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

İlkay Bahçeci

1Recep Tayyip Erdogan University Faculty of Medicine Department of Medical Microbiology, Ordu, Turkey

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

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Address for correspondence/reprints:
İlkay Bahçeci

Telephone number: +90 (505) 713 1865
ORCID-ID 0000 0003 3662 1629

E-mail: bahceciie@hotmail.com
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Introduction
The physical integrity of the skin is the most important barrier that prevents colonization and preventing infection by pathogenic microorganisms 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 normal 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 samples 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...
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*Pseudomonas aeruginosa* (*P. aeruginosa*), Coagulase negative staphylococci (CNS), *Acinetobacter baumannii* (*A. baumannii*), *Streptococcus* spp. *Candida* spp. and others. (Table I)

| Microorganism/Unit | Outpatient | Service | Intensive care | Total |
|--------------------|------------|---------|---------------|-------|
| 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 | cep | 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|
| Staphylococcus 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: Gentamicin, 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 | pcp | caz | cep | atm | mem | ipm | fox | gn | ak | cep | 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: Pipercillin, TZP: Pipercillin / 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*
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Table 4. Resistance rate in Gram-negative non-fermentative bacteria

| Microorganism       | pip  | tzp  | caz  | fep  | ipm  | me  | gn  | ak  | cip  | 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 |

PPIP: Piperacillin, TZP: Piperacillin / tazobactam, CAZ: Cefazidime, FEP: Cefepime, IPM: Imipenem, MEM: Meropenem, GN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin, SXT: Trimetoprim / sulphonetoxazole, TG: Tigecycline

**Discussion**

Correct determination of microorganisms in wound infections and accurate reporting of their antibiograms 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 Izmir, 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) (Avcigol 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 Avcigol 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; Avcigol 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%; Avcigolu 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; Avcigolu 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%, ceftriaxone resistance 59%, 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%, ceftriaxone 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. aeroginosa 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.

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