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Study on PVL, blaOXA-23 and blaOXA-51 Genes in Drug Resistant *Staphylococcus aureus* Causing Surgical-Sites and Traumatic Wounds Infections, Sudan

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Abstract

Background: The characteristics of *Staphylococcus aureus* that made it the most important cause of wound infections are environmental spread antimicrobials resistance and virulence. Absence of molecular detection of drug resistance and virulence factors in many developing countries limits the epidemiological information. This study conducted to identify PVL virulence gene, and blaOXA-23 and blaOXA-51 drug resistance genes of *Staphylococcus aureus* isolated from surgical-sites infections (SSIs) and traumatic wounds. **Methods:** A cross-sectional study was conducted from 2019 to 2021, in which 70 cefepime resistant *Staphylococcus aureus* were used, the strains were isolated from patients of SSIs and traumatic wounds admitted to the department of General Surgery in Wad Medani Teaching Hospital. Mannitol salt agar was used for primary culture followed by biochemical identification and Kirby Bauer susceptibility testing. Single and multiplex PCR protocols performed for bacterial confirmation and target genes detection. **Results:** *Staphylococcus aureus* strains from SSIs constituted 56% (39/70) from which 41% (16/39) possessed PVL gene while 42% (13/31) of wound infections strains were positive for PVL gene. Presence of PVL gene was significantly associated with resistance to meropenem (P. value 0.023) and ceftriaxone (P. value 0.037). blaOXA-23 was significantly detected with resistance to meropenem, augmentin and ceftriaxone. While blaOXA-51 was significantly identified among *Staphylococcus aureus* strains that showed resistance to meropenem and ci-

profloxacin. **Conclusion:** This is the first study in Sudan that identified blaOXA-23 and blaOXA-51 in *Staphylococcus aureus* and correlated them to resistance to commonly used antimicrobials. Meropenem resistant *Staphylococcus aureus* were significantly positive for PVL, blaOXA-23 and baOXA-51 genes.

Keywords

Staphylococcus aureus, PVL, blaOXA-23, blaOXA-51, Wounds, Sudan

1. Introduction

Staphylococcus aureus is a pathogen contributed in both types of wound infections; surgical-sites infections (SSIs) and traumatic wounds [1] [2]. Morbidity and mortality due to SSIs have been increased in developing countries [3] especially in the presence of resistant microorganisms [4]. External skin injury is the main cause of wound infections, which can lead to deep installation of infectious agents such as bacteria and fungi [5].

During infection *Staphylococcus aureus* elaborates various virulence factors for adhesion, invasion process and avoidance of immune system [6]. In addition to enterotoxins and supertoxins [7], one of the most known virulence factors evolved in staphylococcal invasion mechanism called Panton-Valentine leukocidin (PVL) which is a cytotoxin [8] [9] that exhibits properties against human leucocytes [10] [11]. Importantly, it has been documented that, skin and soft tissues infections are associated with *Staphylococcus aureus* PVL producers [11]. Treatment of wound infections caused by *Staphylococcus aureus* could be highly complicated with the presence of resistance to used antimicrobials [12].

Molecular detection of antimicrobial resistant genes gives rapid and more reliable results when compared to phenotypical methods [13]. Identified elements for drug resistance gene transferring of *Staphylococcus aureus* include plasmids and transposons [14] [15] [16].

From literature PVL *Staphylococcus aureus* producers are frequently isolated from community setting [8] [17], and methicillin sensitive *Staphylococcus aureus* MSSA [18]. Looking at the published data related to the frequency of PVL among clinical isolates of *Staphylococcus aureus*, a marked variation in countries such as China, Germany and Japan was documented, with most remarkably of 97% in United States of America [19].

Due to the limited toxicity and high efficacy, carbapenems have been used as drug of choice for dealing with resistance situations [20]. However, resistance to carbapenems is attributable to carbapenemases, that hydrolyze carbapenems and other β-lactams drugs, production by certain bacterial agents [21]. Carbapenemases including enzymes of Ambler classes, A, B, C and D OXA-type have been encoded by correspondence genes [22] [23]. Some of OXA enzyme overexpression is the reason for increased resistance to carbapenems such as blaOX-

A-23 and blaOXA-51 [24]. Carbapenems had been used in Sudan for the treatment of serious infections in the past two decades and its use has been increasing gradually, so it is advisable to monitor the resistance status. From our knowledge, OXA-type genes were not detected in *Staphylococcus aureus*, on the other hand, many studies have identified the OXA-48 genes in *Pseudomonas aeruginosa* [25], *Escherichia coli* [26] *Acinetobacter baumannii* [27] [28]. The present study aim to assess PVL, blaOXA23 and blaOXA 51 genes from clinical isolates of *Staphylococcus aureus* obtained from SSIs and traumatic wounds.

2. Methods

2.1. Study Settings

Cross-sectional laboratory based study was conducted in the period from 2019 to 2021. Seventy strains of *Staphylococcus aureus* which expressed resistant to cefepime by disc diffusion technique were used [29]. The isolates were from patients admitted to Wad Medani Teaching Hospital in the Department of General Surgery with clinical manifestation of surgical-sites or traumatic wound infections. SSIs were diagnosed during hospitalization and before discharge while traumatic wounds were diagnosed after admission. The study was approved by the Faculty of Medical Laboratory, University of Gezira.

2.2. *Staphylococcus aureus* Isolates and Susceptibility Testing

Swabs from infected surgical-sites and infected wounds were cultured on blood agar and incubated overnight at 37˚C. Gram's reaction used for morphological demonstration and biochemical tests for identification were catalase, coagulase and Dnase enzymes production, and mannitol fermentation tests. Kirby Bauer antimicrobial sensitivity testing was done according to the Clinical Laboratory Standard Institute (CLSI) guidelines.

2.3. Isolation of Genomic DNA and Used Primers

Molecular testing was accomplished in the Molecular Epidemiology Laboratory, Department of Epidemiology, Tropical Medicine Research Institute, Sudan. Genomic DNA was extracted using (G-spin™ Total DNA extraction kit) iNtron (South Korea, Soul), Lot. No. 105251551. Extracted DNA was stored at −80˚C until used. Primers for 16S rRNA amplification, PVL, blaOXA-23 and blaOXA-51 were shown in (**Table 1**). Quality measurement of DNA concentration and purity was accomplished by NanoDrop spectrophotometer (Bibby Scientific, UK).

2.4. PCR and Agarose Electrophoresis

Three different molecular tests were performed (PCR BIO RAD, USA); a single PCR reaction for the detection of bacterial 16S rRNA gene, a single protocol for PVL and multiplex protocol for blaOXA-23 and blaOXA-51 genes detection. For the 16s rRNA, the initial denaturation done at 95˚C for 6 minutes, 40 cycles at 95˚C for 30 seconds is the denaturation, annealing for 1 minute at 50˚C, and

Table 1. Primers used in the study.

Primer	Sequence	Fragment size	Reference
16s r 27 F 16s r 1492 R	5'-AGAGTTTGATCCTGGCTCAG-3' 5'-GGTTACCTTGTTACGACTT-3'	1500	[30]
Pvl F Pvl R	5'-GCTGGACAAAACTTCTTGGAATAT-3' 5'-GATAGGACACCAATAAATTCTGGATTG-3'	85 bp	[31]
Oxa 23 F Oxa23 R	5-GAT CGG ATT GGA GAA CCA GA-3 5-ATT TCT GAC CGC ATT TCC AT-3	501 bp	[32]
Oxa 51 F Oxa51 R	5-TAA TGC TTT GAT CGG CCT TG-3 5-TGG ATT GCA CTT CAT CTT GG-3	353 bp	[32]

extension at 72˚C for one minutes. The final extension was 10 minutes at 72˚C. For PVL, an initial denaturation done for 1 minute at 94˚C followed by 30 seconds of denaturation as 30 cycles at 94˚C, 59˚C of annealing temperatures for 1 minute, and an extension period of 1minute at 72˚C. Final extension accomplished at 72˚C for 10 minutes. The multiplex PCR performed at a denaturation temperature of 94˚C for 3 minutes, then 35 cycles for 45 seconds at 94˚C, whereas annealing done at 57˚C for 45 seconds, and with extension at 72˚C for 1 minute, and a final extension for 5minutesat 72˚C. The PCR products were visualized in 1% agarose gel after ethidium bromide staining.

2.5. Data Analysis

The collected qualitative data were analyzed descriptively to obtain frequency and association. Significant P. value was less than 0.05. SPSS version 20 was used for analysis process.

3. Results

A total of 70 strains of *Staphylococcus aureus* that were isolated from SSIs and traumatic wounds were examined, all strains expressed resistance to cefepime and vary degree of resistance to commonly used antimicrobials. 16s rRNA gene as confirmatory for bacteria was detected with approximately 1500 bp (**Figure 1**). Each of PVL, blaOXA-23 and blaOXA-51 genes were successfully identified with characteristic band size as presented in (**Figure 2**) and (**Figure 3**). The frequency of PVL virulence gene, was observed in 41.4% (29/70) of *Staphylococcus aureus* strains. Destribution of PVL gene in study subject according to the characters is showed in (**Table 2**). Presence of PVL gene was significantly associated with resistance to meropenem and ceftriaxone as shown with Chi-Square of 0.023 and 0.037 respectively (**Table 3**). Presence of blaOXA-23 gene gave signeficant association among strains that were resist to; meropenem, augmentin

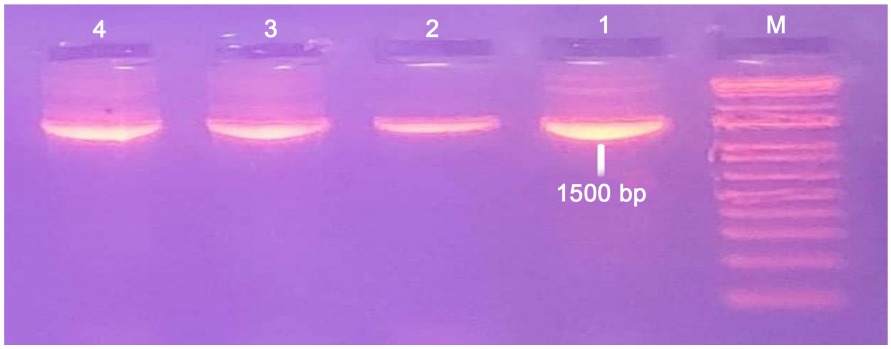

Figure 1. Amplified bacterial 16rRNA gene (1500 bp). M = DNA ladder, 1 - 4 = Positive detection.

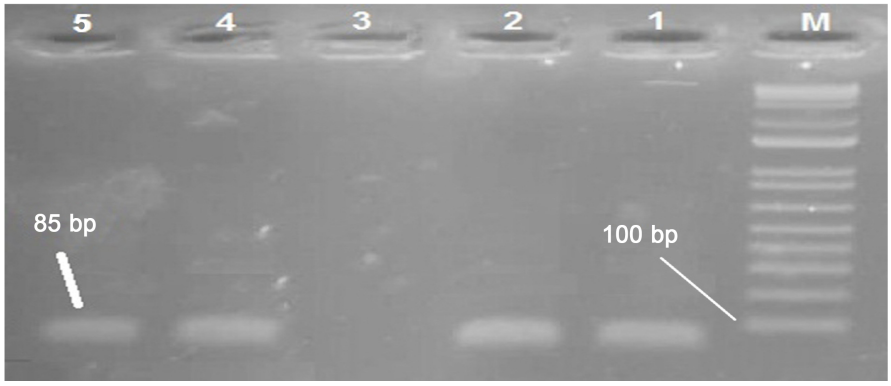

Figure 2. PCR amplification of PVL gene (85 bp). M = 100 bp DNA ladder, 1, 2, 4 and 5 = positive samples. 3 = negative sample.

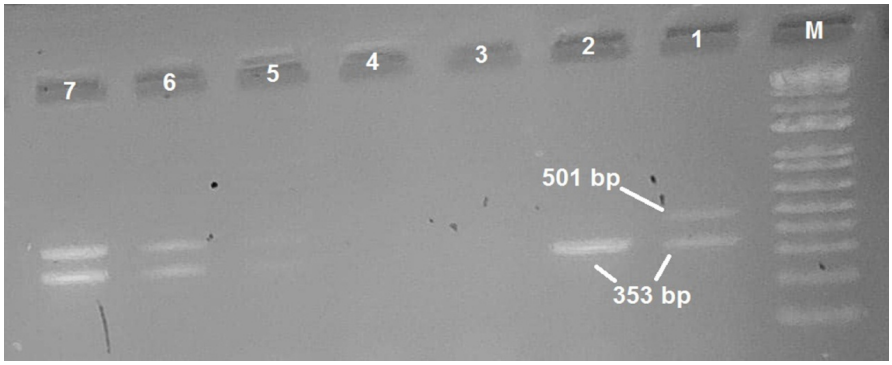

Figure 3. Amplified blaOXA-23 (501 bp) and blaOXA-51 (353 bp) genes. M = 100 bp DNA ladder, 1 = blaOXA-23 and blaOXA-51 positive, 2 = blaOXA-51 positive, 3 and 4 = negative samples, 5 - 7 = positive samples.

and ceftriaxone (Table 4), and also blaOXA-51 gene gave significant association among strains that were resist to meropenem (Table 5).

4. Discussion

It is widely recognized that *Staphylococcus aureus*, is a major cause of both hospital- and community-acquired infections e.g. wound infections, bacteremia, pneumonia and sepsis [25] [33] [34]. Strains associated with wounds infections are

Table 2. Frequency and association of PVL gene of *Staphylococcus aureus* strains in study subjects.

	PVL positive	PVL negative	P. value
Gender			
Male	16	21	0.744
Female	13	20	
Age groups			
16 - 40	7	14	0.655
41 - 60	12	14	
>60	10	13	
Wound type			
SSI	16	23	0.939
Traumatic	13	18	
Wound location			
Abdomen	14	11	
Hand	9	17	0.179
Foot	6	10	
Back	0	3	

frequently reported to develop certain virulence factors as well as multi-drug resistance against commonly used antimicrobials [34] [35]. Recently, these resistant strains of *Staphylococcus aureus* had been documented in both hospitalized and community patients [36] [37]. Accurate identification of bacterial drug resistance is one of health challenges in poor countries where, molecular tagging as a diagnostic tool is not available especially in many developing countries [25].

Tested strains of *Staphylococcus aureus* in this study showed high positivity for PVL gene, this finding may explain the magnitude of wound infections by *Staphylococcus aureus.* Hence, *Staphylococcus aureus* that harbor PVL is potentially skin invaders [11]. From the fact that, expression of PVL gene is motivated by beta-lactam antibiotics with sub-MIC values [19], this study showed significant relationship between PVL gene detection and resistance to beta-lactam antimicrobials such as meropenem and ceftriaxone. Interestingly, PVL gene was markedly observed in strains of *Staphylococcus aureus* isolated from SSIs, which could be interpreted by the movement of PVL *Staphylococcus aureus* producer from community settings into hospitals [38]. On the other hand, infection with PVL *Staphylococcus aureus* producers involves severe inflammatory responses, tissues necrosis, tissue damage and in addition to higher ability for abscess formation and risk of lower antibiotic diffusion [33] [39]. The existence of PVL gene in the current study was not associated with age and gender of patients, and same result had been considered by other author [17].

Unlike other studies done before, this research focus on the detection of beta-lactamase gene D; blaOXA-23 and blaOXA-51 in *Staphylococcus aureus*, and this result was not recorded before at the level of Sudan. Basically, blaOXA-23 and blaOXA-51genes are detected as evidence of resistance to carbapenems and

Table 3. Positive and negative PVL gene of *Staphylococcus aureus* according to susceptibility to commonly used antimicrobials.

	PVL positive	PVL negative	P. value
Meropenem			
Sensitive	19	37	0.023
Intermediate	2	2	
Resistant	8	2	
Gentamycin			
Sensitive	17	19	0.356
Intermediate	1	5	
Resistant	11	17	
Ciprofloxacin			
Sensitive	8	15	0.703
Intermediate	3	3	
Resistant	18	23	
Augmentin			
Sensitive	5	12	0.138
Intermediate	3	9	
Resistant	21	20	
Cefuroxime			
Sensitive	6	11	0.730
Intermediate	2	4	
Resistant	21	26	
Ceftriaxone			
Sensitive	6	17	0.037
Intermediate	2	7	
Resistant	21	17	
Cefotaxime			
Sensitive	6	14	0.243
Intermediate	3	7	
Resistant	20	20	

some beta-lactam drugs in Gram-negative bacteria [40]. One of the possible scenarios for the acquisition of the plasmid mediated blaOXA-23 gene in *Staphylococcus aureus* is the transferring of genetic material through what is known as mobile genetic elements as had been documented with other bacterial species [41]. Importantly, in this study all studied blaOXA-23 producer *Staphylococcus aureus* strains were resistant to meropenem, augmentin and cefuroxime, as well as, 90% of ceftriaxone and cefotaxime resistant. This was indicated by other studies [42] [43], which considered the mechanism of blaOXA-23 gene production to be a major reason for the resistance against meropenem and other beta-lactams antibacterials. Moreover, blaOXA-23 positivity was significantly associated in the current study with resistance against meropenem, augmentin and ceftriaxone.

Table 4. Distribution and association of blaOXA-23 gene of *Staphylococcus aureus* according to susceptibility to commonly used antimicrobials.

	blaOXA-23 positive	blaOXA-23 negative	P. value
Meropenem			
Sensitive	0	56	0.000
Intermediate	0	4	
Resistant	10	0	
Gentamycin			
Sensitive	7	29	0.355
Intermediate	0	6	
Resistant	3	25	
Ciprofloxacin			
Sensitive	5	18	0.108
Intermediate	2	4	
Resistant	3	38	
Augmentin			
Sensitive	0	17	0.016
Intermediate	0	12	
Resistant	10	31	
Cefuroxime			
Sensitive	0	17	0.058
Intermediate	0	6	
Resistant	10	37	
Ceftriaxone			
Sensitive	1	22	0.047
Intermediate	0	9	
Resistant	9	29	
Cefotaxime			
Sensitive	1	19	0.071
Intermediate	0	10	
Resistant	9	31	

In this study the chromosomal mediated blaOXA-51gene in *Staphylococcus aureus* was less detected when compared to blaOXA-23, and at the same time expressed similar feature against tested antibacterials. In line, recent findings have indicated that transferring of resistant genes via plasmids is higher than chromosomal genes [44]. In the current study each of blaOXA-23 and blaOXA-51 resistant genes were found among SSIs isolates of *Staphylococcus aureus* rather than traumatic wounds, by looking to probable source of infection, SSIs are more likely caused by resistant strains.

This study showed that a considerable proportion of *Staphylococcus aureus*, especially those found in hospital, possessed PVL gene. The occurrence of PVL was frequently recorded in the community, which indicate the spreading from

Table 5. Distribution and association of blaOXA-51 gene of *Staphylococcus aureus* according to susceptibility to commonly used antimicrobials.

	blaOXA-51 positive	blaOXA-51 negative	P. value
Meropenem			
Sensitive	0	56	0.000
Intermediate	0	4	
Resistant	4	6	
Gentamycin			
Sensitive	3	33	0.588
Intermediate	0	6	
Resistant	1	27	
Ciprofloxacin			
Sensitive	1	22	0.000
Intermediate	2	39	
Resistant	1	5	
Augmentin			
Sensitive	0	17	0.223
Intermediate	0	12	
Resistant	4	37	
Cefuroxime			
Sensitive	0	17	0.354
Intermediate	0	6	
Resistant	4	43	
Ceftriaxone			
Sensitive	0	23	0.168
Intermediate	0	9	
Resistant	4	34	
Cefotaxime			
Sensitive	0	20	0.204
Intermediate	0	10	
Resistant	4	36	

the community into the hospitals [45].

4.1. Conclusion

Significant proportion of isolated *Staphylococcus aureus* possessed PVL gene as virulence factor contributed to wound infections. Each of blaOXA-23 and baOXA-51 drug resistance gens were detected among isolated *Staphylococcus aureus* strains and correlated to resistance against commonly used antimicrobials; meropenem, ceftriaxone, augmentin and ciprofloxacin.

4.2. Limitation of Study

More information about these resistance genes, as analysis of the genetic code

may help in comparing similar genes, which did not be accomplished in this study. We also did not classify wounds into acute and chronic, which may help in the epidemiological values.

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Conflicts of Interest

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

References

[1] Shettigar, K. and Murali, T.S. (2020) Virulence Factors and Clonal Diversity of *Staphylococcus aureus* in Colonization and Wound Infection with Emphasis on Diabetic Foot Infection. *European Journal of Clinical Microbiology & Infectious Diseases*, **39**, 2235-2246. https://doi.org/10.1007/s10096-020-03984-8

[2] Pal, S., Sayana, A., Joshi, A. and Juyal, D. (2019) *Staphylococcus aureus*: A Predominant Cause of Surgical Site Infections in a Rural Healthcare Setup of Uttarakhand. *Journal of Family Medicine and Primary Care*, **8**, 3600-3606. https://doi.org/10.4103/jfmpc.jfmpc_521_19

[3] Rajkumari, N., Sharma, K., Mathu, P., Kumar, S. and Gupta, A. (2014) A Study on Surgical Site Infections after Trauma Surgeries in a Tertiary Care Hospital in North India. *Indian Journal of Medical Research*, **140**, 691-694.

[4] Guo, Y., Song, G., Sun, M., Wang, J. and Wang, Y. (2020) Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus aureus*. *Frontiers in Cellular and Infection Microbiology*, **10**, Article No. 107. https://doi.org/10.3389/fcimb.2020.00107 https://www.frontiersin.org/articles/10.3389/fcimb.2020.00107/full

[5] Negut, I., Grumezescu, V. and Grumezescu, A.M. (2018) Treatment Strategies for Infected Wounds. *Molecules*, **23**, Article No. 2392. https://doi.org/10.3390/molecules23092392

[6] Cheung, G.Y.C., Bae, J.S. and Otto, M. (2021) Pathogenicity and Virulence of *Staphylococcus aureus*. *Virulence*, **12**, 547-569. https://doi.org/10.1080/21505594.2021.1878688

[7] Hu, D.L., Li, S., Fang, R. and Ono, H.K. (2021) Update on Molecular Diversity and Multipathogenicity of Staphylococcal Superantigen Toxins. *Animal Diseases*, **1**, Article No. 7. https://doi.org/10.1186/s44149-021-00007-7

[8] Shallcross, L.J., Fragaszy, E., Johnson, A.M. and Hayward, A.C. (2013) The Role of the Panton-Valentine Leucocidin Toxin in Staphylococcal Disease: A Systematic Review and Meta-Analysis. *The Lancet Infectious Diseases*, **13**, 43-54. https://doi.org/10.1016/S1473-3099(12)70238-4

[9] Jiang, B., Wang, Y., Feng, Z., Xu, L., Tan, L., Zhao, S., *et al.* (2017) Panton-Valentine Leucocidin (PVL) as a Potential Indicator for Prevalence, Duration, and Severity of *Staphylococcus aureus* Osteomyelitis. *Frontiers in Microbiology*, **8**, Article No. 2355. https://doi.org/10.3389/fmicb.2017.02355

[10] Oogai, Y., Matsuo, M., Hashimoto, M., Kato, F., Sugai, M. and Komatsuzawa, H. (2011) Expression of Virulence Factors by *Staphylococcus aureus* Grown in Serum.

Applied and Environmental Microbiology, **77**, 8097-8105. https://doi.org/10.1128/AEM.05316-11

[11] Li, X., Huang, T., Xu, K., Li, C. and Li, Y. (2019) Molecular Characteristics and Virulence Gene Profiles of *Staphylococcus aureus* Isolates in Hainan, China. *BMC Infectious Diseases*, **19**, Article No. 873. https://doi.org/10.1186/s12879-019-4547-5

[12] Misha, G., Chelkeba, L. and Melaku, T. (2021) Bacterial Profile and Antimicrobial Susceptibility Patterns of Isolates among Patients Diagnosed with Surgical Site Infection at a Tertiary Teaching Hospital in Ethiopia: A Prospective Cohort Study. *Annals of Clinical Microbiology and Antimicrobials*, **20**, Article No. 33. https://doi.org/10.1186/s12941-021-00440-z

[13] Duran, N., Ozer, B., Duran, G.G., Onlen, Y. and Demir, C. (2012) Antibiotic Resistance Genes & Susceptibility Patterns in Staphylococci. *Indian Journal of Medical Research*, **135**, 389-396.

[14] Hope, D., Ampaire, L., Oyet, C., Muwanguzi, E., Twizerimana, H. and Apecu, R.O. (2019) Antimicrobial Resistance in Pathogenic Aerobic Bacteria Causing Surgical Site Infections in Mbarara Regional Referral Hospital, Southwestern Uganda. *Scientific Reports*, **9**, Article No. 17299. https://doi.org/10.1038/s41598-019-53712-2

[15] Nomura, R., Nakaminami, H., Takasao, K., Muramatsu, S., Kato, Y., Wajima, T., *et al*. (2020) A Class A *β*-Lactamase Produced by Borderline Oxacillin-Resistant *Staphylococcus aureus* Hydrolyses Oxacillin. *Journal of Global Antimicrobial Resistance*, **22**, 244-247. https://doi.org/10.1016/j.jgar.2020.03.002

[16] Bonko, M.D.A., Lompo, P., Tahita, M.C., Kiemde, F., Karama, I., Somé, A.M., *et al*. (2021) Antibiotic Susceptibility of *Staphylococcus aureus* and *Streptococcus pneumoniae* Isolates from the Nasopharynx of Febrile Children under 5 Years in Nanoro, Burkina Faso. *Antibiotics*, **10**, Article No. 444. https://doi.org/10.3390/antibiotics10040444

[17] Darboe, S., Dobreniecki, S., Jarju, S., Jallow, M., Mohammed, N.I., Wathuo, M., *et al*. (2019) Prevalence of Panton-Valentine Leukocidin (PVL) and Antimicrobial Resistance in Community-Acquired Clinical *Staphylococcus aureus* in an Urban Gambian Hospital: A 11-Year Period Retrospective Pilot Study. *Frontiers in Cellular and Infection Microbiology*, **9**, Article No. 170. https://doi.org/10.3389/fcimb.2019.00170

[18] Castellazzi, M.L., Bosis, S., Borzani, I., Tagliabue, C., Pinzani, R., Marchisio, P., *et al*. (2021) Panton-Valentine Leukocidin *Staphylococcus aureus* Severe Infection in an Infant: A Case Report and a Review of the Literature. *Italian Journal of Pediatrics*, **47**, Article No. 158. https://doi.org/10.1186/s13052-021-01105-5

[19] Karmakar, A., Jana, D., Dutta, K., Dua, P. and Ghosh, C. (2018) Prevalence of Panton-Valentine Leukocidin Gene among Community Acquired *Staphylococcus aureus*: A Real-Time PCR Study. *Journal of Pathogens*, **2018**, Article ID: 4518541. https://doi.org/10.1155/2018/4518541

[20] Meletis, G. (2016) Carbapenem Resistance: Overview of the Problem and Future Perspectives. *Therapeutic Advances in Infectious Disease*, **3**, 15-21. https://doi.org/10.1177/2049936115621709

[21] Gurung, S., Kafle, S., Dhungel, B., Adhikari, N., ThapaShrestha, U., Adhikari, B., *et al*. (2020) Detection of OXA-48 Gene in Carbapenem-Resistant *Escherichia coli* and *Klebsiella pneumoniae* from Urine Samples. *Infection and Drug Resistance*, **13**, 2311-2321. https://doi.org/10.2147/IDR.S259967

[22] Sugumar, M., Kumar, K.M., Manoharan, A., Anbarasu, A. and Ramaiah, S. (2014) Detection of OXA-1*β*-Lactamase Gene of *Klebsiella pneumoniae* from Blood Stream

Infections (BSI) by Conventional PCR and *In-Silico* Analysis to Understand the Mechanism of OXA Mediated Resistance. *PLOS ONE*, **9**, e91800. https://doi.org/10.1371/journal.pone.0091800

[23] Mlynarcik, P., Chalachanova, A., Vagnerová, I., Holy, O., Zatloukalova, S. and Kolar, M. (2020) PCR Detection of Oxacillinases in Bacteria. *Microbial Drug Resistance*, **26**, 1023-1037. https://doi.org/10.1089/mdr.2019.0330

[24] Taylor, T.A. and Unakal, C.G. (2021) *Staphylococcus aureus*. StatPearls Publishing, Treasure Island. http://www.ncbi.nlm.nih.gov/books/NBK441868/

[25] Ali, D.O. and Nagla, M.M.A. (2020) Molecular Detection of *bla* OXA-48 Gene Encoding Carbapenem Resistance *Pseudomonas aeruginosa* Clinical Isolates from Khartoum State Hospitals, Sudan. medRxiv. http://medrxiv.org/lookup/doi/10.1101/2020.06.22.20137034 https://doi.org/10.1101/2020.06.22.20137034

[26] Mahmoud, N.E., Altayb, H.N. and Gurashi, R.M. (2020) Detection of Carbapenem-Resistant Genes in *Escherichia coli* Isolated from Drinking Water in Khartoum, Sudan. *Journal of Environmental and Public Health*, **2020**, Article ID: 2571293. https://doi.org/10.1155/2020/2571293

[27] Anane, Y.A., Apalata, T., Vasaikar, S., Okuthe, G.E. and Songca, S. (2020) Molecular Detection of Carbapenemase-Encoding Genes in Multidrug-Resistant *Acinetobacter baumannii* Clinical Isolates in South Africa. *International Journal of Microbiology*, **2020**, Article ID: 7380740. https://doi.org/10.1155/2020/7380740

[28] Zhao, Y., Hu, K., Zhang, J., Guo, Y., Fan, X., Wang, Y., *et al*. (2019) Outbreak of Carbapenem-Resistant *Acinetobacter baumannii* Carrying the Carbapenemase OXA-23 in ICU of the Eastern Heilongjiang Province, China. *BMC Infectious Diseases*, **19**, Article No. 452. https://doi.org/10.1186/s12879-019-4073-5

[29] Yousif, S.M., Abakar, A.D., Nour, B.Y.M., Ibrahim, S.O., Elhasan, O.M.A., Yousif, M.A., *et al*. (2021) Frequency and Antimicrobials Susceptibility Pattern of *Staphylococcus aureus* Associated with Wound Infections in Surgery Department, Wad Madani Teaching Hospital, Sudan. *Pharmacology & Pharmacy*, **12**, 334-343. https://doi.org/10.4236/pp.2021.1212028

[30] Chen, Y.L., Lee, C.C., Lin, Y.L., Yin, K.M., Ho, C.L. and Liu, T. (2015) Obtaining Long 16S rDNA Sequences Using Multiple Primers and Its Application on Dioxin-Containing Samples. *BMC Bioinformatics*, **16**, Article No. S13. https://doi.org/10.1186/1471-2105-16-S18-S13

[31] Dayie, N.T.K.D., Osei, M.M., Opintan, J.A., Tetteh-Quarcoo, P.B., Kotey, F.C.N., Ahenkorah, J., *et al*. (2021) Nasopharyngeal Carriage and Antimicrobial Susceptibility Profile of *Staphylococcus aureus* among Children under Five Years in Accra. *Pathogens*, **10**, Article No. 136. https://doi.org/10.3390/pathogens10020136

[32] Joshi, P.R., Acharya, M., Kakshapati, T., Leungtongkam, U., Thummeepak, R. and Sitthisak, S. (2017) Co-Existence of *bla* OXA-23 and *bla* NDM-1 Genes of *Acinetobacter baumannii* Isolated from Nepal: Antimicrobial Resistance and Clinical Significance. *Antimicrobial Resistance & Infection Control*, **6**, Article No. 21. https://doi.org/10.1186/s13756-017-0180-5

[33] Amin, D.H.M., Guler, E. and Baddal, B. (2020) Prevalence of Panton-Valentine Leukocidin in Methicillin-Resistant *Staphylococcus aureus* Clinical Isolates at a University Hospital in Northern Cyprus: A Pilot Study. *BMC Research Notes*, **13**, Article No. 490. https://doi.org/10.1186/s13104-020-05339-0

[34] Ma, Y., Zhao, Y., Tang, J., Tang, C., Chen, J. and Liu, J. (2018) Antimicrobial Susceptibility and Presence of Resistance & Enterotoxins/Enterotoxin-Likes Genes in

Staphylococcus aureus from Food. *CyTA—Journal of Food*, **16**, 76-84. https://doi.org/10.1080/19476337.2017.1340341

[35] Wolters, M., Frickmann, H., Christner, M., Both, A., Rohde, H., Oppong, K., *et al*. (2020) Molecular Characterization of *Staphylococcus aureus* Isolated from Chronic Infected Wounds in Rural Ghana. *Microorganisms*, **8**, Article No. 2052. https://doi.org/10.3390/microorganisms8122052

[36] Deyno, S., Toma, A., Worku, M. and Bekele, M. (2017) Antimicrobial Resistance Profile of *Staphylococcus aureus* Isolates Isolated from Ear Discharges of Patients at University of Hawassa Comprehensive Specialized Hospital. *BMC Pharmacology Toxicology*, **18**, Article No. 35. https://doi.org/10.1186/s40360-017-0141-x

[37] Azab, K.S.M., Abdel-Rahman, M.A., El-Sheikh, H.H., Azab, E., Gobouri, A.A. and Farag, M.M.S. (2021) Distribution of Extended-Spectrum β-Lactamase (ESBL)-Encoding Genes among Multidrug-Resistant Gram-Negative Pathogens Collected from Three Different Countries. *Antibiotics*, **10**, Article No. 247. https://doi.org/10.3390/antibiotics10030247

[38] Papastergiou, P. and Tsiouli, E. (2018) Healthcare-Associated Transmission of Panton-Valentine Leucocidin Positive Methicillin-Resistant *Staphylococcus aureus*: The Value of Screening Asymptomatic Healthcare Workers. *BMC Infectious Diseases*, **18**, Article No. 484. https://doi.org/10.1186/s12879-018-3404-2

[39] Ahmed, O.B. (2020) Prevalence of Methicillin-Resistant *Staphylococcus aureus* and Classical Enterotoxin Genes among Sudanese Food Handlers. *Cureus*, **12**, e12289. https://www.cureus.com/articles/47546-prevalence-of-methicillin-resistant-staphylococcus-aureus-and-classical-enterotoxin-genes-among-sudanese-food-handlers https://doi.org/10.7759/cureus.12289

[40] Evans, B.A. and Amyes, S.G.B. (2014) OXA β-Lactamases. *Clinical Microbiology Reviews*, **27**, 241-263. https://doi.org/10.1128/CMR.00117-13

[41] Sun, H., Xiao, G., Zhang, J., Pan, Z., Chen, Y. and Xiong, F. (2019) Rapid Simultaneous Detection of $bla_{\text{oxa-23}}$, *Ade-B*, *int*-1, and *ISCR*-1 in Multidrug-Resistant *Acinetobacter baumannii* Using Single-Tube Multiplex PCR and High Resolution Melting Assay. *Infection and Drug Resistance*, **12**, 1573-1581. https://doi.org/10.2147/IDR.S207225

[42] Hou, C. and Yang, F. (2015) Drug-Resistant Gene of *blaOXA*-23, *blaOXA*-24, *blaOXA*-51 and *blaOXA*-58 in *Acinetobacter baumannii. International Journal of Clinical and Experimental Medicine*, **8**, 13859-13863.

[43] Vakili, B., Fazeli, H., Shoaei, P., Yaran, M., Ataei, B., Khorvash, F. and Khaleghi, M. (2014) Detection of Colistin Sensitivity in Clinical Isolates of *Acinetobacter baumannii* in Iran. *Journal of Research in Medical Sciences*, **19**, S67-S70.

[44] Wang, Y., Batra, A., Schulenburg, H. and Dagan, T. (2022) Gene Sharing among Plasmids and Chromosomes Reveals Barriers for Antibiotic Resistance Gene Transfer. *Philosophical Transactions of the Royal Society B*, **377**, Article ID: 20200467. https://doi.org/10.1098/rstb.2020.0467

[45] Bhatta, D.R., Cavaco, L.M., Nath, G., Kumar, K., Gaur, A., Gokhale, S., *et al*. (2016) Association of Panton Valentine Leukocidin (PVL) Genes with Methicillin Resistant *Staphylococcus aureus* (MRSA) in Western Nepal: A Matter of Concern for Community Infections (a Hospital Based Prospective Study). *BMC Infectious Diseases*, **16**, Article No. 199. https://doi.org/10.1186/s12879-016-1531-1