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High prevalence of panton-valentine leukocidin in methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital in Peshawar, Pakistan

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide notorious pathogen. MRSA pose serious threats to the available therapeutic choices. Panton-Valentine Leukocidin (*pvl*) is an important virulence factor usually associated with skin and soft tissue infection while staphylococcal complement inhibitor (*scn*) is considered as a human adaptability marker. Therefore, the given study was conducted to determine the current antibiotic resistance trends, prevalence of induclible clindamycin resistant phenotype, *pvl* gene, and *scn* gene in MRSA strains prevailing in Peshawar, Pakistan. This prospective cross-sectional study was carried out at the Center of Biotechnology and Microbiology, University of Peshawar, Pakistan from December 2017 to May 2018. Non-double consecutive MRSA isolates were anonymously enrolled in the study, isolated from different clinical specimens. Antibiotic susceptibility testing was determined by disc diffusion method, while inducible clindamycin resistance was detected by D-test. Moreover, all the phenotypically identified MRSA were subjected to multiplex PCR for the detection of *mecA, mecC, pvl* and *scn* genes. A total of 178 MRSA were included in the study, wherein, none of the isolate was either resistant or sensitive to all the 10 tested antibiotics. The resistance frequency of different antibiotics was: ciprofloxacin 89.8%, erythromycin 80.3%, cotrimoxazole 72.5%, gentamycin 71.9%, fusidic acid 63.4%, tetracycline 60.1%, clindamycin 46.1%, doxycycline 25.8%, and quinupristin/dalfopristin 6.7%, while linezolid was 100% susceptible. Prevalence of inducible clindamycin resistance, *pvl* and *scn* were 14.6%, 46.7%, and 97.1% respectively.The studied MRSA strains showed significant resistance toward the common therapeutic choices, and the prevalence of inducible clindamycin resistance and *pvl* is considerably high.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global challenge for infection control strategies and therapeutic management since its discovery in 1960 (O'Neill, 2016). MRSA can cause a spectrum of diseases spanning from mild skin and soft tissue infections to invasive fatal conditions encompassing pneumonia, meningitis, toxic shock syndrome, scalded skin syndrome, osteomyelitis, and septicemia (Tong *et al*., 2015).

Several reports on the surveillance of antimicrobial resistance in MRSA describe that antibiotic resistance is limiting our ability to treat the common MRSA infections in hospital and community (WHO, 2014; Ventola, 2015).

The antibiotic resistance determinants continuously transfer through different mechanism among the circulating clone of MRSA and subsequently alter the antibiotics resistance trends of the endemic pathogens (Frieri *et al*., 2017).

Macrolide, lincosamide and streptogramin B (MLS_B) are the common therapeutic agents used against MRSA infections, but clindamycin is a drug of choice for the treatment of community acquired-MRSA (CA-MRSA) because of the pharmacokinetic properties (Ansari *et al*., 2014). However, MRSA usually offers cross resistance to the MLS_B antibiotics mediated by erythromycin ribosomal methylase (*erm*) determinant and the resultant phenotype is known as MLS_B resistant phenotype. MLS_B resistant phenotype could be the result of either constitutive ($cMLS_B$) or inducible ($iMLS_B$) expression of *erm* gene, wherein, the $iMLS_B$ resistant phenotype is *in-vitro* susceptible to clindamycin but *in-vivo* resistant and often lead to treatment failure (Woods, 2009).

Panton-Valentine Leukocidin (PVL) is a virulence factor of MRSA, act as a potent cytotoxin. PVL is composed of two exoprotein components encoded by a lysogenic phage carrying cotranscribed genes *LukS-PV* and *LukF-PV*. It enhances virulence by selectively disrupting the leucocytes membrane and causes tissue necrosis (Kaneko & Kamio, 2004). PVL harboring MRSA are usually associated with skin and soft tissue infections, however, they can also cause invasive fatal conditions like necrotizing fasciitis and necrotizing hemorrhagic pneumonia (McGrath *et al*., 2008).

The severity and mortality of the infections caused by PVL producing MRSA strains are higher in contrast to the PVL non producing strains (Zhang *et al*., 2016), several other studies also advocate for the vital role of PVL in pathogenicity of MRSA (Melles *et al*., 2006). However, studies in animal models report that the role of PVL is indeterminate in pathogenicity (Bazzi *et al*., 2015). Worldwide increasing prevalence of PVL is worrisome but the reported burden is variable in different parts of the world (Karmakar *et al*., 2018).

Staphylococcal complement inhibitor protein prevents complement pathway and enhances the virulence of MRSA. it is encoded by *scn* gene located in the immune evasion cluster on a bacteriophage converting β-hemolysin.

The *scn* gene is very specific with human immune cascade, therefore, it is considered as a specific marker for human adaptation (Worthing *et al*., 2018).

We do not have a surveillance program for monitoring the antibiotic resistance pattern of MRSA, and no study has particularly determined the prevalence of *pvl* gene and *scn* gene in the prevailing clone of MRSA in Peshawar, Pakistan. Therefore, the current study was conducted to determine the current antibiotic resistance trends of MRSA, the prevalence of $iMLS_B$ resistant phenotypes, the prevalence of *pvl* gene and *scn* gene in the circulating regional clone of MRSA.

Materials and methods

This prospective cross-sectional study was conducted at the Center of Biotechnology and Microbiology, University of Peshawar, Pakistan. A total of 178 non-double consecutive MRSA isolated from different clinical specimens received to the laboratory of

Microbiology, Lady Reading Hospital (LRH), Peshawar, were anonymously included in the study from December 2017 to May 2018. LRH is the largest public sector hospital comprised of 30 departments and 1751 beds, located into the center of Peshawar in the Province of Khyber Pakhtunkhwa, Pakistan.

Isolation and Identification of MRSA

All the MRSA isolates included in the study were identified by culturing on Mannitol salt agar, colonial morphology, and tube coagulase test. All the isolates were screened for methicillin resistance by growing on Brilliance MRSA 2 agar (Thermo Fisher Scientific), cefoxitin disc testing by disc diffusion technique. Subsequently, identified MRSA were evaluated by measuring minimum inhibitory concentration (MIC) against cefoxitin by using E-test according to the instructions of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2018).

In E-test, a bacterial suspension was prepared in normal saline and adjusted at 0.5 McFarland standard with nephelometer followed by preparation of bacterial lawn on Muller Hinton agar and finally E-test strip (Biomerieux, Germany) was placed on the surface of the agar and results were recorded after 16-18 hours aerobic incubation at 35°C. *Staphylococcus aureus subsp. aureus* ATCC 25923 was included in the disc diffusion technique and E-test as a control in each run.

Determination of Antibiotic Reistance Pattern

The antibiotic resistance pattern was determined by disc diffusion method against ten commonly used antibiotics encompassing gentamicin, clindamycin, erythromycin, fusidic acid, linezolid, cotrimoxazole, ciprofloxacin, doxycycline, quinupristin-dalfopristin, and tetracycline. Briefly, 3 to 5 isolated colonies of the overnight culture were emulsified in normal saline and adjusted against 0.5 McFarland standard, bacterial lawn was prepared by dipping a sterile cotton stick into the bacterial suspension, all the discs were applied and zone of inhibition was measured after 16-18 hours aerobic incubation at 35°C as outlined in the document of CLSI (CLSI, 2018).

Moreover, 20 MRSA strains selected on the base of their antibiogram were screened by Thermo Fisher Scientific Sensititre for *Staphylococcus species* EUSTAPH AST plate for MIC against the 16 antibiotics used for the treatment of MRSA according to the instructions of the manufacturer. Among the 16 antibiotics, 8 were the antibiotics not tested by disc diffusion method while 8 antibiotics were retested for the evaluation of the results of disc diffusion method as an internal control. While *Staphylococcus aureus subsp. aureus* ATCC 29213 was included in the batch as a control strain.

Detection of Inducible Clindamycine Resistance

The $iMLS_B$ resistant phenotype was detected by double disc diffusion test. In detail, the disc of erythromycin and clindamycin were placed 15 to 20 millimeters apart from each other on a lawn of a target isolate and the resultant zone was observed for D-shape phenomenon or hazy growth within the zone of inhibition around clindamycin after 16-18 hours aerobic incubation at 37°C according to CLSI protocol (CLSI, 2018). *Staphylococcus aureus subsp. aureus* ATCC BAA-977 was included in each batch as a positive control.

Multiplex PCR

MRSA determined by phenotypic methods were subjected to the multiplex PCR for the detection of *mecA*, *mecC, pvl* gene , and *scn* gene as previously reported (Stegger *et al.*, 2012).

The sets of primer used are listed in Table 1. The DNA from MRSA isolates was extracted by treating with lysostaphin, Proteinase K followed by boiling for 5 minutes as published before (Al-Talib *et al.*, 2013). A PCR reaction mixture was prepared by adding 0.4µM of each primer to a Qiagen Multiplex PCR Master Mix kit (Qiagen, Valencia, CA, USA), and amplification was carried out at these conditions: 15 min at 94°C, followed by 30 cycles of 30s at 94°C, 1 min at 59°C, and 1 min at 72°C, with a final 10 min elongation step at 72°C. PCR products were visualized on 2% E-Gels (Invitrogen,Grand Island, CA, USA) (Stegger *et al.*, 2012).

Results

A total of 178 MRSA identified were included in the study, wherein, 174 (97.7%) isolates were obtained from pus or wound swab, 1 (0.6%) from blood, 2 (1.1%) from body fluid and 1 (0.6%) from sputum. All the specimens were collected from 106 male and 72 female, and age of the participants was ranged from 2 to 86 years.

Table 1. Primer used in the study.

Primer name	Gene	Sequence (5´-3´)	Amplicon size
mecAP4 Oliveira	*mecA*	TCCAGATTACAACTTCACCAGG	162bp
mecA P7		CCACTTCATATCTTGTAACG	
lga 251 opt FP	*mecC*	GAA AAA AAG GCT TAG AAC GCC TC	138bp
lga 251 opt RP3		GAA GAT CTT TTC CGT TTT CAG C	
scnFW3	*Scn*	ATATTTTGCTTCTGACATTTTCT	112bp
scnRev2		AGCTACTGGAAGTTTAAACACT	
pvl-FP	*Pvl*	GCTGGACAAAACTTCTTGGAATAT	~ 85bp
pvl-RP		GATAGGACACCAATAAATTCTGGATTG	

All the tested strains produced ≤ 21mm zone of inhibition against cefoxitin disc and turned denim blue on Brilliance MRSA 2 agar with MIC value of ≥ 8µg/ml against cefoxitin E-strips were phenotypically considered MRSA. The amplification product of 162bp, 138bp, 112bp, and 85bp for *mecA, mecC, scn* gene*, and pvl* gene was produced respectively as shown in Fig. 1.

Table 2. Antibiotic resistance pattern of MRSA stains.

Antibiotics	Resistance n (%)	Sensitive n (%)
Ciprofloxacin	160 (89.8)	18 (10.2)
Erythromycin	143 (80.3)	35(19.6)
Cotrimoxazole	129 (72.5)	49 (27.5)
Gentamicin	128 (71.9)	50 (28.1)
Fusidic acid	113 (63.4)	65 (36.6)
Tetracycline	107 (60.1)	71 (39.9)
Clindamycin	82 (46.1)	96 (53.9)
Doxycycline	46 (25.8)	132 (74.2)
Quinupristin/Dalfopristin	12 (6.7)	166 (93.2)
Linezolid	00 (0.0)	178 (100)

All the 178 phenotypically identified isolates of MRSA were positive for *mecA* gene and *mecC* was not detected in any of the studied strain. The prevalence of *pvl* gene and *scn* gene was 46.7% (83/178) and 97.1% (173/178) respectively. Out of total 178 isolates, none is resistant and/or susceptible to all the 10 tested antibiotics, whereas only 2 (1.1%) strains were resistant to 9 tested antibiotics, 14 (7.8%) strains to 8 tested antibiotics, 33 (18.5%) strains to 7 tested antibiotics, 31 (17.4%) strains to 6 tested antibiotics, 39 (21.9%) strains to 5 tested antibiotics, 26 (14.6%) strains to 4 tested antibiotics, 16 (8.9%) strains to 3 tested antibiotics, 7 (3.9%) strains to 2 tested antibiotics and 10 (5.6%) strains were resistant to only 1 of the 10 tested antibiotics. The highest frequency of resistance was shown against ciprofloxacin (89.8%) and least resistance was found against quinupristin/dalfopristin (6.7%), while linezolid showed 100% susceptibility as depicted in Table 2.

A total of 26 (14.6%) isolates were positive for inducible clindamycin resistance, while 30 (16.8%) isolates were sensitive to both erythromycin and clindamycin, 51 (28.6%) isolates were detected with a $cMLS_B$ resistance i.e. *in vitro* resistant to both clindamycin and erythromycin. Interestingly, 66 (37.1%) strains with erythromycin resistance were found sensitive to clindamycin and only 5 (2.8%) strains were resistant to clindamycin but sensitive to erythromycin.

Table 3. EUSTAPH results.

Antibiotics	Resistant	Sensitive
Ceftaroline	00	20
Vancomycin	00	20
Telavancin	00	20
Mupirocin	02	18
Rifampin	02	18
Teicoplanin	01	19
Levofloxacin	18	02
Moxifloxacin	18	02

The EUSTAPH screening showed similar results for the 8 antibiotics already tested by the disc diffusion method. While for the other 8 antibiotics, none of the tested strains was resistant to ceftaroline, vancomycin, and telavancin as presented in table 3.

Discussion

The given study determined the current antibiotic resistance trends of MRSA and prevalence of iMLSB resistant phenotype, the prevalence of pvl gene, and scn gene in the MRSA strains isolated at LRH.

The highest resistance was found against ciprofloxacin (89.8%), followed by erythromycin (80.3%), which is in concordance with the study from Lahore, Pakistan in 2017 reported the similar trends of resistance for these two antibiotics (Sohail & Latif, 2017).

Fig. 1. Line M: DNA Marker Line 1-22: Tested MRSA, Line 23, 24 MRSA control strains.

Among tested MRSA strains, 72.5% strains showed resistance to cotrimoxazole, 71.9% were resistant to gentamycin, and 60.1% were found resistant to tetracycline which extent the previous knowledge (Perveen *et al.*, 2013).

The findings of this study about fusidic acid, clindamycin and doxycycline resistance are

inconsistent with the results of a study published from Peshawar, it might be possible because of the difference in sample size, the origin of the strains and study setting as they enrolled most of the invasive isolates from a private sector hospital (Ullah *et al.*, 2016). Our work also found resistance against quinupristin/dalfopristin, though it is very low but unusual because most of the studies reported 100% susceptibility to quinupristin/dalfopristin in Pakistan (Baysallar *et al.*, 2004; Brohi & Noor, 2017), however, resistance against this antibiotic has been reported in different parts of the world (Yu *et al.*, 2014).

In our study, linezolid kills 100% strains of MRSA which are in line with the previous findings from different parts of the country (Kaleem *et al.*, 2010; Ullah *et al.*, 2016).

The D-test results showed 14.6% prevalence of $iMLS_B$ resistant phenotype which was lower than the $cMLS_B$ resistant phenotype, other studies also reported 15.8% and 18% prevalence of $iMLS_B$ resistant phenotype, which are congruent to our reported prevalence of $iMLS_B$ and also lower to $cMLS_B$ resistance (Afridi *et al.*, 2014; Ullah *et al.*, 2016), hence, it is utmost important to include D-test in routine culture and sensitivity testing of *S. aureus* and/or MRSA. The $cMLS_B$ resistance and resistance to erythromycin with clindamycin susceptibility was 28.6% and 37.1% respectively, which is comparable with the previous study (Khodabandeh *et al.*, 2019).
The numbers of strains tested by EUSTAPH are too limited to obtain useful inference except for evaluation of the disc diffusion testing. Though, the result showed 100% susceptibility of vancomycin, ceftaroline, and telavancin which gives better hope about these last resort against MRSA but still we could not deduce a meaningful conclusion at this stage.

The overall resistance pattern is consistent with the other studies reported from different parts of Pakistan for the MRSA strains isolated from skin or soft tissue infections (Perwaiz *et al.*, 2007; Kaleem *et al.*, 2010), but there is a finding worthy of further consideration encompassing the resistance toward quinupristin/dalfopristin.

The prevalence of two virulence genes *pvl* and *scn* is 46.7% and 97.1% respectively, *pvl* carrying *S. aureus* and/or MRSA can cause a more severe infection of the skin and soft tissue, or rarely fatal invasive infections, therefore, more stringent infection control and therapeutic strategies are required for *pvl* positive cases (Agency, 2008). Our findings show a higher prevalence of *pvl* but previous studies from Pakistan has been reported similar findings (Madzgalla *et al.*, 2016; Jamil *et al.*, 2018), so it is important to consider this higher prevalence of *pvl* for further investigation. Studies from different parts of Pakistan also reported the prevalence of *scn* gene in more than 95% of the MRSA, which is in line with our results, and it also indicates the host specificity of the circulating MRSA clone because *scn* is considered a human-specific marker of MRSA, thus, this high prevalence of *scn* gene suggests that the circulating MRSA strains are originated from human.

The limitation of the study included that it is unicentric with consecutive samples, and we could not associate the studied MRSA strains with their origin like hospital-associated, community-associated or live-stock associated.

Conclusion

In conclusion, MRSA confers resistance to many of the available therapeutic agents like more than 50% of the tested strains were resistant to ciprofloxacin, erythromycin, gentamycin, cotrimoxazole, tetracycline, and fusidic acid, while none of the studied strain was resistant to linezolid, therefore, still remained potential candidates for the MRSA treatment. The prevalence of $iMLS_B$ and *pvl* gene is also significantly high. Moreover, higher prevalence of scn gene indicate the host adabtibility of the studied MRSA strains.

References

Afridi FI, Zeb M, Hussain A, Farooqi BJ, Murtuza G. 2014. Inducible clindamycin resistance

in Staphylococcus species. Journal of the College of Physicians and Surgeons--Pakistan **24(7),** 481-484. http://dx.doi.org/07.2014/JCPSP.481484

Agency HP. 2008. Guidance on the diagnosis and management of PVL-associated Staphylococcus aureus infections (PVL-SA) in England.

Al-Talib H, Yean CY, Al-Khateeb A, Ravichandran M. 2013. Comparative evaluation of three different methods of genomic DNA extraction for Staphylococcus aureus. World Applied Sciences Journal **21,** 424-427.

Ansari S, Nepal HP, Gautam R, Rayamajhi N, Shrestha S, Upadhyay G, Chapagain ML. 2014. Threat of drug resistant Staphylococcus aureus to health in Nepal. BMC Infectious Diseases **14(1),** 157. http://dx.doi.org/10.1186/1471-2334-14-157

Baysallar M, Kilic A, Aydogan H, Cilli F, Doganci L. 2004. Linezolid and quinupristin/dalfopristin resistance in vancomycin-resistant enterococci and methicillin-resistant Staphylococcus aureus prior to clinical use in Turkey. International Journal of Antimicrobial Agents **23(5),** 510-512.

Bazzi AM, Rabaan AA, Fawarah MM, Al-Tawfiq JA. 2015. Prevalence of Panton-Valentine leukocidin-positive methicillin-susceptible Staphylococcus aureus infections in a Saudi Arabian hospital. Journal of Infection and Public Health ***8*(4),** 364-368. http://dx.doi.org/10.1016/j.jiph.2015.01.010

Brohi NA, Noor AA. 2017. Frequency of the Occurrence of Methicilin Resistant Staphylococcus aureus (MRSA) Infections in Hyderabad, Pakistan. Pakistan Journal of Analytical and Environmental Chemistry **18(1),** 84-90.

CLSI. 2018. Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA.

Frieri M, Kumar K, Boutin A. 2017. Antibiotic resistance. Journal of Infection and Public Health **10(4),** 369-378. http://dx.doi.org/10.1016/j.jiph.2016.08.007

Jamil B, Gawlik D, Syed MA, Shah AA, Abbasi SA, Muller E, Monecke S. 2018. Hospital-acquired methicillin-resistant Staphylococcus aureus (MRSA) from Pakistan: molecular characterisation by microarray technology. European Journal of Clinical Microbiology and Infectious Diseases **37(4),** 691-700. http://dx.doi.org/10.1007/s10096-017-3161-y

Kaleem F, Usman J, Hassan A, Omair M, Khalid A, Uddin R. 2010. Sensitivity pattern of methicillin resistant Staphylococcus aureus isolated from patients admitted in a tertiary care hospital of Pakistan. Iranian Journal of Microbiology ***2***(3), 143-146. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22347563

Kaneko J, Kamio Y. 2004. Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. Bioscience Biotechnology and Biochemistry **68(5),** 981-1003. http://dx.doi.org/10.1271/bbb.68.981

Karmakar A, Jana D, Dutta K, Dua P, Ghosh C. 2018. Prevalence of Panton-Valentine Leukocidin Gene among Community Acquired Staphylococcus aureus: A Real-Time PCR Study. Journal of pathogens **2018,** 4518541. http://dx.doi.org/10.1155/2018/4518541

Khodabandeh M, Mohammadi M, Abdolsalehi MR, Alvandimanesh A, Gholami M, Bibalan MH, Rajabnia R. 2019. Analysis of Resistance to Macrolide-Lincosamide-Streptogramin B Among mecA-Positive Staphylococcus Aureus Isolates. Osong Public Health Res Perspect **10(1),** 25-31. http://dx.doi.org/10.24171/j.phrp.2019.10.1.06

Madzgalla S, Syed MA, Khan MA, Rehman SS,

Muller E, Reissig A, Monecke S. 2016. Molecular characterization of Staphylococcus aureus isolates causing skin and soft tissue infections in patients from Malakand, Pakistan. European Journal of Clinical Microbiology and Infectious Diseases **35(9),** 1541-1547.
http://dx.doi.org/10.1007/s10096-016-2695-8

McGrath B, Rutledge F, Broadfield E. 2008. Necrotising pneumonia, Staphylococcus aureus and Panton-Valentine leukocidin. Journal of the Intensive Care Society **9(2),** 170-172.

Melles DC, van Leeuwen WB, Boelens HA, Peeters JK, Verbrugh HA, Van Belkum A. 2006. Panton-Valentine leukocidin genes in Staphylococcus aureus. Emerging Infectious Diseases ***12*(7),** 1174-1175.
http://dx.doi.org/10.3201/eid1207.050865

O'Neill. 2016. Tackling drug-resistant infections globally: final report and recommendations. Review of antimicrobial resistance. London: HM Government and Wellcome trust; 2016.

Perveen I, Majid A, Knawal S, Naz I, Sehar S, Ahmed S, Raza MA. 2013. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus and coagulase-negative Staphylococci in Rawalpindi, Pakistan. Journal of Advances in Medicine and Medical Research **3(1),** 198.

Perwaiz S, Barakzi Q, Farooqi BJ, Khursheed N, Sabir N. 2007. Antimicrobial susceptibility pattern of clinical isolates of methicillin resistant Staphylococcus aureus. Journal of the Pakistan Medical Association ***57***(1), 2-4. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17319409

Sohail M, Latif Z. 2017. Prevalence and antibiogram of methicillin resistant Staphylococcus aureus isolated from medical device-related infections; a retrospective study in Lahore, Pakistan. Revista da Sociedade Brasileira de Medicina Tropical **50(5),** 680-684.
http://dx.doi.org/doi:10.1590/0037-8682-0352-2016

Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Larsen AR. 2012. Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecA(LGA251). Clinical Microbiology and Infection **18(4),** 395-400.
http://dx.doi.org/10.1111/j.1469-0691.2011.03715.x

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. 2015. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews **28(3),** 603-661.
http://dx.doi.org/10.1128/CMR.00134-14

Ullah A, Qasim M, Rahman H, Khan J, Haroon M, Muhammad N, Muhammad N. 2016. High frequency of methicillin-resistant Staphylococcus aureus in Peshawar Region of Pakistan. Springerplus **5(1),** 600.

Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. P T **40(4),** 277-283. Retrieved from
https://www.ncbi.nlm.nih.gov/pubmed/25859123

WHO. 2014. *Antimicrobial resistance: global report on surveillance*: World Health Organization.

Woods CR. 2009. Macrolide-inducible resistance to clindamycin and the D-test. The Pediatric Infectious Disease Journal **28(12),** 1115-1118.
http://dx.doi.org/10.1097/INF.0b013e3181c35cc5

Worthing KA, Abraham S, Pang S, Coombs GW, Saputra S, Jordan D, Norris JM. 2018. Molecular Characterization of Methicillin-Resistant Staphylococcus aureus Isolated from Australian Animals and Veterinarians. Microbial Drug Resistance **24(2),** 203-212.
http://dx.doi.org/10.1089/mdr.2017.0032

Yu F, Lu C, Liu Y, Sun H, Shang Y, Ding Y, Huang X. 2014. Emergence of quinupristin/dalfopristin resistance among livestock-associated Staphylococcus aureus ST9 clinical isolates. International Journal of Antimicrobial Agents **44(5),** 416-419.

Zhang C, Guo L, Chu X, Shen L, Guo Y, Dong H, Van der Veen S. 2016. Presence of the Panton-Valentine leukocidin genes in methicillin-resistant Staphylococcus aureus is associated with severity and clinical outcome of hospital-acquired pneumonia in a single center study in China. PloSOne **11(6),** e0156704.