n = 42) showed resistance to erythromycin (zone size ≤ 13 mm) and sensitive to clindamycin (zone size ≥ 21 mm) without "D" zone of inhibition around clindamycin and were designated as *MS* phenotypes as shown in Figure 1.

Among the MRSA with iMLSB phenotypes, 46% (n = 92) isolates were from humans, 33% (n = 33) from swine. In addition, cMLSB phenotype, 34% (n = 68) originated from human while 47% (n = 47) were from swine. However, among the MS phenotypes, 15% (n = 30) and 12% (n = 12) were from human and swine respectively whereas among the S phenotype, 5% (n = 10) were from human and 8% (n = 8) were from swine (Figure 2). D-test was used to rule out cMLSB and iMLSB (D zone present) as shown in Figure 3.

Results of mecA gene PCR where an Amplicon of approximately 180 bp was expected using positive and negative controls are shown in Figure 4.

4. Discussion

The increasing frequency of the *Staphylococcal* infections and their respective
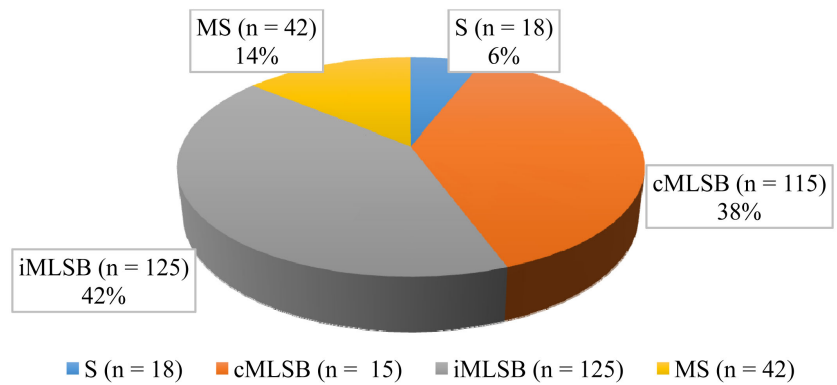
Figure 1. Clindamycin and Erythromycin resistance pattern of MRSA isolates. Footnote: S = Sensitive to both clindamycin and erythromycin, cMLSB = constitutive clindamycin; iMLSB = inducible clindamycin resistance (D zone present); MS = Erythromycin resistant but clindamycin sensitive (D zone absent).

Figure 2. MLSB resistance profile among MRSA isolates. Footnote: cMLSB = constitutive clindamycin; iMLSB = inducible clindamycin resistance (D zone present); MS= Erythromycin resistant but clindamycin sensitive (D zone absent).
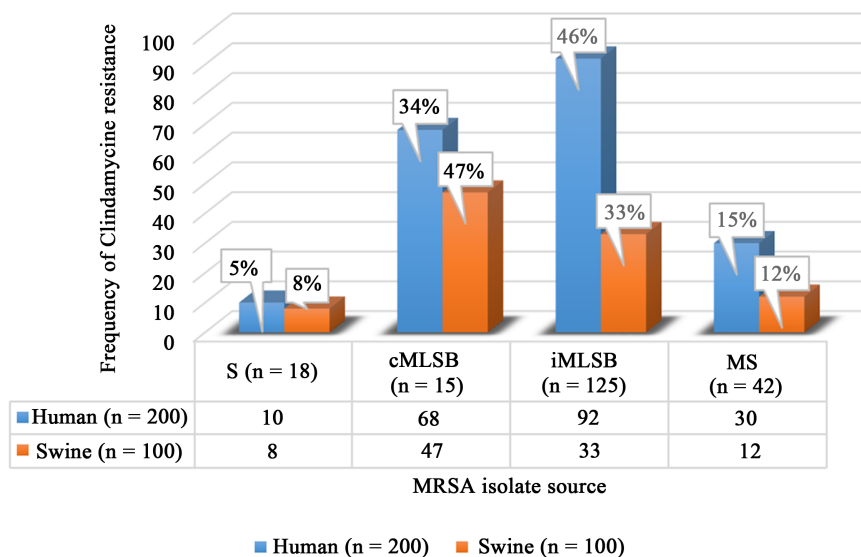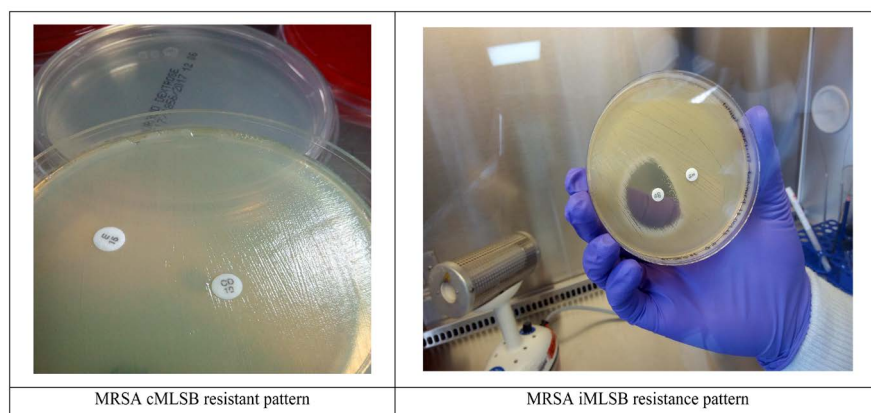

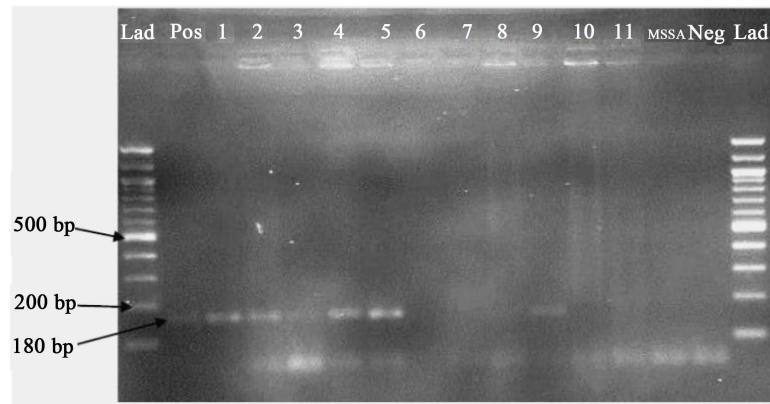**Figure 3.** D-test showing inducible clindamycin resistance.

Figure 4. Results of mecA gene PCR. where an Amplicon of approximately 180bp was expected. Lad is 100bp ladder, Lane Pos. contains the Positive control (MRSA ATCC 43300), and Lanes 1, 2, 3 MRSA isolated from Human 4, 5, and 9 MRSA isolated from swine. Lane 6, 7, 8 MSSA from Human and 10 and 11 were MSSA isolates from swine. MSSA contains a Methicillin Susceptible *S. aureus* strain ATCC 25923, whereas Neg. is the Negative amplification control.

changes in antimicrobial resistance patterns has led to a renewed interest in clindamycin as therapeutic choice. In fact, MRSA prevalence is now regarded as a pandemic scourge with varying prevalence across different countries and among the hospitals as well as livestock population [18] [19]. Indiscriminate use of antibiotics in both human and animal coupled with poor clinical practices may be contributing factors leading to the emergence of MRSA and other antimicrobial resistances(AMRs). Currently, Macrolide-Lincosamide-Streptogramin B (MLS$_B$) resistance is on the rise and catching public health interest [12]. The Macrolide–Lincosamide–Streptogramin B (MLS$_B$) resistance may be of the constitutive (cMLS$_B$) or the inducible (iMLS$_B$) type. The isolates with cMLS$_B$ are resistant to both erythromycin (ER) and clindamycin (CL) and they are readily detected by in vitro testing. This family of antibiotics (MLS$_B$) is increasingly used because of their excellent oral absorption and decent tissue penetration as well as their ability to accumulates in abscesses. This has made it the best choice in both veterinary and human medicine [19] [20] [21]. Clindamycin is an antimicrobial agent which belong to the Macrolide–Lincosamide–Streptogramin B (MLS$_B$) family. The wide spread use of the MLS$_B$ family of antimicrobials has led to the emergence of resistance. Our study showed prevalence of induced Macrolide-Lincosamide-Streptogramin B (iMLS$_B$) among MRSA isolated from both Human and swine 42%, which is in agreement with other reports elsewhere [21] [22] [23]. Higher rates of iMLS$_B$ have been reported in other studies conducted in Uganda [24] and Kenya [25]. Molecular studies have indicated that some SCCmec elements on MRSA may carry transposon Tn554 which contains the gene ermA mediating MLS resistance [23]. This could be a probable reason for higher rate of resistance reported in our study. MRSA isolates from human had higher iMLSB compared to those isolated from swine. This suggests an increasing usage of this class of antibiotics resulting selective pressure and consequently

multidrug resistance [26]. Probable widespread empirical use of erythromycin and clindamycin today and increasing consumption are the drivers of selective pressure [27]. We suggest reduction in macrolide usage to reverse such resistance pattern as it has been described elsewhere [28].

Clindamycin resistance can develop in the *Staphylococcal* bacteria with the inducible phenotype and spontaneous constitutive resistant mutants can also be selected from iMLSB isolates. This may happen both in vitro and in vivo during the CL treatment and this phenomenon is faster in the MRSA strains [6] [7]. We report a high prevalence of cMLSB [48%] among all the MRSA isolates with high percentage registered swine isolates [47%]. These findings are in agreement with several other studies by Almasri *et al.* (2016) and Ganesh *et al.* (2016) who reported that constitutive phenotype is predominant higher than inducible phenotype in MRSA isolates [29] [30]. However, different results have been reported in India, where Kumari *et al.* (2016) reported $cMLS_B$ (35.2%) and $iMLS_B$ (15.9%) showing higher constitutive resistance among MRSA [31]. In similar note, Das *et al.* (2016) showed that cMLSB was 36.8% compare to iMLSB of 31.8% in MRSA isolates [19]. Also, Mohammad, 2012 reported 32.5% of MRSA were cMLSB phenotypes and 10% were iMLSB phenotype. These variations could probably be due to differences in the circulating clones or due to the variations in infection prevention practices and trends of antibiotics prescriptions in the community and veterinary practice [32].

The current study also reveals 6% of MS phenotype (E-R, Cl-S) among MRSA isolates. In this case, clindamycin can be used as treatment option only for less number of MRSA which are erythromycin resistant. While treating Erythromycin MRSA infection with Clindamycin antibiotic, there is always minimum chance of clinical efficacy compared to vancomycin antibiotic therapy [33]. We therefore emphasize routine use of D-test in diagnostic laboratories to avoid clinical failure while using clindamycin as an alternative to anti-MRSA antibiotics like vancomycin and linezolid [34] [35].

5. Conclusion

The prevalence of inducible and constitutive clindamycin resistance among MRSA Isolates from both humans and swine is high. The D test is a simple and affordable technique that can be used in low resourced settings to define precisely MLSB, both inducible and constitutive resistance patterns in addition to MSB in *Staphylococcus aureus* in the routine clinical laboratories. This can be an important strategy for good antibiotic stewardship in under resourced settings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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