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ARTICLES

Antibiotics resistance patterns of Panton-Valentine leukocidin-positive methicillin-resistant staphylococci isolated from clinical samples in Abidjan, Côte d’Ivoire
Sylvie M. Kouamé-Sina, N’Golo D. Coulibaly, Fernique Konan, Yakoura K. Ouattara, Kan S. Kouassi, Solange Ngazoa-Kakou, Nathalie K. Guessennd and Adjéhi Dadié

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Full Length Research Paper

Antibiotics resistance patterns of Panton-Valentine leukocidin-positive methicillin-resistant staphylococci isolated from clinical samples in Abidjan, Côte d’Ivoire

Sylvie M. Kouamé-Sina¹*, N’Golo D. Coulibaly¹, Fernique Konan², Yakoura K. Ouattara³, Kan S. Kouassi¹, Solange Ngazoa-Kakou¹, Nathalie K. Guessennd² and Adjéhi Dadié³

¹Platform of Molecular Biology, Pasteur Institute, 01 BP 490 Abidjan 01, Côte d’Ivoire.
²National Reference Center for Antibiotics, Pasteur Institute, 01 BP 490 Abidjan 01, Côte d’Ivoire.
³Department of Food Science and Technology, Nangui Abrogoua University, Abidjan, Côte d’Ivoire.

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Methicillin-resistant staphylococci have emerged as significant pathogens which cause various infections and its multidrug resistance is a major concern. This study aimed to determine the prevalence of Panton-Valentine leukocidin (PVL) gene and antibiotic resistance patterns of staphylococci isolated from clinical infections in Abidjan. A total of 35 staphylococci strains was obtained from 35 clinical samples (pus, blood, pleural fluid, sputum, wound, and urine), then, characterized by polymerase chain reaction (PCR) to differentiate S. aureus from coagulase-negative staphylococci (CNS) and to detect the presence of PVL genes (LukS). The antimicrobial susceptibility was performed using disk diffusion method and the phenotype of resistance to macrolides-lincosamides-streptogramin B (MLSB) was detected. Out of 35 strains, 80% (28/35) were methicillin-resistant Staphylococcus aureus (MRSA) and 20% (7/35) were methicillin-resistant CNS (MR-CNS). S. aureus were isolated from 75% of outpatient samples and 84.2% of inpatient samples. However, CNS were isolated from 25% of outpatient samples and 15.8% of inpatient samples. LukS were detected in 68.6% of strains (20 MRSA and 4 MR-CNS) and both inpatients and outpatients. The highest resistance rates were observed for penicillin (100%), cefoxitin (100%), ciprofloxacin (66%), tobramycin (66%), tetracyclin (66%), sulphamethoxazole-trimethoprim (63%), erythromycin (60%), kanamycin (57%) and gentamicin (54%). In addition, S. aureus strains were subdivided into five antibiotics resistance phenotypes: 57.1% belonged to phenotype 1 (Methicillin-resistant and susceptible to Kanamycin-Tobramycin-Gentamicin) followed by 25% of phenotype 4 (Resistant to Methicillin-Kanamycin-Tobramycin-Gentamicin), 7.1% of phenotype 2 (MR with constitutive MLSb), 7.1% of phenotype 5 (MR and resistant to Kanamycin-Tobramycin-Gentamicin with inducible MLSB) and 3.6% of phenotype 3 (MR with inducible MLSb). CNS strains were grouped in three phenotypes (1, 4 and 5). 100% of LukS positive MRSA were multi-drug resistant, with 45% of strains resistant to 6 or more antibiotics. The high level of multi-drug resistance of clinical PVL positive staphylococci with inducible MLSb, suggest increasing the monitoring of these pathogens in Côte d’Ivoire.

Key words: Methicillin-resistant Staphylococcus aureus (MRSA), Panton-Valentine Leukocidin, inducible MLSb, multi-drug resistance.
INTRODUCTION

Staphylococcus aureus and coagulase-negative staphylococci (CNS) are Gram-positive opportunistic pathogens that cause various diseases, ranging from localized mild infections to invasive life-threatening diseases (Pedroso et al., 2018; Tong et al., 2015). However, CNS have emerged as significant pathogens causing nosocomial infections (Lenart-Boron et al., 2016; Nanoukon et al., 2017). S. aureus, especially methicillin-resistant S. aureus (MRSA) strains increase the occurrence of serious infections (Skov et al., 2012) which are among the most frequent bacteria in healthcare-associated infections. Skin and soft tissue infections due to S. aureus are most common, whereas pneumonia, osteomyelitis, endocarditis, and sepsis, although less usual, account for greater morbidity and mortality (Tong et al., 2015; Dong et al., 2013). S. aureus possesses an arsenal of virulence factors that contribute to evasion of host defenses. These virulence factors, including toxins, exoenzymes, and adhesins, which are secreted or linked to cell membrane and fight against the action of antibiotics. Antibiotic resistance in both hospital-acquired methicillin-resistant S. aureus and community-acquired MRSA strains has increased the difficulty to treat these infections (Messina et al., 2016; Shopsin et al., 2016). Most MRSA strains can also produce a leukotoxin as Panton-Valentine leukocidin (PVL), a bicomponent cytotoxin encoded by prophage that increases their virulence and can cause skin and soft tissue infection and necrotizing pneumonia (Shallcross et al., 2013; van der Meer et al., 2014; Yanagihara et al., 2009). PVL is a member of pore forming toxins that targets host leukocytes. Two open reading frames are responsible for coding PVL, that is, LukS-PV and lukF-PV (Abdulgader et al., 2015). The MRSA strains are widely distributed around the world with quick evolution of antimicrobial resistance and it is a high priority according to WHO to found new antibiotics (Willyard, 2017). WHO global report on surveillance of antimicrobial resistance lacked information from the majority of the countries in sub-Saharan African countries or lacked data on priority pathogens such as MRSA (WHO, 2014). However, a recent retrospective review performed on wound infection between 2004 and 2016, had shown the prevalence of MRSA in Benin (34.6%), Congo (31.9%), Togo (14.3%) and Madagascar (14.5%) (Lai et al., 2018). S. aureus colonizes about one third of healthy humans and is most often found in the nose (Kaspar et al., 2016). A study in Burkina Faso, showed that the rate of S.aureus nasal carriage was 32.9% with 29% in healthy volunteers and 37% in hospital patients in Bobo Dioulasso. In addition, the percentage of MRSA strains isolated from hospital patients was very low (2.3%) with high prevalence of strains harboring PVL-encoding genes (45%) (Ouedraogo et al., 2016). Nosocomial infections are a major threat in most of the hospitals and as high as 19% in the developing countries, where number of direct contacts between the hands of Health Care Workers and the patient occurs, which mandates the strict adherence to infection control practices and standards (Maheshwari et al., 2014). In Ghana, Saba et al. (2017) isolated MRSA (17%) from handles and other points of contact in a public hospital environment. In the Burn Center of Korle Bu Teaching Hospital in Ghana, 50% of patients were infected with S. aureus including MRSA (Amissah et al., 2017). In Côte d’Ivoire, previous studies have reported the occurrence of S. aureus strains harboring PVL gene. During 2004 and 2005, two outbreaks caused by the same clone of doxycycline resistant, PVL-positive methicillin-susceptible S. aureus (MSSA) were characterized in two French military companies (Lesens et al., 2007). In the Cocody University Teaching Hospital, Kacou et al. (2011) have reported high rate of PVL positive S. aureus strains (67.7%) from clinical samples in hospitalized patients. However, Zinzendorf et al. (2012) showed a high rate (77.4%) of clinical S. aureus strains from patients expressed PVL gene in Abidjan Military Hospital. The increasing prevalence of staphylococcal virulence factor, PVL, and MRSA strains isolated from patients is a growing problem. Constant surveillance and adequate infection control measures for S. aureus may reduce their roles in the incidence of nosocomial diseases and other infections. Update data on S. aureus resistances are particularly important to know the type of antibiotic resistance for establishing adequate therapeutic approaches. The objective of this study was to determine the prevalence of PVL gene and antibiotic resistance patterns of Staphylococcus species isolated from patients in Abidjan, Côte d’Ivoire.

MATERIALS AND METHODS

Sample collection

Pasteur Institute of Côte d’Ivoire is a laboratory where patients from different hospitals in Côte d’Ivoire are referred for medical tests. The clinical samples (n=35) were collected from 16 outpatients referred to Pasteur Institute and 19 inpatients of Cocody University Teaching Hospital. Samples of inpatients came from surgery (n=5), maternity/neonatal (n=2), neurology (n=1), obstetrics/gynecology (n=2), pediatrics (n=3), pulmonology (n=2), emergency (n=3) and urology (n=1) services of Cocody University Teaching Hospital. All samples were collected from Pasteur Institute between February and September, 2017 (Table 1).

*Corresponding author. E-mail: kouameslyiemireille@yahoo.fr. Tel: 00225 58030673.

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Table 1. The source of clinical samples.

<table>
<thead>
<tr>
<th>Type of clinical samples</th>
<th>Outpatients clinical samples</th>
<th>Inpatients clinical samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus (n=19)</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Blood (n=11)</td>
<td>4</td>
<td>7</td>
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<tr>
<td>Pleural fluid (n=1)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sputum (n=2)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Wound (n=1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Urine (n=1)</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Total (n=1)</td>
<td>16</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 2. List of primers used in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Sequence of primers (5’-3’)</th>
<th>Size of amplified product (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>16S rRNA-F</td>
<td>GCAAGCGTTATCCGGGATTT</td>
<td>597</td>
<td>Al-Talib et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>16S rRNA-R</td>
<td>CTTAATGATGGCAACTAAGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FemA</td>
<td>FemA-F</td>
<td>CGATCCATATTACCATATCA</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FemA-R</td>
<td>ATCACGCTCTTCGTATTGTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LukS</td>
<td>LukS-F</td>
<td>CAGGAGGTAATGGTGTCATT</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LukS-R</td>
<td>ATGTCACAGACTTTACCTAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Staphylococci isolation

Bacteria were isolated and identified in the Department of Bacteriology at Pasteur Institute of Côte d'Ivoire. The phenotypic identification of staphylococci species was carried out by standard microbiology methods including Gram staining, catalase activity, mannitol fermentation and the ability to coagulate rabbit plasma (Kateete et al., 2010; Amini et al., 2012). Molecular characterisation of strains was carried out in the Molecular Biology Laboratory at Pasteur Institute of Côte d'Ivoire.

Molecular characterization of strains

DNA extraction

Bacterial DNA was extracted by boiling method (Kacou et al., 2011; Oliveira et al., 2014). An overnight culture of staphylococci strains in Brain-Heart Infusion (1.5 mL) was centrifuged (12000 rpm, 5 min) and the supernatant was discarded. The pellet was dissolved in 400 µL deionized water and the suspension was heated at 100°C for 10 min and then immediately frozen at -20°C for 10 min. Finally, after centrifugation for 10 min at 12000 rpm, the supernatant containing bacterial DNA was stored at -20°C for further analysis.

Detection of staphylococcal genes

All strains were screened for the presence of three Staphylococcus genes by modified method of PCR amplification previously described by Kacou et al. (2011). DNA extracts were used as template in the PCR. The multiplex PCR was performed to identify the Staphylococcus genus (16S rRNA gene), to differentiate S. aureus from CNS (FemA gene), and to detect PVL toxin (LukS gene) simultaneously. The PCR was performed in a final volume reaction of 25 µL containing 9.75 µL nuclease-free water (Ambion), 5 µL PCR buffer (5X), 1.5 µL magnesium chloride (MgCl₂, 25 mM) (Promega Corporation, Madison, USA), 0.5 µL Deoxynucleotides Triphosphates (dNTPs; 10 mM), 0.5 µL of each primer (20 mM) (Table 2), 0.25 µL Go Tag®G2 Flexi DNA polymerase 5 U/µL (Promega Corporation, Madison, USA) and 5 µL of template DNA. The PCR method was performed according to the following program: initial denaturation (94°C, 5 min), 35 cycles each composed of initial denaturation (94°C, 30 s), primer annealing (60°C, 1 min) and extension (72°C, 1 min) and a final extension (72°C, 5 min). Primers used in this PCR were previously reported by Al-Talib et al. (2009) (Table 2). A previously known (Kacou et al., 2011) LukS gene positive S. aureus strain was used as a control strain. PCR amplification products were revealed on a gel Doc EZ® imager (Bio-Rad) after electrophoresis in 2% agarose gel containing Syber safe (Invitrogen).

Antimicrobial susceptibility

Susceptibilities were determined using the disk diffusion method in accordance with the performance standards for antimicrobial susceptibility testing, recommended by the Committee of Antiibiogram of French Society of Microbiology (CA-SFM/EUCAST, 2016). The susceptibility testing was carried out by culturing strains on Mueller-Hinton agar (Bio-rad, Marne-la- coquette, France). Antibiotics used for susceptibility testing included penicillin 6 µg, cefoxitin 30 µg, ciprofloxacin 5 µg, norfloxacin 5 µg, gentamicin 10 µg, kanamycin 30 µg, tobramycin 10 µg, netilmicin 10 µg,
Table 3. *Staphylococci* strains isolated in clinical samples.

<table>
<thead>
<tr>
<th>Type of clinical samples</th>
<th>Number of strains from outpatients</th>
<th>Number of strains from inpatients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>CNS</td>
</tr>
<tr>
<td>Pus (n=19)</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Blood (n=11)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Pleural fluid (n=1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sputum (n=2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wound (n=1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Urine (n=1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total (n=35)</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

CNS: Coagulase-negative staphylococci.

eromycin 15 µg, clindamycin 2 µg, tetracyclin 30 µg, minocyclin 30 µg, tigecyclin 15 µg, chloramphenicol 30 µg, fusidic acid 10 µg, sulphamethoxazole-trimetoprim 25 µg, rifampicin 30 µg, vancomycin 30 µg, and teicoplanin 30 µg. Susceptibility to methicillin was screened with the cefoxitin disk diffusion result. All antibiotics were obtained from Bio-rad. It should be indicated that the reference strain *S. aureus* ATCC 29213 were used as positive-control and provided by the National Reference Center for Antibiotics, Pasteur Institute of Côte d’Ivoire. The macrolide-lincosamide-streptogramin B (MLS$_B$) group of antibiotics can be used to treat less severe skin and soft tissue infections (Liu et al., 2011). Strains resistant to both erythromycin and clindamycin in routine antibiotic susceptibility testing were considered constitutive MLS$_B$ phenotypes. Inducible MLS$_B$ phenotypes were identified using a double-disk diffusion test (D-test). Erythromycin 15 µg and clindamycin 2 µg disks were placed on a Mueller-Hinton agar plate containing a lawn culture of the test isolate at a distance of 15 mm edge to edge. After incubation at 35°C for 16 to 18 h, strains that showed no flattening of the inhibition zone around the clindamycin disk were reported as susceptible to clindamycin (negative D test); strains that showed flattening of the inhibition zone around the clindamycin disk adjacent to erythromycin disk (D zone) indicated inducible clindamycin resistance (positive D test). Strains with a D-shaped zone of inhibition were considered iMLS$_B$ Phenotype (resistant to erythromycin and susceptible to clindamycin) (Kumari et al., 2016; Pereira et al., 2016). Multidrug resistance (MDR) was defined as resistance to at least three distinct antimicrobial classes or being MRSA (Magiorakos et al., 2011).

RESULTS

Clinical specimens and staphylococci strains

In this study, a total of 35 staphylococci were isolated from 35 patient samples. Patients were 7 days to 62 years old and 17 patients were female and 18 were male. *S. aureus* strains isolated from patients samples were identified by Gram-positive cocci, catalase positivity, mannitol fermentation, and coagulase production. However, strains that were Gram-positive cocci, catalase positive, and coagulase negative were considered as CNS. This phenotypic identification was confirmed by PCR results. Among 35 bacteria tested, 28 (80%) were *S. aureus* (16S rRNA positive and femA positive) and 7 (20%) were CNS (16S rRNA positive and femA negative).

*S. aureus* strains were recovered from 75% of outpatients samples (12/16) and 84.2% of inpatients samples (16/19). However, CNS were isolated from 25% of outpatients samples (4/16) and 15.8% of inpatients samples (3/19). *S. aureus* was reported in all type of clinical specimens but CNS were isolated only in blood (54.5%; 6/11) and pus (5.3%; 1/19) (Table 3). CNS strains were isolated from hospitalized patients provided from maternity/neonatal and pediatrics.

Detection of PVL gene

PVL-encoding gene (*LukS*) was detected in 24 (68.6%) of the 35 strains. The PVL gene was present in all type of clinical samples (blood, pus, pleural fluid, sputum, wound, and urine). Among all *S. aureus* strains (28), 71.4% carried the *LukS* gene (20/28) (Figure 1). In addition, the *LukS* gene was present in 57.1% (4/7) of CNS. *LukS* positive CNS (*LukS+CNS*) strains were only detected from blood samples of inpatients and outpatients. In contrast, *LukS* positive *S. aureus* strains were detected from blood (4/11), pus (12/19), pleural fluid (1/1), sputum (1/2), wound (1/1) and urine (1/1) samples.

Antibiotic susceptibility of bacteria isolated

Antibiotic susceptibility tests were performed on the 35 *Staphylococcus* spp. isolated from clinical samples. The highest resistance rates were observed for penicillin (100%) and cefoxitin (100%). Resistance rates of 50-70% were recorded for the antibiotics ciprofloxacin (66%), tobramycin (66%), tetracyclin (66%), sulphamethoxazole-trimetoprim (63%), erythromycin (60%), kanamycin (57%) and gentamicin (54%). The lower resistance rate <50% were observed for rifampicin (26%), netilmicin (26%), fusidic acid (23%), norfloxacin (14%), minocyclin (11%), clindamycin (11%) and chloramphenicol (11%). All the strains were susceptible to tigecyclin tgc (100%), vancomycin (100%) and teicoplanin (100%) (Table 4). The strains resistant to cefoxitin were classified as those
strains methicillin-resistant staphylococci. In this study, 100% (35/35) of staphylococci strains were methicillin-resistant of which 80% were MRSA and 20% were methicillin-resistant CNS (Table 4).

**Antibiotic phenotypes of methicillin-resistant strains**

In this study, staphylococci strains were subdivided into five phenotypes according to the antibiotics resistance...
<table>
<thead>
<tr>
<th>Antibiotic profiles</th>
<th>Number (% of staphylococci strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em> (n=28; %)</td>
</tr>
<tr>
<td>MR + KTG S</td>
<td>16 (57.1)</td>
</tr>
<tr>
<td>MR + cMLS_B</td>
<td>2 (7.1)</td>
</tr>
<tr>
<td>MR + IMLS_B</td>
<td>1 (3.6)</td>
</tr>
<tr>
<td>MR + KTG R</td>
<td>7 (25)</td>
</tr>
<tr>
<td>MR + KTG R + iMLS_B</td>
<td>2 (7.1)</td>
</tr>
</tbody>
</table>

MR: Methicillin-resistant; KTG S: Kanamycin-Tobramycin-Gentamicin Susceptible; KTG-R: Kanamycin-Tobramycin-Gentamicin Resistant; cMLS_B: Constitutive MLSB; IMLS_B: inducible MLSB.

Antibiotics resistance pattern of positive PVL strains

Among the 24 LukS positive strains, 20 were MRSA with four different phenotypes of antibiotic resistance. The majority of LukS positive strains (70%) were phenotype 1. Two (2/20) of LukS+S. aureus strains were phenotype 2, also two strains (2/20) were phenotype 4 and two (2/20) other were phenotype 5 (Table 6). Of the four LukS positive CNS (LukS+CNS), phenotype 4 was observed in two (50%) strains and phenotype 5 in one (25%) strain and also one other (25%) isolate was phenotype 1. The highest prevalence of resistance of LukS+MRSA strains was for penicillin (100%), cefoxitin (100%) followed by tobramycin (60%), ciprofloxacin (57%), erythromycin (47%), tetracyclin (43%), sulphamethoxazole-trimethoprim (40%), gentamicin (36%) and kanamycin (37%). Lower percentage of resistance was observed for rifampicin (21%), norfloxacin (15%), chloramphénicol (10%), fusidic acid (11%), minocyclin (7%) and netilmicin (6%). All the LukS+MRSA strains were susceptible to tigecyclin (100%), fosfomycin (100%), vancomycin (100%) and teicoplanin (100%).

In addition, multi-drug resistant strains were classified as those resistant to three or more antibiotics classes. All LukS+MRSA were multi-drug resistant (100%; 20/20), of which 9 (45%) strains were resistant to 6 or more antibiotics, 2 (10%) strains were resistant to 5 antibiotics, 7 (35%) were resistant to 4 antibiotics and 2 (10%) were resistant to 3 antibiotics.

DISCUSSION

The present study reports the molecular characteristics and antibiotics resistance of staphylococci isolated from patients specimens in Abidjan, Côte d’Ivoire. A high prevalence of *S. aureus* was found in clinical samples (pus, blood, pleural fluid, sputum, wound, and urine) compared to the prevalence of CNS was lower. The strains of CNS was isolated in only 7 (20%) clinical samples. These prevalences were different than those reported in Nigeria where *S. aureus* and CNS were isolated at 56 and 43.9%, respectively in clinical infections (wounds, skin and soft tissue infections, osteomyelitis, burns, genitourinary tract infection, septicaemia, urinary tract infection, otitis media, and bronchitis) (Shittu et al., 2012). With regard to the virulence factors of these strains, PVL (a leukotoxin associated with human clinical diseases) encoding gene (*LukS*) was sought. This research showed that, the high rate of *LukS* gene prevalence in 68.6% of staphylococci strains was obtained from clinical infections of inpatients and outpatients. The *LukS* gene was detected at a high rate in *S. aureus* strains (71.4%) than in CNS strains (57%). The *LukS* positive CNS strains were detected only from blood. In the hospitalised patients, *LukS+CNS* strains were found in patients of neonatalar and pediatrics unit and maybe due to nosocomial infections. Indeed, CNS have emerged as one of the main microorganisms causing nosocomial infections and clinically (Nanoukon et al., 2017). The prevalence of PVL gene in *S. aureus* (71.4%) in the present study was high compared with the rate (67.8%) reported in a previous study conducted in Côte d’Ivoire in 2009 in clinical infections (Kacou et al., 2011). The prevalence of *LukS* carriage in *S. aureus* in clinical sample in Abidjan increased from 67.8 to 71.4% between 2009 and 2017. The high frequency of PVL-positive *S. aureus* in patients provide important insights...
Table 6. Antibiotic resistance phenotype of PVL gene positive strains.

<table>
<thead>
<tr>
<th>Antibiotic profiles</th>
<th>Number (%) of LukS positive strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LukS+ S. aureus (n=20; %)</td>
</tr>
<tr>
<td>MR + KTG S</td>
<td>14 (70)</td>
</tr>
<tr>
<td>MR + cMLS_S</td>
<td>2 (10)</td>
</tr>
<tr>
<td>MR + iMLS_S</td>
<td>0 (0)</td>
</tr>
<tr>
<td>MR + KTG R</td>
<td>0 (0)</td>
</tr>
<tr>
<td>MR + KTG R + iMLS_S</td>
<td>2 (10)</td>
</tr>
</tbody>
</table>

MR: Methicillin-resistant; KTG S: Kanamycin-Tobramycin-Gentamicin Susceptible; KTG-R: Kanamycin-Tobramycin-Gentamicin Resistant; cMLS_S: Constitutive MLS_S; iMLS_S: Inducible MLS_S; LukS+ CNS: LukS Positive CNS; LukS+ S. aureus: LukS Positive S. aureus.

to evaluate the risk of infection and dissemination of this bacteria in Abidjan, Côte d’Ivoire and in West Africa. In other sub-Saharan Africa countries, the frequency of PVL-positive S. aureus in clinical samples is high. In Ghana, PVL-encoding genes were detected in 75% (42/56) of S. aureus blood culture (Dekker et al., 2016) and in 27% (17/62) of carriage of PVL-positive S. aureus in burn patients (Amissah et al., 2017). This gene carriage rate was higher (90.7%; 68/75) in community-acquired-S. aureus isolated in Mozambique (van der Meeren et al., 2014), in the Democratic Republic of the Congo (49.1%) (Vandendriessche et al., 2017) and in Nigeria (33.3%; 17/51) in clinical infections (Shittu et al., 2012). The main findings of this study are a high prevalence of MRSA and PVL-positive gene strains. PVL is mostly associated with community-associated methicillin resistance in S. aureus infections. According the antibiotics resistance profile, 100% of staphylococci strains were methicillin-resistant, of which 80% were methicillin-resistant S. aureus (MRSA) and 20% were methicillin-resistant CNS. MRSA prevalence rate in the present study is very higher than MRSA prevalence rate (11.8%) obtained by Kacou et al. (2011) in 2009 in Abidjan. In addition, PVL gene (LukS) was detected only among methicillin-sensitive S. aureus (MSSA) strains. In contrast, in this present study, the PVL gene was commonly detected among MRSA as well as in methicillin-resistant CNS. The increase of prevalence of PVL-positive MRSA in Abidjan could present a significant challenge in disease management and infection control. The rate of PVL positive MRSA strains in Abidjan is higher than those reported from Ghana (63.8%) (Kpeli et al., 2016), Ouganda in pastoral communities (69.4%) (Asiimwe et al., 2017), Zambie (9.4%) (Samutela et al., 2017) and Nigeria (6.6%) (Shittu et al., 2012). However, the rate of PVL+MRSA was high than the rate (56.8%) reported by Bhatta et al. (2016) in MRSA isolated from clinical specimens in Nepal. The staphylococci isolated from patients in this study exhibit resistance to many antibiotics (Table 4). These results are in agreement with those reported by Chen et al. (2017) in China which the resistance rates of S. aureus to penicillin and cefoxitin were all 100%. However, all strains were susceptible (100%) to tigecyclin, vancomycin and teicoplanin. The MRSA strains collected from 13 different hospitals in Kuwait, were susceptible to vancomycin (100%), teicoplanin (100%) and linezolid (100%). Tigecyclin was not tested by the authors (Boswih et al., 2018). The occurrence of MRSA is on the rise since the previous study (Kacou et al., 2011) in Abidjan. The threat posed by MRSA was vividly demonstrated in this study, as 100% of LukS+MRSA were multi-drug resistant and 45% of strains were resistant to 6 or more antibiotics tested. This might reflect the frequent and repeated administration of antibiotics, thus selecting for resistance and resulting in high frequencies of MDR. The MDR in staphylococci strains could lead to failure in treatment therapy, prolonged illnesses, increased expenses for health care, and in serious cases, risk of death (Tanwar et al., 2014). The prevalence of antimicrobial resistance in Abidjan clinical staphylococci strains was higher than those observed in other African countries. Indeed, the prevalence of antimicrobial resistance was below 5% for all antibiotics agents tested except for penicillin (97%), tetracycline (42%) and erythromycin (6%) in MRSA strains in Ghana (Egyir et al., 2014). Comparatively, the prevalence of MDR (9%) was lower than those reported in the present study, Bhatta et al. (2016) have reported overall 73% of MRSA from clinical specimens were multidrug resistant with 50% were PVL positive which indicates a lower prevalence than the present findings. The high antimicrobial resistance in Abidjan is due to the overconsumption of antibiotics in the populations. Indeed, there is a huge prescription of antibiotics and because of poverty people turn to self-medication by sourcing from street drug sellers. It is important to note that there is a large informal market in Abidjan for medicines and antibiotics that are visible to everyone. Indeed, the prevalence of antimicrobial resistance was below 5% for all antibiotics agents tested except for penicillin (97%), tetracycline (42%) and erythromycin (6%) in MRSA strains in Ghana (Egyir et al., 2014). Comparatively, the prevalence of MDR (9%) was lower than those reported in the present study, Bhatta et al. (2016)
have reported that overall 73% of MRSA from clinical specimens were multidrug resistant with 50% which were PVL positive indicating a lower prevalence than the present findings. Otherwise, according to resistance of antibiotics, clinical strains were grouped into 5 antibiotic phenotypes (Table 5). The frequency of cMLSβ and iMLSβ of the present study was lower than those found in previous studies. Two other previous Brazil studies (Bottega et al., 2014; Pereira et al., 2016) have documented the rate of cMLSβ and iMLSβ in clinical MRSA strains ranging from 14.3 to 37.9% and 2.1 to 4.9%, respectively; while the KTG-R phenotype was not detected. In India, the MLSβ phenotypes of MRSA in clinical samples were higher than those reported in this study. The iMLSβ phenotype rate ranged from 19.1 to 35.2% and the cMLSβ phenotype rate ranged from 11.4 to 31.9% in MRSA strains (Bhattacharya et al., 2015; Kumari et al., 2016). The antibiotic profile of CNS was not consistent with the results of Abdullahi et al. (2016) where 58.2% of 110 strains were methicillin resistant CNS to which 54.6% were cMLSβ and 6.25% were iMLSβ phenotype. It was important to know the type of MLSβ resistance for establishing adequate therapy. The vancomycin is used for the treatment of serious infections and MLSβ group are usually used to treat less severe skin and soft tissue infections (Liu et al., 2011). Reporting the susceptibility to clindamycin without verifying the presence of inducible resistance, may lead to inadequate therapy with this drug. In contrast, a negative result for inducible resistance to clindamycin, confirms the susceptibility of this antimicrobial, providing a very good therapeutic option (Pereira et al., 2016). Clindamycin is the preferred agent for the treatment of MRSA due to its excellent pharmacokinetic properties, such as optimal tissue penetration and accumulation in abscesses. However, the indiscriminate use of MLSβ antibiotics has led to an increase in the number of Staphylococcus spp. strains that are resistant to these drugs (Bottega et al., 2014).

Conclusion

The present analysis of S. aureus strains isolated from clinical specimens in Abidjan, showed a high level of multidrug resistance staphylococci strains both among inpatients than outpatients. These strains harboring a high rate of PVL gene with inducible resistance to MLSβ antibiotics. Therefore, frequent monitoring of this pathogen, its antibiotic susceptibility and determining their virulence factors is of great importance in control and treatment of infections. This study provides an important data to increase the country-wide monitoring of methicillin resistant staphylococci.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to staff of Department of Bacteriology, Plateform of Molecular Biology and National Reference Center for Antibiotics of Pasteur Institute of Côte d’Ivoire for their cooperation while carrying out this work.

REFERENCES


Phenotypic and molecular characterization of inducible clindamycin resistance among Staphylococcal strains isolated from cancer patients with febrile neutropenia

Rasha A. Alm El-Din1, Hanan El-Bassat2, Mohamed El-Bedewy3 and Mohamed A. Alm El-Din4

1Department Medical Microbiology and Immunology, Faculty of Medicine, Tanta University, Egypt.
2Department of Tropical Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt.
3Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt.
4Department of Clinical Oncology, Faculty of Medicine, Tanta University, Tanta, Egypt.

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This work aimed at the phenotypic and molecular characterization of inducible clindamycin resistance among strains of Staphylococcus aureus isolated from cancer patients with febrile neutropenia. Out of 231 clinical specimens Staphylococci were isolated from 179 (77.48%) cases. Isolates were identified by conventional microbiological methods. Antimicrobial sensitivity to all isolates was done using disc diffusion methods. For strains that were erythromycin resistant, D-test was performed to screen the presence of inducible clindamycin resistance. Multiplex polymerase chain reaction (multiplex-PCR) was done for strains with constitutive or inducible resistance to detect the distribution of erm (e), and erm (C) genes. Out of 231 clinical specimens, staphylococci were isolated from 179 (77.48%). Staphylococcal isolates were tested for susceptibility to erythromycin; 100 (55.8%) of them were erythromycin resistant. Of these 100, erythromycin resistant isolates (8, 4.46%) were resistant to both erythromycin and clindamycin indicating constitutive MLSB Phenotype; 92 isolates were erythromycin resistant, and clindamycin sensitive. Out of these, 45 (25.1%) isolates showed positive D test indicating inducible MLSB phenotype while 47 (26.2%) gave negative D test indicating MS phenotype. Molecular study revealed that 8 strains (100%) of staphylococci with constitutive MLSB phenotype and 23 strains (51.1%) of staphylococci with inducible MLSB phenotype had both erm(e) and erm(c) gene, erm (e) gene was present in 33.3% and erm (c) gene present in 15.6% of staphylococcus isolates with inducible MLSB phenotype. Inducible resistance and MS phenotype were found to be higher in MRSA as compared to MSSA (27.6, 24.3 and 1.6 and 4% respectively). Rapid dissemination of inducible clindamycin-resistant S. aureus isolates is worrisome and calls for judicious use of antibiotics. Therefore, the D-test should be added as a routine procedure on each staphylococcal isolates to detect inducible clindamycin resistance to avoid failure of antibiotic therapy.

Key words: Clindamycin, Staphylococcus aureus, antimicrobial susceptibility.

INTRODUCTION

Febrile neutropenia is defined as fever with other signs of infection, in a patient with neutropenia, which is considered with abnormal low concentration of neutrophils granulocytes (<500 cells/mm³), the most
abundant circulating white blood cells that is considered the first line of the organism defence against infections (Viscoli et al., 2005). Percentage of patients with malignancy experience a decrease in the cells and other elements of the immune systems that make them more liable to different types of infections (Lustberg, 2012). Neutropenia could be considered as an oncology emergency and can lead to serious adverse consequences such as serious infection complications and death (Villafuerte et al., 2014). Bacteria, including Gram-positive and Gram-negative species, viruses, and fungi; all of these may be a causative agent of febrile neutropenia. The incidence and epidemiology of febrile neutropenia depends on different factors including type of cancer, the age, and sex of the patient, the type and cycle of treatment (Kristjanson, 2015).

*Staphylococcus aureus* (S. aureus) is considered as one of the most common organisms causing infections in patients with malignancy with increasing the risk of resistance to wide range of antibacterial drugs. Macrolide-Lincosamide-Streptogramin B (MLS <sub>B</sub>) is a group of antibiotics that are nowadays used in treatment of S. aureus infections. Clindamycin is the most preferred antimicrobial drug in this group due to its proper pharmacokinetic properties that led to increase development of clindamycin resistance among strains of *S. aureus* (Yilmaz et al., 2007).

Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible (Deotale et al., 2010). It is difficult to detect strains of *S. aureus* that have inducible clindamycin resistance by routine laboratory methods as they appears as erythromycin-resistant and clindamycin sensitive in routine laboratory in vitro disc diffusion tests when the two discs of erythromycin and clindamycin not placed adjacent to each other (Lim et al., 2006). When these cases are treated with clindamycin; the target will be constitutive erm genes that lead to failure of treatment (Drinkovic et al., 2001). Strains of *S. aureus* that have msrA genes (efflux genes) have with another mechanism of resistance that presented clinically as erythromycin-resistant and clindamycin-sensitive both in vivo and in vitro, with no resistance to clindamycin resistance during therapy (Laclercq, 2002).

The aim of this study was to detect the incidence of *staphylococcus aureus* causing infection among cancer patients with febrile neutropenia with phenotypic and molecular characterization of inducible and constitutive clindamycin resistance among these strains.

**MATERIALS AND METHODS**

A prospective study was carried out in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University over a period of 6 months from September 2017 to March 2018 on 273 cancer patients with febrile neutropenia admitted to Oncology Department in Tanta University Hospital with suspected clinical sepsis after the approval of ethical committee in Tanta Faculty of Medicine, and a written consent from the participated patients. Clinical sepsis was defined as per the criteria established by American College of Physicians and Society of Critical Care Medicine (ACOM, SCCM) which included temperature >38°C, heart rate >90/min and respiratory rate >20/min. Neutropenia was defined as an absolute neutrophilic count (ANC) of 500 mm<sup>3</sup> or less or a count that is expected to fall to that level in the next 1-2 days (Steven and John, 2008).

Out of 273 samples 100 staphylococci strains could be isolated that were eligible for the study.

Different patient samples (pus, throat swabs, blood, urine, sputum) were collected from the patients under complete asepsis and transferred immediately to microbiology laboratory. Samples were cultured on blood agar and mannitol salt agar. *Staphylococci* were identified using conventional microbiological methods and biochemical reactions; coagulase test and catalase test according to the standards of Clinical and Laboratory Standards Institute (2007).

**Antibiotic susceptibility testing**

Antibiotic susceptibility testing was performed using the disk diffusion method. The antibiotics chosen were Erythromycin, Clindamycin, vancomycin, gentamicin, oxacillin, cefotaxime, ciprofloxacin, cefepime and meropenem. Interpretation of the results according to the standards of Clinical and Laboratory Standards Institute (2007).

**Phenotypic identification of clindamycin resistance (D-test)**

All erythromycin-resistant isolates were further examined by double-disc test with erythromycin (15 μg) and clindamycin (2 μg) discs and the results were interpreted according to Clinical and Laboratory Standards Institute (2007) guidelines, to determine the resistance phenotype. According to the results of D-test; 3 phenotypes could be identified MS<sub>β</sub> phenotype, showed circular zone around clindamycin disc, MLS<sub>β</sub> (inducible resistance) phenotype, demonstrated a flat zone around clindamycin disc, and cMLS phenotype were resistant to both discs of erythromycin and clindamycin (constitutive resistance).

**Multiplex PCR for Genotypic identification of staphylococcal strains with clindamycin resistance**

**DNA extraction**

QiAamp DNA Mini Kit (Qiagen) was used for isolation of genomic presence of erythromycin resistance methylase erm genes *erm* (e), and *erm* (C) PCR using the primer pairs as described by Lim et al. (2006). Multiplex PCR for *erm* (a) and *erm* (c) was performed in final volume of 20 μl by DFS Master Mix Kit (Cinagen) including Taq polymerase enzyme, MgCl<sub>2</sub>, dNTP, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, TrisHCl, Tween – 20. Reaction mixtures consisted of 12.5 μl Master Mix, 1 μl MgCl<sub>2</sub>,

*Corresponding author. E-mail: almrasha@yahoo.com.

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0.25 μl of each primer, 2 μl distilled water and 1 μl DNA template. Cycling conditions were primary denaturation at 94°C for 4 min, denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 1 min, then 30 cycle, followed by a final extension at 72°C for 5 min. Primers used for ermA (a) gene were: 5’ TCA GGA AAA GGA CAT TTT ACC3; 3’ATA TAGTGG TGG TAC TTT TTT GAG C5’detected at 118 bp, erm(e) gene 5’TGG TGG TAC TTT TTT TTT GAG C5’detected at 495bp, (Matthew et al., 2006).

RESULTS

Table 1 shows the demographic and clinical characteristics studied cases where among 179 cases from which S. aureus were isolated, 95 cases were male patients and 84 were female patients, with their age from 18 to 72 (33±24.66), samples were collected from different sites according to the site of infection; pus 33 (18.4%), urine 33 (18.4%), blood 31 (17.3%), sputum 47 (26.2), throat swab 35 (19.4%). Colorectal cancer represents 3.3%, lymphoma 11%, Breast 10.6%, lung 19.5%, Hepatocellular carcinoma (HCC) 11.7%, Prostate 10.6% and Metastasis of unknown origin (MUO) 10.6%.

Table 2 shows pattern of clindamycin resistance among studied cases where among 179 S. aureus that were eligible for the study, 79 (44.1%) strains were erythromycin sensitive, and 100 (55.8%) of them were erythromycin resistant. Of these 100 erythromycin resistant strains, 8 (4.46%) isolates were resistant to both erythromycin and clindamycin indicating constitutive MLSB phenotype; 92 isolates were erythromycin resistant, and clindamycin sensitive. Out of these, 45 (25.1%) isolates showed positive D test indicating inducible clindamycin resistance (MLSβ phenotype) while 47(26.2%) gave negative D test indicating MS phenotype.

Table 3 shows relation between pattern of clindamycin resistance and type of staphylococci as regard sensitivity to methicillin where constitutive MLSB phenotype was found in higher percentage in MRSA [2 strains (25%)] of MSSA and 6 strains (70%) of MRSA; inducible MLSB phenotype found also in higher percentage in MRSA (10 strains (22.2%) of MSSA and 35 strains of MRSA (77.8%). As regard MS phenotype was equally distributed in MSSA and MRSA [22 strains (46.8%) and 25 strains (53.2)] respectively.

Table 4 shows the distribution of erm(e) and erm(c) genes among different phenotypes of clindamycin resistance where it was found that 100% of the strains with constitutive resistance phenotype contain both erm(e) and erm(c) gene, and as regard strains with inducible resistance phenotype, 15 strains (33%) contain erm(e) gene, 7 strains (15.8%) contain erm(c) gene, 23 strains (51%) contain both erm(e) and erm(c) genes.

Table 5 shows the antimicrobial sensitivity and resistance pattern of the S. aureus isolates with constitutive resistance phenotype where highest sensitivity was for vancomycin (98.7%), oxacillin (97.4%), cephalin and ciprofloxacin (83.5%) each, and all of the erythromycin sensitive strains were clindamycin sensitive.

Table 6 and 7 shows the antimicrobial sensitivity and resistance pattern of staphylococcal strains with inducible resistance phenotype where 75% of the strains were resistant to meropenem, gentamycin, and ciprofloxacin, 62% of the strains were resistant to cefotaxim, 50% were resistant to cefepime, and 100% of the strains were vancomycin sensitive. Agarose gel electrophoresis showing positive amplification of 118 and 495 base fragments is shown in Figure 1.

DISCUSSION

Cancer patients receiving cytotoxic antineoplastic therapy are at risk for invasive infection due to colonizing bacteria or fungi that translocate across intestinal mucosal surfaces. Since the magnitude of the neutrophil-mediated component of the inflammatory response may be muted in neutropenic patients. The first-line empirical treatment should cover the prevalent microorganism of the institute.

This study performed on 231 cases of febrile neutropenia from which Staphylococcus aureus could be

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>84</td>
</tr>
<tr>
<td>Sample</td>
<td>Pus</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Sputum</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Throat swab</td>
<td>35</td>
</tr>
<tr>
<td>Type of cancer</td>
<td>Colorectal</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Lymphoma</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Breast</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>HCC</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Prostate</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>MUO</td>
<td>19</td>
</tr>
</tbody>
</table>

Patients (n = 179).

<table>
<thead>
<tr>
<th>Pattern of clindamycin resistance</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin Sensitive</td>
<td>79</td>
<td>44.1</td>
</tr>
<tr>
<td>CR (E resistant, CL resistant)</td>
<td>8</td>
<td>4.46</td>
</tr>
<tr>
<td>IR (E resistant, CL sensitive ) +ve D test</td>
<td>45</td>
<td>45 (25.1)</td>
</tr>
<tr>
<td>MS (E resistant , Cl sensitive ) -ve D test</td>
<td>47</td>
<td>47 (26.2)</td>
</tr>
</tbody>
</table>
Table 3. Relation between pattern of clindamycin resistance and type of staphylococci as regard sensitivity to methicillin.

<table>
<thead>
<tr>
<th>Type of staph</th>
<th>Pattern of clindamycin resistance</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CR</td>
<td>IR</td>
</tr>
<tr>
<td>MSSA</td>
<td>N</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>25.0%</td>
</tr>
<tr>
<td>MRSA</td>
<td>N</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>75.0%</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Chi-square</td>
<td>$X^2$</td>
<td>6.507</td>
</tr>
</tbody>
</table>

Table 4. Distribution of $erm$ (e) and $erm$ (c) genes among different phenotypes of clindamycin resistance.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pattern of clindamycin resistance</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constitutive MLS$_B$</td>
<td>Inducible MLS$_B$</td>
</tr>
<tr>
<td>$erm$ (e)</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0%</td>
</tr>
<tr>
<td>$erm$ (c)</td>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>0%</td>
</tr>
<tr>
<td>$erm$ (e)+$erm$ (c)</td>
<td>N</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>N</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Chi-square</td>
<td>$X^2$</td>
<td>112.616</td>
</tr>
</tbody>
</table>

Table 5. The antimicrobial sensitivity and resistance pattern of the $S. aureus$ isolates with constitutive resistance phenotype.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Cefepime</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

$S$=sensitive; $R$=resistant.

coli was the commonest isolate. Gram positive microorganisms were isolated in 12 (15%) cases and most common was $S. aureus$. This study reported only one staphylococcus isolate (1%) with constitutive clindamycin resistance; resistant to vancomycin and 12 strains with inducible clindamycin resistance (12%) were

isolated from 179 (77.4%). On reverse to this work, a study performed by Taj et al. (2015) found that Gram negative infections accounted for 68 (85%) and Escherichia
Table 7. Antimicrobial sensitivity and resistance of the *S. aureus* isolates with MS phenotype.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S</th>
<th>N</th>
<th>R</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>0.00</td>
<td>0.00</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Cefotaxime</td>
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<td>Oxacillin</td>
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<td>Vancomycin</td>
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<td>Cefepime</td>
<td>23</td>
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<td>Ciprofloxacin</td>
<td>40</td>
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Figure 1. Agarose gel electrophoresis showing positive amplification of 118 and 495 base fragments specific for *erm* (e) and *erm* (c), respectively. Lane 1, 100-1500 bp ladder; lane 2, negative control; lane 3, control *erm* (e) (382 bp); lane 4, control *erm* (c) (188 bp); lanes 5-8, test strains.

resistant to vancomycin. Taj et al. (2015) also reported that the commonest Gram positive isolate is *S. aureus* and 4 cases of MRSA were isolated: all were sensitive to vancomycin; however, 2 cases of vancomycin resistant enterococci (VRE) were documented and were treated successfully with linezolid. In this study, sputum samples represent 26%, pus and urine 18.4 and 18.4% respectively, throat swab 19.4%, blood 17.3% of the clinical samples of cancer patients febrile neutropenia. Anderson et al. (2006) and Nasehi et al. (2010) reported that the most common infections that complicate cancer patients with febrile neutropenia include urinary tract infections, soft tissue infection, pneumonia, and sepsicaemia.

In this study, out of 179 *S. aureus* that were eligible for the study, 79 (44.1%) strains were erythromycin sensitive, and 100 (55.8%) of them were erythromycin resistant. Of these 100 erythromycin resistant strains, 8 (4.46%) isolates were resistant to both erythromycin and clindamycin indicating constitutive MLS<sub>B</sub> Phenotype; 92 isolates were erythromycin resistant, and clindamycin sensitive. Out of these, 45 (25.1%) isolates showed positive D test indicating inducible MLS<sub>B</sub> phenotype while 47 (26.2%) gave negative D test indicating MS phenotype.

The study of Deotale et al. (2010) showed high percentage of erythromycin resistant isolates [80 (32.4%)]. Amongst them 36 (45%) isolates tested positive for inducible clindamycin resistance by D test while the rest of the isolates were negative for D test, out of which 9 (11.25%) were shown to have constitutive clindamycin
resistance and 35 (43.75%) showed true sensitivity to clindamycin (MS phenotype). They reported that these results indicate that D test must be done as a routine laboratory procedure otherwise most of the erythromycin resistant isolates will be misidentified as clindamycin sensitive resulting in failure of treatment. As regard the relation of pattern of clindamycin resistance to the type of sensitivity of S. aureus to mecitheline, the results of this study showed that constitutive MLS B Phenotype was found in higher percentage in MRSA (2 strains (25%) of MSSA and 6 strains (70%) of MRSA), inducible MLS B phenotype were found also in higher percentage in MRSA [10 strains (22.2%) of MSSA and 35 strains of MRSA (77.8%)]. As regard MS, phenotype was equally distributed in MSSA and MRSA [22 strains (46.8%) and 25 strains (53.2)] respectively. These results are in accordance with the results of Kavitha et al. (2011) who observed that percentages of inducible resistance and constitutive clindamycin resistance were higher amongst MRSA as compared to MSSA (20, 16.66, 6.15, and 6.15%, respectively).

As regard the antimicrobial sensitivity and resistance pattern of the S. aureus, isolates with constitutive resistance phenotype in this study showed that highest sensitivity was to vanomycin (98.7%), oxacillin (97.4%), cefpime and ciprofloxacin (83.5% each), and all of the erythromycin sensitive strains were clindamycin sensitive. Moreover and as regard the antimicrobial sensitivity and resistance pattern of staphylococcal strains with inducible resistance phenotype, the results of this study showed that 75% of the strains were resistant to meropenem, gentamycin, and ciprofloxacin, 62% of the strains were resistant to cefotaxim, 50% were resistant to cefepime, and 100% of the strains were vancomycin sensitive.

**Conclusion**

Rapid dissemination of inducible clindamycin-resistant among isolates of S. aureus is worrisome and calls for judicious use of antibiotics. Therefore, the D-test should be added as a routine procedure of antimicrobial susceptibility tests on S. aureus isolates to detect inducible clindamycin resistance to avoid failure of antibiotic therapy with improvement of the mortality rate among cancer patients with febrile neutropenia.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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