Middle Black Sea Journal of Health Science December 2020; 6(3):358-363

RESEARCH ARTICLE

Microorganisms that Reproduce in Wound Cultures in Rize Region and Their Antimicrobial Susceptibility

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Received: 10 October 2020, Accepted: 12 December 2020, Published online: 31 December 2020

Abstract

Objective: Among nosocomial infections, wound infections are one of the important factors causing mortality and morbidity after urinary tract infections. In this study, it was aimed to guide the empirical treatment and to contribute to epidemiological data by determining the microorganism and antimicrobial susceptibility which have been isolated from cultures of wounds and abscesses, which came from outpatient clinics, services and intensive care units, that grow in wound site and abscess cultures that comes from outpatient clinics, services and intensive care units.

Methods: This study was conducted on the basis of cultures in which at least one microorganism grew in the wound site and abscess samples from the polyclinic, service and intensive care units during routine application at the Recep Tayyip Erdoğan University Training and Research Hospital Microbiology Laboratory, between January 2011 and December 2016. Samples were taken with two sterile swabs in the form of superficial swabs or deep aspiration and delivered to the laboratory with transport medium as soon as possible. Gram staining preparation was prepared and inoculated on 5% sheep blood agar, eosin-methylene-blue agar, chocolate agar and sabouraud dextrose agar. The gram-stained preparation was evaluated by Q scoring. Evaluation of the culture and antibiogram susceptibility were made according to the Clinical and Laboratory Standards Institute (CLSI) criteria.

Results: A total of 2202 samples were received, and reproduction was observed in 930 samples. Among the 793 wound and 137 abscess samples, the most common microorganism was *Staphylococcus aureus* (224 cultures) and the second was *Escherichia coli* (135 cultures).

Conclusion: Surgery clinics, especially orthopedics, provided the most common wound infections, from which *S. aureus* was the most isolated microorganism. The fact that bacterial, most notably *S. aureus*, propagation occurred from surgery samples indicates that surgical site infections are generally caused by endogenous flora. It has, thus, become apparent in our study that patients and hospital staff should pay more attention to hygiene, especially hand washing. Due to the changing of the distribution and resistance patterns of microorganisms that are frequently seen in hospitals at certain time intervals, their antibiotic susceptibility will be a guide in the rational use of antibiotics. Thus, specific treatment will contribute to the saving on the cost and reduce mortality.

Key words: Abscess, antimicrobial susceptibility, microorganism, wound culture

Suggested Citation: Bahceci I. Microorganisms that Reproduce in Wound Cultures in Rize Region and Their Antimicrobial Susceptibility. Middle Black Sea Journal of Health Science, 2020; 6(3):358-368.

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DOI: 10.19127/mbsjohs.823556

Not: Presented as a poster statement at the 4th KLİMUD Congress, (8-12 November 2017) Antalya/Turkey

Introduction

The physical integrity of the skin is the most important barrier that prevents colonization and preventing infection by pathogenic microorganism in the skin and underlying tissues (Atiyeh et al., 2002). Breaching of this barrier by pathogens may result in infection (Broughton et al., 2006).

Infection development may also result if pathogenic microorganism colonization's occur other than normal flora members (Schultz et al., 2003). Since wound colonization is mostly polymicrobial, the wound is likely to become infected (Akinjogunla et al., 2009). Infections of the skin and subdermal tissue occur when the microorganisms settle, proliferate, and spread in the wound and also overcome the immune system by virulence factors (Yurtseven et al., 2009).

Wound infections are the second source of nosocomial infections after urinary tract infections and are among important causes of mortality and morbidity (Owens et al., 2008). Wound infection factors vary depending on the infected area, its clinical characteristics and the underlying disease of the person (Sesli et al., 2006).

Although different microorganisms are among the infectious agents, the most common are gram positive bacteria, which are also found in normal flora. (Citil et al., 2015). The correct identification of the microorganism and antimicrobial susceptibility will increase the success of the treatment, increase the quality of life of the person, decrease the costs by shortening the duration of hospital stay, and reduce the use of unnecessary antibiotics, in addition the development of antimicrobial resistance will be minimized (Cirit et al., 2014).

In this study, it was aimed to guide symptomatic treatment and contribute to epidemiological data by identifying the microorganism species and antimicrobial susceptibilities isolated from wound site and abscess samples sent to our laboratory from outpatient clinics, services, and intensive care units of our hospital, retrospectively.

Methods

This study was carried out retrospectively scanning the wound and abscess cultures from the outpatient, wards, and intensive care units during routine referral to the Microbiology Laboratory of the Training and Research Hospital (Faculty of Medicine, Recep Tayyip Erdogan University) between January 2011 and December 2016. The cultures in which at least one microorganism grew were evaluated. Samples were taken in the form of superficial swab or deep aspiration with sterile eucvyon rod and delivered to the laboratory as soon as possible with the transport medium. All samples were inoculated to 5% sheep bloody agar, eosin-methylene-blue (EMB) and sabourand dextrose agar and chocolate agar media then under aerobic conditions incubated at 37°C for 24-48 hours. Gram-stained preparats were prepared simultaneously from wound samples. Morphologies of leukocyte, epithelium and microorganisms were evaluated using x100 magnification. At the end of the 48th hour, the plaques were examined together with gram-stained results. The Gram stained preparat was evaluated in terms of epithelial cells, leukocytes and microorganisms by Q scoring. In the microscopic examination, if there is little / no epithelium as well as leukocytes, the samples were evaluated as quality samples. It was decided whether the microorganisms in the reproductive plaques were causative or contaminant, based on direct microscopic examination. Conventional methods and the automated system VITEK 2 (BioMérieux / France) were used for the assessment of the culture and determination of antimicrobial susceptibilities. Evaluation of the culture and antimicrobial susceptibilities were made according to the Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI; 2011-2016). TMC-ADTS Restricted Notification Table was used in the evaluation.

Ethics committee approval was obtained from the Recep Tayyip Erdoğan University Non-Interventional Ethics Committee (Approval no: 40465587-140).

Statistical data were evaluated using the SPSS 21.0 program.

Results

A total of 2202 wound, and abscess samples were sent from the outpatient, wards and intensive care units between January 2011 and December 2016 during routine referral to the Microbiology Laboratory. 856 samples were reported as "no growth", 348 were "evaluated as contamination with skin flora members", 68 samples were reported as "normal flora members have grown", 930 samples were reported as "growth detected".

Of 930 samples with growth, 793 were wound site and 137 were abscess samples. Of the 930 samples, 523 (56.2%) were derived from males and 407 (43.8%) from females. Of the microorganisms grown, 435 (46.8%) were Gram positive bacteria, 484 (52.1%) were Gram negative bacteria, while 11 (1.1%) were fungal agents. *Staphylococcus aureus (S. aureus)* was the most common microorganism isolated (224 specimens), followed by *Escherichia coli (E. coli)* isolated (135 specimens). The rest were

Pseudomonas aeruginosa (P. aeruginosa), Coagulase negative staphylococci (CNS), *Acinetobacter baumannii (A. baumannii)*, *Streptococcus* spp. *Candida* spp. and others. (Table I)

Tablo 1. Clinical Distribution of Microorganisms

Microorganism/Unit	Outpatient 390(%41.9)	Service 445(%47.9)	Intensive care 95(%10.2)	Total 930(%100)
S. aureus	135(%14.5)	83(%8.9)	6 (%0.6)	224 (% 24)
E. coli	38 (%4.1)	83(%8.9)	14(%1.5)	135 (% 14.5)
CNS	58 (%6.3)	49(% 5.3)	13 (% 1.4)	120 (% 13)
A. baumannii	11(%1.1)	36(%4)	35(3.9)	82 (% 9)
P. aeruginosa	61 (%6.6)	55(%6)	7(%0.7)	123 (%13.3)
Proteus spp.	14 (%1.5)	25(%2.7)	2(%0.2)	41 (% 4.4)
Enterococcus spp.	6 (% 0.6)	26(% 2.8)	7(%0.7)	39 (% 4.1)
Candida albicans	5(%0.5)	5(%0.5)	0 (%0)	10 (% 1)
Non-*Candida albicans* yeast	0 (%0)	0 (%0)	1 (%0.1)	1 (% 0.1)
Klebsiella spp.	31(%3.3)	39(%4.2)	8(%0.8)	78 (% 8.3)
Streptococcus spp.	18 (%1.9)	34 (%3.7)	0 (%0)	52 (%5.6)
Enterobacter spp.	13 (%1.4)	10 (%1.1)	2 (%0.2)	25 (% 2.7)

Methicillin resistance was 26.3% in *S. aureus*, while it was 69.6% in CNS. ESBL (+) positivity was 41.7% among the agents in the Enterobacteriaceae family; In terms of organisms, it was found as 59.7% in *E. coli*, 44.2% in *Klebsiella* spp. 14.3% in Proteus spp. and 16.7% in *Enterobacter* spp. Antifungal susceptibility tests were not performed on fungi. 390 (41.9%) of the wound cultures with reproduction outpatient clinic patients, 445 (47.9%) are service patients and 95 (10.2%) intensive care patients. When the distribution of reproductive specimens by clinics was examined, the orthopedics polyclinic had the largest share with 11.3%. By grouping microorganisms, antibiotic sensitivities are shown in Table II, III and IV.).

Table 2. Antimicrobial resistance rate in Gram-positive bacteria

Microorganism	p	amp	fox	van	tec	lzd	gn	ak	cıp	hlgn	f	sxt
Enterococcus spp.	-	18,9	-	0	0	0	-	-	60	15	8,3	-
CNS	90,4	60	69,6	0	0	0	31,1	25	57,3	-	50	44,4
Stafhyilococcus aureus	90,2	80	26,3	0	0	0	4,4	0	16,8	-	1,4	33,3
Streptococcus spp.	8,3	5,9	-	0	0	0	-	-	8,3	-	-	0

P: Penicillin, AMP: Ampicillin, FOX: Cefoxitin, VAN: Vancomycin, TEC: Teicoplanin, LZD: Linezolid, GN: Gentamycin, AK: Amikacin, CIP: Ciprofloxacin, HLGN: High Level Gentamicin, F: Nitrofurantoin, SXT: Trimethoprim/sulfamethoxazole

Table 3. Antimicrobial resistance rate in Gram-negative bacteria

Microorganism	amp	amc	pip	tzp	caz	cro	fep	atm	mem	ıpm	fox	gn	ak	cıp	f	sxt
E. coli	83,2	40,2	72,5	22,4	39,2	59,7	36,8	53,3	0,8	0,8	3,6	27,9	2,4	41,2	8,8	53,8
Klebsiella spp.	100	50	100	16,7	36,4	42,6	15,4	44,2	0	0	33,3	25,4	0	31,1	25	47,9
Proteus spp.	78,9	42,5	16,7	0	5,7	14,3	0	6,5	0	5,6	-	10,5	0	12,8	100	48,7
Enterobacter spp.	100	100	23,1	14,3	16,7	4,5	0	10,5	0	0	100	0	0	4	33,3	60

AMP: Ampicillin, AMC: Amoxicillin / clavulanic acid, PIP: Piperacillin, TZP: Piperacillin / tazobactam,CAZ: Ceftazidime, CRO: Ceftriaxone, FEP: Cefepime, ATM: Aztreonam, MEM: Meropenem, IPM: Imipenem, FOX :Cefoxitin, GN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin F: Nitrofurantoin, SXT: Trimethoprim / sulfamethoxazole

Table 4. Resistance rate in Gram-negative non-fermentative bacteria

Microorganism	pip	tzp	caz	fep	ıpm	mem	gn	ak	cıp	sxt	tg
P.aeruginosae	26,7	13,9	13	12,1	17,3	19,8	7,1	3,3	14,7	-	-
A. baumannii	97,2	92,3	88,9	92,3	87,2	86,5	65,9	65,9	88,9	80,5	7,3

PIP: Piperacillin, TZP: Piperacillin / tazobactam, CAZ: Ceftazidime, FEP: Cefepime, IPM: Imipenem, MEM: Meropenem, GN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin, SXT: Trimetoprim / sulphametoxazole, TG: Tigecycline

Discussion

Correct determination of microorganisms in wound infections and accurate reporting of theirantibiograms are the key points in both reducing the duration of hospital stay and health costs (Dogan et al., 2010).

Misuse of antimicrobials can cause significant side effects and lead to the development and spread of antimicrobial resistance (Wong et al., 2015). Considering the fact that microorganisms can be transmitted from own flora or the environment, paying attention to the personal hygiene and healthcare personnel will help reduce the infection rates (Copeland-Halperin et al., 2016). The importance of this is that wound-site infections tend to be polymicrobial (Espejo et al., 2018).

Isolates in wound site cultures vary in studies depending on region. In our study, Gram-negative bacteria isolation rate was 52.1%, Gram positive 46.8%. In a similar study conducted in İzmir, 21.8% of the isolates were Gram positive bacteria and 78.2% were Gram negative bacteria (Yurtsever et al., 2009). In another study conducted in Duzce, it was reported that 46.4% of the microorganisms isolated were Gram positive bacteria, 53% Gram negative bacteria and 0.6% were fungi (Candida albicans) (Avcioglu et al., 2019). In another study by Turhanoglu et al. (2018) 52.5% of the isolates were Gram positive bacteria, 42.8% Gram negative bacteria and 4.61% fungal agents. Similarly, in a study conducted in Erzincan city, the rates were found to be 67.6% Gram positive, 32.4% Gram negative bacteria (Gundem et al., 2012).

When microorganisms isolated in wound culture are examined, while Avcioglu et al. determined S. aureus as the most common 21%, CNS as the second most frequent 16%, *E. coli* as the third 15%; Gundem et al. *S. aureus* 32.4%, CNS 25.3%, *E. coli* 11.3%, *Klebsiella* spp. 9.9% *Streptococcus* spp. 9.9%, *P. aeruginosa* 7% and *Acinetobacter* spp. 4.2% (Gundem et al., 2012; Avcioglu et al., 2019). In their retrospective study spanning study six years, found CNS 58.5%, *S. aureus* 41.4%, *Pseudomonas* spp. 18.2%, *E. coli* 13.1%, *Klebsiella* spp. 5.48%, Enterobacter cloacae complex 4.9% and *Acinetobacter* spp. 4.3%. *E. coli* and *S. aureus* are the most frequently isolated organisms from wound infections in the study of Davarci et al. (2018). A few wound infection reports from different parts of the world have shown that *S. aureus* and *E. coli* are the most common agents (Bhatt et. al., 2006 and Mulu et al., 2012). Schnuriger et al. (2010) reported *E. coli* especially in wound infections that develop after surgery. In our study, the most frequently isolated agent was *S. aureus* with a rate of 23.5%, followed by *E. coli* with a rate of 14.1%, P. aeruginosa with a rate of 12.8%, CNS with a rate of 12.5%, and *A. baumannii* with a rate of 8.6%.

In studies examining the distribution of species, S. aureus and CNS draw attention in terms of rates and have a large share in hospital infections especially in the presence of methicillin resistance (Cirit et al., 2014). In studies investigating methicillin resistance in *S.aureus* and CNS in wound cultures, methicillin resistance was found as follows: Dogan et al. 18.3%, 54.5%; Avcioglu et al. 16.7%, 58.8%, Gundem et al. 21.8%, 33.3%; Turhanoglu et al. 35.8%, 71,1%. In our study, while 26.3% methicillin resistance was detected in *S. aureus* and 69.6% in CNS, no glycopeptide resistance was found in *S. aureus* isolate as in many previous studies (Dogan et al., 2010; Gundem et al., 2012; Turhanoglu et al., 2018; Avcioglu et al., 2019). Ozturk et al. (2020) also did not find glycopeptide resistance in their study. In our study, resistance to antibiotics such as Linezolid, Vancomycin and Teicoplanin was not observed in Gram positive bacteria.

The fact that the isolated agents are strains producing ESBL (Expanded Spectrum Beta-Lactamase) causes limitations in antibiotics to be used for treatment, resulting in serious economic losses due to increased mortality and high costs (Cirit et al., 2014). Cirit et. al. (2014) found ESBL positivity in *E. coli* and *Klebsiella pneumoniae* strains, respectively, 55% and 39% and Turhanoglu et al. (2018). Found 63.3% and 72.2% for the same agents. In this study, ESBL positivity was 41.7% for the Enterobacteriaceae family and 59.7% in *E. coli*, 44.2% in *Klebsiella* spp. 14.3% in *Proteus* spp. and *Enterobacter* spp. 16.7%. Davarci et al. (2018) found that colistin is the most effective antimicrobial against Gram negative in their study. In addition, they found that 86.9% of *E. coli* strains were resistant to ampicillin, 60.7% to ceftriaxone, 60% to cefepime, 54.1% to trimethoprim/ sulfamethoxazole and 0.6% to imipenem. Bessa et al. (2015) found ampicillin resistance 94.1%, ceftazidime resistance 5.9%, cefepime resistance 11.8%, ciprofloxacin resistance 52.9% in *E. coli* strains. They also reported that all strains were susceptible to meropenem and ertapenem. In our study, ampicillin resistance was 83.2%, ceftazidime resistance was 39.2%, ciprofloxacin resistance was 41.2%, and carbapenem resistance was 0.8% in *E. coli* strains. Fosfomycin/trometamol resistance was found around 3%, making it one of the antimicrobials with the least resistance. Our results are like other studies.

In a worldwide study piperacillin/tazobactam resistance was 52.2%, ciprofloxacin resistance 45.6%, meropenem resistance 30.4%, and ceftazidime resistance 50% in Pseudomonas spp. strains (Bessa et al, 2015). In our study, the resistance in *P. aerigosae* was determined as 13.9% for piperacillin/tazobactam, 14.7% for ciprofloxacin, 19.8% for meropenem, 13% for ceftazidime. The resistance profile of *A. baumannii* strains was determined to be higher. Piperacillin and piperacillin/tazobactam resistance is over 90%, carbapenem resistance is over 80%. Tigecycline resistance was found to be 7.3%.

Many studies found that surgical clinics constitute the largest group in the distribution of cultures with reproduction according to clinics (Yurtsever et al., 2009; Dogan et al., 2010; Altan et al., 2017). Similarly, in our study, it was observed that surgical clinics constituted the largest group, especially the orthopedic clinic.

Conclusion

In conclusion, in our study, it was determined that the clinics that wound infection is most common are surgical clinics, especially orthopedics clinics, and *S. aureus* was the most frequently isolated from wound cultures. The fact that surgical clinics are the most common units may be an indicator of post-traumatic development. At the same time, since the area where the sample is taken is a region with flora, it is important that both the patient and the person taking the sample pay attention to the hygiene rules in defining the correct factor. It is very important to determine infectious agents and susceptibilities through regular surveillance studies conducted at regular intervals, and to contribute to the rational use of antibiotics with the information obtained

The data determine the resistance rates and will contribute both to infection control and management strategies which will be carried out in our institution and also to national and international epidemiological data.

Ethics Committee Approval: Clinical Studies Ethics Committee of Recep Tayyip Erdoğan University, Faculty of Medicine, Decision number :2017/140 Date: 22.09.2017.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept -I.B; Design- I.B; Supervision- I.B; Materials- I.B; Data Collection and/or Processing- I.B; Analysis and/or Interpretation- I.B; Literature Review- I.B; Writing- I.B; Critical Review- I.B.

Conflict of Interest: No conflict of interest was declared by the author.

Financial Disclosure: The author declared that this study hasn't received no financial support.

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