Research Article

Microbiological Diagnosis of Osteoarticular Infections and their Antibiogram

Shumaila Jabbar[1], Fiaz Ahmad[2], Maryam Khan[3], Tania Ahmad Shakoori[4], Saba Shamim[5]

[1-2,3]Mphil Student, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore; [4-5]Associate Professor, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore.

Abstract

Background: Osteoarticular infections in adults and children are a significant cause of elevated morbidity and may lead to restrictive mobility of various stages.

Objective: Isolation and determination of the occurrence, pathomorphological and antibiotic susceptibility patterns of isolated microorganisms from the patients with osteoarticular infections.

Methods: This research work was carried out at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL). Pus specimens (n = 120) were gathered from patients of osteoarticular infections. Bacterial isolates were purified and identified biochemically. Antibiotic resistance of the bacterial isolates was investigated by the criterion set by Clinical and Laboratory Standards Institute (CLSI). All experiments were run in triplicate using randomized study design. The mean, standard error and standard deviation values were determined using SPSS (v. 23.0).

Results: Out of 120 samples, 111 isolated samples (93%) were tested positive for total viable count. The isolated bacterial species were observed to be *Streptococcus pyogenes*, *Staphylococcus hemolyticus*, *S. aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus* sp., *Serratia* sp., *Klebsiella* sp., *Enterobacter* sp., and *Proteus* sp. Antibiogram results also yielded *S. pyogenes* and *S. hemolyticus* to be erythromycin resistant, while *S. aureus* was vancomycin resistant. *E. coli* and *Klebsiella* sp. were found to be resistant to tobramycin while *Proteus* and *Enterobacter* sp. were both sensitive to it.

Conclusion: *P. aeruginosa*, *E. coli*, and *S. aureus* were prevalent in all groups of age, while *Micrococcus* and *Serratia* sp. were common in 16-55 years. Patient hygiene, immune health and the course of medications are all factors that should be kept in consideration while treating the disease.

Corresponding Author | Dr. Saba Shamim, Assistant Professor, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore **Email:** sabashamimgenetics@gmail.com

Key Words: Osteoarticular infections, Antimicrobial Susceptibility, *Pseudomonas aeruginosa*, *Serratia*, *Proteus*, *Enterobacter*, *Streptococcus pyogenes*, *Staphylococcus hemolyticus*

Introduction

Osteoarticular infections present themselves as a challenge pertaining to its diagnosis and treatment.[1] These bone and joint infections can affect both children and adults, with osteoarticular infections being widely reported in children, with an elevated rate of morbidity and affected mobility development.[2] These infections may be localized to one part of the body or may spread to other areas like the joints or other bones and tissues. Gram-positive cocci are reported to be prevalent in osteoarticular infections, though current studies also report Gramnegative bacteria to be ubiquitous, with respect to infections arising due to the placement of orthopaedic materials.[3,4] Among all microorganisms that are etiological agents of these infections, *Stap-hylococcus aureus*, *Streptococcus pyogenes* and *S. pneumoniae*, respectively are those which affect adults while

recent studies elucidate *Kingella kingae* to be a significant cause of osteoarticular infections that affect children.[1, 5]

The respiratory tract is considered to be the most suitable pathway for entry of pathogens. Microorganisms are found to be ubiquitous in dust particles present in air which is why many pathogens invade through this route of entry. Some other pathogens use this direct entry to bones via trauma, soft tissue infection etc. Acute osteoarticular infections can last upto a fortnight which can turn advance into chronic stages if symptoms tend to persist. Swift diagnosis and subsequent treatment is imperative for downsizeing the symptoms and other infection-related complications.[6] Classic culture methods have been the gold standard for successful diagnosis of osteoarticular infections in children, but negative culture can act as a hinderance in correct diagnosis and treatment, especially in paediatric cases.[7] The incidence of the pat-hogen as a causative agent may be more attributable to specific and sensitive detection techniques like PCR, rather than the prevalence of the pathogen in the case of infection.[8]

The severity of infections is dependent upon the age, the causative agent and the type of bone structure affected. The clinical features, test results and the antibiogram findings all should be considered jointly for the treatment to achieve a favourable recovery rate. Typically, bone cultures, exudates of sub periosteal areas and joint fluids contribute for the microbialogical diagnosis in 50-70 % of the cases.[9] Children are usually treated with an antimicrobial therapy which spans over a month, with doctors recommending intravenous therapy if S. *aureus* is the causative agent. However, recent studies have discouraged the practice.[10]

This research work aimed to investigate and examine the prevalence and frequency of the isolated microbial flora from patients of osteoarticular infections. of the bacterial isolates obtained, their antibiogram was performed which revealed the sensitivity and resistance pattern of these isolates against many broad-spectrum antibiotics.

Methods

Samples were collected from various government hospitals of Lahore, Punjab, which were selected according to the stated inclusion and exclusion criteria. The inclusion criteria contained all age groups and genders infected with osteomyelitis whereas the latter contained patients who were not stable haemodynamically, had any traumatic head injury and patients diagnosed with tuberculosis (TB). Patient proforma were filled for registering their consents, while the approval from Ethical Review Committee of UOL was duly gained. Prior to the collection of samples, cleansing of the wound was performed in a gentle way to eliminate contamination. by using sterile cotton wool swabs (Amies®), which were extended to lesion area for sufficient amount of sample collection from the infected site.

To culture aerobic bacteria from pus specimens, selective and non-selective agar media were used. After the swab samples were proceeded on nutrient agar, the isolates were selectively cultured on Mannitol salt agar (MSA), MacConkey agar, Blood agar**,** Cetrimide agar and Eosin Methylene Blue (EMB) agar following completely randomized design and conditions of incubation at 37 °C for 24 h. If a sample was suspected to be infected or foul-smelling, the presence of anaerobic bacteria was speculated. All bacterial strains were identified based on their cultural characteristics, and morphology patterns including size, pigmentation, texture, elevation, boundary, opacity, surface and margin. Gram staining was performed to determine Gram morphology and subsequent biochemical characterization was carried out by oxidase, triple sugar iron (TSI) test, catalase, hydrogen sulfide, citrate utilization, Voges-Proskauer, indole, uease, nitrate reduction and methyl red tests, respectively. The results for the respective tests were observed after 24 h.[11]

Purified bacterial isolates were also preserved in glycerol buffered saline solution. Each isolate was suspended in the autoclaved PBS solution and the suspension was centrifugated at 6000 rpm for 5 minutes. The supernatant and the pellet were discarded and resuspended, respectively, in 1.2 mL of 10 % glycerol PBS sterilized solution and stored in a sterile Eppendorf tube (1.5 mL) at 4 °C for 12 hours and then shifted to - 20 °C.[11]

Kirby-Bauer disc diffusion method was employyed for the determination of the antibiotic resistance pattern of the bacterial species. Some of the

Aminoglycosides including streptomycin (10µg), tobramycin (10-µg), amikacin (30 µg), *penicillin* (β-lactamase ring) mainly penicillin G (2 units), amoxicillin (10µg), mpicillin (10 µg), cephalosporins including ceftazidime (10µg), cephradine (30µg), cefotaxime (30µg), cefparazon (30µg) were used. Carbapenems including imipenem (10µg), erythromycin (30µg), clindamycin (10µg), and quinolones including ciprofloxacin (10µg), levofloxacin (5µg), gentamycin (30µg), piperacillin/tazobactam (40µg), were used. For the experimentation, MHA agar (Mueller-Hinton agar) was prepared under standard conditions. The bacterial isolates were then streaked onto the agar surface and antibiotic discs were applied onto the agar. The results were measured in the form of zones of inhibition the next day. All experiments were per-formed in triplicate, where the mean, standard error and standard deviation was calculated, observed and tabulated using SPSS (v. 23.0) (Significance level 0.05 %).

Results:

In the study, 120 samples were collected to identify the microorganisms causing osteoarticular infections. Out of 120 samples, 93% (111 patients) were found to be positive for bacterial species, with the CFU count of more than 30-300 colonies per plate (Fig. 1). Bacterial species like *S. pyogenes*, *Staphylococcus haemolyticus*, *S. aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus* sp., *Serratia* sp., *Kle-bsiella* sp., *Enterobacter* sp., and *Proteus* sp. were identified in the positive samples, as shown in Table 1. Biochemical tests were conducted to identify bacterial species, the results of which are tabulated in Table 2. In positive patients, the major incidence was of *S. aureus*, which was found to be 75 %. *P. aeruginosa* was found to be the second major pathogen, occurring in 55 % of the total patients. Other Gram-negative bacteria such as *Enterobacter*, *K. pneumoniae*, and *E. coli* were the third most common pathogens affecting the patients, whereas bacterial species of *Serratia* and *Micrococcus* sp. were found in lesser number of patients, respectively (Table 1).

Different groups of age were formed for ease of sampling and documentation. The first group consisted of patients of 2-15 years of age, while the second group ranged from 16-30 years old patients. The third and last group was designated to patients of 31-55 years of age. Results accumulated with respect to age demonstrated the prevalence of *S. aureus*, *P. aeruginosa*, *E. coli* and *S. haemolyticus* in the first and second age groups, respectively, while *K. pneumoniae* was found to be prevalent in the second and third age groups, respectively. Likewise, *Micrococcus* and *Serratia* were also seen to be dominant in 16-30-and 31 -55-years old patients (Table 3). Results for the anti-biogram for respective bacterial isolates are elucidated in Table 4. Sensitivity and resistance patterns of Gram-positive cocci, Enterobacteriaceae, and *P. aeruginosa* are further discussed in the next section.

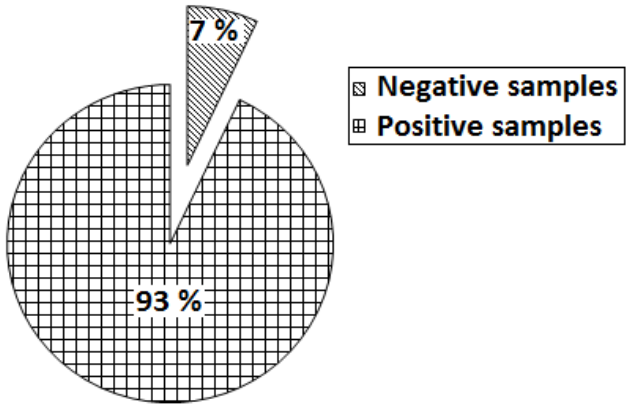

Figure 1: *Pie-chart showing total sample percentage of positive and negative samples*

Table 1: *Categories of bacterial isolates in 111 samples*

Sr. No.	Bacterial strains	Sample-wise percentages
1.	*S. aureus*	75 %
2.	*Enterobacter*	28%
3.	*P. aeruginosa*	55%
4.	*E. coli*	50%
5.	*K. pneumoniae*	40%
6.	*Proteus*	25%
7.	*S. pyogenes*	39%
8.	*Serratia*	3%
9.	*Micrococcus*	10%

Table 2: *Biochemical tests implemented to confirm bacterial isolates*

Biochemical Tests	Bacterial isolates								
	S. aureus	**P. aeruginosa**	***E.coli***	**K. pneumoniae**	**S. pyogenes**	**Micro-coccus**	**Entero-bacter**	**Proteus**	**Serra-tia**
Gram stain	+ cocci	- bacilli	- rods	- rods	+ cocci	+ cocci	- rods	- bacilli	- rods
Catalase	+	+	+	NA	+	+	-	-	+
Coagulase	+	-	-	NA	-	-	-	-	-
Oxidase	-	+	NA	-	-	+	-	-	-
V-P	NA*	-	-	+	NA	NA	+	-	+
MR	NA	-	+	-	NA	NA	NA	NA	-
Citrate	NA	+	-	+	NA	-	-	+	+
Indole	NA	--	+	-	NA	-	-	+	-
Urease	NA		-	+ (slow)	NA	NA	-	+	NA
Motility	NA	+	NA	-	NA	NA	+	+	+

*NA= not applicable

Table 3: *Categories of each bacterial isolate from total number of 464 isolates*

Sr. No.	Groups	Bacterial isolate									
		S. aureus	***P. aeruginosa***	***E. coli***	***K. pneumoniae***	***S. pyogenes***	***Proteus***	***Serratia***	***S. haemolyticus***	***Micrococcus***	***Enterobacter***
1.	First (2-15yrs)	40	30	35	Nil	10	Nil	Nil	7	Nil	Nil
2.	Second (16-30 yrs)	33	36	40	23	Nil	Nil	10	Nil	9	17
3.	Third (31-55yrs)	29	40	28	32	Nil	17	13	Nil	15	Nil
	Total	**102 (22%)**	**106 (23%)**	**103 (22%)**	**55 (12%)**	**10 (2%)**	**17 (3%)**	**23 (5%)**	**7 (1%)**	**24 (5%)**	**17 (3%)**

Table 4: *Antibiogram of bacterial isolates*

Bacterial sp.	Drugs (cm)										
	Ampicillin	**Piperacillin Tazobactam**	**Amikacin**	**Cefradoxil**	**Cefepime**	**Levofloxacin**	**Ceftriaxone**	**Imipenem**	**Ciprofloxacin**	**Cefixitin**	**Tobramycin**
E.coli	0.5	0.2	0.4	0.6	0.5	0.3	0.8	0.2	0.8	0.7	0.3
Klebsiella	0.4	0.3	0.2	0.6	0.4	0.8	0.3	0.1	0.9	0.8	0.4
Proteus	0.3	0.3	0.8	0.2	0.4	0.5	0.2	0.8	0.9	0.1	0.7
Enterobacter	0.3	0.1	0.2	0.3	0.6	0.6	0.1	0.9	0.7	0.5	0.5
P. aeruginosa	0.7	0.2	-	0.6	0.5	-	0.5	0.4	-	0.8	-

	Penicillin G	**Vancomycin**	**Amikacin**	**Clindamycin**	**Ciprofloxacin**	**Levofloxacin**	**Erythromycin**	**Gentamycin**	**Moxifloxacin**
S. aureus	1	0	0.8	0.6	0.8	0.5	0.7	0.9	1
S. haemolyticus	0.9	0.6	0.4	0.8	0.8	0.7	1	0.4	0.9
S. pyogenes	0.5	1	0.2	0.4	0.7	0.9	0.1	0.1	0.6

Discussion:

Osteoarticular infection is a medical condition in which patients undergo many transitional changes with respect to risk factors, prognosis as well as treatment. Cases of these infections have been somewhat arduous to examine due to the diverse ranges of infection. Osteoarticular infection poses an alarming challenge to the orthopaedic medical community. It constitutes potential risks to the hospitalized patients in conditions of bigger healthcare expenditures, morbidity and mortality. There are many reasons for not attaining high success rate in many bacterial diseases with antibiotic therapy in osteoarticular infection, such as the complex anatomical and physiological features of bone, poor health, under nourishment and a compromised immune system. Maleb et al.[12] reported around 52 % of positive cultures from collected samples. However, in our study we found 93 % of the samples (111 out of 120 samples) to be positive for total viable count reaching to 30-300 colonies per plate. The results of positive samples are elucidated in Figure 1. The most commonly observed causative agents of osteomyelitis are *S. aureus*, and Gram-negative bacteria such as *E. coli* and *P. aeruginosa*, and group B *Streptococcus* sp. The results of our study (Table 1 and 2) agreed with the findings of Maleb et al. [12]. In our study, *S. aureus* was observed to be the most frequent (75 %) whereas *Serratia* was the least occurring bacteria, with just 3 % of incidence in 111 positive samples (Table 2). Results were also in agreement to the study of Chaudhry et al. [13] where *S. aureus* was reported to be the most common causative agent of osteoarticular infections. A similar prevalence of *S. aureus* was also observed in a study determining septic arthritis in children. [14]

The study of Tariq [15] elucidated the presence of *Klebsiella*, *E. coli*, *Serratia* sp., *Pseudomonas* sp., along with coagulase negative Staphylococci, *S. aureus* and *Streptococcus* sp. causing infections in children, which agreed with our study. In our study, *S. aureus* (22%), *P. aeruginosa* (23%), *E. coli* (22%), *K. pneumoniae* (12%) and *Micrococcus* (5 %) were found in adults (second and third age group) with *E. coli* and *P. aeruginosa* recovered the most from middle aged patients and older aged patients, respectively (Table 3). The incidence of *S. pyogenes* (2%) and *S. haemolyticus* (1%) was only found in the first age group and whereas *Proteus* was found only in the age group of 31-55 years of age (3 %) (Table 3). The presence of *Klebsiella* sp. in osteomyelitis infection in children was reported for the first time in the study of Qadir et al. [16], which was also found to be sensitive to gentamicin and imipenem. The study findings of Malik.[17] revealed the presence of Enterobacteriaceae, *S. aureus*, and *P. aeruginosa* in osteomyelitis patients, highlighting the presence of these microbial pathogens at infection sites, which seemed to be in agreement with our study.

The results of the antibiogram were described in Table 4, respectively. Out of Gram-positive cocci, *S. aureus* was found to be resistant to vancomycin, while being sensitive to penicillin, amikacin, clindamycin, ciprofloxacin, levofloxacin, erythromycin, gentamycin and moxifloxacin, respectively. This result demonstrated the chance of effective treatment by these antibiotics. *S. haemolyticus* was found to be resistant to amikacin, and gentamycin, while being sensitive to the rest of the antibiotics. *S. pyogenes* was found to be resistant to four antibiotics, namely amikacin, clindamycin, erythromycin and gentamycin, respectively. Results of Tariq [13] did not agree with our study as *Staphylococcus* was found to be resistant to penicillin and sensitive to vancomycin. In group Enterobacteriaceae, resistance to ampicillin was found in *Klebsiella*, *Proteus*, and *Enterobacter* sp. Whereas *E coli* was found to be sensitive to it (Table 6). *E. coli* and *Klebsiella* were found to be resistant to imipenem sensitivity was found in the other bacteria. The same pattern of resistance and sensitivity was reported for tobramycin. Ciprofloxacin sensitivity and piperacillin/tazobactam resistance was found in all four species, respectively. The study findings of Hariharan et al.[18] stated that *E. coli* demonstrated high level of resistance against ampicillin, and ciprofloxacin. *Klebsiella* also demonstrated high resistance to ampicillin, gentamycin and piperacillin/tazobactam. *P. aeruginosa* was found to be resistant to piperacillin/ tazobactam and imipenem, whereas it demonstrated high sensitivity to cefixitin, ceftriaxone, significant sensitivity to ampicillin, and cefradoxil and low sensitivity to piperacillin/ tazobactam in our study. The study findings of Hariharan et al.[18] presented both agreeable and disparate results with respect to our own, as in their study *P. aeruginosa* was found to be resistant to imipenem, piperacillin and ciprofloxacin, respectively.

Conclusion

This study was successful in reporting the various bacterial species that are causative agents of osteoarticular infections in patients of various ages. The presence of both Gram-positive and Gram-negative bacteria demonstrate that although Gram-positive bacteria have been established as the main pathogen involved in causing osteoarticular infections, evolution and changes in epidemiology, community behaveiour, and other environmental factors have rendered Gram-negative bacteria as equally pathogenic for causing these infections.

The antibiogram revealed the antibiotic susceptibility patterns of the bacterial species, indicating effective treatment options for infection. However, care must be taken while treating bone and orthopaedic ailments, wounds, and replacement materials as pathogenesis can easily occur through the entry of microorganism into the body. Moreover, a good hygiene must be practised when treating wounds by patients and people alike, so that the risk of infection is minimized.

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