Clinical Research

Prevalence of methicillin-resistant *Staphylococcus aureus* and other pathogens in pus samples of orthopedic department at a tertiary care hospital in Pakistan

Ashfaq Ahmed, Rizwan Akram, Saeed Ahmad, Atiq Uz Zaman, Naeem Ahmad, Shahzad Javed, Amer Aziz

pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.v28i3.2634
Med J Indones. 2019;28:252–7

Received: April 04, 2018
Accepted: June 25, 2019

Authors' affiliations:
Department of Orthopaedic and Spine Surgery, Ghurki Trust Teaching Hospital, Lahore, Pakistan

Corresponding author:
Ashfaq Ahmed
Department of Orthopaedic and Spine Surgery, Ghurki Trust Teaching Hospital, Lahore, Pakistan
Tel/Fax: +92-03339881342
E-mail: ashfaqjadoon40@yahoo.com

ABSTRACT

BACKGROUND Orthopedic infections are difficult to manage. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most dangerous and harmful bacteria and is difficult to eradicate because of its changing strains as well as sensitivity to different antibiotics. The main aim of this study was to determine the prevalence of pathogens, especially MRSA, associated with orthopedic wounds and their sensitivity to different antibiotics.

METHODS A prospective study was conducted from September 2015 to August 2016. Pus samples of 1,350 patients who presented at the out-patient department or admitted with a wound infection after an orthopedic intervention were taken with the help of culture swab and were sent for culture and sensitivity according to hospital protocol. Data analyses were made using the SPSS software, version 17 (IBM).

RESULTS Adults aged between 15 and 30 years were most affected, with 444 (32.9%) cases. Of the patients, 268 (19.9%) had negative cultures. Among the patients with positive cultures, the gram-positive cocci and gram-negative rods were 497 (36.8%) and 377 (27.9%), respectively. The most common pathogen was MRSA (240; 17.8%), followed by *Escherichia coli* and methicillin-sensitive *S. aureus*.

CONCLUSIONS Multiple pathogens are involved in patients having an orthopedic surgical intervention. The high occurrence of MRSA and *E. coli* has an increasing economic burden on patients because of these pathogens high resistance to antibiotics. Thus, proper preventive measures should be done to decrease the occurrence of such infections as well as their associated morbidity.

KEYWORDS MRSA, orthopedics, prevalence, *Staphylococcus aureus*

Orthopedic infection is a devastating disease because of its physical and emotional trauma[1] as well as its association with high morbidity and cost. The recurrence rate of methicillin-resistant *Staphylococcus aureus* (MRSA) is about 10–20% and is difficult to manage.[2] Several factors differ MRSA from methicillin-sensitive *S. aureus* (MSSA) including antibiotic use at presentation, pulse rate, blood picture, and infective markers.[3] The story behind such resistance of the pathogens is because of the slimy layer called biofilm, which is made by bacteria when they adhere to damaged tissue or implanted medical devices.[4] Many efforts had been made to prevent these biofilm formations like the placement of an inert or tissue-derived implant deep within the body cavity, but still, only a small percentage develops a biofilm layer that harbors invasive microorganisms.[5] The formation of this slimy layer and production of toxins are two different mechanisms. *Staphylococcus* infection is a complex regulatory mechanism that involved cell-to-cell communication through the release and response to chemical signals in a

process known as quorum sensing. To prevent this mechanism, the antibiotics should be given 30 min to 1 hour before incision or before tourniquet inflation.[6]

Reducing the adhesion of a broad range of bacteria could be an attractive means to decrease infection and allow for subsequent appropriate tissue integration with the biomaterial surface.[6,7] *S. aureus* and *Staphylococcus epidermidis* cause at least half of all orthopedic surgical infections. Gram-negative bacilli are involved to a much lesser extent (10–30%).[8] The diagnosis of orthopedics associated with implant infections can be achieved by gathering and evaluating patient's sign and symptoms, microbiology, and laboratory analyses as well as histopathology and imaging studies, among which radiolabeled leukocyte scanning seems to be suitable for early diagnosis.[9]

The only methods that are Food and Drug Administration-approved for detecting and identifying bacterial infections are cultures and selected DNA-based polymerase chain reaction (PCR) methods that detect only specific pathogens (e.g., MRSA). New DNA-based technologies enable to detect and identify all bacteria present in a sample and to determine the antibiotic sensitivities of the organisms.[10] The culture methods still used in microbiology laboratories were developed by Robert Koch in Berlin back in 1884.[9] The culture and sensitivity of the pus samples obtained from the wound are the readily available tests and more cost effective. There is a literature gap in among Pakistani population in infected orthopedics patients. There is no previous literature available to the best of our knowledge to determine the trend of different microorganisms, especially MRSA and their sensitivity to different drugs in orthopedic infections. The main aim of this study was to determine the prevalence of different microorganisms, especially MRSA in Pakistani population and their sensitivity as well as resistance to various antibiotics.

METHODS

This was a prospective study that enrolled 1,350 patients of either sex and any age who presented with wound infections related to any type of orthopedic surgery managed whether for open fracture or closed fracture after initial trauma or for any type of elective surgery like arthroplasty through either the out-patient department or the emergency department between September 2015 and August 2016 at the Department of Orthopedics and Spine Centre, Ghurki Trust Teaching Hospital, Pakistan. The patients were classified into three socioeconomic status i.e. low (poor in the society), middle (the patients relied on their salaries and have no savings), and upper (the wealthy families who had accumulation of their wealth). The patients were also asked for any alcohol or other drugs addiction like heroine, cannabis, cocaine, tobacco, and others. The diagnosis classification was determined to be acute if there was an infection from total knee replacement before four weeks but there was no need of removal of the implant; and chronic was determined if the time period was after four weeks. Moreover, the location of pus was determined based on the wound made after the intervention was done; it would be considered superficial if the wound was only limited to muscular and deep if the wound was exposed to the bone. The comorbidites were retrieved from diagnosis written in medical records.

The patients who presented with infection without any previous culture and sensitivity report were asked to stop antibiotics for 3 days, and then, pus samples were taken for culture and senstivity. The antibiotics were stopped for 3 days to prevent any bias in report. The patients who did not give consent, were discharged before their laboratory reports came, and were lost in follow-up were excluded from the study. Of the patients, 1,100 (81.5%) were operated at other orthopedic centers, whereas 250 (18.5%) were operated at Department of Orthopedics, Ghurki Trust Teaching Hospital. The average duration of patients presented with wound infection was 23.5 days (range 5–88 days). The approval for this study was taken from the Hospital Ethical Committee with reference number GTTH:2015/31 and after proper patient consent. After taking a detailed history and thorough examination of infected wound side, samples of pus from infected side were taken using sterile cotton-tipped swabs.Swabs were transported to the laboratory within 30 min of collection. The culture and sensitivity were done according to the hospital and laboratory protocols. All samples were sent to the same diagnostic laboratory, and patients were given ceftriaxone and amikacin as empirical treatments as per the hospital protocol until the

Table 1. Patient demographic characteristics in all patients and MRSA-infected patients

Sample characteristics	All patients, n (%) (N = 1,350)	MRSA-infected patients, n (%) (N = 240)
Sex		
Male	1,096 (81.2)	201 (83.8)
Female	254 (18.8)	39 (16.3)
Socioeconomic status		
Low	681 (50.4)	172 (71.7)
Middle	506 (37.5)	45 (18.8)
Upper	163 (12.1)	32 (13.3)
Area		
Urban	326 (24.2)	108 (45.0)
Rural	1,024 (75.9)	132 (55.0)
Age (years)		
<15	160 (11.9)	67 (27.9)
15–30	216 (16.0)	73 (30.4)
31–45	444 (32.9)	55 (22.9)
46–60	358 (26.5)	45 (18.7)
>60	172 (12.7)	
Comorbidity		
No comorbidity	1,107 (82)	175 (72.9)
DM	97 (7.2)	38 (15.8)
CRF	12 (0.9)	5 (2.1)
HBV, HCV	15 (1.1)	7 (2.9)
HTN	47 (3.5)	9 (3.5)
Others	72 (5.3)	6 (2.5)
Addiction (smoking, others)		
Yes	291 (21.6)	35 (14.6)
No	1,059 (78.4)	205 (85.4)
Diagnosis		
Acute (≤4 weeks)	768 (56.9)	-
Chronic (>4 weeks)	582 (43.1)	-
Location		
Superficial	415 (30.7)	-
Deep	935 (69.2)	-

MRSA=methicillin-resistant *Staphylococcus aureus*; DM=diabetes mellitus; CRF=chronic renal failure; HBV=hepatitis B virus; HCV=hepatitis C virus; HTN=hypertension

culture and sensitivity reports came, which were also changed if needed. Culture method was used rather than PCR because of affordability issues of the patients. Almost all of the patients were from poor families. Data were initially entered on a preformed proforma, and then, SPSS software, version 17 (IBM) was used for data analysis. Descriptive statistics were applied, and the results were presented as numbers and percentages. Graphs were used where necessary.

RESULTS

The patient demographic characteristic are shown in Table 1. A total of 1,350 patients with a mean (SD) age of 37.1 (18.1) years were included in the study. Of the patients, 1,096 (81.2%) were men, and 254 (18.8%) were women, with a men to women ratio of 4.31:1. There were 160 (11.9%) patients less than 15 years old, 216 (16.0%) between 15 and 30 years old, 444 (32.9%) between 31 and 45 years old,

Table 2. Frequency of different pathogens according to gram stain and bacterial shape

Pathogen	Frequency, n (%) (N = 1,350)
Gram-positive cocci	497 (36.8)
Gram-negative rods	377 (27.9)
Gram-negative rods and positive cocci	92 (6.8)
Gram-negative cocci	78 (5.8)
Gram-negative rods and cocci	16 (1.2)
Fungus	8 (0.6)
Gram-positive rods and cocci	6 (0.4)
Gram-positive rods and negative cocci	4 (0.3)
Gram-positive rods	4 (0.3)
No microorganism (negative culture)	268 (19.9)

Table 3. Frequency of different pathogens species

Pathogen	Frequency, n (%) (N = 1,350)
MRSA	240 (17.8)
Escherichia coli	214 (15.9)
MSSA	214 (15.9)
Streptococci species	92 (6.8)
Acinetobacter species	82 (6.0)
Klebsiella species	74 (5.5)
Proteus spp.	64 (4.7)
Pseudomonas aeruginosa	58 (4.3)
Others	22 (1.6)
Proteus mirabilis	14 (1.0)
Fungus	8 (0.60)
No pathogens	268 (19.9)

MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-sensitive *S. aureus*

358 (26.5%) between 46 and 60 years old, and 172 (12.7%) above 60 years old.

The frequency of pathogens was different; of the pus samples, 268 (19.9%) were negative for any type of microorganisms. Most of the microorganisms were gram-positive cocci 497 (36.8%) followed by gram-negative rods 377 (27.9%). Some samples showed growth of more than one organism, which was also included in this study. The gram-negative rods and gram-positive cocci were found in 92 (6.8%) of the cultures. Other pathogens and their frequency with percentages are presented in Table 2. The different microorganisms were in various percentages. The most common were MRSA 240 (17.8%) followed by *Streptococci* species and MSSA with equal incidence 214 (15.9%). The fungal infection was found only in 8 (0.6%) patients. The summary of the occurrence of other organisms is shown in Table 3. The characteristics of patients suffering from MRSA is summarized in Table 1.

The summary of different organisms susceptible to various antibiotics is presented in Table 4. Vancomycin and doxycycline were the most common drugs sensitive against MRSA followed by amikacin and fusidic acid. *Proteus mirabilis*, *Klebsiella* species, *Pseudomonas aeruginosa*, and *Escherichia coli* were most sensitive to amikacin. MSSA were mostly sensitive to vancomycin followed by ampicillin and amoxicillin. *Streptococcus* species showed most and equal sensitivity to amoxicillin and ampicillin.

DISCUSSION

Healthcare-associated infections are relatively rare in orthopedic and trauma surgery compared with other surgical wards.[2] The principles of treatment depend on different factors, including determining appropriate indications for antibiotic administration, choosing the correct antibiotic based on known or expected pathogens, determining the correct dosage, and determining the appropriate duration of treatment.[11,12] The infections caused by *S. aureus* are highly variable ranging from relatively mild infections of the skin and soft tissue to life-threatening sepsis. The changing strains of this pathogen to methicillin and other antimicrobial agents have become a serious concern, especially in the hospital environment, because of the higher mortality due to systemic MRSA infections.[13] The epidemiology of MRSA has continued to evolve since its first appearance more than three decades ago.[14] The emergence of MRSA in 1961 occurred mostly in hospitalized patients, whereas community-acquired MRSA isolates were identified in the 1990's.[15] Individuals previously colonized with MRSA are more at risk of surgical site infections compared with other patients, especially those undergoing joint replacement surgeries of the lower limbs.[11] MRSA infections are associated with increased mortality, costs, and length of stay compared with non-MRSA infections.[16]

In this study, the mean (SD) age of patients suffering from MRSA was 37.1 (18.1) years. Most of the patients were suffering from MRSA 240 (17.8%), with a higher proportion of men 187 (77.9). Men

Table 4. Sensitivity of antibiotics to different pathogens*

Antibiotics	Microorganisms, n (%)					
	MRSA	Streptococci	*E. coli*	MSSA	Others	Total
Amoxicillin	5 (0.37)	38 (2.8)	12 (0.9)	82 (6.1)	29 (2.0)	164 (12.2)
Ampicillin	5 (0.37)	38 (2.8)	10 (0.74)	84 (6.2)	25 (1.8)	162 (12.0)
Amikacin	77 (5.7)	16 (1.2)	121 (9.0)	69 (5.1)	121 (6.0)	808 (59.9)
Gentacin	37 (2.7)	20 (1.5)	68 (5.0)	65 (4.8)	81 (6.0)	271 (20.1)
Doxycycline	79 (5.9)	28 (2.1)	66 (4.9)	56 (4.2)	89 (6.2)	318 (23.6)
Ciprofloxacin	15 (1.1)	13 (0.96)	32 (2.4)	50 (3.7)	46 (3.4)	156 (11.6)
Cefoperazone + sulbactam	31 (2.3)	5 (0.37)	92 (6.8)	17 (1.3)	84 (11.1)	229 (16.9)
Piperacillin	35 (2.6)	5 (0.37)	94 (7.0)	13 (0.96)	101 (7.4)	248 (18.3)
Fucidin	66 (4.9)	9 (0.67)	27 (2.0)	62 (4.6)	47 (3.0)	204 (15.1)
Vancomycin	201 (14.9)	17 (1.3)	9 (0.67)	145 (10.7)	17 (1.2)	389 (28.8)
Ecasil	1 (0.07)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.07)
Tygacil	2 (0.14)	0 (0)	6 (0.44)	5 (0.37)	33 (2.0)	40 (3.0)
Polymyxin	-	2 (0.14)	26 (1.9)	5 (0.37)	41 (3.1)	75 (5.6)
Colistin	-	2 (0.14)	25 (1.9)	5 (0.37)	37 (2.5)	66 (4.9)

MRSA=methicillin-resistant *Staphylococcus aureus*; *E. coli=Escherichia coli*; MSSA, methicillin-sensitive *S. aureus*
*Most of pathogens are sensitive to more than one antibiotic

were mostly infected because of their high outdoor activities compared with women among Pakistani population. Kelly et al[17] reviewed 12,259 orthopedic patients over 3 years. The mean age of MRSA-infected patients in their study was 71 years, with a higher proportion of female patients than male patients.[17]

According to this study, there is a high occurrence of MRSA in orthopedic wounds because of overcrowding, workload, and understaffing of wards. The total prevalence rate of MRSA in the current study was 17.8%, whereas the study conducted in the United Kingdom found that 1.6% of the total admission was diagnosed to be either MRSA-infected or colonized, with an average of three new MRSA cases detected per month.[18] Similarly, in a study in Ireland, MRSA represented 0.76% of all admissions.[19] The correlation of different risk factors like overcrowding has not been fully established in the literature. Prevalence rates of MRSA varied over a wide range, from less than 1% to greater than 20% in different studies.[13] In the current study, 454 (33.7%) patients were found infected with *S. aureus*. Of which, 240 (17.8%) were identified as MRSA, whereas 214 (15.9%) were MSSA. Vidhani et al[14] conducted a study on high-risk patients and found that 188 (41.8%) patients were positive for *S. aureus*. Out of which, the proportion of MRSA was found to be 51.6%, that is 97 out of 188. Similarly, Kaur and Wankhede[20] found that the common pathogens were MRSA 85 (32.4%) and MSSA 60 (22.9%). Chen and Zhu[21] also revealed that *P. aeruginosa* (17.1%) and *S. aureus* (14.8%) were the leading microorganisms.

The sensitivity of pathogens to various drugs were varied. In the current study, MRSA (14.9%) and MSSA (10.7%) were mostly sensitive to vancomycin. Kumar et al[22] did a study in a tertiary care hospital and found that the sensitivity of MRSA to vancomycin and clindamycin were 100% and 78.0%, respectively, whereas the resistance of MRSA was very high for co-trimoxazole (88.1%) and ciprofloxacin (81.4%). MRSA with intermediate resistance to vancomycin was first isolated in 2006 from a surgical wound of a patient hospitalized at the orthopedic ward of Hospital de São Marcos-Braga, in the town of Braga.[23] Economedes et al[24] found that only 2 (11%) who were previously decolonized changed antibiotics sensitivity after retesting. The study of Dar et al[25] concluded that in case of both methicillin-resistant and methicillin-sensitive *Staphylococcal* isolates, zero resistance was found to vancomycin, whereas the highest resistance was found to penicillin G followed by ampicillin. The exact mechanism of changing antibiotics sensitivity is still unknown.

There are some limitations to this study. The patients were not regularly followed after managing the wounds and presenting with reinfection. Moreover, the pathogens associated with different procedures of orthopedics were not mentioned separately to give a better understanding of various pathogens and their sensitivity to different drugs. Furthermore, highly sensitive tests like PCR were not used for further analysis because of the cost issue for the patients. The exact cause of the high negative culture rate was also not sorted out. So, further studies are needed especially among Pakistani population to give more feasible results.

In developing countries like Pakistan, there is an increased incidence of MRSA and other pathogen infections. Several factors are associated with these such as overburden patients, nontechnical staff, ward understaffing, poor sterilization techniques, and poor hygiene patients. All the risk factors which contribute to orthopedics infections especially MRSA should be sort out properly because of the changing sensitivity of these pathogens to different antibiotics as well as for the formulation of a definite antibiotic policy.

Conflict of Interest

The authors affirm no conflict of interest in this study.

Acknowledgment

The authors would like to thank all members of the Department in Ghurki Trust Teaching Hospital for providing help in this study.

Funding Sources

None.

REFERENCES

1. Ehrlich GD, Stoodley P, Kathju S, Zhao Y, McLeod BR, Balaban N, et al. Engineering approaches for the detection and control of orthopaedic biofilm infections. Clin Orthop Relat Res. 2005;(437):59–66.
2. Uckay I, Hoffmeyer P, Lew D, Pittet D. Prevention of surgical site infections in orthopaedic surgery and bone trauma: state-of-the-art update. J Hosp Infect. 2013;84(1):5–12.
3. Ju KL, Zurakowski D, Kocher MS. Differentiating between methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* osteomyelitis in children: an evidence-based clinical prediction algorithm. J Bone Joint Surg Am. 2011;93(18):1693–701.
4. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;358(9276):135–8.
5. Hickok NJ, Shapiro IM. Immobilized antibiotics to prevent orthopaedic implant infections. Adv Drug Deliv Rev. 2012;64(12):1165–76.
6. Trampuz A, Widmer AF. Infections associated with orthopedic implants. Curr Opin Infect Dis. 2006;19(4):349–56.
7. Puckett SD, Taylor E, Raimondo T, Webster TJ. The relationship between the nanostructure of titanium surfaces and bacterial attachment. Biomaterials. 2010;31(4):706–13.
8. Periti P, Mini E, Mosconi G. Antimicrobial prophylaxis in orthopaedic surgery: the role of teicoplanin. J Antimicrob Chemother. 1998;41(3):329–40.
9. Arciola CR, Montanaro L, Costerton JW. New trends in diagnosis and control strategies for implant infections. SAGE Publications Sage UK: London, England; 2011.
10. Palmer M, Costerton W, Sewecke J, Altman D. Molecular techniques to detect biofilm bacteria in long bone nonunion: a case report. Clin Orthop Relat Res. 2011;469(11):3037–42.
11. Murphy E, Spencer SJ, Young D, Jones B, Blyth MJ. MRSA colonisation and subsequent risk of infection despite effective eradication in orthopaedic elective surgery. J Bone Joint Surg Br. 2011;93(4):548–51.
12. Campbell KA, Stein S, Looze C, Bosco JA. Antibiotic stewardship in orthopaedic surgery: principles and practice. J Am Acad Orthop Surg. 2014;22(12):772–81.
13. Dulon M, Haamann F, Peters C, Schablon A, Nienhaus A. MRSA prevalence in European healthcare settings: a review. BMC Infec Dis. 2011;11:138.
14. Vidhani S, Mehndiratta PL, Mathur MD. Study of methicillin resistant *S. aureus* (MRSA) isolates from high risk patients. Indian J Med Microbiol. 2001;19(2):13–6.
15. Truong H, Shah SS, Ludmir J, Tawananana EO, Bafana M, Wood SM, et al. *Staphylococcus aureus* skin and soft tissue infections at a tertiary hospital in Botswana. S Afr Med J. 2011;101(6):413–6.
16. Edmundson S, Hirpara K, Bennett D. The effectiveness of methicillin-resistant *Staphylococcus aureus* colonisation screening in asymptomatic healthcare workers in an Irish orthopaedic unit. Eur J Clin Microbiol Infect Dis. 2011;30(9):1063–6.
17. Kelly J, O'Briain D, Walls R, Lee SI, O'Rourke A, Mc Cabe JP. The role of pre-operative assessment and ringfencing of services in the control of methicillin resistant *Staphlococcus aureus* infection in orthopaedic patients. Surgeon. 2012;10(2):75–9.
18. Tai CC, Nirvani AA, Holmes A, Hughes SP. Methicillin-resistant *Staphylococcus aureus* in orthopaedic surgery. Int Orthop. 2004;28:32–5.
19. Roche SJ, Fitzgerald D, O'Rourke A, McCabe JP. Methicillin-resistant *Staphylococcus aureus* in an Irish orthopaedic centre: a five-year analysis. J Bone Joint Surg Br. 2006;88(6):807–11.
20. Kaur DC, Wankhede S. Biofilm formation and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* from wound infection. Asian Pac J Health Sci. 2014;1(4):322–28.
21. Chen X, Zhu D. Pathogens distribution of hospital infection and their drug resistance in Department of Orthopedics. Chinese Journal of Nosocomiology. 2008;(04):570–3.
22. Kumar S, Joseph NM, Easow JM, Singh R, Umadevi S, Pramodhini S, et al. Prevalence and current antibiogram of Staphylococci isolated from various clinical specimens in a tertiary care hospital in Pondicherry. Internet J Microbiol. 2012;10(1).
23. Gardete S, Aires-De-Sousa M, Faustino A, Ludovice AM, de Lencastre H. Identification of the first vancomycin intermediate-resistant *Staphylococcus aureus* (VISA) isolate from a hospital in Portugal. Microb Drug Resist. 2008;14(1):1–6.
24. Economedes DM, Deirmengian GK, Deirmengian CA. *Staphylococcus aureus* colonization among arthroplasty patients previously treated by a decolonization protocol: a pilot study. Clin Orthop Relat Res. 2013;471(10):3128–32.
25. Dar JA, Thoker MA, Khan JA, Ali A, Khan MA, Rizwan M, et al. Molecular epidemiology of clinical and carrier strains of methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital settings of north India. Ann Clin Microbiol Antimicrob. 2006;5:22.