Original Article

Microbiological profile of septic arthritis in Pakistani population – A prospective study

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ABSTRACT

Objective: The objective of this study is to observe the positive rate of aspirated synovial fluid culture, bacterial isolation and their antibiotic susceptibility against commonly used drugs in a microbiology laboratory.

Material and Methods: During a period of 6 months, 398 synovial fluid samples were analyzed using VITEK-MS for identification and tested for antibiotic susceptibility following standard recommendations.

Results: Only 22% (89) of the samples showed aerobic bacterial growth while 77.6% (309) were negative. The primary pathogen, *Staphylococcus aureus* (49.4%), exhibited resistance to Cefoxitin (56%), Co-trimoxazole (17.1%), and Clindamycin (13.6%). *Escherichia coli* and *Pseudomonas species* (10% each) were the other significant contributors. Concerningly, gram negative exhibited resistance to Ceftriaxone (68%) and Meropenem (20%), indicating a rise in antimicrobial resistance (AMR) in the community. Other less frequently isolated bacteria included *Coagulase negative staphylococcus, Streptococcus pneumoniae, Streptococcus pyogenes, Burkholderia species*, *Acinetobacter* and *Enterobacter species*. Females (57.3%) in our community were more affected than males (42.6%). Elderly patients, more than 60 years of age, are more affected (25%) than any other age group.

Conclusion: The increasing prevalence of MRSA, ESBL, and CRE poses challenges in treatment, leading to higher mortality and morbidity. Early diagnosis through PCR or culture and sensitivity, targeted or combination therapy, and implementation of an "Antibiotic Stewardship Program" can help reduce morbidity and AMR.

Keywords: Synovial fluid (SF) culture, Septic arthritis (SA), Antibiotic susceptibility in joint fluid, prevalence of septic arthritis, Microbiological profile

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INTRODUCTION

Septic arthritis (SA) is a progressive, destructive condition with potentially irreversible consequences, leading to disability, morbidity, and mortality. Incidence and clinical presentation vary depending on patient comorbidities and demographics. Low-income countries like India reports an incidence ranging between 2 to 20 cases per 100,000 people annually, while Western Europe reports 4-10 cases/ 100,000/ year, and Australia reports 29 cases/ 100,000/year [1-3]. The current prevalence in the Pakistani population remains unknown.

The acute presentation of SA necessitates expedited diagnosis. Predisposing factors include old age, diabetics, rheumatoid arthritis, recent joint surgery, prosthetic joints, and the use of immunosuppressive drugs. Diagnosis involves a combination of radiology, synovial fluid analysis, microbiological culture, and non-specific serum inflammatory markers. Global culture positivity rates are reported to be low, with our study also reporting a 22% positive rate. *Staphylococcus aureus* (*S. aureus*, 49%) emerged as the main causative pathogen in our population. Treatment for SA typically involves a 2-6week regimen, while fastidious bacteria like N. gonorrhoeae or fungal infections require extended antimicrobial courses based on history and serology [4,5,10].

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The objective of this cross-sectional study is to observe the synovial fluid culture positive rate, bacterial isolate frequencies, and antibiotic susceptibility during 6-month period (April - September 2023) at the Microbiology Department of the Chughtai Institute of Pathology, Lahore, Pakistan. Future results will be compared with synovial fluid collected in blood culture bottles to enhance pathogen recovery.

MATERIAL AND METHODS

A cross-sectional, observational study was conducted for 6 months (April to September '23) at the Chughtai Institute of Pathology (CIP) Microbiology BSL-2 laboratory after obtaining the IRB number. The sample size was calculated by using Cochran formula that allowed us to calculate the ideal minimum sample size from unknown population with a desired confidence level (Z), level of precision (e) and estimated proportion of the disease in the population. In this study by reviewing the literature (7) and expert opinion the proportion of *Staphylococcus aureus* the population is 38% with e=5% and 95% confidence level. By using this value in formula, the minimum sample size is 362 was obtained ($\eta_0 = \frac{Z^2 pq}{e^2}$).

Clinical data (Gram stain result, bacterial isolate name, antibiotic susceptibility result) and demographic data (patient age, gender, location) were collected and recorded at Microsoft excel sheet and a unique identification (serial number) was assigned to each sample. No patient identifiable variable (name, contact number etc.) was documented in data to keep confidentiality of patient. Data analysis was performed using Microsoft Excel. Graphs and tables were fabricated considering frequency and/or percentage of categorical data. Antibiotic resistance was calculated by the following formula:

$$Resistance\ percentage = \frac{No.\ of\ resistant\ isolates}{Total\ no\ of\ isolates\ tested} \times 100$$

So, for the current study total of 398 synovial fluid aspirates in a period of 6 months were processed aerobically and anaerobically for culture and sensitivity in both genders and all age groups. Anaerobic chamber (BACTRON300, Sheldon Manufacturing USA) was used to process samples anaerobically. Duplicate samples of the same patient and swabs were excluded. Bacterial isolates after incubation period of 18-24 hours with 5% CO2 at 37°C, were identified using MALDI-TOF. Antimicrobial sensitivities were tested using disc diffusion/minimum inhibitory concentration (MIC) methods on Mueller Hilton Agar (MHA) or SBMHA, following CLSI M100 guidelines, with QC strains *Escherichia coli* (ATCC25922 and ATCC35218), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923 and ATCC29213) for testing standardization. Antibiotic breakpoints were species-specific, and results were reported as sensitive (S), resistant (R), or intermediate (I), with 'I' falling between the S and R categories.

RESULTS

A total of 398 synovial fluid samples were processed in the microbiological laboratory, with 89 samples (22%) showing positive results and 309 (77.6%) were negative. Aerobic and facultative anaerobes were isolated, while no obligate anaerobic bacteria were found. All samples exhibited monobacterial growth, with 66% gram-positive cocci (GPC) and 33% gram-negative rods (GNR). Among GPCs, *Staphylococcus aureus* (49.4%) was the primary pathogen, followed by *Streptococcus viridans group* (4.4%), *Streptococcus pyogenes* (3.3%), and *Streptococcus pneumoniae* (2.2%). *S. aureus* was isolated in 49% of cases and among those 56% were found to be MRSA. Moreover *S. aureus* exhibited resistance to Clindamycin (13.6%) and Trimethoprim-sulfamethoxazole (17.1%) in our population.

Among GNR, *E. coli* and *Pseudomonas species* (10% each) made significant contributions to SA. *Klebsiella, Burkholderia species* (3% each), and *E. cloacae* (2%) were also isolated (chart-1). Resistance to Ceftriaxone (ESBLs) was observed in 68% of GNR, while 20% exhibited resistance to Meropenem (CRE). Ciprofloxacin, a commonly used drug for both GPCs and GNR, showed 77% resistance (Table-I & II). Additionally, 25%

of adults over 60 years, 7.8% of children under 10 years, and 6.8% of young adults aged 31-40 were affected, with females being more affected than males (1.3:1).

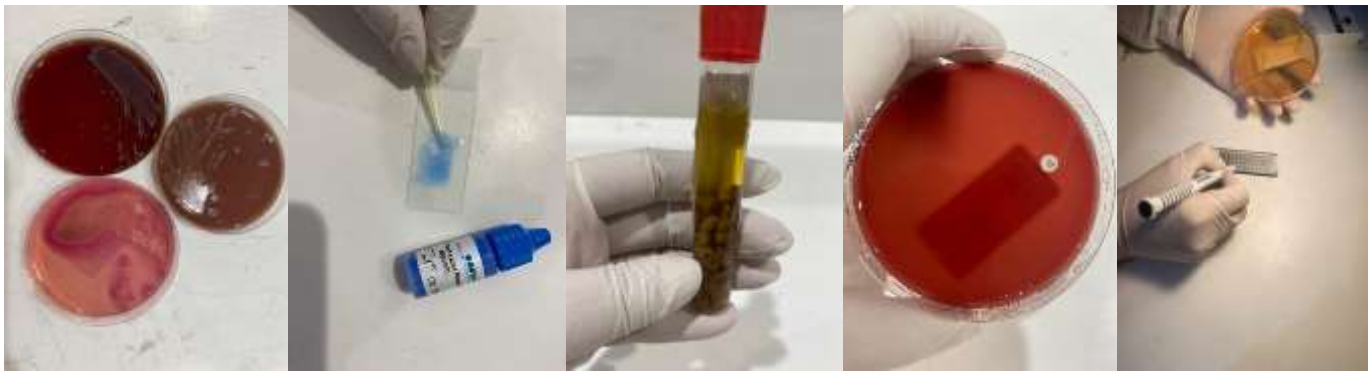

Figure-I: From left to right; growth *of E.coli*, slide coagulase, RCM, plate showing no growth of anaerobe and slide for identification on MALDI-TOF.

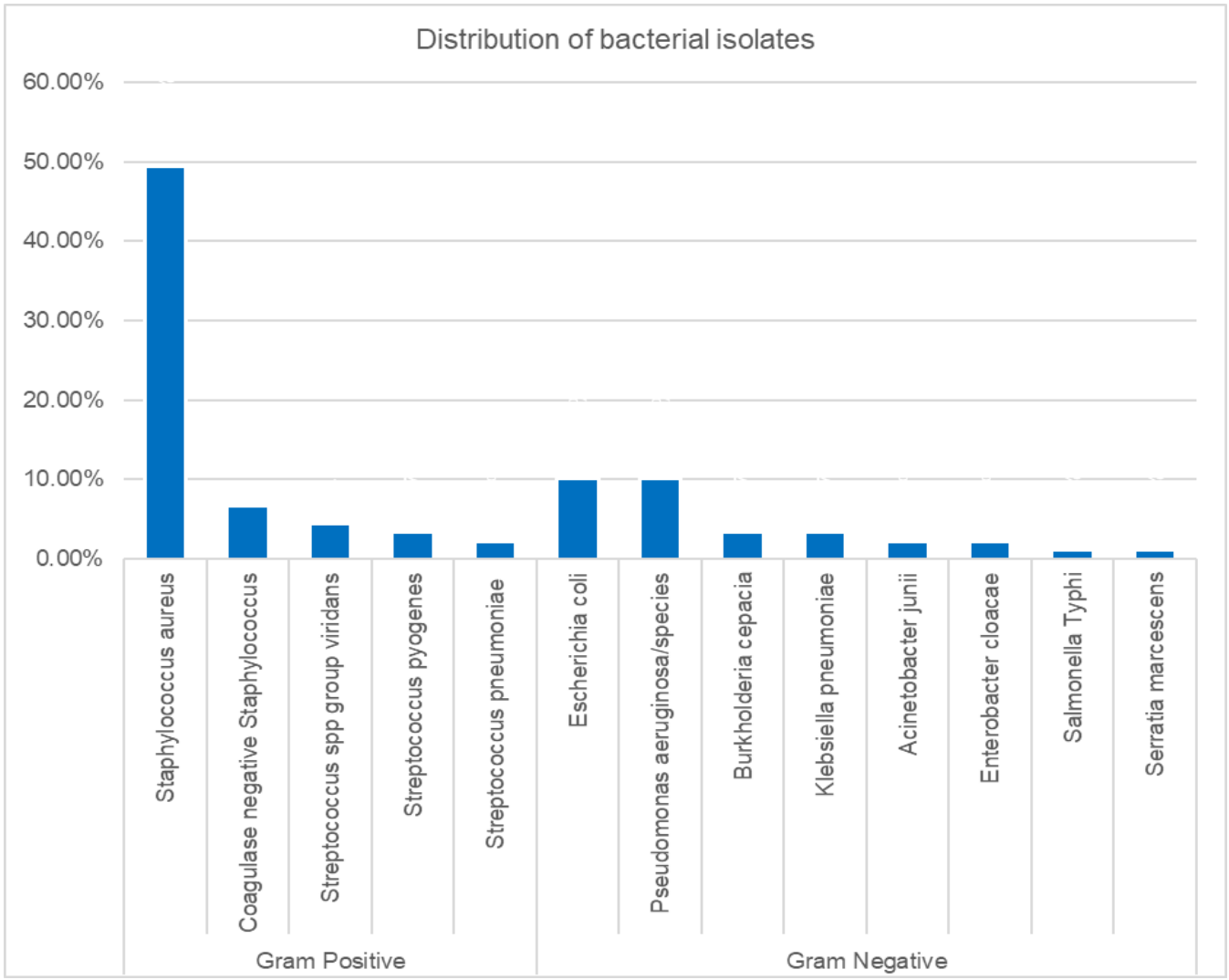

Chart-1: Bacterial isolates in SF culture.

Table-I: Antibiotic resistance profile in Gram positive cocci

Antibiotic resistance profile, Gram positive bacterial isolates	Amikacin	Ampicillin	Ceftriaxone	Ciprofloxacin	Clindamycin	Doxycycline	Erythromycin	Fusidic Acid	Gentamicin	Levofloxacin	Linezolid	Oxacillin	Penicillin	Trime-Sulphamethoxazole	Vancomycin
Staphylococcus aureus	6.7%	NT	NT	88.4%	13.6%	25.0%	79.5%	6.8%	23.3%	NT	0.0%	56.8%	NT	17.1%	0.0%
Coagulase negative Staphylococcus	0.0%	NT	NT	66.7%	0.0%	50.0%	33.3%	16.7%	0.0%	NT	0.0%	0.0%	NT	50.0%	NT
Streptococcus spp group viridans	NT	0.0%	0.0%	NT	0.0%	NT	0.0%	NT	NT	50.0%	NT	NT	NT	NT	0.0%
Streptococcus pyogenes	NT	0.0%	0.0%	NT	33.3%	NT	33.3%	NT	NT	100.0%	NT	NT	NT	NT	0.0%
Streptococcus pneumoniae	NT	NT	0.0%	NT	0.0%	0.0%	0.0%	NT	NT	0.0%	0.0%	NT	0.0%	100.0%	0.0%

Table-II: Antibiotic resistance profile in Gram Negative rods.

Antibiotic resistance profile, Gram negative bacterial isolates	Amikacin	Amoxicillin-Clavulanic acid	Cefepime	Cefixime	Ceftazidime	Ceftriaxone	Ciprofloxacin	Doxycycline	Gentamicin	Imipenem	Levofloxacin	Meropenem	Piperacillin-Tazobactam	Tobramycin	Trime-Sulphamethoxazole
Escherichia coli	11.1%	66.7%	NT	85.7%	NT	88.9%	88.9%	77.8%	44.4%	11.1%	88.9%	11.1%	11.1%	33.3%	44.4%
Pseudomonas aeruginosa/species	22.2%	NT	44.4%	NT	33.3%	NT	44.4%	NT	22.2%	22.2%	44.4%	22.2%	22.2%	22.2%	NT
Burkholderia cepacia	NT	NT	NT	NT	0.0%	NT	NT	NT	NT	NT	33.3%	33.3%	NT	NT	0.0%
Klebsiella pneumoniae	33.3%	66.7%	NT	66.7%	NT	66.7%	66.7%	100.0%	33.3%	66.7%	66.7%	66.7%	66.7%	66.7%	100.0%
Acinetobacter junii	0.0%	NT	0.0%	NT	0.0%	NT	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Enterobacter cloacae	0.0%	NT	NT	100.0%	NT	50.0%	50.0%	100.0%	50.0%	0.0%	50.0%	0.0%	0.0%	50.0%	NT
Salmonella Typhi	NT	NT	NT	100.0%	NT	100.0%	100.0%	NT	NT	NT	NT	0.0%	NT	NT	NT
Serratia marcescens	0.0%	NT	NT	100.0%	NT	100.0%	100.0%	100.0%	100.0%	0.0%	100.0%	0.0%	0.0%	100.0%	0.0%

DISCUSSION

In this prospective study, we have analyzed synovial fluid samples from various regions of Pakistan to identify the bacterial cause of infection and assess antibiotic susceptibility. Septic arthritis diagnosis is based on clinical findings supported by laboratory evidence. Of the 89 (22%) culture-positive synovial fluid (SF) samples, 59 isolates (66%) were gram-positive cocci, and 30 (33%) were gram-negative rods (GNR). *Staphylococcus aureus* was the most frequent (49%) isolate among GPCs. Other studies have also identified *S. aureus* as a major pathogen in septic arthritis, often followed by *Streptococci species*. Abid *et al*, in his research reported polymicrobial and anaerobic co-infections with *E.coli* (1.1%) from SF culture [6]. In contrast, our study noted *E. coli* and *Pseudomonas* species as the second most prevalent pathogens. Notably, the prevalence of MRSA (56%) in our study, as opposed to the reported 11% in a 2020 Indian study, is particularly concerning [7].

There has been limited research on the antibiotic profile in synovial fluid. A study in Nepal in 2023 documented high resistance to Clindamycin (39.6%) and Cotrimoxazole (39.5%) in *S. aureus*, in contrast to our findings of 13.6% (Clindamycin) and 17.1% (Cotrimoxazole) (8). The variation could be due to the difference in sample type and size, as we specifically selected synovial fluid for the research purposes. We have observed 100% sensitivity to Vancomycin and Linezolid; however, an increase in minimum inhibitory concentration (MIC) for vancomycin raises concern. Furthermore, Jin *et al*. in Sweden reported 70% *S. aureus* in SA patients, identifying hematogenous causes, rheumatoid arthritis (RA), as the major predisposing factor for SA, along with recent joint surgery, haemodialysis, HIV, skin infections, intra-joint corticosteroid, and immunosuppression [9-12]. Unfortunately, our study lacks this detailed information.

Another important finding was the 68% resistance to CRO (ESBL), 20% resistance to Meropenem (CRE) and 55% resistance to Levofloxacin in gram negative bacilli. *Pseudomonas aeruginosa/species* exhibited resistance of 33.3% and 22.2% to Ceftazidime and Piperacillin/Tazobactam, respectively. Additionally, we have also observed 3.3% of cases with *Burkholderia species* during 6-months. 33.3% of *Burkholderia cepacia* were resistant to Levofloxacin, possibly due to biofilm formation in prosthetic joint infections (PJI), as described by Mathew *et al* [13-15]. Similarly, Wu *et al* carried out a 10-year retrospective study in China on septic arthritis caused by *Bukrholderia pseudomallei* only. They also documented that secondary infection of *B. pseudomallei* caused either septic arthritis, osteomyelitis or both with high mortality rate of 20%. In the current study we did not rule out it as secondary infection due to various limitations. Our study also illustrated female to male ratio as 1.3:1 which was different to Wu *et al*' s findings (F:M of 1:13.7) [16,17].

CONCLUSION

According to our study, the Luminal A subtype was observed in 48 cases (29.9%), followed by Luminal B with 43 cases (26.7%). A significant association between increasing age and breast cancer was found. Further investigations are warranted to explore the clinical implications and therapeutic considerations associated with specific subtypes of breast carcinoma

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Amna Younas: Substantial contributions to conception of the design, data analysis, data interpretations, revisions, final approval of the work

Irim Iftikhar: Substantial contributions to conception of the design, revisions, final approval of the work

Karam Rasool: Data analysis, data interpretations, revisions

REFERENCES

1. Elsissy JG, Liu JN. Wilton PJ, Ikenna N, Gowd AK, Amin NH. Bacterial septic arthritis of the adult native knee joint: A review. JBJS Rev. 2020; 8(1): pe0059. DOI: https://doi.org/10.2106/jbjs.rvw.19.00059
2. Chiappini E, Mastrolia MV, Galli L, De Martino M, Lazzeri S. Septic arthritis in children in resource limited and non-resource limited countries: An update on diagnosis and treatment. Expert Rev Anti Infect Ther. 2016; 14(11): 1087-96. DOI:https://doi.org/10.1080/14787210.2016.1235973
3. McBride S, Mowbray J, Caughey W, Wong E, Luey C, Siddiqui A, *et al*. Epidemiology, management, and outcomes of large and small native joint septic arthritis in adults. Clin Infect Dis. 2020; 70(2): 271-9. DOI: https://doi.org/10.1093/cid/ciz265
4. Gerena LA, Mabrouk A, DeCastro A. Knee Effusion. [Updated 2024 Feb 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/ NBK532279/
5. Umer M, Hashmi P, Ahmad T, Ahmed M, Umer M. Septic arthritis of the hip in children: Aga Khan University Hospital experience in Pakistan. J Pak Med Assoc. 2003; 53(10): 472.
6. Abid N, Bhatti M, Azharuddin M, Islam M. Septic arthritis in a tertiary care hospital. J Pak Med Assoc. 2006; 56(3): 95-8.
7. George J, Chandy VJ, Premnath J, Hariharan TD, Oommen AT, Balaji V, *et al*. Microbiological profile of septic arthritis in adults: Lessons learnt and treatment strategies. Indian J Med Microbiol. 2019; 37(1): 29–33. DOI: https://doi.org/10.4103/ijmm.ijmm_19_134
8. Adhikari P, Basyal D, Rai JR, Bharati L, Budthapa A, Gharti KP, *et al*. Prevalence, antimicrobial susceptibility pattern and multidrug resistance of methicillin-resistant *Staphylococcus aureus* isolated from clinical samples at a tertiary care teaching hospital: An observational, cross-sectional study from the Himalayan country, Nepal. BMJ open. 2023; 13(5): e067384. DOI: https://doi.org/10.1136/bmjopen-2022-067384
9. Jin T, Mohammad M, Pullerits R, Ali A. Bacteria and host interplay in *Staphylococcus aureus* septic arthritis and sepsis. Pathogens. 2021; 10(2): 158. DOI: https://doi.org/10.3390/pathogens10020158
10. Mathews CJ, Weston VC, Jones A, Field M, Coakley G. Bacterial septic arthritis in adults. The Lancet. 2010; 375(9717): 846-55. DOI: https://doi.org/10.1016/S0140-6736(09)61595-6
11. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015; 28(3): 603-61. DOI: https://doi.org/10.1128/cmr.00134-14
12. Geirsson ÁJ, Statkevicius S, Víkingsson A. Septic arthritis in Iceland 1990–2002: Increasing incidence due to iatrogenic infections. Annals Rheumatic Dis. 2008; 67(5): 638-43. DOI: https://doi.org/10.1128/cmr.00134-14
13. Dastgheyb S, Parvizi J, Shapiro IM, Hickok NJ, Otto M. Effect of biofilms on recalcitrance of staphylococcal joint infection to antibiotic treatment. J Infect Dis. 2015; 211(4): 641-50. DOI: https://doi.org/10.1093%2Finfdis%2Fjiu514
14. Dastgheyb SS, Hammoud S, Ketonis C, Liu AY, Fitzgerald K, Parvizi J, *et al*. Staphylococcal persistence due to biofilm formation in synovial fluid containing prophylactic cefazolin. Antimicrob Agents Chemother. 2015; 59(4): 2122-8. DOI: https://doi.org/10.1128/aac.04579-14
15. Alder KD, Lee I, Munger AM, Kwon HK, Morris MT, Cahill SV, *et al*. Intracellular *Staphylococcus aureus* in bone and joint infections: A mechanism of disease recurrence, inflammation, and bone and cartilage destruction. Bone. 2020; 141: 115568. DOI: https://doi.org/10.1016/j.bone.2020.115568
16. Shahpari O, Mousavian A, Elahpour N, Malahias MA, Ebrahimzadeh MH, Moradi A. The use of antibiotic impregnated cement spacers in the treatment of infected total joint replacement: challenges and achievements. Arch Bone Jt Surg. 2020; 8(1): 11-20. DOI: https://doi.org/10.22038/abjs.2019.42018.2141
17. Wu H, Wang X, Zhou X, Chen S, Mai W, Huang H, *et al*. Osteomyelitis and septic arthritis due to Burkholderia pseudomallei: A 10-year retrospective melioidosis study from South China. Front Cell Infect Microbiol. 2021; 11: 654745. DOI: https://doi.org/10.3389/fcimb.2021.654745
18. Spyridakis E, Gerber JS, Schriver E, Grundmeier RW, Porsch EA, St. Geme III JW, *et al*. Clinical features and outcomes of children with culture-negative septic arthritis. J Pediatric Infect Dis Soc. 2019; 8(3): 228-34. DOI: https://doi.org/10.1093/jpids/piy034