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MICROORGANISMS ISOLATED FROM WOUND CULTURES AND THEIR ANTIBIOTIC RESISTANCE PROFILES IN A TERTIARY HOSPITAL

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ABSTRACT

In this study, pathogenic microorganisms were isolated from wound samples of patients, who were followed up in different clinics of Amasya University, Sabuncuoglu Serafettin Training and Research Hospital between January, 2012 and January, 2015. The aim of the study is to assess these microorganisms retrospectively as well as detect their antibiotic resistance status. The wound samples, which were sent to our microbiology laboratory from various clinics of the hospital, were seeded with the help of classical techniques and strains were isolated from them. The isolates were identified and their antibiotic resistances were determined by using VITEK 2 (BioMerieux, French) automatic system. Out of 712 bacteria, the 411 of them (57.7%) were Gram positive bacteria whereas 301 of them (42.3%) were Gram negative bacteria. The isolation frequencies of bacteria were 33%, 21.4%, 13.6%, 9.4%, 8.4% and 4.2% for coagulase-negative staphylococci (CNS), *S. aureus*, *E. coli*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*; respectively. The 7.1% of the *S. aureus* strains and 55.8% of CNS were methicillin resistant whereas they were not resistant against vancomycin. It has been detected that amikacin is the most effective antibiotic for the isolated Gram negative bacteria. While planning the treatment plan of wound infections, it is recommended that the appropriate antibiotic selection can be made mainly by considering the types of infectious agents and their antibiotic sensitivities.

KEY WORDS: Wound culture, Antibiotic resistance.

INTRODUCTION

The primary role of the skin is to ensure the isolation by being a barrier between microorganisms on the skin and the tissue surrounded by the skin. However, tissues surrounding by the skin become vulnerable to invasion and colonization of many microorganisms as a result of the deterioration of the skin integrity. Wound infections are mostly nosocomial and they can occur frequently after any type of surgical intervention. They are the second most common infections observed after the urinary tract infections in hospitals (1-3). The recovery of the wound infections, which occur particularly upon the surgical interventions, takes long time both due to the weakness of

the immune status of the patient and since these infections are nosocomial and resistant against various antibiotics. This extends the length of stay in hospital, induces the anxiety of the patient, increases the mortality and morbidity rates as well as leads to the economical loses (4).

The antibiotic resistance rates of infectious bacteria isolated from wound cultures continuously increasing (5, 6). It has been shown that the use of appropriate microbial agents in the treatment of wound infections both prevents the increase the resistance rates and decrease the high mortality rates as well as the costs of the treatments (7).

The success rate of the treatment will increase and the use of unnecessary antibiotic use as well as the spread of resistant bacteria will be prevented in case the wound infectious agents and their antibiotic sensitivity rates are determined and further used in the treatment plans (8-10).

In this study, the pathogenic microorganisms were sent to the microbiology laboratory upon being isolated from the surgical samples of patients who were followed up in different clinics of Amasya University Sabuncuoglu Serafettin Training and Research Hospital. The aim of the study is to determine the distribution and antibiotic sensitivities of these nosocomial infectious agents in wound samples.

MATERIALS AND METHODS

Pathogenic and infectious 712 microorganisms were isolated from wound cultures of patients in different clinics of Amasya University, Sabuncuoglu Serafettin Training and Research Hospital between January, 2012 and January, 2015. Their isolation frequencies and antibiotic resistance rates were assessed retrospectively. The wounds were taken from its place with a sterile swab and brought to our laboratory in a with the carrier medium.

The Gram-stained preparations were evaluated in terms of leukocytes, epithelial cells and dominant bacteria and the samples which had more leukocytes compared to epithelial cells were cultured. Samples were plated in 5% sheep blood agar and eosin methylene blue agar (EMB) at 37°C and they were incubated for 18-24 hours. Microorganisms, which propagated in cultures, were identified with the help of conventional methods according to the colony morphologies and Gram-staining properties. The antibiotic resistance status of the microorganisms was

specified by using VITEK 2 (BioMerieux France) automated systems.

RESULTS

In our study, totally 712 bacteria were examined in terms of the isolation frequency and antibiotic resistance. Out of these 712 bacteria, 411 of them (57.7%) were Gram positive bacteria and 301 of them (42.3%) were Gram negative bacteria. According to the isolation frequencies, it has been detected that there were 235 (33%), 153 (21.4%), 97 (13.6%), 67 (9.4%), 60 (8.4%) and 30 (4.2%) CNS, *S. aureus*, *E. coli*, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*; respectively (Table 1).

When the antibiotic resistance of the isolated Gram positive bacteria was examined, all of the staphylococcal isolates were linezolid, vancomycin and teicoplanin sensitive. The antibiotic resistance rates of the *S.aureus* isolates were detected as 86.2% for penicillin, 7.1% for oxacillin, 10.5% for clindamycin and 10.2% for ciprofloxacin whereas these rates for CNS were 85.8% for penicillin, 55.8% for oxacillin, 34.1% for clindamycin and 48.9% for ciprofloxacin.

When the antibiotic resistance of the isolated Gram negative bacteria was examined, *E. coli* strains did not show imipenem and meropenem resistance whereas *K. pneumoniae* isolates indicated their lowest resistance rates against imipenem (25%) and meropenem (25%). All of the *A. baumannii* isolates were found as imipenem, meropenem, ceftazidime and piperacillin-tazobactam resistant. Besides, these isolates showed the least resistance against amikacin (31.6%). The antibiotics that *P. aeruginosa* isolates showed the highest and the lowest resistance were ceftazidime (10.6%) and piperacillin-tazobactam (46%); respectively.

Table 1. Distribution of isolated organisms

Factor	n	%
CNS	235	33
<i>S.aureus</i>	153	21.4
<i>E.coli</i>	97	13.6
<i>P.aeruginosa</i>	67	9.4
<i>A.baumannii</i>	60	8.4
<i>K.pneumoniae</i>	30	4.2
<i>Enterococcus spp.</i>	23	3.3
Other bacteria*	47	6.7
Total	712	100

**Proteus* spp, *Citrobacter*, *Morganella*, *Serratia*, *Stenotrophomonas*

Table 2. Resistance rates of isolated Gram positive bacteria against antibiotics (%)

Antibiotics	<i>S.aureus</i>	CNS*
Penicillin	86.2	85.8
Oxacillin	7.1	55.8
Erythromycin	15.1	56
Clindamycin	10.5	34.1
Ciprofloxacin	10.2	48.9
Linezolid	0	0
Teicoplanin	0	0
Vankomycin	0	0

*CNS: Coagulase-negative staphylococci

Table 3. Resistance rates of isolated Gram negative bacteria against antibiotics (%)

Antibiotics	<i>E. coli</i>	<i>P.aeruginosa</i>	<i>A.baumannii</i>	<i>K.pneumoniae</i>
Imipenem	0	22.9	100	25
Meropenem	0	26.2	100	25
Amikacin	13	26.9	31.6	25.9
Gentamicin	31.3	40.2	53.4	26.6
Ciprofloxacin	56.7	38.8	100	44.8
Levofloxacin	54.2	41.6	96.2	55
Ceftazidime	62.1	10.6	100	65.1
Piperacillin/tazobactam	42.1	46	100	60.7

DISCUSSION AND CONCLUSION

As long as the skin integrity is not deteriorated, the skin prevents the possible infections since it is a barrier between the inner tissues and the environmental microorganisms. The wound infections are the most common ones among the nosocomial infections and it occurs due to the deterioration of the skin integrity and colonization of the infectious microorganisms in the wound directly or indirectly. These microorganisms are particularly the resistant bacteria in the patient's own flora emerging upon the surgical interventions or the pathogens colonized in the hospital. These infections extend the healing process of the patient as well as they causes fairly high increase in the mortality, morbidity and financial expenses (10-12). The resistance rates of the isolated infectious bacteria from the wound are increasing continuously and the resistant bacteria and fungi can colonize on the wound and worsen the infection when the infection is not appropriately treated on time (6, 13, 14). While planning the treatments of wound infections, paying attention to the culture and antibiogram results increases the success of the therapy and decreases the expenses and costs(15). This shows that being aware of the types of infectious agents and their antibiotic resistance profiles is very important in the planning of the treatment.

In our study in which the isolated pathogenic bacteria from wounds were examined, it has been observed that the significant part of the infectious bacteria were Gram positive ones. Similar to our study, staphylococci are mostly detected in various studies performed in Turkey. Sumer et al. (8) have detected that the bacteria which were mostly isolated after the surgeries were CNS (26.7%), *S.aureus* (24.9%) and *Pseudomonas* spp. (13.6%). Sesli et al. (10) examined the wound samples in their study and they detected *S.aureus* (29.1%), CNS (24%), *E.coli* (11.3%), *Enterococcus* spp. (6.7%), *P. aeruginosa* (5.9%) and *A.baumannii* (5.6%); respectively. On the other hand, according to the study performed by Ciftci et al. (16) in order to determine the microorganism profiles in surgical wound infections, they isolated *S.aureus* (27%), *Enterococcus* spp. (19%), *Pseudomonas* spp. (16%) and *E.coli* (13.5%); respectively. Unlike our work, there are also studies in which the Gram negative bacteria were mostly isolated. Dogan et al. (17) have shown that the 421 of the 525 infectious agents were Gram negative bacteria whereas only 104 of them were Gram positive bacteria in wound

samples. In their study, they isolated mostly *E. coli* (28.5%). Furthermore, they also detected *E. aerogenes* (15.6%), *S.aureus* (14.8%) and *P.aeruginosa* (14%) from the wound samples; respectively. Yurtsever et al. (15) isolated *E. coli* (26.8%), *P. aeruginosa* (18.3%), *S. aureus* (18%), *A. baumannii* (11.6%), *K. pneumoniae* (8.9%) and *Enterococcus* spp. (2.7%); respectively. Asik et al. (18) detected mostly *E. coli* (39.8%), *S. aureus* (11.9%), *A. baumannii* (9.1%), *P. aeruginosa* (7.5%), *Candida* spp. (7.8%), *Enterobacter cloacae* (7.1%), *K. pneumoniae* (6.1%), *Enterococcus* spp. (6.9%) and CNS (2.4%). Fisgin et al. (19) performed a study with patients who had colorectal surgery and they mostly isolated *E. coli* as an infectious agent. They also specified that the age, gender, the length of stay in hospital increased the infection risks.

In our study, the antibiotic resistance profiles of the isolated Gram positive bacteria were examined and it has been shown that there were linezolid, vancomycin and teicoplanin sensitive. *S. aureus* isolates were resistant against penicillin (86.2%), oxacillin (7.1%), clindamycin (10.5%) and ciprofloxacin (10.2%); respectively. On the other hand, CNS was resistant against penicillin (85.8%), oxacillin (55.8%), clindamycin (34.1%) and ciprofloxacin (48.9%).

When we examined the antibiotic resistance of isolated Gram negative bacteria, *E. coli* strains did not show resistance against imipenem and meropenem whereas we detected that *K. pneumoniae* showed resistance against imipenem (25%) and meropenem (25%) at the lowest level. Besides, all of the *A. Baumannii* isolates were imipenem, meropenem, ceftazidime and piperacillin-tazobactam resistant whereas the antibiotic that *A. Baumannii* showed the least resistance was amikacin (31.6%). The antibiotics which *P. aeruginosa* isolates showed the highest and the lowest resistances were ceftazidime (10.6%) and piperacillin-tazobactam (46%); respectively.

Similar to our study, Asik et al. (18) showed that staphylococci isolates were vancomycin, teicoplanin and linezolid resistant. Out of the enteric bacteria, the maximum resistances of *E. coli* and *K. pneumoniae* were detected against cefotaxime (82.4%) and ceftazidime (74.6%), the maximum resistance of *P. aeruginosa* was detected against piperacillin-tazobactam (77%) and ceftazidime (70%), the maximum resistance of *A. baumannii* was resistant against

ceftazidime (92%) and cefoperazone-sulbactam (78%). Ciftci et al. (16) did not detect glycopeptide resistance in *S.aureus* strains. They stated that Pseudomonas isolates showed 33% quinolone resistance and Gram negative bacilli had 8% carbapenem, 33% aminoglycosides and 50% quinolone resistance. Unlu et al. (20) specified that all of the staphylococci were sensitive to vancomycin and teicoplanin.

In our study, CNS isolates had higher resistance rates against antimicrobial agents compared to *S. aureus*. The methicillin resistances of the staphylococci isolates, which are among the nosocomial infections, increase the severity of the infection. Methicillin resistance is a very important issue since it complicates the treatment and increases costs (21). The methicillin resistance of *S. aureus* isolates was 7.1% whereas this resistance rate was 55.8% for CNS. In different studies performed in Turkey, Yurtsever et al. (15), Polat et al. (22) and Dogan et al. (17) detected that *S.aureus* and CNS were resistant against methicillin as 29%-50%, 19.7%-7.6%, 18.3%-54.5%; respectively. Furthermore, Diler et al. (23) specified (37.5% - 39.8%) oxacillin resistance in *S. aureus* strains which were isolated from the hospital environment and devices. Oguz et al. (24) detected (15%) oxacillin resistance in *S. aureus* isolates. Ciftci et al. (16) detected 40% methicillin resistance. It is quite worrying to have high rates of methicillin resistance (55.8%) in the mostly isolated bacteria group, which is CNS (33%), in our hospital. Different types and antibiotic resistance rates of bacteria isolated from wound samples can be due to the differences in antibiotic use policies, clinics which provide the samples, population groups and the origins of the colonized bacteria

in hospitals. The success of the treatment can be increased as well as the spread of resistant strains can be prevented when health professionals pay attention on the types of strains and the antibiotic resistance profiles of them while planning the treatments of these infections (25).

In our study, we determined the distribution as well as the antibiotic sensitivities of the wound-derived microorganisms in our hospital and we compared our results with the others which were performed in different regions of Turkey. Mostly isolated bacteria groups and their antibacterial resistance rates were found to be very different. It has been shown that CNS group bacteria which were mostly isolated from the wounds were more resistant against antimicrobials compared to *S. aureus* isolates.

Besides, it was interesting that CNS group bacteria had high methicillin resistance. Isolating high amount of these bacteria from wound samples lead us to think that they can be colonized in our hospital. While planning the treatment process of these infections, it is recommended that the appropriate antibiotic selection can be made regarding the infectious agents as well as their antibiotic sensitivities.

Furthermore, taking precautions for the infections, paying attention on the wound care and providing the training to the health professionals accelerate the healing process and prevent the spread of resistant strains and high treatment costs.

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CONFLICT OF INTEREST: NIL

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