Preoperative and Intraoperative Culture Results of Patients with Chronic Otitis Media; Is Culture Swab Reliable?

Abstract

Objective: The aim of this study was to analyze the culture antibiogram results of samples obtained directly from the middle ear and/or mastoid cells in the operating room, and from the external auditory canal in the outpatient.

Methods: This study was conducted between 2016-2018 in Microbiology and Otorhinolaryngology Department. Swab cultures were obtained through the external ear canal preoperatively in outpatients. Middle ear swabs and mastoid granulation tissue were collected intraoperatively, respectively. Demographic datas, bacterial identifications and antibiotic susceptibilities were compared in both groups.

Results: Two hundred thirty eight patients with chronic otitis media were enrolled in the study. Out of the 238 cases, 86 patients had negative culture results. *P. aeruginosa* (n=44), Coagulase Negative Staphylococcus (n=33), *S. aureus* (n=27), *Proteus mirabilis* (n=16) and *E. coli* (n=10) were the most common grown bacteria in both groups. Most common identified groups were Gram-positive bacteria (n=92), non-fermenter Gram-negative bacteria (n=55) and Enterobactericeae (n=42). Gram-positive cocci were higher in outpatient group, whereas Enterobactericeae were higher in intraoperative group ($p<0.05$). Antibiotic susceptibilities of *P. aeruginosa* and *S. aureus* were found to be lower especially in the preoperative group ($p <0.05$).

Conclusion: *P. aeruginosa* was the most common bacteria in both preoperative and intraoperative cultures and high ciprofloxacin resistance of the isolates were remarkable. Although contamination is an issue in samples collected from the external ear canal of outpatients using cotton swabs, similar microorganisms grew in outpatient and intraoperative cultures

Key Words: Antibiotic susceptibility, chronic otitis media, intraoperative, microbiology, preoperative

MAİN POİNTS

P. aeruginosa was the most frequently isolated bacterium in this study, and its resistance to ciprofloxacin was high.

Similar microorganisms grew in outpatient and intraoperative cultures.

Contamination from the external ear canal and/or failure to reach the middle ear discharge may have resulted in negative culture results.

The frequency of MRSA was lower overall than in prior reports, it was higher in our operation versus outpatient group

INTRODUCTION

Chronic suppurative otitis media (CSOM) is characterised by chronic histopathological changes in the middle ear and mastoid cells, and ear discharge from the external auditory canal lasting for more than 3 months (1).

Medical and surgical treatments are both used for CSOM. Antibiotic treatment is necessary before and after surgery to control infection and prevent complications. Choosing the most appropriate antibiotic is important, for which accurate identification of the causative microorganism is a prerequisite. In the outpatient clinic, culture is performed on middle ear discharge from the external auditory canal, to identify the causative microorganism in the middle ear and mastoid.

Samples obtained through the outer ear can be contaminated by the skin flora, leading to false-positive results. Furthermore, it is unknown whether the results for samples obtained through the external auditory canal reflect the pathogen in the middle ear. Therefore, clinicians tend to prescribe empirical antibiotics.

We analysed the culture antibiogram results of samples obtained directly from the middle ear and/or mastoid cells in the operating room, and from the external auditory canal in the outpatient clinic. We also compared the culture results, i.e. bacteria and antibiotic susceptibility, of samples obtained from the external auditory canal and middle ear.

METHODS

This study was carried out from 2016 to 2018 in the Departments of Otorhinolaryngology (ENT) and Microbiology of the Faculty of Medicine of Dicle University. The culture results of chronic otitis media patients operated on in the otorhinolaryngology clinic were assessed. Chronic otitis media was diagnosed by anamnesis, otoscopic examination, and radiological study. Age, gender, culture sampling methods, culture results, and antibiotic susceptibilities were recorded.

Inclusion criteria: This study included only patients with suppurative chronic otitis media. Cases with discharge from the middle ear were included, as were those from whom aerobic and/or facultatively anaerobic bacteria were isolated.

Exclusion criteria: Patients with external ear canal infection were excluded, as were those with inactive chronic otitis media, chronic granulomatous disease, or tumoral lesions, and those receiving immunosuppressive treatment. Cases with fungal or anaerobic growth were excluded.

Approval was received from the Ethics Committee of the Faculty of Medicine of Dicle University (02.26.2016-No: 1107)

Sampling of the external ear canal

Using culture swabs, external ear canal samples were obtained from chronic otitis media patients admitted to the otorhinolaryngology outpatient clinic and subjected to microbiological evaluation. No patient received antibiotherapy in the 48–72-hour period prior to culture. To prevent contamination, the external auditory canal was cleaned with disinfectant. After about 5 minutes, the

disinfectant was removed, and the discharge in the medial part of the external ear canal was swabbed. The cotton swabs were then placed in sterile culture tubes and sent to the microbiology laboratory (group 1) (Figure 1).

Sampling from the middle ear and mastoid cells

Intraoperative specimens were obtained from patients who were operated on in our ear, nose, and throat clinic. A retroauricular incision was made under sterile conditions and general anaesthesia in the operating room, and mastoid cells were reached by drilling the mastoid bone (figure 2a). The middle ear was reached by elevating the skin of the external ear canal. Infected tissue was obtained from mastoid cells and the middle ear, transferred to thioglycollate broth medium, and transported to the microbiology laboratory for culturing (group 2) (Figure 2b).

Statistical analysis

The Shapiro-Wilk test was used to determine the normality of the data distribution. The non-parametric Mann-Whitney U test was used to analyse data that were not normally distributed, and the *t*-test was used to determine whether the two groups differed with respect to the various outcome variables. A P-value < 0.05 was taken to indicate statistical significance. The data are provided as means ± standard deviation.

Informed consent was obtained from children and parents who volunteered to participate in the study.

RESULTS

A total of 238 patients with chronic otitis media were included in the analysis. The age range of the patients was 3–69 years and the mean age was 27.6 ± 16.3 years. The male/female ratio was 126/112. A total of 189 aerobic and/or facultative anaerobic bacteria were isolated from 238 cultures. There was no bacterial growth in 86 of the samples, while more than one bacterium was isolated in 20 samples. The isolated bacteria comprised 70 Gram-positive cocci, 22 Gram-positive rods, 42 Gram-negative rods (Enterobacteriaceae), and 55 non-fermenting Gram-negative rods (Table 1). The most frequently isolated bacteria were *Pseudomonas aeruginosa* (n = 44), coagulase-negative *Staphylococci* (CoNS) (n = 33), *Staphylococcus aureus* (n = 27), *Proteus mirabilis* (n = 16), and *Escherichia coli* (n = 10) (Table 2).

S. aureus and CoNS expression levels were significantly higher in the outpatient group (group 1) than the operation group (group 2). Amikacin, piperacillin-tazobactam, meropenem,

ceftazidime, cefepime, and piperacillin were the most effective antibiotics against *P. aeruginosa* isolates (susceptibility rates of 95.4%, 86.3%, 86.3%, 75%, 79.5%, and 79.5% respectively). The least effective antibiotics were ciprofloxacin and gentamicin (susceptibility rates of 52.2% and 56.8%, respectively) (Table 3, Graphic 1).

All *S. aureus* isolates were resistant to penicillin G and ampicillin, likely due to penicillinases. Eight (29.6%) of the *S. aureus* isolates were resistant to methicillin. No *S. aureus* isolate was resistant to trimethoprim-sulfamethoxazole, vancomycin, or clindamycin (Table 4, Graphic 2).

P. mirabilis was sensitive to cefepime, amikacin, meropenem and ciprofloxacin. The rates of sensitivity of *P. mirabilis* were as follows: ceftriaxone, 93.7%; ampicillin, 56.2%; amoxicillin-clavulanate, 62.5%; and piperacillin-tazobactam, 25%. All *Proteus* isolates had intrinsic resistance to colistin (Table 5, Graphic 3).

E. coli was fully sensitive to imipenem, gentamicin and meropenem, whereas the sensitivity rates to the other antibiotics varied (Table 6, Graphic 4). The frequency of negative culture results was significantly higher in the operation versus outpatient group ($P < 0.05$). In the outpatient group, the rate of isolation of Gram-positive cocci was significantly elevated relative to the operation group ($P < 0.05$).

Analysis results of samples obtained in the outpatient clinic

In the outpatient clinic, samples were obtained from 136 patients. The male/female ratio was 78/58 and the age range was 7–54 years. The mean age was 31.3 ± 18.5 in female patients and 27 ± 18.9 in males. Aerobic/facultatively anaerobic bacteria were detected in 124 samples, and more than one microorganism was cultured from 12 samples. No growth was detected in the samples from 36 patients. The microorganisms identified were *P. aeruginosa* (n = 28), CoNS (n = 27), *S. aureus* (n = 21), *Corynebacterium* (n = 13), and *Proteus mirabilis* (n = 9) (Table 2).

P. aeruginosa isolated from the external ear canal showed sensitivity to amikacin (96.4%), piperacillin-tazobactam (92.8%), piperacillin (85.7%), cefepime (78.5%), ceftazidime (75%), gentamicin (64.2%), and ciprofloxacin (53.5%) (Table 3).

S. aureus showed no resistance to clindamycin, tobramycin, trimethoprim-sulfamethoxazole, teicoplanin, vancomycin, rifampin, tetracycline, or linezolid. The sensitivity rates of *S. aureus* to erythromycin, gentamicin, fusidic acid, ciprofloxacin, and levofloxacin were 95.2%, 95.2%, 95.2%, 90.4%, and 90.4%, respectively (Table 4).

All *E. coli* isolates were susceptible to gentamicin, meropenem, imipenem, and ertapenem. The rates of susceptibility of *E. coli* were as follows: cefepime, 60%; trimethoprim-sulfamethoxazole, 60%; ceftriaxone, 40%; piperacillin, 40%; piperacillin tazobactam, 40%; ciprofloxacin, 40%; and amoxicillin-clavulanate, 20% (Table 5).

No resistance to amikacin, ciprofloxacin, meropenem, or trimethoprim-sulfamethoxazole was detected among *P. mirabilis* isolates. The sensitivity rates to other antibiotics were as follows: ceftriaxone, 87.5%; gentamicin, 75%; piperacillin-tazobactam, 62.5%; and ampicillin and amoxycillin, 50% (Table 6).

Analysis results of samples obtained in the operating room

In the operating room, samples were obtained from the infected middle ear tissue and/or mastoid cells of 101 patients. Fifty-two patients were female and forty-nine were male; the mean age was 25.3 ± 14.8 years.

Mastoid surgery and broad-spectrum antibiotics (cephalosporins) were applied 2 hours before surgery, for surgical prophylaxis, in all patients. No growth was detected in 50 cultures. The most frequently isolated bacteria were *P. aeruginosa* (n = 16), *S. aureus* (n = 7), *P. mirabilis* (n = 7), *Corynebacterium* spp. (n = 6), CoNS (n = 6), *E. coli* (n = 5), and *Klebsiella pneumonia* (n = 3) (Tables 1 and 2). The susceptibility rates of the most frequently isolated microorganisms are listed in Tables 3–6.

Cultures were obtained from 23 patients preoperatively and intraoperatively. The same microorganism was isolated from samples taken from the outer and middle ear of nine patients. In four patients showing no bacterial growth in samples from the external ear canal preoperatively, the middle ear culture was positive. Five patients exhibited bacterial growth in external ear canal cultures but not middle ear cultures (Table 7).

DISCUSSION

Chronic otitis media is a serious condition that is typically treated surgically. Application of an appropriate antibiotic before surgery increases the likelihood of successful surgery and reduces the risk of complications. Therefore, it is crucial to determine the most appropriate antibiotic.

The reliability of culture results obtained by conventional methods (i.e. culture swabs) is controversial (2, 3). Few studies have evaluated direct mastoid or middle ear culture (4). Therefore, whether the microorganisms isolated from samples obtained by conventional methods reflect the pathogens in the middle ear and mastoid is unclear. Because antibiotic sensitivity test results take at least 48 hours to produce, treatment tends to be delayed. However, if the patient's condition is

critical, empirical treatment is initiated without waiting for culture results. Culturing and antibiotic sensitivity tests are both time-consuming and costly. We compared culture results between samples obtained in the outpatient clinic and operating room. Also, preoperative and intraoperative culture inoculation was performed in the 27 same each patients. Ahn *et al*. (4) reported that *S. aureus* and *P. aeruginosa* were the most frequently detected bacteria, and were isolated at similar frequencies preoperatively and postoperatively. However, although preoperative and intraoperative bacteria may be similar, different microorganisms may be present in the external ear canal (preoperatively) versus intraoperative mastoid granulation tissue (4). Culture from the external ear canal obtained using cotton swabs is not sufficient to isolate causative agents, because the source of infection is not reached (3).

P. aeruginosa, CoNS, *S. aureus*, and *P. mirabilis* were the most common microorganisms isolated in our outpatient group, but were also detected in the operation group, albeit that there was a difference in their relative frequencies. The frequency of *S. aureus* and CoNS was significantly higher in the outpatient group, suggesting the efficacy of perioperative antibiotics against this organism. The large number of CoNS in the outpatient group also suggests possible contamination of the samples.

Saunders *et al*. (5) isolated, in order of frequency, *S. aureus*, *Corynebacterium* spp. and *P. aeruginosa*, while Yeo *et al*. (6) identified *P. aeruginosa*, *S. aureus* and CoNS. In this study, *P. aeruginosa*, CoNS, *S. aureus*, *P. mirabilis* were common to both groups. *P. aeruginosa* and *S. aureus* were significantly more resistant, especially in the operation group. This may be due to elimination of susceptible microorganisms by prophylactic antibiotics, thereby increasing the number of resistant bacteria. Surgery may be needed when resistant bacteria are present. Overall, the rate of resistance in both groups was high.

The frequency of methicillin-resistant *S. aureus* (MRSA) has increased recently (7, 8). The incidence of MRSA among otology patients was reported as 6–12% (9, 10). However, Ahn *et al*. reported a higher rate of resistance (4). In this study, the frequency of MRSA was 4.2%, which was lower than reported previously (4, 10), although the rate was higher in the operation group.

There was no difference between the two groups in terms of negative culture results, possibly due to an inadequate sampling methodology, antibiotic prophylaxis, or inappropriate or delayed transport of samples to the laboratory. The culture material should not be contaminated and should be delivered to the laboratory rapidly; moreover, antibiotics should not be used in the prior 24 hours. In this study, the number of members of the normal skin flora was remarkable, and included CoNS

and *Corynebacterium* spp. Due to the high rate of contamination during sampling of outpatients, culture methods need to be reviewed. In our operation group, samples were obtained from the surgical area aseptically, without skin contact. Despite the appropriate culture method, the growth of normal flora in the operation group suggests that these microorganisms may have been the causative pathogen. Saunder (5) reported isolation of resistant *S. epidermidis* and *Corynebacterium* species.; these two microorganisms have the ability to form biofilm, which is associated with complications of chronic otitis media (11).

Samples were obtained from both the external auditory canal (preoperatively) and surgical site in 27 patients, of whom 9 had identical results between the two sampling locations. The preoperative cultures were negative, but the intraoperative cultures were positive, in four patients; this may have been because cultivation in the operating room increases the growth rate. Contamination from the external ear canal and/or failure to reach the middle ear discharge may have resulted in negative culture results. However, preoperative antibiotic prophylaxis may prevent the growth of bacteria. In five patients, no bacteria were present in the surgical cultures despite the preoperative cultures being positive.

Antibiotic resistance was detected frequently. *P. aeruginosa* and *S. aureus* are sensitive to ciprofloxacin and gentamicin (1, 12). In this study, *S. aureus* and *P. mirabilis* were sensitive, while *P. aeruginosa* and *E. coli* exhibited significantly reduced sensitivity (~ 50%). Overall, the ototoxic effect of ciprofloxacin was low and the bacteria were highly sensitive to it (13, 14). Therefore, ciprofloxacin should be applied locally as drops as a first-line treatment (13). The sensitivity of ciprofloxacin was significantly reduced. Jang *et al*. (15) reported high bacterial resistance to ciprofloxacin in patients with persistent chronic otitis media, likely due to widespread use of this antibiotic.

We also detected *Turicella otitidis*, *Kerstersia gyiorum*, *Arthobacter* and *Achromobacter* species in patients with chronic otitis media.

Similar microorganisms grew in outpatient and intraoperative cultures. Contamination is an issue in samples collected preoperatively from the external ear canal of outpatients using cotton swabs. *P. aeruginosa* was the most frequently isolated bacterium in this study, and its resistance to ciprofloxacin was high. Although the frequency of MRSA was lower overall than in prior reports, it was higher in our operation versus outpatient group.

Conflicts of interest

The authors have no competing interests.

Patient consent

Informed consent was obtained from children and parents who volunteered to participate in the study

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All authors have contribution in preparation of this study

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Table 1. Distribution of aerobic/facultative anaerobic bacteria in outpatient and operating room conditions

	Outpatients (Group 1) (n, %)	**Intraoperative (Group 2) (n,%)**	**P**	**Total**
Gram(+) cocci *Streptococcus spp.* (n=9) *S.aureus* (n=27) Coagulase Negative Staphylococcus (n=33) *Enterococcus spp.* (n=1)	56(%45,1)	14(%21,5)	**<0.05****	70(%37)
Gram(+) rods *Corynebacterium spp.*(n=19) *Bacillus spp.*(n=3) *Arthrobacter spp.*(n=1)	15(%12)	7(%10,7)	>0.05	22(%11,6)
Enterobactericae *E.coli* (n=10) *Klebsiella* spp.(n=4, *Proteus* and *Providencia spp.* (n=19), *Morganella* morganii(n=3), *Enterobacter spp.*(n=1), *Serratia marcescens*(n=3), *Citrobacter freundi* (n=2)	20(%16,1)	22(%33,8)	**<0.05****	42(%22,2)
Non-fermentative Gram-negative bacteria *P.aeruginosa* (n=44) *Acinetobacter* baumannii (n=1) *Achromobacter* spp.(n=2) *Alcaligenes* spp.(n=2)	33(%26,6)	22(33,8)	>0.05	55(%29,1)

Turicella otitidis (n=1) *P.putida* (n=1) *Oligella urethralis* (n=2) *Kerstersia gyiorum* (n=2)				
Total	124	65		189*

* A total of 189 aerobic/facultative anaerobic bacteria were produced in the culture results of all patients.

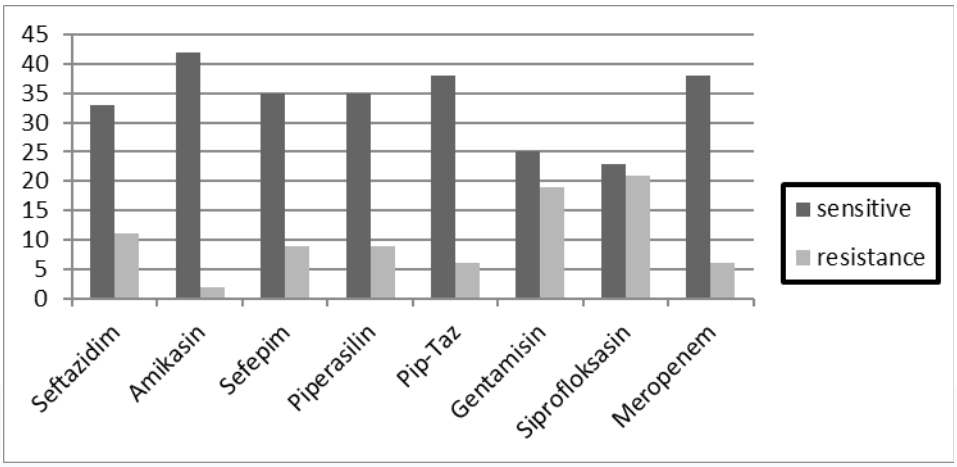
** Statistically significant

Table 2. Analysis of the four most common bacteria in two groups

	Outpatient (group 1) (n, %)	**Intraoperative (group 2) (n, %)**	**p**
P.aeruginosa	28 (%16)	16 (%13,2)	>0,05
S.aureus	21 (%12)	6 (%4,9)	<0,05**
Proteus	9 (%5,1)	10 (%8,2)	>0,05
E.coli	5 (%2,8)	5 (2,8)	>0,05
CoNS	27 (%15,5)	6 (4,9)	<0,05**

Table 3. *P.aeruginosa* susceptibility in both groups ($p<0.05$)

	Outpatient (group 1) (n=28)	**Intraoperative (gruop 2) (n=16)**
Ceftazidime	21(%75)	12(%75)
Cefepime	22(%78.5)	13(%81,2)
Piperacillin	24(%85.7)	11(%68.7)
Pip-taz	26(%92.8)	12(%75)
Amikasin	27(%96.4)	15(%93.7)
Gentamicin	18(%64.2	7(%43.7)
Ciprofloxacine	15(%53.5)	8(%50)
Meropenem	24(%85.7)	14(%87.5



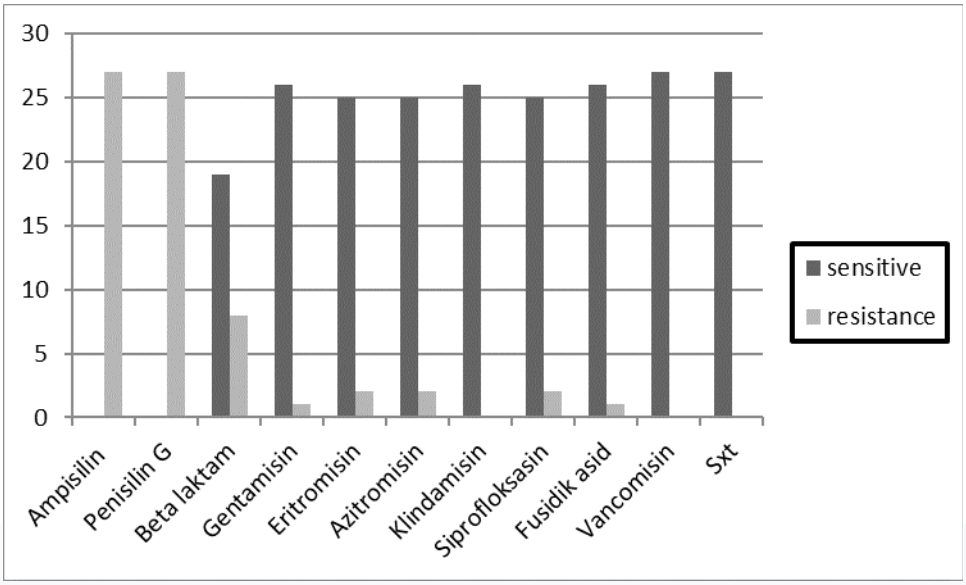
Graphic 1. Antibiotic resistance-susceptibility graph of all breeding *P.aeruginosa* species (n = 44)

Table 4. *S.aureus* susceptibility in two groups ($p<0.05$)

	Outpatient (group 1) (n=21)	**Intraoperative (group 2) (n=6)**
Ampicilin*	0(%0)	0(%0)
Peniciline G*	0(%0)	0(%0)
Clindamycin	21(%100)	5(%83,3)
Erythromycin	20(%95,5)	5(%83,3)
Ciprofloxacine	19(%90,4)	6(%100)
Levofloxacin	19(%90,4	6(%100)
Fusidic acid	20(%95,5)	6(%100)
Gentamycin	20(%95,5)	6(%100)
Tobramycin	21(%100)	6(%100)
Sxt	21(%100)	6%100)
Teicoplanin	21(%100)	6(%100)
Vankomycin	21(%100)	6(%100)
Rifampin	21(%100)	6(%100)
Tetracycline	21(%100)	5(%83,3)
Linezolid	21(%100)	6(%100)
Quino/Dalfopristin	21(%100)	6(%100)
Cefoxitin**	17(%80,9)	2(%33,3)

* Penicillin and ampicillin resistance in *S.aureus* depends on penicillinase enzyme

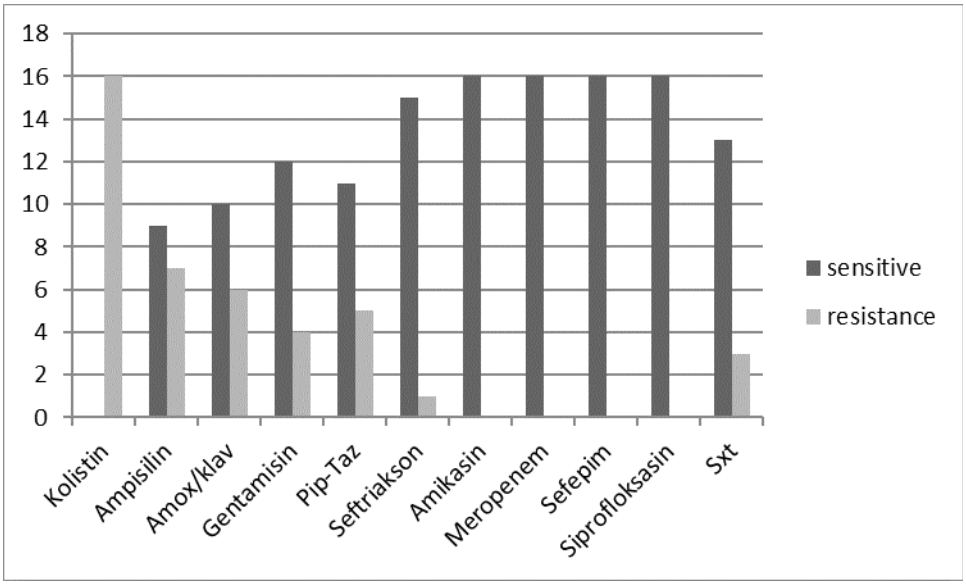
** Antibiotic used to detect methicillin susceptibility



Graphic 2. Antibiotic resistance-susceptibility graph of all isolated *S.aureus* (n = 27) species

Table 5. *P.mirabilis* susceptibility in two groups (p>0.05)

	Outpatient (group 1) (n=8)	**Intraoperative(group 2) (n=8)**
Ampicillin	4(%50)	5(%62.5)
Amc/Klv	4(%50)	6(%75)
Cefepime	8(%100)	8(%100)
Ceftriaxone	7(%87.5)	8(%100)
pip-taz	5(%62.5)	6(%75)
Gentamycin	6(%75)	6(%75)
Amikacin	8(%100)	8(%100)
Ciprofloxacin	8(%100)	8(%100)
Meropenem	8(%100)	8(%100)
Sxt	8(%100)	5(%62.5)

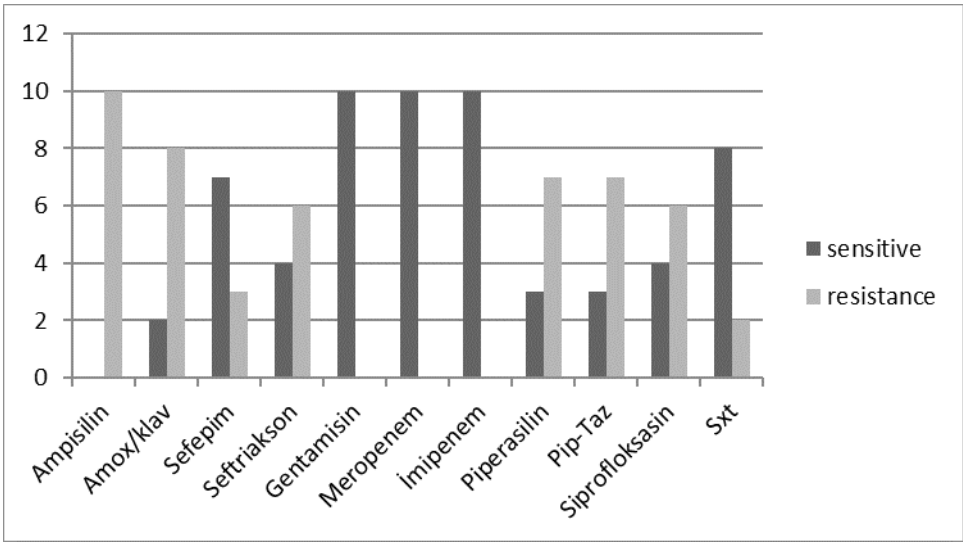


Graphic 3. Antibiotic resistance-susceptibility graph of all isolated *P.mirabilis* species (n = 16)

Table 6. *E.coli* susceptibility in two groups (p>0.05)

	Outpatient (group 1) (n=5)	**Intraoperative (group 2) (n=5)**
Ampicilin	0(%0)	0(%0)
Amx/Klv	1(%20)	1(%20)
Ceftriaxone	2(%40)	2(%40)
Cefepime	3(%60)	4(%80)
Gentamycin	5(%100)	5(%100)
Meropenem	5(%100)	5(%100)
Imipenem	5(%100)	5(%100)
Ertapenem	5(%100)	5(%100)
Piperacillin	2(%40)	1(%20
Pip-taz	2(%40)	1(%20)
Ciprofloxacin	2(%40)	2(%40)

Sxt	3(%60)	5(%100)



Graphic 4. Antibiotic resistance-susceptibility graph of all *E.coli* (n = 10) species

Table 7. Preoperative and intraoperative results both from the same patients

Patients	**Preoperative**	**Intraoperative**
1.Patient	*Citrobacter koseri*	no reproduction
2. Patient	*Enterococcius faecium*	*Corynebacterium amycolatum, S. epidermidis*
3. Patient	*Proteus mirabilis*	*Corynebacterium amycolatum (kont?)*
4. Patient	*Proteus mirabilis*	*P. mirabilis*
5. Patient	*Proteus mirabilis*	*Oligella ürethialis*
6. Patient	*E.coli*	*E. coli*
7. Patient	*E.coli*	*Corynebacterium amycolatum +C.coyleae*
8. Patient	*E.coli*	*E.coli (esbl)*
9. Patient	*E.coli*	no reproduction
10. Patient	*S. Capitis (KNS)*	no reproduction
11. Patient	*Achromacter species*	*Psedomonas aerigonosa*
12. Patient	*C. amycolatum +Fusobacterium varium*	*Prevotella disiens*
13. Patient	no reproduction	*Proteus spp-providencia rustigiai*
14. Patient	no reproduction	*P. aeruginosa*
15. Patient	no reproduction	*S. aureus (mrsa)*
16. Patient	no reproduction	*C. amycolatum, Stafilococcus hominis*
17. Patient	*Achromacter species*	*Alcaligenes spp.*
18. Patient	*S.aureus*	*S.epidermidis (mskns)*
19. Patient	*S. aureus*	no reproduction
20. Patient	*S. aureus*	no reproduction

21. Patient	*S. simulans*	*S. aureus(mssa)*
22. Patient	*P.aeruginosa*	*P. aeruginosa*
23. Patient	*P.aeruginosa*	*P. aeruginosa*
24. Patient	*P.aeruginosa*	*P. aeriginosa*
25. Patient	*P.aeruginosa*	*P.aeruginosa*
26. Patient	*P.aeruginosa*	*P. aeruginosa*
27. Patient	*P.aeruginosa*	*P. aeruginosa*

Figure Legends:

Figure 1. Using culture swabs, external ear canal samples were obtained in outpatients (group 1).

Figure 2a. Surgical area and infected mastoid tissue were seen in intraoperatively (group 2)

Figure 2b. Infected tissue was obtained from mastoid cells, and transferred to thioglycollate broth medium.