Egyptian Journal of Medical Microbiology Volume 30 / No.4 / October 2021 157-162 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Septic Arthritis: Microbiological Etiology and Molecular Detection of the Most Resistant Etiological Agents

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Key words:
Septic arthritis, MRSA, mecA gene

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ABSTRACT

Background: *Septic arthritis is a serious emergency causing remarkable morbidity and mortality worldwide. Expeditious diagnosis and effective treatment are necessary to achieve better clinical outcomes and avoid devastating joint consequences.* ***Objective:*** *we focused to detect the most common etiological agent and associated resistance to commonly used antibiotics in Nariman Hospital in Alexandria, Egypt. Molecular detection of mecA gene which causes resistance in methicillin-resistant Staphylococcus aureus and results in treatment failure was studied among our isolates.* ***Methodology:*** *One hundred and fifty joint fluid aspirates were included in the study. Identification of the isolates was done by conventional microbiological methods and BACTEC MGIT 960 TM system. Antibiotic susceptibility tests were done to detect resistance in our isolates. Molecular amplification of mecA gene was done in isolated methicillin-resistant Staphylococcus aureus (MRSA).* ***Results:*** *It was noted that most the of specimens were collected from males. Culture results showed monomicrobial bacterial growth in 90.2% of samples tested. Staphylococcus aureus was the major organism isolated. 88.9% of methicillin-resistant Staphylococcus aureus (MRSA) were positive for mecA gene.* ***Conclusion:*** *As far as we know, this is the first research in Alexandria investigating the most common etiological agent and associated resistance resulting in treatment failure in the leading orthopedic hospital in Alexandria.*

INTRODUCTION

Septic arthritis is a serious medical emergency in both developing and developed countries, it is linked to severe morbidity and mortality [1-3]. Rapid diagnosis of joint infections and implementation of appropriate therapy is necessary to limit joint destruction and associated systemic infections [4, 5]. In spite of treatment improvement and the development of advanced innovative methods for diagnosis, sepsis is still the ultimate cause of death, especially among patients in intensive care units [6].

Identifying the etiological pathogen is the benchmark in targeting effective antimicrobial therapy [4]. Septic arthritis is mainly caused by a number of bacterial pathogens, mostly occurring as a single organism [1]. *Staphylococcus aureus* is the leading cause of all septic arthritis cases in Europe and all nongonococcal infections in the United States. Streptococci are less abundant and commonly isolated from patients with chronic skin infections [7]. Among Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* are the most frequently detected pathogens in septic arthritis accounting for about 10-20% of all cases. Anaerobes are rarely reported, mostly linked to hematogenous spread from a distant anaerobic infection [7, 8].Incidence of MRSA among orthopedic septic arthritis patients has increased in recent years and resulted in poor clinical outcome [2]. It has been postulated that *mecA* gene encoding penicillin binding protein 2a in all MRSA is the main cause of resistance to Beta-lactam [5]. Molecular amplification of the highly conserved *mecA* gene is considered the cornerstone in identification MRSA which is the main cause of treatment failure.

The present work focused on investigating the main etiologic pathogen in orthopedic patients with septic arthritis Nariman Hospital in Alexandria. Besides, identification of antimicrobial susceptibilities and associated antimicrobial resistance resulting in treatment failure in the hospital. The study also focused to highlight the contribution of *mecA* gene to cause resistance in isolated MRSA Accordingly, it would facilitate the suggestion of the most appropriate empiric treatment for orthopedics patients with acute bacterial arthritis.

METHODOLOGY

Between July 2019 and September 2020, 150 joint fluid aspirates were obtained aseptically from orthopedics patients admitted to Nariman hospital in Alexandria, Egypt. All procedures were carried out in accordance with ethical guidelines by Ethics Committee. Specimens were aseptically divided into 3 aliquots for Gram stain, conventional culture media and

inoculation in BACTEC MGIT 960 ™ system. Simultaneously, blood culture samples were taken and empirical treatment of intravenous injection of Oxacillin, Nafcillin or Ceftriaxone was started. The aspirates were cultured in Mannitol salt agar, Mackonkey agar, blood agar, and chocolate agar. The microorganisms were incubated for 48 hours and results were analyzed. The identification of *Staphylococcus aureus* isolates was confirmed using MALDI-TOF/MS; Bruker, Billerica, MA, USA.

Antimicrobial susceptibility

Antibiotic susceptibility according was assessed according to CLSI 2019 guidelines. Antibiotic susceptibility testing was carried out by using the antibiotic discs including: Amikacin (AK 30 µg); Ampicillin/sulbactam (SAM 10 µg /10 µg); Ceftriaxone (CRO 30 µg); Gentamicin (CN 10 µg); Linzolid (LZD 30 µg); Methicillin (MET 5 µg); Oxacillin (OX 1 µg); Clindamycin (DA 10 µg) Trimethoprim/ sulfamethoxazole (SXT 1.25 µg /23.75 µg); Vancomycin (VA 30 µg); Cefoxitin (FOX 30µg); Nafcillin (NF 1µg). Oxoid (Cambridge, UK) provided all of the needed culture media and antibiotic discs.

Phenotypic Detection of MRSA

S. aureus strains isolates were tested by cefoxitin and oxacillin and disc diffusion methods according to Clinical and laboratory standards institute (CLSI) (9).

Molecular detection of *mecA* gene

The MRSA isolates' DNA was extracted using the boiling technique [10]. DNA amplification was done in a DNA thermal cycler (Tpersonal Thermocycler biometra, applied biosystem -USA) using Master mix (Intron biotechnology -Korea). The primers for *mecA* gene (SBS -China) [11, 12] and PCR conditions are in table 1. 2% agarose gel in TBE buffer was used for separation of the PCR products. Gels were stained with 2 µg/ml ethidium bromide, then visualized under ultraviolet transillumination (BIORAD, Italy).

Table 1: The primers and PCR conditions for *mec*A gene

Resistant gene	Primers sequence	Band Size(bp)	Annealing temptature
mec A	F: 5′-GTA GAA ATG ACT GAA CGT CCG ATA A R: 5′-CCA ATT CCA CAT TGT TTC GGT CTA A	310	55°c for 60 s,72°c for 60 s, final elongation: 72°c for 5 min

RESULTS

Patient demographics and lab results

Patients with complicated sepsis were found to be men in this study. However, there were no differences in abundance among groups based on patient age, CRP, or WBC count. Table 2 shows that the kind of bacteremia was not substantially different between the two patient groupings.

Table 2: Comparison of clinical and laboratory findings obtained at admission of 150 patients regarding sepsis severity

	Group 1 (non septic)		Group 2 (septic)	
	Non septic	Non-septic with local infection	Severe sepsis	Septic shock
Number	37	22	50	41
Sex (M:F)	21:16	13:9	25:25	30:11
CRP (mg/dL)	1.01	1.20	19.93	11.68
WBC ($\times 10^9$/L)	5.6	6.12	10.5	10
BACTEC MGIT 960 ™ system for Bacteremia type	Negative	Negative	Positive	Positive
Total	59		91	

Microbiological Culture Results

The culture results of the 150 joint fluid aspirates from orthopedics patients yielded monomicrobial bacterial growth in 104 samples (90.2%) and polymicrobial bacterial growth (2-3 microorganisms) in 9 samples (9.8%) as in table 3.

Table 3: Relevance of hospital acquisition and association within 150 from synovial fluid samples

Growth pattern in culture	No.	%
Bacterial growth		
Monomicrobial	104	90.2
Polymicrobial microorganism (2 – 3)	9	9.8
Total	**113**	**100.0**

113 isolates from non-septic with local infection, Severe sepsis and Septic shock were obtained from 150 orthopedics patients admitted to Nariman Hospital in Alexandria. Majority of isolates were gram positive (68.3 %), *Staphylococcus aureus* isolates were the predominant isolated microorganism (56.9%), followed by *Streptococcus pyogenes* (7.9%), and *coagulase-negative staphylococci* (3.5%). *E.coli* were the most predominant isolated among Gram negative bacteria (15.9%) followed by *Pseudomonas aeruginosa* (12.3%) then *Klebsiella spp.* (3.5%).(Table 4)

Table 4: Frequency of bacterial isolates from synovial fluid samples

Isolated bacteria		No.	%
Gram negative Bacteria	*Pseudomonas aeruginosa*	14	12.3
	E. coli	18	15.9
	Klebsiella spp.	4	3.5
Gram positive Bacteria	*Staphylococcus aureus*	64	56.9
	coagulase-negative staphylococci	4	3.5
	Streptococcus pyogenes	9	7.9
Total		**113**	**100.0**

Antibiotic Susceptibility

Sixty-four (80%) *Staphylococcus aureus* culture isolates showed resistance to Amikacin, Nafcillin, Cefoxitin, Methicillin, Oxacillin, Ceftriaxone, Ampicillin/sulbactam (64 %) with varied degree to the others. The fourteen *Pseudomonas aeruginosa* isolates were resistant Trimethoprim/sulfamethoxazole, Ceftriaxone, Ampicillin/sulbactam (100 %) and Gentamicin (92.9%) and the resistance percent for Clindamycin & Amikacin were (85.7 % & 71.4%) respectively. Most of eighteen *E. coli* culture isolates were resistant to Ampicillin/sulbactam (100 %,), Ceftriaxone (100%) and AMK (100%) with varied degree to the rest. Klebsiella spp. has four culture isolates which were resistant mostly to Trimethoprim/sulfamethoxazole (100%), Ceftriaxone (100%), Ampicillin/sulbactam (100%), Gentamicin (100%), Amikacin (100%) and linezolid (1%). (Table 5)

Table 5: Resistance profile of the tested isolates to different antimicrobial agents

Name of microorganism	Total No. of isolates	Antibiotics % of Resistance											
		vancomycin	Nafcillin	Cefoxitin	Methicillin	Trimethoprim/ sulfamethoxazole	Clindamycin	linezolid	Oxacillin	Ceftriaxone	Ampicillin/ sulbactam	Gentamicin	Amikacin
Pseudomonas aeruginosa	14	–	–	–	–	14(100%)	12 (85.7%)	–	–	14 (100%)	14 (100%)	13 (92.9%)	10 (71.4%)
E. coli	18	–	–	–	–	16 (88.9%)	–	3 (16.7%)	–	18 (100%)	18 (100%)	8 (44.4%)	5 (27.8%)
Klebsiella spp.	4	–	–	–	–	4 (100%)	–	1 (25%)	–	4 (100%)	4 (100%)	4 (100%)	4(100%)
Staphylococcus aureus	64	0 (0%)	41 (64%)	41 (64%)	41 (64%)	40 (62.5%)	40 (62.5%)	1 (1.6%)	41 (64%)	41 (64%)	41 (64%)	45 (70.3%)	51(80%)
coagulase-negative staphylococci	4	-		-	-	-	-	-	-	-	-	-	-
Streptococcus pyogenes	9	–		–	–	–	–	–	–	–	6 (66.7%)	–	–

Prevalence of *mec A* gene

Out of the 45 *S.aureus* isolates were confirmed to be MRSA according to the cefoxitin and oxacillin disc diffusion method, 40 isolates (88.9%) showed band positivity for *mecA* gene. (Figure 1)

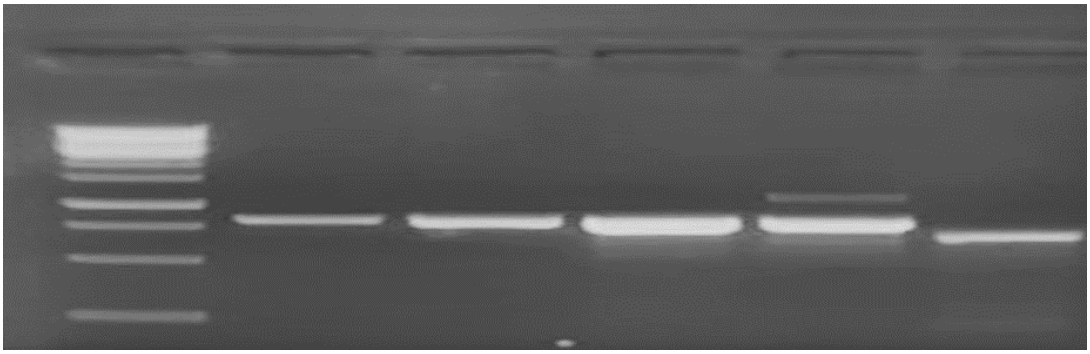

Fig. 1: MRSA isolates showing positive PCR bands for *mecA* gene showing positive bands at 310 bp.

DISCUSSION

Septic arthritis is a serious clinical health emergency. Rapid diagnosis and speedy initiation of targeted therapy are necessary to prevent devastating joint consequences and to prevent further systemic spread [13, 14]. Despite advancement in laboratory diagnostic methods and improvement in treatment options, Septic arthritis still results in notable mortality[4,15].

The clinical history, clinical parameters examination along with the demographic profile of the patients are the necessary to guide treatment [3]. Septic arthritis is generally more common among older adults [16-20]. This was also noted in the present study, where all our patients were in 50-70 age group. *Lafi, Al-Mashhadani (19)* and Nair, Schweizer (20) assumed that higher incidence among geriatrics can be attributed to pre-existing medical conditions and other age-related joint abnormalities can be used to explain the higher incidence. Several demographic studies have illustrated male predominance [17, 21, 22] this was in agreement with our findings. In this study 85(56.7%) of the specimens were males, while those collected from female patients represent 65 (43.3%). This is possibly because males are more liable to exposure to occupational hazards.

Identifying the etiological agent is the benchmark in determining the effective optimal antimicrobial treatment [4]. In attempts to identify the main etiological agent, specimens collected were identified by standard conventional methods and inoculation in BACTEC MGIT 960 ™ system. Bactec method has proved efficacy in identification of the tested specimens. Superiority of Bactec was also supported in a study by Nutt, Orth (23).

Culture results showed that monomicrobial infections were the most predominant. Previous reports also demonstrated predominance of single organism infection as the sole etiological agent in septic arthritis [1]. Staphylococci were the main prominent cause of septic arthritis. *Staphylococcus aureus* were in 56.9% of the cases and coagulase-negative staphylococci were present in 3.5% of the cases tested. This has been described in previous reports in various areas around the world [1-4, 20, 24-29]. A three-decade study showed that *Staphylococci* were the major trend accounting for 55% of septic arthritis [24]). Previous reports suggested Gram-negative organisms account 20% of cases of septic arthritis [1, 4]. A changing trend showing higher incidence of gram-negative pathogens was shown in previous studies[1]. Our results show that Gram negative pathogens were the etiological agent in 31.8% of our cases. *Escherichia coli* (15.9%) was the most abundant Gram-negative bacteria among our isolates followed by *Pseudomonas aeruginosa* (12.3%). Nair *et al.* [20] showed that 23% to 30% of septic arthritis in elderly can be caused by *Escherichia coli*. He justified the higher incidence of *Escherichia coli* by the occurrence of bacteremia following urosepsis. Gonococcal septic arthritis is of scarce occurrence among elderly. Other reports agreed with our finding showing no gonococcal septic arthritis occurrence [3, 24]. The variation in bacteriological profile may be due to the geographic differences, use of antibiotics, vaccinations and empirical therapy implied.

Several reports in literature confirmed the use of ESR, CRP and leukocytosis which can be used as reliable prognostic markers in septic arthritis [3, 30, 31]. Antibiotic treatment can significantly decrease these markers. However, the unjustifiable antibiotics use can obscure symptoms and results in delayed diagnosis. In this study blood as well as joint fluid culture were sterile in 24.6 % of the cases, other reports showed (50–80%) of their samples being sterile [3, 32, 33].

Susceptibility testing is the key step to optimize antibiotic therapy for septic arthritis and achieve better clinical outcomes [34]. *S.aureus* isolates showed higher resistance to empiric antibiotic treatment and increased susceptibility to higher classes of antibiotics linezolid (98.5%) and vancomycin (100%). Methicillin resistance among *staphylococcus aureus* isolates tested was as high as 70.3%. Similar patterns of resistance to first line drugs were detected with increased prevalence of MRSA in Egypt (54%) [35], Japan (45%) [36], Sudan (61.5%) [37, 38], India (35%)[22] and USA (62%) [39].

Methicillin-resistant *S aureus* is a notable cause of septic arthritis among older adults. The rising incidence of MRSA strains, in addition to high resistance noted to first line drugs is an alarming signal worldwide and can result in devastating joint consequences [24-29].

Previous studies postulated the contribution of *mecA* gene to cause resistance to betalactams and encode penicillin binding protein 2a, accounting for MRSA abundance [5]. Molecular detection of *mecA* gene is the proof for the detection of MRSA isolate [5, 37, 38]. In the light of this fact *mecA* gene was investigated in MRSA isolates. 40 out of 45 MRSA isolates (88.9%) showed *mecA* gene. This percentage was midway between the percentage detected by Elhassan, Ozbak (5) and Yang, Choe (40) who detected 13 positive *mecA* in 26 samples of synovial fluid.

CONCLUSIONS

Septic arthritis is a serious medical emergency. Delayed diagnosis and inappropriate treatment results in devastating consequences. In this study Bactec method has proved efficacy in identification of the specimens. *Staphylococcus aureus* was the major striking cause of septic arthritis. The increased prevalence of MRSA harboring *mecA* gene among our isolates is an alarming signal. This sheds the light on the necessity to steadily assess resistance patterns to select the most appropriate empirical antibiotic treatment. In Egypt, further investigations are still needed regarding different resistance mechanisms among MRSA to guide treatment selection.

Acknowledgments:

The author thanks Pharos University in Alexandria and Medical technology center for their technical support.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

Ethical Approval

Regarding ethical clarification required concerning the isolation of bacterial isolates form clinical specimens, this was carried out by nurses and technicians in the hospitals not by us. We only collected the sub cultured isolates from the Microbiology Laboratories in these Hospitals. Privacy and confidentiality of all patients' information was anonymized.

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