DOI: https://doi.org/10.53350/pjmhs202317438

ORIGINAL ARTICLE

Prevalence of Methicillin Resistance *Staphylococcus aureus* in Community Cases of Pyoderma

BAHRAM KHAN KHOSO[1], FAKHUR UDDIN[2], FAIZA ZEESHAN[3], MUHAMMAD ANWAR[4], MIR ALAM KHAN[5]
[1]*Assistant Professor of Dermatology Department, JPMC/JSMU, Karachi*
[2]*Medical Technologist, Department of Microbiology, BMSI, JPMC, Karachi*
[3]*Lecturer, Department of Pathology, JSMU, Karachi*
[4]*Associate Professor of Pathology, JSMU/JPMC, Karachi*
[5]*Medical Technologist, Department of Microbiology, BMSI, JPMC, Karachi*
Correspondence to Dr. Bahram Khan Khoso, Email: drbahramk@gmail.com, Cell: 03337343450

ABSTRACT

Aim: To determine the susceptibility pattern of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates of pyoderma cases.
Study design: Cross-sectional.
Place and duration: Research was conducted in the department of Microbiology in collaboration with the Department of Dermatology, Jinnah Postgraduate Medical Centre, Karachi, during the period of 1.8.2022 to 25.11.2022.
Methodology: Total232 pus samples were collected from the clinically suspected patients of pyoderma. Pus swab was cultured on blood and MacConkey's agar plates. Isolates were identified by routine assay and antimicrobial susceptibility was determined by disc diffusion method.
Results: Out of 232 specimens, 196 (84.5%) were culture positive; 170(86.7%) were Gram-positive, 25(12.7%) were Gram-negative and 1(0.6%) was *Candida* sp. *S.aureus* was the prevalent offender (119; 51.2%), followed by *S.pyogenes* (51; 22%) and *S.epidermidis* (3; 1.3%). *S.aureus* showed 73(61.3%) MRSA. The highest rate of resistance among all *S. aureus* was recorded for, penicillin, cefoxitin and ciprofloxacin, 89; 74.7% 73; 61.3% and 72; 60.5% respectively.
Conclusion: *S.aureus* is the prevalent in pyoderma and a significant number was MRSA. Therefore it is mandatory for dermatologist to start the treatment after culture and sensitivity testing is mandatory for failure of treatment and recurrences.
Keywords: Methicillin-Resistant Staphylococcus aureus, Pyoderma, S. aureus,Streptococcus pyogenes.

INTRODUCTION

Pyoderma or pyogenic infection of the skin is the purulent skin disease considered as one of the most common conditions observed in dermatological outpatient department.[1]Pyoderma can be classified as primary and secondary on the basis of mode of acquisition. The primary pyoderma is caused by direct invasion of intact and healthy skin[2]. Impetigo, ecthyma, carbuncle, folliculitis, furunculosis, peri-poritis and infected scabies are some of the clinical types of primary pyoderma[3]. The secondary type is associated with superimposed bacterial infection on an underlying skin disorder; include infected superficial ulcer, infected pemphigus and contact dermatitis, scabies associated with superimposed bacterial infections, and various other dermatoses. Protein deficiency, poor hygiene, under privileged socioeconomic status, obesity, immunosuppressive conditions such as diabetes mellitus, malignancies can augment the risk for pyoderma infections.[4] Most of the pyodermic infections are caused by *Staphylococcus aureus* and Group A *Streptococci.* However, the clinical, profile is in constant momentum which is responsible for increasing resistance to antibiotics[5].

Staphylococcus aureus is a potential pathogen in pyoderma.[2] Emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA) has now become a burning issue. All beta-lactams including carbapenems, high-end cephalosporins, piperacillin-tazobactam,etc have become abortive against MRSA.[6] The prevalence of MRSA with pyoderma is between 6 to 16% in various parts of the globe.[7] The association of MRSA with pyoderma has now become a night mare as far as its chronicity, recurrence, and complications are concerned. Therefore, prompt bacterial diagnosis with antimicrobial susceptibility is pivotal for the fruitful management and treatment of pyoderma. Bacterial profile and antibiotic sensitivity is variable from region to region. Knowledge of prevalence of MRSA and their current anti-microbial profile becomes necessary in the selection of appropriate treatment of this cumbersome condition.

Received on 19-10-2022
Accepted on 29-03-2023

MATERIAL AND METHODS

Study setting: This cross sectional prospective study was conducted in the Department of Microbiology in collaboration with the Department of Dermatology, Jinnah Postgraduate Medical Centre, Karachi. The study was conducted after getting approval from the Institutional Review Board (IRB) of Jinnah Postgraduate Medical Centre (NO. F.2-81/2022-GENL/212/JPMC). The procedure was thoroughly explained to the patients prior to specimen collection. It was a cross sectional cross-sectional non-probability convenient sampling technique was implied.
Sample size and duration: The sample size was calculated by using Open EPI menu (version 3) which is found to be 232 by taking the frequency of CA-MRSA as 19%, at the confidence interval of 95% and with 5% bound of error.[8]The samples were collected during the 1.8.2022 to 25.11.2022.
Inclusion and exclusion criteria: The study subjects were the individuals who visited dermatology outpatient department with any form of the pyoderma. The hospitalized patients and patients on antibiotics were excluded from this study.
A pre designed questionnaire was filled by asking questions related to demographics including age, gender, residence, marital status and occupation. Relevant clinical history was also recorded comprising of comorbidity, type of pyoderma, duration and location of lesions and associated signs and symptoms.
Microbial methods: The samples were collected by using sterile swabs. Two swabs were taken from the representative lesion. Intact pustules were first cleaned with 70% alcohol. They were then ruptured with a sterile needle and the expressed pus was collected on two sterile cotton swabs. In case of ulcers and crusted lesions, normal saline was used to clean the wound. Smears were prepared using pus from the first swab and stained by Gram's staining method. They were examined for the type and number of bacteria. Catalase and coagulase tests were performed. The pus from the second swab was inoculated on blood agar and MacConkey's agar. All isolates were tested for their antibiotic susceptibility by Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standard Institute guidelines (CLSI, 2022).[8] To check sensitivity against methicillin resistant *Staphylococcus aureus* (MRSA), cefoxitin disk of 30µg was placed on Mueller-Hinton agar, and plates were incubated for 24 hours. The sensitivity was checked by considering the sensitivity zone. For

cefoxitin a zone of ≥25µg was considered as sensitive and ≤24µg will be taken as resistant. The sensitivity for gentamicin, erythromycin, tetracycline, ciprofloxacin, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol, rifampicin and linezolid were also observed according to the proposed cut-off points by CLSI (2022)[8].

Statistical analysis: The data was recorded in SPSS version 21. For calculation of frequencies, percentages, mean and standard deviation, descriptive statistics were used. Association between variables was determined by chi square test.

RESULTS

Out of 232 patients of pyoderma, 157(67.7%) were males and 75(32.3%) were females with male to female ratio of 2:1. Majority of the individuals were unmarried 126(54.3%). A majority of patients fall in the age group of 11-20 years 67(28.9%), while least affected age group was elderly above 60 years 3(1.3%) (Table 1). Pyoderma was commonly observed among students 86(37.1%) and labours 52(22.4%), Localized lesions were noted in 204(87.9%) individuals, the most common lesion was superficial folliculitis 64(27.6%), followed by furuncle 73(27.2%). The most commonly affected site was lower limb and foot 88(37.9%).

Upon processing the samples, 196(84.5%) samples were culture positive and 36(15.5%) did not show any bacterial growth. Out of culture positive samples, 170(86.7%) were Gram-positive bacteria, 25(12.7%) were Gram-negative and 1(0.6%) was a fungal isolate. Among Gram-positive bacteria, *Staphylococcus aureus* was the most common offender 119(51.2%), followed by *Streptococcus pyogenes* 51(22%) and *Staphylococcus epidermidis* 3(1.3%). Among the Gram-negative *Pseudomonas aeruginosa* 13(5.6%) and *E. coli* 12(5.2%) were isolated. *Candida* species was observed in 1(0.4%) case.

The antibacterial susceptibility testing of *Staphylococcus aureus* showed 73(61.3%) methicillin resistant *Staphylococcus aureus* (MRSA) and 46(38.6%) methicillin susceptible *Staphylococcus aureus* (MSSA). The highest rate of resistance among all *S. aureus* was recorded for, penicillin, cefoxitin and ciprofloxacin, 89; 74.7% 73; 61.3% and 72; 60.5% respectively. The resistance against rifampin, linezolid, chloramphenicol, clindamycin, gentamicin and ceftaroline was, 8(6.7%), 8(6.7%), 13(10.9%), 13(10.9%), 20(16.8%) and 22(18.4%) respectively (Figure 2). The resistance pattern of MRSA and MSSA was analysed that showed the resistance to all antimicrobial agents was statistically higher in MRSA in comparison with MSSA ($P \leq 0.05$), however there was no significant difference in case of gentamicin (Table 2).

Figure 1: Age group distribution of study population (n=232)

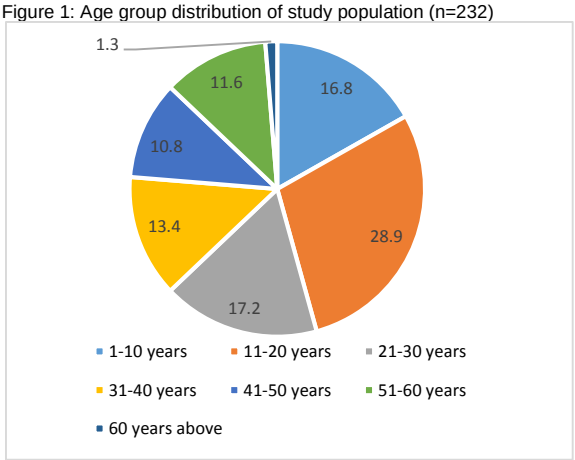

Figure 2: Resistant pattern of *S. aureus* against common antimicrobial agents (n=119)

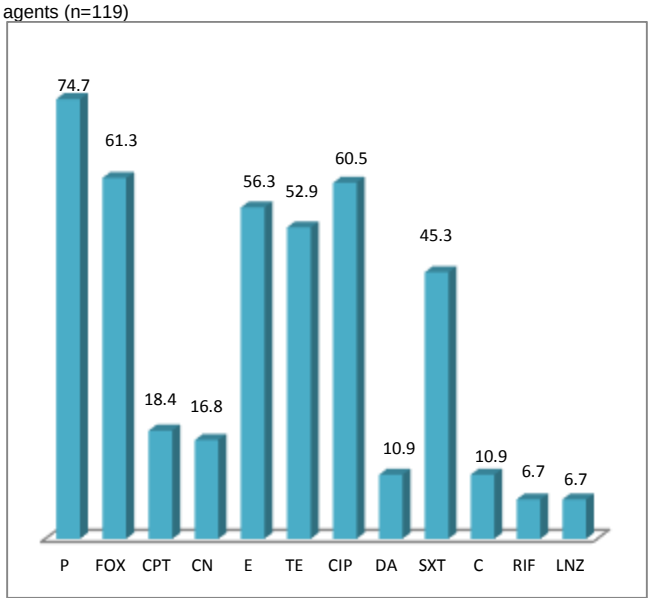

Table 1: Baseline traits of study population (n=232)

Baseline traits		**n**
Gender	Male	157 (67.7%)
	Female	75 (32.3%)
Residence	Karachi	152 (65.5%)
	Baluchistan	45 (19.4%)
	Sukkur	14 (6.0%)
	Other	21 (9.0%)
Marital status	Married	106 (45.7%)
	Unmarried	126 (54.3%)
Occupation	Students	86 (37.1%)
	Labor	52 (22.4%)
	Office staff	32 (13.8%)
	Factory workers	12 (5.2%)
	Others	50 (21.5%)
Type of lesions	Generalized	28 (12.1%)
	Localized	204 (87.9%)
Primary pyoderma	Superficial folliculitis	64 (27.6%)
	Furunculosis	69 (27.6%)
	Impetigo	24 (10.3%)
	Carbuncle	18 (7.8%)
	Cellulitis	10 (4.3%)
	Open wounds and ulcer	9 (3.8%)
Secondary pyoderma	Infected leishmaniasis	13 (5.6%)
	Infected scabies	13 (5.6)
	Dermatophytoses	7 (3.0%)
	Eumycetoma	5(2.2%)
Site of lesion	Low limb	92(39.6%)
	Back	41(17.6%)
	Upper lim	22(9.4%)
	Scalp	18(7.7%)
	Other	59(25.4%)

Table 2: Comparative analysis of antibiotic susceptibility pattern of MRSA and MSSA (n=119)

Antimicrobial drugs	Drug Susceptibility	MRSA	MSSA%	Total%	p-value*
Ceftaroline	Susceptible	54(74.0)	43(93.5)	97(81.51)	0.007
	Resistant	19(26.0)	3(6.5)	22(18.49)	
Gentamicin	Susceptible	58(79.5)	41(89.1)	99(83.19)	0.130
	Resistant	15(20.5)	5(10.9)	20(16.81)	
Erythromycin	Susceptible	27(37.0)	25(54.3)	52(43.70)	0.04
	Resistant	46(63.0)	21(45.7)	67(56.30)	
Tetracycline	Susceptible	28(38.4)	28(60.9)	56(47.05)	0.01
	Resistant	45(61.6)	18(39.1)	63(52.95)	
Ciprofloxacin	Susceptible	15(28.8)	32(18.2)	47(39.50)	0.0001
	Resistant	58(44.2)	14(27.8)	72(60.50)	
Clindamycin	Susceptible	60(82.2)	46(100)	106(89.07)	0.001
	Resistant	13(17.8)	0(00)	13(10.93)	
Trimethoprim-sulfamethoxazole	Susceptible	28(38.4)	37(80.4)	65(54.62)	0.0001
	Resistant	45(33.1)	9(20.9)	54(45.38)	
Chloramphenicol	Susceptible	60(82.2)	46(100)	106(89,07)	0.001
	Resistant	13(17.8))	0(00)	13(10.93)	
Rifampicin	Susceptible	65(89.0)	46(42.9)	111(93.28)	0.01
	Resistant	8(11.0)	0(00)	8(6.72)	

*P value ≤0.05 is considered as statistically significant

DISCUSSION

According to the results of current study, the pyoderma was frequently seen in male population. The adolescents falling in age group of 11-20 years were more commonly affected. Our results are in complete agreement former study showing the male dominancy and majority of the subjects were under the age of 20 years. Primary pyoderma cases are common in patients under the age of 20 years. This has been related to under developed epidermal barrier in children and adolescents. A male predominance was noted, consistent with other studies[9-10]. This could be due to higher probability of occupational hazards and exposure to bacteria in males. There is evidence that males harbour higher numbers of aerobes than females[7]. Furunculosis and superficial folliculitis were the most commonly observed lesions in our setting. This is in accordance with the results reported from India[9], Africa[11] and Iran[12].

The emerging threat of antibiotic resistance from multiple drug resistant (MDR) to extremely drug resistance (XDR) and pan drug resistance (PDR) has arisen serious concerns about local epidemiology and clinical practices. Our study focuses the antibacterial susceptibility patterns of various antibiotics for MRSA which is involved in community acquired infections. The current study highlights the increased prevalence of MRSA. Similar results were observed by Thomas et al[13], Kamarehei et al[12] and Patil et al[2.] The highest rate of resistance in MRSA and MSSA was seen with erythromycin. According to our results, 63% MRSA and 45.7% MSSA isolates were resistant to erythromycin. Similar results were reported by Regmi et al from Nepal[14], according to which 63.9% *Staphylococcus aureus* were resistant to erythromycin. However, Azimi et al[15] reported contradictory results; according to their observation only 8% isolates were resistant to erythromycin. Rapid transmission of *erm* genes responsible for macrolides resistance, this has plunged down the use of macrolides particularly erythromycin[13].

In the current study, resistance to ciprofloxacin was found to be 44.2% among MRSA and 27.8% MSSA isolates. These results are in accordance with Cabrera et al who reported moderate level of resistance[16]. On the other hand, a study by Elsyedet al[17] demonstrated contradictory results, in their study, 76% MRSA were resistant to ciprofloxacin. The resistance to ciprofloxacin has been associated with mutated efflux pump *norA*, *norB*, and *norC* genes[17]. Ciprofloxacin is an efficacious drug which is still the choice of treatment for several infections; nonetheless its effect on MRSA has now become questionable.

Another paramount therapeutic agent for soft tissue infections is Trimethoprim-sulfamethoxazole (SXT). Among MRSA group, around 33.1% isolates showed resistance to SXT while 20.9% MSSA isolates were resistant to SXT. According to Gitau et al, higher level of resistance (56.9%) to SXT was observed in their study from Kenya[18]. However, Klein et al from Germany reported contradictory results with low rate of resistance for SXT[19]. SXT resistance in *S. Aureus* is predominantly mediated by the accession of an extra-chromosomal dihydrofolatereductase (DHFR) encoding genes [*dfrA (synonym dfrS1)*, *dfrG*, *dfrK*], whereas intrinsic mechanisms for SXT resistance are mutations on the chromosomal dihydropteroate synthase (DHPS) encoding gene (*folP*)[20].

In comparative analysis of the MRSA and MSSA resistance pattern, the resistance to anti-staphylococcal agent was higher in MRSA isolates. Similar findings are reported in a recent study of Horváth et al[22]. The linezolid, rifampin, chloramphenicol and ceftaroline are the good choice of treatment against MRSA. The limitations of this study is that the molecular aspect of the isolates were not evaluated, which are essential and strengthening of this study. Therefore large scale with molecular determination of the virulence factors and *mecA* studies are required in future.

CONCLUSION

Staphylococcus aureus is the major pathogen of primary pyoderma in the community. The frequency of MRSA was higher and resistance to the commonly in practice antibiotics was ≥50 percent. Pus culture sensitivity and screening is essential in primary pyoderma cases. This may reduce the recurrences and spread of CA-MRSA strains.

Acknowledgement: We are thankful to the staff of Department of Microbiology, BMSI, JPMC, for their support for providing reagents and media preparation.

Disclaimer: None to declare.

Conflict of interest: None to declare.

Funding disclosure: None to declare.

Author contribution: BKK: Conception of study, Designing, Planning, Clinical and demographic data, Critical review. **FU:** Conception of study, Designing, Planning, Manuscript writing, Analysis. Interpretation, Discussion, Critical review, **FZ:** Conception of study, Manuscript writing, Critical review/Data analysis, **MA:** Conception of study, Manuscript writing, Critical review, Data analysis, **MAK:** Experimentation, Study conduction, Analysis. Interpretation, Discussion. Facilitated for reagents, Material analysis

REFERENCES

1. Parveen S, Saqib S, Ahmed A, Shahzad A, & Ahmed N. Prevalence of MRSA colonization among healthcare-workers and effectiveness of decolonization regimen in ICU of a Tertiary care Hospital, Lahore, Pakistan. **Adv Life Sci 2020;8(1):38-41.**
2. Patil S, Suresh T, Murthy C, &Ireddy S. Bacteriological study of pyodermas with an emphasis on Methicillin Resistant *Staphylococcus aureus* (MRSA) at a tertiary care centre in North Karnataka. Int J

DermatolVenereolLepSci 2021;4(1):99-102https://doi.org/10.33545/26649411.2021.v4.i1b.75.
3. Chavan K, Doshi BR, &Dohe V. Clinicobacteriological study of pyoderma with trends in antibiotic sensitivity at a tertiary care center in western India. ClinDermatol Rev 2021;*5*(2):161-67.DOI: 10.4103/CDR.CDR_67_20
4. Thomas N &Girisha BS. Bacteriological study of community-acquired pyoderma with special reference to methicillin-resistant *Staphylococcus aureus*. ClinDermatol Rev 2018; 2(1):13-18.
5. Powell FC, Su WD, & Perry HO. Pyodermagangrenosum: classification and management. J Am AcadDermatol 1996;34(3): 395-99. DOI: 10.1016/s0190-9622(96)90428-4
6. Agostino JW, Ferguson JK, Eastwood K & Kirk MD. The increasing importance of community-acquired methicillin-resistant *Staphylococcus aureus* infections Med J Austra 017; 207(9):388-93.
7. Kumar A, Soni R, Babita SS. Pyoderma-Bacteriological Profile and its Antibiotic Sensitivity Pattern: A Retrospective Study. Int J Pharmaceuti Clini Res 2022; 14(3); 447-51
8. Clinical laboratory standard institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*, M100 32nd Edition. Clinical and Laboratory Standards Institute, 2022, Wayne, PA.
9. Ghosh S, Sengupta M, Sarkar S, et al. Bacteriologic Profile Along With Antimicrobial Susceptibility Pattern of PediatricPyoderma in Eastern India. Cureus 2022;14(6): e25716. doi:10.7759/cureus.25716.
10. Jeevannavar R, Ranugha PS, Betkerur JB, Kulkarni M. *Staphylococcus aureus* nasal colonization and strain concordance in patients with community associated Staphylococcal primary pyoderma-A cross-sectional study. J Pak Ass Dermatol 2020;30(4):608-17.
11. Furtado S, Bhat RM, Rekha B, Sukumar D, Kamath GH, Martis J, et al. The clinical spectrum and antibiotic sensitivity patterns of staphylococcal pyodermas in the community and hospital. Indian J Dermatol 2014;59(2):143-49.doi: 10.4103/0019-5154.127674.
12. Armitage EP, Senghore E, Darboe S, Barry M, Camara J, Bah S, et al. High burden and seasonal variation of paediatric scabies and pyoderma prevalence in The Gambia: A cross-sectional study. PLoSNegl Trop Dis 2019;13(10):e0007801doi: 10.1371/journal.pntd.0007801
13. Kamarehei F, Rahimi-Alang S, Vaez H and Ghaemi E. Prevalence of Panton-valentine gene in *Staphylococcus aureus* isolated from clinical samples and healthy carriers in Gorgan city, north of Iran. Minerva biotecnol 2015; 27(1):51-54.
14. Thomas N, Girisha BS. Bacteriological study of community-acquired pyoderma with special reference to methicillin-resistant *Staphylococcus aureus*. ClinDermatol Review 2018; 2(1):13-19.DOI: 10.4103/CDR.CDR_21_17
15. Regmi RS, Khadka S, Sapkota S, Magar ST, Adhikari S, Subedi S, et al. Phenotypic detection of inducible clindamycin resistance among clinical isolates of Staphylococcus aureus in Bharatpur hospital. J Coll Med Sci Nepal 2020;16(3):178-83.DOI: https://doi.org/10.3126/jcmsn.v16i3.28490
16. Azimi A, Moosavi ME, Peymani A. Phenotypic and Genotypic Characterization of Methicillin and Erythromycin Resistance in *Staphylococcus aureus* Collected from Nasal Samples in Qazvin Medical Students. Electron Physician 2021; (1):7803-12 DOI: http://dx.doi.org/10.19082/7803
17. Cabrera R, Fernández-Barat L, Motos A, López-Aladid R, Vázquez N, Panigada M, et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* clinical strains from the endotracheal tubes of patients with nosocomial pneumonia. Antimicrob Resist Infect Control 2020; 9(1):1-0.https://doi.org/10.1186/s13756-020-0679-z
18. ElSayed N, Ashour M, Amine AE. Vancomycin resistance among *Staphylococcus aureus* isolates in a rural setting, Egypt Germs 2018; 8(3):134-39.doi: 10.18683/germs.2018.1140.
19. Gitau W, Masika M, Musyoki M, Museve B, Mutwiri T. Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates from clinical specimens at Kenyatta National Hospital. BMC Res Notes 2018;11(1):1-5.doi: 10.1186/s13104-018-3337-2.
20. Klein S, Menz MD, Zanger P, Heeg K, Nurjadi D. Increase in the prevalence of Panton–Valentine leukocidin and clonal shift in community-onset methicillin-resistant *Staphylococcus aureus* causing skin and soft-tissue infections in the Rhine-Neckar Region, Germany, 2012–2016. Int J Antimicrob Agents 2019;53(3):261-7.doi: 10.1016/j.ijantimicag.2018.10.026.
21. Khamash DF, Voskertchian A, Tamma PD, Akinboyo IC, Carroll KC, Milstone AM. Increasing clindamycin and trimethoprim-sulfamethoxazole resistance in pediatric*Staphylococcus aureus* infections. J Pediatric Infect Dis Soc 2019;8(4):351-3.doi: 10.1093/jpids/piy062
22. Udo EE, Boswihi SS, Mathew B, Noronha B, Verghese T. Resurgence of chloramphenicol resistance in methicillin-resistant *staphylococcus aureus* due to the acquisition of a variant florfenicol exporter (Fexav)-mediated chloramphenicol resistance in Kuwait hospitals. Antibiotics 2021;10(10):1250-56.https://doi.org/10.3390/antibiotics10101250
23. Horváth A, Dobay O, Sahin-Tóth J, Juhász E, Pongrácz J, Iván M, et al. Characterization of antibiotic resistance, virulence, clonality and mortality in MRSA and MSSA bloodstream infections at a tertiary-level hospital in Hungary: a 6-year retrospective study. Ann ClinMicrobiolAntimicrob 2020; 19(17): https://doi.org/10.1186/s12941-020-00357-z