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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

BİR ÜÇÜNCÜ BASAMAK HASTANESİNDE PRİMER VE REVİZYON DİZ PROTEZİ AMELİYATLARINDAN SONRA İZOLE EDİLEN MİKROORGANİZMALARIN SIKLIĞININ VE ANTİBİYOTİK DİRENÇLERİNİN ZAMAN İÇERİSİNDE DEĞİŞİMİNİN DEĞERLENDİRİLMESİ

ASSESSMENT OF THE FREQUENCY OF ISOLATED MICROORGANISMS AND CHANGE OF THEIR ANTIBIOTIC RESISTANCE OVER TIME AFTER PRIMARY AND REVISION KNEE ARTH- ROPLASTIES AT A TERTIARY REFERRAL HOSPITAL

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ÖZET

AMAÇ: Artmış total diz artroplasti sayısı zaman içerisinde periprostetik eklem enfeksiyonları (PEE) ve diğer komplikasyonların artmasına neden olmuştur. Enfeksiyon ve mikrobiyoloji konusundaki güncel trendlerin anlaşılması PPE'nin tedavisi ve önlenmesi için gereklidir. Bu nedenle çalışmada zaman içerisinde revizyon total diz protezi ameliyatı sonrasında izole edilen bakteri türlerindeki ve bu bakterilerin gösterdiği antibiyotik dirençlerindeki değişimi ortaya koymayı amaçladık.

GEREÇ VE YÖNTEM: İki hasta grubu retrospektif olarak değerlendirildi. Birinci grup enfeksiyon gelişimine neden olan ameliyatını 2005 ve 2011, ikinci grup ise 2012 ve 2018 yılları arasında olan hastalardan oluşmaktaydı. İzole edilen mikroorganizma türlerinin ve bu organizmaların antibiyotik dirençlerinin zaman içerisindeki değişimi incelendi. Sefazolin (allerji varlığında da klindamisin) 2005 ile 2018 yıllarında rutin profilakside kullandığımız antibiyotiktir.

BULGULAR: Çalışmaya 42 hasta (43 diz eklemi) dahil edildi. En sık izole edilen bakteri *Staphylococcus epidermidis* idi. *Staphylococcus aureus* birinci ve ikinci grupta en sık izole edilen ikinci bakteriydi. Zamanla gram-negatif (*Pseudomonas aeruginosa*) bakteri izolasyon oranlarında artış olduğu gözlendi. Yapılan istatistiksel analize göre siprofloksasine (p=0.0021), gentamisine (p=0.0001), tetrasikline (p=0.043) ve trimetoprim/sulfomethoksazole (p=0.0016) karşı antibiyotik direncinde zaman içerisinde artış olduğu gözlendi. Sefazolin allerjisi durumunda kullandığımız klindamisine karşı (p=0.88) zaman içerisinde artmış bir antibiyotik direnci gözlenmedi. Sefazolin direncinin 2012 ve 2018 yılları arasında belirgin olarak azaldığı gözlendi. Sadece bir hastada vankomisin direnci olduğu görüldü.

SONUÇ: Literatür ve bizim sonuçlarımız PEE olan hastalarda gram negatif bakteri izolasyon oranlarında istikrarlı bir artış olduğunu göstermektedir. Bu yüzden, diz artroplastisinde gram negatif bakterileri de kapsayacak antibiyotik protokollerinin kullanılması zamanla daha da gerekli hale gelecektir. Yapılan çalışmada siprofloksasine, gentamisine, tetrasikline ve trimetoprim/sulfomethoksazole karşı antibiyotik direncinde zaman içerisinde artış olduğu gözlenmiştir fakat bu antibiyotikler bizim rutin profilakside kullandığımız antibiyotikler değildir. Profilaksi için tek başına sefazolin veya klindamisin kullanmak yerine bu antibiyotiklerin gentamisinle kombine edilmesi gereklilik arz etmektedir.

ANAHTAR KELİMELER: Diz, Artroplasti, Enfeksiyon, Mikroorganizma, Antibiyotik direnci

ABSTRACT

OBJECTIVE: Increased number of total knee arthroplasty has led to an increases the risk of periprosthetic joint infection (PJI) and other complications over time. Therefore, the current trends in infection and microbiology data are necessary to be understood to prevent and treat knee PJI. For this reason, we aimed to identify the course of bacterial species isolated after revision total knee arthroplasty and to investigate the change of antibiotic resistance over time.

MATERIAL AND METHODS: Two groups of patients were evaluated, retrospectively. Group 1 consisted of patients who had surgery that caused the development of infection performed between 2005 and 2011 while group 2 consisted of patients between 2012 and 2018. The variation of isolated microorganisms species and their antibiotic resistances over time were investigated. Cefazolin (clindamycin in case of allergy) was the antibiotic used for routine prophylaxis between 2005 and 2018.

RESULTS: Overall, 42 patients (43 knee joints) were included in the study. The most frequently isolated bacterium was *Staphylococcus epidermidis* while *Staphylococcus aureus* was the second most isolated bacterium in groups 1 and 2. An increased rate of gram-negative bacteria (*Pseudomonas aeruginosa*) isolation was observed in time. According to statistical analysis, a significant increase in antibiotic resistance to ciprofloxacin (p=0.0021), gentamicin (p=0.0001), tetracycline (p=0.043) and trimethoprim/sulfamethoxazole (p=0.0016) were observed over time. No increased antibiotic resistance observed over time against clindamycin (p=0.88) which we used in case of cefazoline allergy. Cefazoline resistance significantly decreased during 2012 and 2018 (p<0.0001). Vancomycin resistance was observed in only one patient.

CONCLUSIONS: Our results and the literature has showed a steady increase in gram-negative bacteria isolation rates in patients with PJI. Therefore, it will become more necessary to use prophylactic antibiotic regimens including gram-negative bacteria in knee arthroplasty surgery. Although an increased resistance to ciprofloxacin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole was found over time in the current study, these were not the antibiotics we used for routine prophylaxis. Instead of using cefazolin or clindamycin alone for prophylaxis, it is necessary to combine these anatibiotics with gentamicin.

KEYWORDS: Knee, Arthroplasty, Infection, Microorganism, Antibiotic resistance

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INTRODUCTION

Total knee arthroplasty (TKA) is a satisfying operation for reducing pain, improving joint function, and enhancing the quality of life in patients with knee osteoarthritis. Notably, an increased number of primary TKAs has been reported in the United States (1) and European countries (2) over the last few decades. However, an increased number of TKAs results in elevated periprosthetic joint infection (PJI) and other complications. Notably, PJI is a severe and sophisticated complication after surgery. Periprosthetic joint infection is the most common etiology for revision TKA in the United States (3).

Periprosthetic joint infection is associated with numerous challenges such as the need for multiple operations, a prolonged period of disability for the patient and occasionally, suboptimal outcomes (4, 5), which results in an economic burden for the society and psychological and biological burden for patients. The aim of the present study was to identify the course of bacterial species isolated during revision TKA over a time duration and to investigate the transition of antibiotic resistance over time.

MATERIALS AND METHODS

This retrospective study was conducted in accordance with the Helsinki Declaration Principles. After approval of the local ethics committee, a retrospective search was performed using the online database of our tertiary referral hospital for the period between 2005 and 2018. Demographics of all patients diagnosed with infection were collected. Patients older than 18 years and with a primary TKA or revision knee due to septic etiology were included in this study. Patients with unicondylar knee or tumor arthroplasty, patients with missing antibiogram record and patients whose index operation performed in another hospital were excluded in the study.

Patients with infected TKA, who were treated with one of the following three options were included, as only-debridement, debridement plus polyethylene insert replacement, and two-stage revision arthroplasty depending on the setting of infection. In addition, the number of spacer implantations per patient was evaluated. Cefazolin was the antibiotic used for routine prophylaxis. Clindamycin was used in case of cephalosporin allergy for prophylaxis. Workgroup of the Musculoskeletal Infection Society infection criteria was used to diagnose PJI as seen in **Table 1** (6).

Table 1: Definition of periprosthetic joint infection. LE: leukocyte esterase, PMN: polymorphonuclear, WBC: white blood cell. For the patients who had 2-5 scores based on the intraoperative minör criteria or who had dry tap, intraoperative diagnostic criteria can be used.

Major criteria (One of the following enough for diagnosis)
Visualization of prosthesis or evidence of a sinus
Two positive cultures with the same organism

		Minor criteria	**Score**	**Decision,**
Preoperative Diagnosis	**Serum**	Elevated CRP or D-dimer	2	
		Elevated ESR	1	≥ 6 infected
	Synovial	Elevated synovial WBC count or LE	3	
		Positive alpha-defensin	3	
		Elevated synovial PMN (%)	2	2-5 possibly infected
		Elevated synovial CRP	1	
				0-1 not infected

	Inconclusive pre-op score or dry tap	**Score**	**Decision**
Intraoperative Diagnosis	Preoperative score	-	≥ 6 infected
	Intraoperative purulent material	3	
	Positive culture with an organism	2	
	Positive frozen biopsy	3	4-5 inconclusive
			≤ 3 not infected

In the operation room, before prophylactic antibiotic administration, synovial fluid samples and periprosthetic soft tissue with inflammatory changes were collected for microbiological and histopathological examination. Samples were transferred in dry, sterile, plastic containers for gram staining and culture. They were inoculated directly onto a conventional blood agar plate (5% of bovine blood) or liquid thioglycolate medium. Blood agar and thioglycolate medium cultures were incubated at 37 ºC. Blood agar cultures were incubated in 5% CO2 atmosphere and thioglycolate medium cultures were incubated in an air atmosphere. Cultures were checked daily for 7 days. If any growth was suspected in the thioglycollate medium, it was cultivated on a Schadler agar medium with 5% sheep blood and incubated in an anaerobic atmosphere. Cultures were accepted negative if no growth was seen within 7 days. Isolated microorganisms were identified by conventional and metabolic tests (catalase, oxidase etc). Antibiotic susceptibility was assessed by the disk-diffusion susceptibility test. ARB and mycobacteria cultures were also performed in one patient because of a previous history of tuberculosis. *Mycobacterium tuberculosis* was isolated in this patient. A second operation was performed on the patient, whose postoperative cure could not be achieved and no microorganism was isolated from the cultures taken during debridement surgery made for PJI. In the second operation ARB, mycobacteria and fungal cultures were obtained and *Candida albicans* were isolated.

Antibiogram tests of these patients were analyzed retrospectively and isolated microorganisms were noted. Antibiotic resistance was determined according to the culture antibiogram tests of isolated agents. Also, the index operation time (minute), the time duration from index surgery to the infection diagnosis date (days) and whether the index operation was a primary or revision TKA operation were noted.

We aimed to investigate whether there were changes over time in the isolated microorganism species. Therefore, patients were divided into two groups: Group 1 included patients who had the index surgery performed between 2005 and 2011 and group 2 included patients with the index surgery performed between 2012 and 2018.

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA) software. Descriptive statistics were calculated as appropriate for the variables. The chi-square test was used to perform the intergroup comparison of changes in the type and rate of bacteria isolated during the two time periods in terms of age, sex, type of index surgery (revision or primary), duration of surgery (minutes) and time until infection (postoperative days). The chi-square test was used to compare the changes in the prevalence of the isolated bacteria types during the two time periods in terms of the total number of surgeries in the hospital, the total number of knee surgeries and the number of infected knees.

The antibiotic resistance of the isolated bacteria was compared using the chi-square test, too. Differences were evaluated using the chi-square test with the significance level set at 0.05.

Ethical Committee

Ministry of Health Metin Sabanci Bone and Joint Diseases Education and Research Hospital, protocol number 55/388.

RESULTS

Patient search on the online database resulted in 4557 knee replacement operations conducted on 4352 patients between 2005 and 2018. Revision surgeries included 319 surgeries of 305 patients. Overall, 172 revision TKAs owing to infection were performed in 168 patients. One hundred and twenty-nine knee of one hundred and twenty-six patients were excluded from the study **(Figure 1)**.

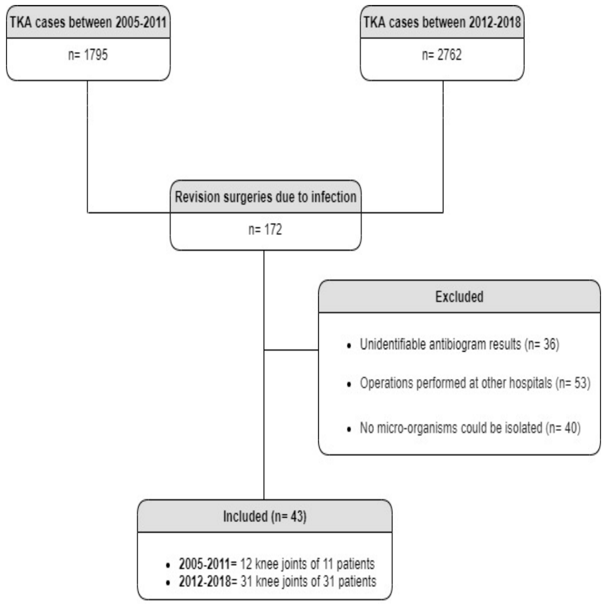

Figure 1: Flowchart showing the selection of patients to be included in the study

Consequently, 43 kneeS of 42 patients were included in the study. The average age of patients was 66.74 years (39-94 years). Overall, there were 12 knees of 11 men (26.19%) and 31 knees of 31 women (73.81%). Nevertheless, no significant differences were noted related to age, gender, type of index surgery, duration of surgery, time of infection diagnosis between the two time periods ($p<0.05$). The distribution of these data is given in **(Table 2)**. Overall, 16 microorganisms were isolated in 11 patients (12 knees) in group 1, and 39 microorganisms from 31 patients (31 knees) in group 2. The most frequently isolated bacterium was Staphylococcus epidermidis (43.75%) with Staphylococcus aureus

(25%) being the second, in group 1. The other microorganisms isolated are listed in Table III. Notably, 2 different microorganisms were isolated from 4 patients in group 1 **(Table 3)**.

Table 2: Distribution of surgery related, infection related and demographic parameters.

Parameters	Group I (patients n:11, 12 knees)	Group II (patients n: 31, 31 knees)	Total (patients n:42,43 knees)
Age	72 (48-84)	67.03 (39-94)	66.74 (39-94)
Sex (Female/male)	5/6	26/5	31/11
Side of surgery (Right/left)	3/9	9/22	12/31
Type of index surgery (revision/primary)	3/9	6/25	9/34
Duration of Surgery (min)	168.19 (90-200)	134.2 (75-165)	145.57 (75-200)
Time of infection (postoperative days)	61 (15-109)	343.4 (16-594)	225.2 (15-594)

Table 3: Number of isolated micro-organisms depending on two different time periods. (MR: Methicillin resistance, MS: Methicillin sensitive, Sp: Species).

Micro-organism	Time duration 2005-2011		2012-2018		2005-2018 (whole period)	
	Patient	%	Patient	%	Patient	%
1) *Candida albicans*	1	6.25	1	2.57	2	3.64
2) *Escherichia coli*	1	6.25	1	2.57	2	3.64
3) *Enterobacter cloacae*	0	0	2	5.13	2	3.64
4) *Enterococcus faecalis*	0	0	1	2.57	1	1.82
5) *Enterococcus faecium*	0	0	1	2.57	1	1.82
6) *Micrococcus sp*	1	6.25	1	2.57	2	3.64
7) *Koagülase negative Staphylococcus*	0	0	1	2.57	1	1.82
8) *Staphylococcus haemolyticus*	0	0	1	2.57	1	1.82
9) *Staphylococcus Aureus*	4	**25**	10	**25.62**	14	**25.45**
9a) MRSA	2	12.5	2	5.13	4	7.27
9b) MSSA	2	12.5	8	20.49	10	18.18
10) *Streptococcus epidermidis*	7	**43.75**	11	**28.19**	18	**32.71**
10a) MRSE	5	31.25	8	20.49	13	23.63
10b) MSSE	2	12.5	3	7.7	5	9.08
11) *Methicillin-sensitive Staphylococcus sp (undefined)*	0	0	1	2.57	1	1.82
12) *P. aeruginosa*	1	6.25	5	**12.8**	6	**10.9**
13) *Proteus mirabilis*	0	0	1	2.57	1	1.82
14) *Pseudomonas stutzer*	0	0	1	2.57	1	1.82
15) *Staphylococcus intermedius*	0	0	1	2.57	1	1.82
16) *Mycobacterium Tuberculosis*	1	6.25	0	0	1	1.82

In group 2, the most frequently isolated bacterium was S. epidermidis (28.19%), followed by S. aureus (25.62%) and Pseudomonas aeruginosa (12.8%). Notably, 3 different microorganisms were isolated from 2 patients while 2 different microorganisms were isolated from 4 patients in group 2 **(Table 4)**.

Table 4: Detailed analysis of ten patients with multiple microorganism growth.

Time duration	Patients	Micro-organism 1	Micro-organism 2	Micro-organism 3
GROUP 1 (2005-2011)	1	*M. tuberculosıs*	*Micrococcus sp.*	
	2	MSSA	MSSE	
	3	MRSA	*P. aeruginosa*	
	4	MRSE	MSSE	
GROUP 2 (2012-2018)	5	MSSA	*P. aeruginosa*	MRSE
	6	*Enterococcus faecium*	*Enterobacter cloacae*	MRSE
	7	MSSE	MRSE	
	8	*Enterococcus faecalis*	*Proteus mirabilis*	
	9	MSSA	*P. aeruginosa*	
	10	MSSE	*C. albicans*	

S. epidermidis (32.71%) was the most frequently isolated bacterium for the entire study period, with S. aureus being the second and *P. Aeruginosa* being the third. The recent period revealed an increased rate of gram-negative bacteria (*P. Aeruginosa*) isolation as evidenced by 2 knees (12.5%) in group 1 and 10 knees (25.64%) in group 2. Regarding methicillin-resistance, methicillin-resistance to *S. epidermidis* (MRSE) was the most frequently isolated bacterium followed by methicillin-sensitive *S. aureus* (MSSA) during the entire study period. *P. aeruginosa* and MSSA infection rates were increased in group 2.

Hospital records were evaluated to analyze how the overall hospital surgery rate, number of knee arthroplasties and number of infected knees affected microorganism isolation over a time duration. Notably, the total hospital surgeries and knee replacement surgeries were increased between 2012 and 2018. Moreover, the PJI rate was noted to be increased **(Table 5)**.

Table 5: Number of infected knees, number of knee arhtroplasty operations (tkp and revision), PJI rates, hospital infection rates, number of all surgeries.

	2005-2011	2012-2018
Number of infected knees	19	64
Number of knee prostheses operations	1795	2762
*PJI rates	1.06 %	2.32 %
Number of all surgeries	34884	66069

Furthermore, the prevalence of microorganisms during the two time periods was calculated **(Table 6)**. Notably, the number of isolated microorganisms per the total number of knee replacement surgeries significantly decreased during 2012-2018 for MRSE and MSSA, whereas an increasing trend was observed for *P. aeruginosa* ($p<0.005$). In addition, the number of isolated microorganisms per the total hospital surgeries was significantly decreased during 2012 - 2018 for *Candida albicans, Escherichia coli, Micrococcus sp*, methicillin-resistant *S. aureus*, MSSA and methicillin-sensitive *S. epidermidis*, whereas *P. aeruginosa* isolation was significantly increased ($p<0.005$). Antibiotic resistance based on the antibiogram results for groups 1&2 were given in **(Table 7)**.

Table 6: Prevalence of the microorganisms in two different time periods.

Micro-organism	2005- 2011 Number of isolated microorganisms/ infected knee	Number of isolated microorganism/ Total number of knee surgery	Number of isolated microorganism/ Total number of surgeries in the hospital	2012-2018 Number of isolated microorganism/ infected knee	Number of isolated microorganism/ Total number of knee surgery	Number of isolated microorganis/ Total number of surgeries in the hospital
Candida albicans	0.05	5x10^{-4}	28x10^{-6}	0.015	3x10^{-4}	15x10^{-6}*
E. coli	0.05	5x10^{-4}	28x10^{-6}	0.015	3x10^{-4}	15x10^{-6}*
Enterobacter cloacae	0	0	0	0.03	7x10^{-4}	30x10^{-6}
Enterococcus faecalis	0	0	0	0.015	3x10^{-4}	15x10^{-6}
Enterococcus faecium	0	0	0	0.015	3x10^{-4}	15x10^{-6}
Micrococcus sp	0.05	5x10^{-4}	28x10^{-6}	0.015	3x10^{-4}	15x10^{-6}*
Koagulase negative Staphylococcus	0	0	0	0.015	3x10^{-4}	15x10^{-6}
Staphylococcus haemolytıcus	0	0	0	0.015	3x10^{-4}	15x10^{-6}
MRSA	0.1	5x10^{-4}	57x10^{-6}	0.03	7x10^{-4}	30x10^{-6}*
MRSE	0.25	25x10^{-4}	14x10^{-5}	0.125	2x10^{-2}**	12x10^{-5}
Methicillin-sensitive Staphylococcus sp	0	0	0	0.015	3x10^{-4}	15x10^{-6}
MSSA	0.1	1x10^{-3}	57x10^{-6}	0.125	0.2x10^{-3}*	12x10^{-5} **
MSSE	0.1	1x10^{-3}	57x10^{-6}	0.04	1x10^{-3}	45x10^{-6}*
P. aeruginosa	0.05	5x10^{-4}	28x10^{-6}	0.07	18x10^{-4}**	75x10^{-6}*
Proteus mirabilis	0	0	0	0.015	3x10^{-4}	15x10^{-6}
Pseudomonas stutzer	0	0	0	0.015	3x10^{-4}	15x10^{-6}
Staphylococcus intermedius	0	0	0	0.015	3x10^{-4}	15x10^{-6}
M. tuberculosis	0.05	5x10^{-4}	28x10^{-6}	0	0	0

* $p<0.05$ ** $p<0.01$

Table 7: Antibiotic resistance rates (C. albicans and M. tuberculosis didn't included. N: number of isolated microbial agent). * $p<0.05$

		Total (n:53)		Group 1 2005-2011 (n:14)		Group 2 2012-2018 (n:39)		p value
Penicillins	Penicillin	37	69.81 %	10	71.43 %	27	69.23 %	0.99
	Methicillin/Oxacillin	15	28.3 %	4	28.57 %	11	28.21 %	0.99
	Ampicillin	23	43.4 %	11	78.57 %	12	30.77 %	**<0.0001 ***
Cephalosporins	Cefazolin	13	24.52 %	7	50 %	6	15.38 %	**<0.0001 ***
	Cefuroxime Axetil	4	7.55 %	3	21.43 %	1	2.56 %	**<0.0001 ***
	Ceftriaxone	4	7.55 %	2	14.29 %	2	5.13 %	**0.031***
	Ceftazidime	2	3.77 %	1	7.14 %	1	2.56 %	0.089
	Cefepime	0	0 %	0	0 %	0	0 %	NA
	Cefoxitin	1	1.89 %	0	0 %	1	2.56 %	0.99
B-lactams/ B-lactams	Sulbactam ampicillin	13	24.52 %	6	42.86 %	7	17.95 %	**0.0035***
	Amoxicillin Clavulonic Acit	12	22.64 %	4	28.57 %	8	20.51 %	0.461
	Erythromycin	24	45.28 %	8	57.14 %	16	41.03 %	0.151
	Clindamycin	12	22.64 %	3	21.43 %	9	23.08 %	0.88
	Ofloxasin	7	13.21 %	2	14.29 %	5	12.82 %	0.91
Fluorokinolon	Levofloxacin	1	1.89 %	1	7.14 %	0	0 %	**0.023***
	Ciprofloxacin	11	20.75 %	1	7.14 %	10	25.64 %	**0.0021***
	Norfloxacin	1	1.89 %	1	7.14 %	0	0 %	**0.023***
Aminoglycoside	Gentamicin	7	13.21 %	0	0 %	7	17.95 %	**0.0001 ***
	Tobramycin	1	1.89%	0	0 %	1	2.56 %	0.25
	Rifampicin	6	11.32 %	2	14.29 %	4	10.26 %	0.34
	Tetracycline	8	15.09 %	1	7.14 %	7	17.95 %	**0.043***
	Fucidic Acid	2	3.77 %	1	7.14 %	1	2.56 %	0.17
	Chloramphenicol	1	1.89 %	0	0 %	1	2.56 %	0.99
	Trimethoprim sulphamethoxazole	5	9.43 %	0	0 %	5	12.82 %	**0.0016 ***
Others	Vancomycin	1	1.89 %	0	0 %	1	2.56 %	0.99
	Aztreonam	1	1.89 %	0	0 %	1	2.56 %	0.99

According to statistical analysis antibiotic resistance to ciprofloxacin ($p=0.0021$), gentamicin ($p=0.0001$), tetracycline ($p=0.043$) and trimethoprim/sulfamethoxazole($p=0.0016$) significantly increased over time. No significant intergroup difference was noted regarding resistance to clindamycin (used in case of cefazolin allergy) ($p=0.88$). Cefazolin resistance significantly decreased during 2012 and 2018 ($p=0.0001$).Notably, vancomycin resistance was observed in only one patient. Nevertheless, to evaluate the efficiency of our routine antibiotic prophylaxis, we reassessed the antibiotic sensitivity of 12 gram-negative bacteria **(Table 8)**.

Table8: Antibiotic sensitivities of isolated gram-negative bacteria

ANTIBIOTIC SENSITIVITY	2005-2011 Pt 1 *E. coli*	Pt 2 *P. aeruginosa*	Pt 3 *E. coli*	Pt 4 *P. aeruginosa*	2012-2018 Pt 5 *E. cloace*	Pt 6 *P. aeruginosa*	Pt 7 *P. aeruginosa*	Pt 8 *P. mirabilis*	Pt 9 *E. Cloace*	Pt 10 *P. Stutzer*	Pt 11 *P. aeruginosa*	Pt 12 *P. aeruginosa*
Ampicillin			x				x	x				
Amoxicillin Clavulonic Acit							x					
Cefazolin								x				
Ceftriaxone									x			
Cefotaxime		x			x					x		
Cefoxitin	x						x					
Cefepime		x				x	x			x		x
Cefuroxime Axetil			x		x			x				
Ceftazidime		x		x		x	x				x	x
Cefotetan	x											
Amikacin	x	x					x	x		x		x
Gentamicin	x	x	x	x	x	x	x		x	x	x	
Ciprofloxacin		x	x	x	x	x	x			x		x
Levofloxacin							x					
Trimethoprim sulphamethoxazole			x			x	x		x			
Meropenem		x		x			x			x		
Imipenem		x		x			x			x		x
Tobramycin				x								
Tazobactam-piperacillin	x		x	x		x	x		x	x	x	x

DISCUSSION

In the current study, the most frequently isolated bacterium was *S. epidermidis, S. aureus* was the second and *P. aeruginosa* was the third. An increased rate of gram-negative bacteria (*Pseudomonas aeruginosa*) isolation was observed over time. According to statistical analysis antibiotic resistance to ciprofloxacin ($p=0.0021$), gentamicin ($p=0.0001$), tetracycline ($p=0.043$) and trimethoprim/sulfamethoxazole ($p=0.0016$) significantly increased over time. An increased antibiotic resistance was not observed against cefazolin and clindamycin ($p=0.88$).

In fact, cefazolin resistance significantly decreased during 2012 and 2018 ($p<0.0001$). A previous study determined that *S. aureus* and *S. epidermidis* were the most common pathogens isolated in the USA (7). Another study from Europe (8) identified coagulase-negative Staphylococcus to be the most common pathogens, followed by *S.aureus*. In addition, an increased rate of gram-negative bacterial infections, especially multi-drug-resistant gram-negative bacilli was reported (9, 10).

Li and Hou mentioned that *S. epidermidis* and *S. aureus* played a significant role, followed by gram-negative bacteria (11.59%) and fungi (1.45%) in China (11). Wang and Chen conducted a study on early and late-onset knee PJI. They observed that *S. aureus* was the most common species isolated in Taiwan (12). Moreover, their results revealed that early-onset post-TKA infection was associated with a higher risk of gram-negative bacterial infection.

Staphylococcus spp (*S. aureus and S. epidermidis*) were the most common microorganisms grown in periprosthetic infections after knee prosthesis in the current study, as seen in previous studies. A steady increase in gram-negative bacteria isolation rates in patients with PJI has been shown in the present study, similar to the literature. In addition, the growth of different microorganisms was observed, in group 2. This trend might probably be due to the effect of the increased virulence of these microorganisms and the use of more specific and advanced microorganism identification tools in routine diagnosis recently. Another possible reason for this increase might be the use of prophylactic antibiotics before surgery that causes a shift in the microbiological etiology (13). Li and Hou observed that a high rate of antibiotic resistance to penicillin, erythromycin and clindamycin was found, in their study with a resistance rate of 78.57%, 66.67%, and 44.74%, respectively. In addition, the resistance rate of second-generation cephalosporin, typically used as the prophylactic antibiotic was 20% and no vancomycin-resistant bacteria were discovered (11). In the current study, the antibiotic resistance rate was similar to the study of Li and Hou. Therefore, considering the entire study period, the highest antibiotic resistance was against penicillin (69.81%) followed by erythromycin (45.28%) and ampicillin (43.4%). Nonetheless, unlike the study of Li and Hou, the current study evaluated the development of antibiotic resistance over time. Antibiotic resistance to ciprofloxacin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole was significantly increased in group 2. Vancomycin resistance was observed in only one patient. No significant intergroup differences were noted regarding resistance to clindamycin ($p<0.0001$). Notably, cefazolin resistance significantly decreased during the years 2012 - 2018 ($p= 0.88$).

Previous studies have noted that colonization plays an essential role in postoperative infection, and the use of prophylactic antibiotics against colonization might decrease the rate of PJI (14-16). Therefore, some studies in the literature have evaluated the antibiotic resistance of microorganisms isolated from patients with PJI and provided suggestions to prevent colonization. The literature has evidenced a steady increase in gram-negative bacteria isolation rates in patients with PJI. There was an increasing trend for gram-negative bacteria species esp. *P. aeruginosa* ($p<0.05$) over time in the current study. Thus, antibiotic prophylaxis should cover not only gram-positive bacteria, but also gram-negative. Therefore, we decided to change the prophylactic antibiotic regimen that was used in knee replacement surgeries. According to the current local and international guidelines (13, 16), a gram-positive bacterial agent like vancomycin is recommended for prophylaxis before the arthroplasty in addition to cefazolin, particularly for its efficiency against gram-negative and methicillin-resistant bacteria. Notably, vancomycin is a crucial weapon against methicillin-resistant bacteria. Therefore, the development of vancomycin resistance will impede the physician's fight against methicillin-resistant bacteria. Thus, it would be more rational to use other alternatives that are considered effective before using vancomycin as prophylaxis. According to the antibiogram results of 12 gram-negative bacteria isolated in the current study, 10 were sensitive to gentamicin. *Proteus mirabilis*, one of the two gram-negative bacteria resistant to gentamicin was noted to be sensitive to cefazolin. Therefore, using gentamicin with cefazolin would be effective against 11 of these 12 bacteria and most gram-positive bacteria species. Hence, cefazolin and gentamicin prophylaxis instead of vancomycin for knee replacement surgery would be an appropriate option. In addition, the combination of gentamicin and clindamycin would be a suitable prophylactic option in patients with cephalosporin allergy.

Nevertheless, because of fewer patients in the present study, the findings related to the combinations, namely cefazolin-gentamycin or clindamycin-gentamycin, can not be generalised and recommended as a standard prophylactic agent. Hence, each clinic should decide on using appropriate antibiotics for prophylaxis by periodically evaluating the antibiotic susceptibility of bacteria belonging to the clinic's flora.

Nonetheless, this study had some limitations. First, this study had a retrospective design with a small sample size. Second, more than one microorganism was isolated from 10 patients in this study. This situation weakens the study because it is difficult to determine whether the infection is polymicrobial and discern the primary microbial agent responsible for the infection. Furthermore, as stated in previous studies, a low-grade infection could be underdiagnosed because some cases could be diagnosed as aseptic loosening, making it challenging to diagnose PJI (17, 18). In the current study, PJI rates increased in the number of total knee replacement surgeries. Therefore, the rate of knee PJI increases with an increase in patient turnover. So, reducing the number of daily surgical procedures could be effective in decreasing knee PJI rates. However, if it is impossible, the knee PJI rates could be decreased by reducing the daily number of knee arthroplasty operations by performing these operations in an operating room with laminar airflow where no other operations are performed by waiting up for a reasonable time between the operations.

Our results have showed a steady increase in gram-negative bacteria isolation rates in patients with PJI similar to previous reports. It is necessary to use prophylactic antibiotics regimens including gram-negative bacteria in knee arthroplasty surgery.

Although we found an increased resistance to some antibiotics over time in the current study, these were not the antibiotics we used for routine prophylaxis. We propose to use cefazolin and gentamicin or clindamycin (in case of cephalosporine allergy) and gentamicin combination for prophylaxis instead of using cefazolin or clindamycin alone.

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