PREVALENCE OF METHICILLIN-RESISTANT AND SUSCEPTIBLE *STAPHYLOCOCUS AUREUS* AMONG PATIENTS FROM SELECTED HOSPITALS IN JOS. PLATEAU STATE. NIGERIA.

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) presents major health risk for humans causing serious nosocomial and community-acquired infections with high rates of morbidity and mortality among patients worldwide. It is a well-recognized public health problem throughout the world. Since the Coronavirus emerged in China and began spreading around the globe, reports of bacterial co-infections and wide spread antibiotic use in patients has prompted concerns that the unprecedented viral pandemic could fuel a rise in antimicrobial resistance (AMR).This study investigated the prevalence of Methicillin resistant (MRSA) and susceptible (MSSA) *staphylococcus aureus* isolates among patients attending hospitals and its public health implications. This hospital-based cross-sectional study included 200 patients attending three (3) major hospitals in Jos, Plateau state. Standard procedures were employed for the isolation of *Staphylococcus aureus* from ear and cutaneous wound swabs of patients, followed by screening and susceptibility testing of the isolated *Staphylococcus aureus*. *Staphylococcus aureus* was identified by growth on mannitol salt agar (MSA), and MRSA by growth on mannitol salt agar containing 4µg Oxacillin, Gram staining, and a conventional biochemical test. Isolates of *Staphylococcus aureus* were characterized by antibiotic susceptibility testing using the disc diffusion method. A total of 135 isolates were identified as *Staphylococcus aureus* based on morphology, Gram stain reaction and biochemical characteristics. Of the total *Staphylococcus aureus*, 36 (26.7%) were identified as Methicillin-Resistant *Staphylococcus aureus*(MRSA) while 99 (73.3%) were Methicilin-Susceptible *Staphylococcus aureus* (MSSA). The overall prevalence of MRSA and MSSA among the study population were found to be 13.6% and 37.5% respectively The study has established the existence of Methicillin –resistant and susceptible *Staphylococcus aureus* infections in patients attending hospital in the post COVID 19 era with an overall prevalence of 13.3%. The MRSA isolated showed multiple drug resistance to beta-lactams commonly prescribed antibiotics.

Keyword: Methicillin-resistant *Staphylococcus aureus*(MRSA), Methicilin-Susceptible *Staphylococcus aureus* (MSSA), Nosocomial infections, Antimicrobial resistance (AMR), .

Introduction

Staphylococcus aureus is a gram-positive bacterium causing nosocomial infections, surgical wound infections and several opportunistic infections. The organism has been continuously evolving and developing resistance to antibiotics since the medical use of penicillin began in1942 (Lobanovska& Pilla.2017).Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major problem worldwide causing hospital-acquired infections (Balden*at al.,* 2009). Of concern is the high mortality associated with MRSA infections. Methicillin-resistant *Staphylococcus aureus* acquires its resistance via the methicillin resistance gene mecA, which encodes a low affinity penicillin-binding protein (PBP2a) that is absent in susceptible *Staphylococcusaureus* strains (Hiramatsu*et al.,* 2001;Sievert *et al.,* 2008). This resistant penicillin binding protein receptor does not bind well to most β-lactams and therefore allows MRSA to grow in their presence (Hiramatsu et al., 2001).

MRSA isolates became multi-resistant to other classes of antimicrobial. Rates of methicillin resistance increased slowly, but progressively, until the late 1990s when a dramatic surge in MRSA rates began (Carleton, *et al.*, 2004).

Methicillin resistant *staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult –to- treat infections in human and animal. It may also be called multi drug resistant *Staphylococcus aureus* or oxacillin –*resistant Staphylococcus aureus* (ORSA), (Klevens, *et al*., 2007). MRSA is by definition, any strain of *staphylococcus aureus* bacteria that have developed resistance to beta-lactam antibiotics which include the penicillins (methicillin, Dicloxacillin, Nafcillin, oxacillin etc.) and the cephalosorins (Moreno *et al*., 2007). Methicillin- resistant *Staphylococcus aureus*was discovered in 1961 in the United Kingdom. It made it first major appearance in the United States in 1881 among intravenous drug users (carmeli*et al.,* 2005). During the past 2 decades, methicillin –resistant *Staphylococcus aureus* (MRSA) has gained global attention as a human pathogen in hospital and in communities. Recent report of MRSA infection and colonization of patients have involved wound and post-operative infections (Weese& Lefebvre, 2007). Most patients are probably infected or colonized as a result of contact with contaminated environment or affected people. However, once infected or colonized, it can be passed to other pets or to people (Weese*et al*., 2006). Infection with methicillin –resistant *Staphylococcus aureus* may be more difficult to treat and predispose to increase morbidity and mortality in affected patients (Van Duijkeren, *et al*., 2004)

Even though different studies have shown that the burden of MRSA constitutes a major public health problem (Kahsay et al., 2014; Godebo et al., 2013;Dilnessa&Bitew, 2-16), prevention and control strategies are not well established to minimize MRSA. In addition, antibiotics are still widely and inappropriately used resulting in increased prevalence of drug resistance strain bacteria such as MRSA, The study on the prevalence of these pathogens and their antimicrobial

susceptibility patterns will give updated information necessary for the management of patients and timely monitoring of the emergence of resistant bacteria. In general ,the current study results can also be used as baseline data to establish a guideline to minimize the burden of MRSA.

The study was designed to investigate the prevalence of methicillin-resistance and methicillin susceptible *Staphylococcus aureus* (MRSA and MSSA) among patients in post COVID-19 era.

Materials and Method.

Study design

This was a hospital based cross-sectional study conducted in three hospitals located in Jos, Plateau state namely; the National Veterinary Research Institute (NVRI) Clinic, Evangelical Church of West Africa (ECWA) Clinic and Jos University Teaching Hospital (JUTH). The study population comprised of 200 patients attending either of the three aforementioned hospitals.

Sample collection

Ear and cutaneous wound swabs were collected randomly from 200 patient attending either of the three hospitals using sterile swab sticks moistened with buffered peptone broth. Sixty (60) samples were collected from NVRI clinic Vom, eighty (80) samples from ECWA clinic Bukuru and sixty (60) samples from Jos University teaching hospital (JUTH). All collected specimens were labeled and transported immediately to the laboratory for processing.

Cultural procedure

Each swab sample was inoculated into brain heart infusion broth and incubated at 37^{o}C for 24 hours. After 24 hours, each broth culture sample was aseptically applied to a small area of nutrient plates plus 7% Nacl and blood agar plate whose surface has been dried in the incubator shelf at 37^{o}C for ten minutes prior to use. Each inoculum was aseptically streaked out from the well to obtain discrete colonies. The plates were then incubated aerobically at 37^{o}C for 24 hours. The characteristics golden yellow colonies were aseptically isolated and sub-cultured on mannitol salt agar. All plates were incubated in aerobic atmosphere at 35–37°C for 24h. *Staphylococcus aureus* was identified based on established microbiological methods that include colonial morphology, Gram stain, hemolysis and biochemical characteristics (Cheesbrough, 2004). Isolates that were Gram positive cocci in clusters, catalase positive, coagulase positive and DNase test positive were considered as *Staphylococcus aurerus*.

Detection of MRSA

The MRSA isolates were identified by growth on mannitol salt Agar (MSA) containing 4µg/ml oxacillin (CLSI, 2014).Four oxacillin-resistant *Staphylococcus aureus* isolates were tested for the mecA gene–a molecular marker of methicillin resistance in *Staphylococcus aureus* by modified KirbyBauer disc diffusion method using cefoxitin (30µg) disc (CLSI, 2014). Isolates resistant to cefoxitin were noted as MRSA and susceptible one as MSSA.

Antimicrobial susceptibility by disc diffusion method

The antimicrobial susceptibility testing was carried out using Kirby–Bauer's disc diffusion method according to clinical and laboratory standards institute (CLSI) 2015 guidelines (CLSI, 2015). All identified MRSA isolates were subjected to in vitro antibiotic susceptibility tests by the modified Kirby-Bauer disc diffusion method recommended by CLSI guidelines (CLSI, 2014). The antibiotics tested were penicillin G (10U), gentamicin (10µg), erythromycin (15µg), ciprofloxacin (5µg), tetracycline (30µg), clindamycin (2µg), cotrimoxazole (1.25/23.75µg), chloramphenicol (30µg) and linezolid (30µg). A standard inoculum was prepared by direct colony suspension in saline and compared with 0.5 McFarland standard turbidity and inoculated on Muller Hinton agar plate (OXOID UK). Antibiotic discs were applied to the inoculated MHA plates and incubated at 35 °C for 18–24 h. After incubation, the zone of inhibition around the discs were noted, and the results were interpreted according to the CLSI guidelines as sensitive, intermediate, or resistant (CLSI 2014). Standard strains of *Staphylococcus aureus* (ATCC25923) obtained from NAFDAC JOS were used as controls on the biochemical tests and agar plates including MHA with antimicrobial discs to assure the testing performance of antimicrobial discs.

Data analyses

Data obtained were analysed using SPSS version 22.0 for Windows. Chi square test or Fisher's exact test was used where applicable to compare the proportions of categorical variables. P value <0.05 was considered as statistically significant.

Results

Socio-demographic Characteristics of Study Participants.

A total of 200 participants were included in this study. Of these, 112 (56%) were male while 88 (44%) were female. The mean ages of the study participants were 32.8 ± 12.4 years (range from 5 to 70 years). A greater percentage (74, 37%) had primary education, majorities also lived in urban areas (128, 64%) (Table 1).

Table 1: Socio-demographic characteristics of the study participants

Characteristics		Frequency	Percentage (%)
Sex	Male	112	56
	Female	88	44
Age (years)	5 – 14	16	8
	15 – 24	51	25.5
	25 – 34	60	30
	35 – 44	35	17.5
	45 – 54	18	9

	≥55	20	10
Residence	Rural	72	36
	Urban	128	64
Educational status	No formal	26	13
	Primary	74	37
	Secondary	63	31.5
	Tertiary	37	18.5

Prevalence of MRSA and MSSA.

Out of 200 patients, a total of 135 isolates were identified as *Staphylococcus aureus*. Of the total *Staphylococcus aureus*, 36 (26.7%) were identified as Methicillin-Resistant *Staphylococcus aureus* (MRSA) while 99 (73.3%) were Methicilin-Susceptible *Staphylococcus aureus* (MSSA). The overall prevalence of MRSA and MSSA among the study population were found to be 13.6% and 37.5% respectively. According to socio-demographic characteristics of the study participants, MRSA and MSSA were highest among patients aged 25-34 years. Male patients also recorded higher prevalence of MRSA and MSSA (72.22% and 68.69% respectively). Patients who resided in urban areas had higher prevalence of MRSA and MSSA than those in rural areas. MRSA and MSSA were also highest among patients with primary education (33.33% and 36.36% respectively) and those with no history of antibiotic use. There was no significant difference in the proportion of MRSA and MSSA according to demographic characteristics ($p>0.05$). The overall prevalence of MRSA and MSSA among the study population were found to be 13.6% and 37.5% respectively. According to sample source, the prevalence of MRSA and MSSA was higher in cutaneous wound swab (55.6% and 52.5% respectively). With respect to hospital, ECWA church clinic Bukuru showed the highest prevalence of MRSA (38.9%) while Jos University Teaching Hospital showed the highest prevalence of MSSA (37.4%) (Table 3). There was no significant difference in the proportion of MRSA and MSSA according to sample source and hospital type ($p>0.05$).

Table 2: Prevalence of MRSA and MSSA among study participants according to their socio-demographic characteristics

Characteristic		MRSA	MSSA	X^2	p-value
Age	5 – 14	1 (2.78)	14 (14.14)	8.68	0.1225
	15 – 24	8 (22.22)	17 (17.17)		
	25 – 34	13 (36.11)	23 (23.23)		
	35 – 44	9 (25)	16 (16.16)		

	45 – 54	2 (5.56)	11 (11.11)		
	≥55	3 (8.33)	18 (18.18)		
Sex	Male	26 (72.22)	68 (68.69)		0.8330
	Female	10 (27.78)	31 (31.31)		
Residence	Rural	12 (33.33)	32 (32.32)		>0.9999
	Urban	24 (66.67)	67 (67.68)		
Education	No formal	6 (16.67)	14 (14.14)	0.3598	0.9484
	Primary	12 (33.33)	36 (36.36)		
	Secondary	10 (27.78)	30 (30.30)		
	Tertiary	8 (22.22)	19 (19.19)		
History of antibiotic use	Yes	11 (30.56)	38 (38.38)		0.4272
	No	25 (69.44)	61 (61.62)		

Table 3: Prevalence of MRSA and MSSA among study participants according to ample source and hospital type

Characteristic		MRSA	MSSA	X^2	p-value
Sample source	Ear swab	16 (44.44)	47 (47.47)		0.8461
	Wound swab	20 (55.56)	52 (52.53)		
Hospital	NVRI clinic Vom	12 (33.33)	28 (28.28)	1.079	0.5831
	ECWA clinic Bukuru	14 (38.89)	34 (34.34)		
	Jos University Teaching Hospital	10 (27.78)	37 (37.37)		

Antibiotic Resistance Pattern of MRSA and MSSA

Out of the 36 MRSA isolates, all the isolates showed total resistance (100%) to penicillin, 17 (47.2%) resisted gentamycin, 14 (38.9%) resisted erythromycin, 20 (55.6%) were resistant to ciprofloxacin, 12 (33.3%) were resistant to tetracycline, 6 (16.7%) clindamycin, 18 (50%) were resistant to cotrimoxazole while 10 (20%) were resistant to chloramphenicol. Out of the 99 isolated MSSA, 82 (82.8%) were resistant to penicillin, 11 (11.1%) were resistant to gentamycin, 12 (12.1%) were resistant to erythromycin, 15 (15.2%) were resistant to ciprofloxacin, 9 (9.1%) were resistant to

tetracycline, 5 (5.1%) were resistant to clindamycin,13 (13.1%)were resistant to cotrimoxazole while 7 (7.1%) were resistant to chloramphenicol. (Table 4)

Table 4: Antibiotic resistance patterns of MRSA and MSSA isolates

Antibiotic	MRSA n=36	MSSA n=99
Penicillin	36 (100%)	82 (82.8%)
Gentamycin	17 (47.2%)	11 (11.1%)
Erythromycin	14 (38.9%)	12 (12.1%)
Ciprofloxacin	20 (55.6%)	15 (15.2%)
Tetracyclin	12 (33.3%)	9 (9.1%)
Clindamycin	6 (16.7%)	5 (5.1%)
Cotrimoxazole	18 (50%)	13 (13.1%)
Chloramphenicol	10 (20%)	7 (7.1%)

Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been proven to be one of the most world wide spread nosocomial and community pathogen of the 21st century (Huijsdens, *et al*., 2000; Cosgrove, *et al*., 2003) and it's increasingly developing resistance to many antibiotics (Lowry, 2003). The present study determined the prevalence of MRSA and MSSA among patients attending hospitals in Jos, Plateau state. The overall prevalence of MRSA and MSSA among the study population were found to be 13.6% and 37.5% respectively. These findings are consistent with reports from other studies (Troille*et al.,* 1998; Davis *et al.,* 2004; Lucet*et al.,* 2005) but higher than the reports of Tsige *et al.*, (2020).

We observed a high proportion of MRSA (72.2%) and MSSA (68.7%) among male patients compared to female patients. The prevalence reported here is comparable with reports from previous studies conducted by Joachim *et al.*, (2017). This could be attributed to gender differences in behavioral practices and hygiene such as hand washing and use of soap or playing contact sports and occupation, which may influence MRSA and MSSA colonization.

Previous studies had reported that exposure to antibiotic was associated with risk of MRSA colonization (Tacconelli*et al.,* 2008; Baraboutis *et al.,* 2011). Our findings showed a trend of but non-significantly higher MRSA and MSSA among patients with previous exposure to antibiotics. This could be due to our small sample size. Alternatively, there is a possibility that some of the patients may have not recalled properly the information on antibiotic use for the past 3 months and even for those who reported some could not mention the name of antibiotic or type of drug used thus underestimating the role of this factor as risk for MRSA and MSSA acquisition.

The resistance profile of MRSA and MSSA recorded in this study is similar to the reports

of other studies (Kahsay et *al.,* 2014). Concerning the antimicrobial resistance profile of the isolates, in the present study, MSSA isolates showed resistance to antibiotics similar to the reports of previous studies by Kahsay *et al.,* (2014). The current study showed MRSA isolates were 100% resistant to penicillin. This finding is in unison with previous studies which reported 100% resistance to penicillin by MRSA (Tsige*et al.,* 2020). Resistance to other antibiotics differed from the reports of previous studies. The main variation in drug resistance patterns among different studies might be due to the indiscriminate use and availability of these antibiotics in a certain area. The variation of resistance rate among different areas indicates that resistance pattern of antibiotics varies according to regional and geographical location and also changes through time.

Conclusion

The study has established the existence of Methicillin –resistant and susceptible *Staphylococcus aureus* infections in patients attending hospital in the post COVID 19 era with an overall prevalence of 13.3%. The MRSA isolated showed multiple drug resistance to beta-lactams commonly prescribed antibiotics.The high prevalence of MRSA and the increased rates of resistance to commonly used antimicrobials among MRSA isolates call for attention to the importance of including the screening of MRSA in our hospitals setting in order to prevent further spread of MRSA strains to other patients and to the communities. Control and prevention strategies should be emphasized including decolonization of careers.

References

Agaba, P., Tumukunde, J., Tindimwebwa, J. V. B., &Kwizera, A. (2017). Nosocomial bacterial infections and their antimicrobial susceptibility patterns among patients in Ugandan intensive care units: a cross sectional study. *BMC research notes*, 10(1), 349.

Baldan, R., Din, C. T., Semeraro, G., Costa, C., Cichero, P., Scarpellini, P. &Cirillo, D. M. (2009). Severe community-onset infections in healthy individuals caused by community-acquired MRSA in an Italian teaching hospital, 2006–2008.*Journal of Hospital Infection*, *72*(3), 271-273.

Baraboutis, I. G., Tsagalou, E. P., Papakonstantinou, I., Marangos, M. N., Gogos, C., Skoutelis, A. T, & Johnson, S. (2011). Length of exposure to the hospital environment is more important than antibiotic exposure in healthcare associated infections by methicillin-resistant Staphylococcus aureus: a comparative study. *Brazilian Journal of Infectious Diseases*, *15*(5), 426-435.

Barrow, G., &Feltham, R. K. A. (1993).Manual for the identification of medical bacteria. SteeleCa, ed.

Boyle-Vavra, S., &Daum, R. S. (2007). Community-acquired methicillin-resistant Staphylococcus aureus: the role of Panton–Valentine leukocidin. *Laboratory investigation*, 87(1), 3-9.

Carleton, H. A., Diep, B. A., Charlebois, E. D., Sensabaugh, G. F., &Perdreau-Remington, F. (2004). Community-adapted methicillin-resistant Staphylococcus aureus (MRSA): population dynamics of an expanding community reservoir of MRSA. *The Journal of infectious diseases*, 190(10), 1730-1738.

Cheesebrough, m. (2004): District Laboratory Practice in tropical countries part 2, Cambridge University Press, United Kingdom. pp; 434.

Cosgrove, S. E., Sakoulas, G., Perencevich, E. N., Schwaber, M. J., Karchmer, A. W., & Carmeli, Y. (2003). Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. *Clinical infectious diseases*, 36(1), 53-59.

Davis, K. A., Stewart, J. J., Crouch, H. K., Florez, C. E., &Hospenthal, D. R. (2004). Methicillin-resistant Staphylococcus aureus (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection.*Clinical Infectious Diseases*, *39*(6), 776-782.

Dilnessa, T. &Bitew, A. (2016). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*isolated from clinical samples at Yekatit 12 hospital medical college, Addis Ababa, Ethiopia," *BMC Infectious Diseases*, 16 (398), 1–9.

Emmerson, M. (1994).Nosocomial staphylococcal outbreaks. *Scandinavian journal of infectious diseases.Supplementum*, 93, 47-54.

Fraimow, H. S., &Abrutyn, E. (1995). Pathogens resistant to antimicrobial agents. Epidemiology, molecular mechanisms, and clinical management. *Infectious disease clinics of North America*, 9(3), 497-530.

Godebo, G., Kibru, G., &Tassew, H. (2013). Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. *Annals of clinical microbiology and antimicrobials*, *12*(1), 17.

Hendaus, M. A., Jomha, F. A., &Alhammadi, A. H. (2015). Virus-induced secondary bacterial infection: a concise review. *Therapeutics and clinical risk management*, 11, 1265.

Hiramatsu, K., Cui, L., Kuroda, M., & Ito, T. (2001).The emergence and evolution of methicillin-resistant Staphylococcus aureus.*Trends in microbiology*, *9*(10), 486-493.

Huijsdens, X. W., van Lier, A. M., Van Kregten, E., Verhoef, L., van Santen-Verheuvel, M. G., Spalburg, E., &Wannet, W. J. (2006). Methicillin-resistant Staphylococcus aureus in Dutch soccer team. *Emerging Infectious Diseases*, 12(10), 1584.

Kahsay, A., Mihret, A., Abebe, T., &Andualem, T. (2014). Isolation and antimicrobial susceptibility pattern of Staphylococcus aureus in patients with surgical site infection at DebreMarkos Referral Hospital, Amhara Region, Ethiopia. *Archives of public Health*, *72*(1), 16.

Kiehlbauch, J. A., Hannett, G. E., Salfinger, M., Archinal, W., Monserrat, C., &Carlyn, C. (2000). Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. *Journal of clinical microbiology*, 38(9), 3341-3348.

Klevens, R. M., Morrison, M. A., Nadle, J., Petit, S., Gershman, K., Ray, S. & Craig, A. S. (2007). Invasive methicillin-resistant Staphylococcus aureus infections in the United States. *Jama*, 298(15), 1763-1771.

Lina, G., Piémont, Y., Godail-Gamot, F., Bes, M., Peter, M. O., Gauduchon, V. & Etienne, J. (1999). Involvement of Panton-Valentine leukocidin—producing Staphylococcus aureus in primary skin infections and pneumonia.*Clinical infectious diseases,* 29(5), 1128-1132.

Lobanovska, M., &Pilla, G. (2017). Focus: drug development: penicillin's discovery and antibiotic resistance: lessons for the future? *The Yale journal of biology and medicine*, *90*(1), 135.

Lowy, F. D. (1998). Staphylococcus aureus infections. *New England journal of medicine*, 339(8), 520-532.

Lowy, F. D. (2003). Antimicrobial resistance: the example of Staphylococcus aureus. The Journal of clinical investigation, 111(9), 1265-1273.

Lucet, J. C., Grenet, K., Armand-Lefevre, L., Harnal, M., Bouvet, E., Regnier, B., & Andremont, A. (2005). High prevalence of carriage of methicillin-resistant Staphylococcus aureus at hospital admission in elderly patients: implications for infection control strategies. *Infection Control & Hospital Epidemiology*, *26*(2), 121-126.

Moreno, F., Crisp, C., Jorgensen, J. H., & Patterson, J. E. (1995).Methicillin-resistant Staphylococcus aureus as a community organism.*Clinical Infectious Diseases*, 21(5), 1308-1312.

Nester, E. W., Denise, G. A., Evan, C., Roberts, J. R., Nancy, N. R., & Martha, T. N. (2001). Microbiology: A human perspective 3rd edition. Published by Churchill Livingstone Pennsylvania, 840-848.

Patel, J. B., Cockerill, F. R., & Bradford, P. A. (2015). Performance standards for antimicrobial susceptibility testing: twenty-fifth informational supplement.

Sievert, D. M., Rudrik, J. T., Patel, J. B., McDonald, L. C., Wilkins, M. J., & Hageman, J. C. (2008). Vancomycin-resistant Staphylococcus aureus in the United States, 2002–2006. *Clinical Infectious Diseases*, *46*(5), 668-674.

Snyder, J. W., McDonald, L. C., & Van, R. E. (2000). Common bacteria whose susceptibility to antimicrobials is no longer predictable. *Le Journal medical libanais. The Lebanese medical journal*, *48*(4), 208-214.

Tacconelli, E., De Angelis, G., Cataldo, M. A., Pozzi, E., &Cauda, R. (2008). Does antibiotic exposure increase the risk of methicillin-resistant Staphylococcus aureus (MRSA)

isolation? A systematic review and meta-analysis. *Journal of antimicrobial chemotherapy*, *61*(1), 26-38.

Troillet, N., Carmeli, Y., Samore, M. H., Dakos, J., Eichelberger, K., DeGirolami, P. C., & Karchmer, A. W. (1998). Carriage of methicillin-resistant Staphylococcus aureus at hospital admission.*Infection Control & Hospital Epidemiology*, *19*(3), 180-185.

Tsige, Y., Tadesse, S., Tefera, M. M., Amsalu, A., Menberu, M. A., &Gelaw, B. (2020). Prevalence of Methicillin-Resistant Staphylococcus aureus and Associated Risk Factors among Patients with Wound Infection at Referral Hospital, Northeast Ethiopia. *Journal of Pathogens*, *2020*.

Tuo, P., Montobbio, G., Vallarino, R., Tumolo, M., Calevo, M. G., Massone, M. L., &Mantero, E. (1995). Nosocomial infection caused by multiresistant Staphylococci in a neonatal and pediatric intensive care unit. *La Pediatriamedica e chirurgica: Medical and surgical pediatrics*, 17(2), 117-122.

Van Duijkeren, E., Box, A. T. A., Heck, M. E. O. C., Wannet, W. J. B., &Fluit, A. C. (2004). Methicillin-resistant staphylococci isolated from animals. *Veterinary microbiology*, 103(1-2), 91-97.

Velavan, T. P., & Meyer, C. G. (2020).The COVID-19 epidemic. *Tropical medicine & international health*, 25(3), 278.

Weese, J. S., & Lefebvre, S. L. (2007). Risk factors for methicillin-resistant Staphylococcus aureus colonization in horses admitted to a veterinary teaching hospital. *The Canadian Veterinary Journal*, 48(9), 921.

Weese, J. S., Dick, H., Willey, B. M., McGeer, A., Kreiswirth, B. N., Innis, B., & Low, D. E. (2006). Suspected transmission of methicillin-resistant Staphylococcus aureus between domestic pets and humans in veterinary clinics and in the household. *Veterinary microbiology*, 115(1-3), 148-155.