

Microbiological Profile and their Antibiotic Susceptibility Results in Patients with Ear Discharge, our Experience at KHMC, Jordan.

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ABSTRACT

Objective: To report the frequency of causative microorganisms in patients with ear discharge and their susceptibility pattern.

Methods: The study was conducted in a retrospective manner during the period from January 2017 to June 2017. A total of 353 specimens were aseptically collected from patients (inpatient and outpatient) with ear discharge. Specimens were cultured according to the standard microbiological procedures on blood, chocolate, MacConkey's and Sabouraud dextrose agar. The clinical isolates were identified using Gram stain, methylene blue stain, manual biochemical reactions and VITEK 2 compact analyzer. Antimicrobial susceptibility testing was performed according to microbiological standards using VITEK 2 compact automated microbiology system. The results were analyzed using Microsoft excel sheet, windows 7 home premium.

Results: 202 specimens (57.2%) had pure single bacterial or fungal growth, 117 (33.1%) no growth and 34 (9.6%) mixed growth. Of 202 isolates, 156 (77.2%) were bacteria and 46 (22.8%) were fungi. Gram negative bacteria showed the highest frequency followed by gram positive bacteria and fungi were the least frequent. The most common microorganisms isolated were *Pseudomonas aeruginosa* (31.7%) followed by *Staphylococcus aureus* (24.2%), *Candida* species (12.3%), *Aspergillus* species (9.4%), and *Proteus* species (3.9%). Antibiotic used for the susceptibility testing of Gram positive bacteria; Vancomycin, Teicoplanin and Linezolid had the highest susceptibility rate. Regarding Gram negative bacteria the highest susceptibility rate was for Imipenem, Ceftazidime and Cefotaxime.

Conclusion: The commonest microorganisms isolated from patients with ear discharge were *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with an increased frequency of methicillin resistant *Staph. aureus*. High antimicrobial susceptibility rate was found among most isolated microorganisms. Ear discharge culture should be requested routinely before starting empirical treatment and waiting for microbiology report.

Key words: Antimicrobial susceptibility, Bacteriology, Ear discharge
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Introduction

Ear discharge is a frequent complaint in ENT clinics. Patients of different ages are candidates for this condition especially children. This is considered as result of inflammation of the auditory canal either the middle or the external one. Acute otitis media and externa, chronic suppurative otitis media, and malignancy are the underlying cause of this condition.⁽¹⁾ Otitis externa affects primarily the external auditory canal. Hot and humid weather is a good condition to develop acute diffuse otitis externa known as (swimmer's ear). In this case the main symptoms are itching and earache. Both redness and edema are frequently

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seen by clinical examination. In addition to these symptoms, the patient of bacterial otitis externa could complain of auricular fullness and discharge.⁽²⁾

Inflammation of the middle ear is called otitis media and is caused by bacteria, fungi and viruses. There are three types of inflammation: chronic suppurative otitis media, otitis media with effusion, and acute otitis media.⁽³⁾ Discharge in chronic suppurative otitis media is recurrent and persistent for more than three months.⁽⁴⁾ Infection enters to the ear through Eustachian tube from nasopharynx, nose, sinuses, or tonsils.⁽⁵⁾ Untreated infection can lead to serious complications including meningitis, intracranial abscesses, deafness, facial nerve paralysis, mastoid abscess, and lateral sinus thrombosis.⁽⁶⁾

Risk factors that lead to development of chronic suppurative otitis media include recurrent respiratory tract infections, poor living conditions, nasal disease, inadequate antibiotic therapy, and poor medical care ⁽⁷⁾. The isolated microorganisms from patients with ear discharge can be anaerobes, aerobes, mixed or fungal.⁽⁸⁾ The most frequent bacterial organisms isolated from ear discharge are *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus*, *Haemophilus influenza*, *Peptostreptococcus*, and *Bacteroides*.⁽⁹⁾ Bacteria are the commonest cause of ear infection, while fungi are a less common but important cause.⁽²⁾

The treatment of otitis media is based on identifying the type of microorganisms and their antibiotic sensitivity. The resistance of microorganisms depend on frequency of resistant bacterial strains, geographical locations, and policy for antibacterial prescribing practices.⁽¹⁰⁾

The aim of our study is to isolate the microbial agents in patients with ear discharge and to study the antibiotic sensitivity pattern of these organisms to commonly used antibiotics.

Methods

Our study was approved by ethics committee of the Royal Medical Services, Amman-Jordan. The study was conducted in a retrospective manner during the period of January 2017 to June 2017. A total of 353 specimens were aseptically collected from patients (inpatient and outpatient) with ear discharge using cotton swab, 147 (41.6%) were female and 206 (58.4%) were male. The specimens were transported within 30 minutes to the microbiology laboratory at Princess Iman Centre for research and laboratory sciences. The patients age ranged from 1 year to 70 years.

Specimens were cultured according to the standard microbiological procedures on blood, chocolate, MacConkey's and Sabouraud dextrose agar. Blood and chocolate media are primary isolation media for obtaining clinical isolates from specimens, MacConkey's media is selective for Gram negative bacteria and is used to differentiate lactose-fermenting from non-lactose-fermenting bacteria, whereas the Sabouraud dextrose media is selective for fungi (figure 1).

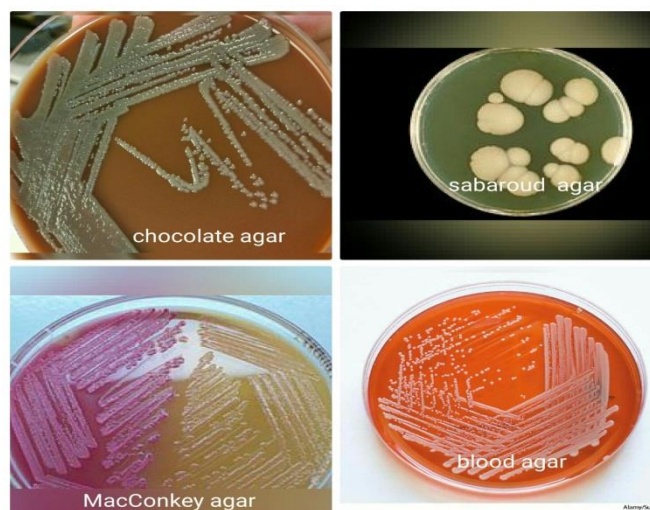


Fig 1: different media types used in ear swab culture.

The clinical isolates were identified using Gram stain for bacteria and yeast, methylene blue stain used for molds, manual biochemical reactions and vitek 2 compact analyzer. The Gram stain was performed on 24-hour old cultures and used mainly to differentiate Gram positive bacterium which are purple in color from Gram negative which are red, while *Candida* yeast cells appear as Gram positive budding yeast cells. Biochemical reactions used for identification of Gram negative bacteria included triple sugar iron test, indole test, hydrogen sulfide test, motility test, citrate test, urease test and oxidase test. For gram positive bacteria catalase test, coagulase test, optochin and Bacitracin susceptibility tests were used. For fungi, Vitek 2 system was used for Identifying yeast, while molds were identified according to their morphology under light microscope.

Antimicrobial susceptibility testing was performed according to standard microbiological standards using Vitek 2 automated microbiology system. The pure bacterial isolates were diluted in 0.45% saline and incubated with a specific substrate card for identification and antimicrobial susceptibility testing. Antibiotics used for Gram positive bacteria included penicillin, Ampicillin, amoxicillin-clavulanic acid, Clindamycin, Lincomycin, erythromycin, Vancomycin, Floxacillin, Linezolid, Teicoplanin, Cephazoline, Cephalothin and Cefuroxime. For Gram negative bacteria Imipenem, Tazocin, Ceftazidime, Cefepime, Cefuroxime, Cefotaxime, Trimethoprim-sulfamethoxazole, Amikacin, Gentamycin, and Ciprofloxacin were used.

The results were analyzed using Microsoft excel sheet, windows 7 home premium.

Results

A total of 353 patients were included in the study, 202 specimens (57.2%) had pure single bacterial or fungal growth, 117 (33.1%) no growth and 34 (9.6%) mixed growth. Mixed growth specimens and anaerobes were not included in this study. Of the 202 isolates, 156 (77.2%) were bacteria and 46 (22.8%) were fungi. Gram negative bacteria showed the highest frequency followed by Gram positive bacteria and fungi were the least frequent (Table I). Most common microorganisms isolated were *Pseudomonas aeruginosa* (31.7%) followed by *Staphylococcus aureus* (24.2%), *Candida* species (12.3%), *Aspergillus* species (9.4%), and *Proteus mirabilis* (3.9%) (Figure 2).

Table I: Frequency of growing microorganism in ear discharge.

Pathogen	Total (202)	
	Number	Percentage
Gram negative bacteria	97	48%
Gram positive bacteria	59	29%
Fungi	46	23%

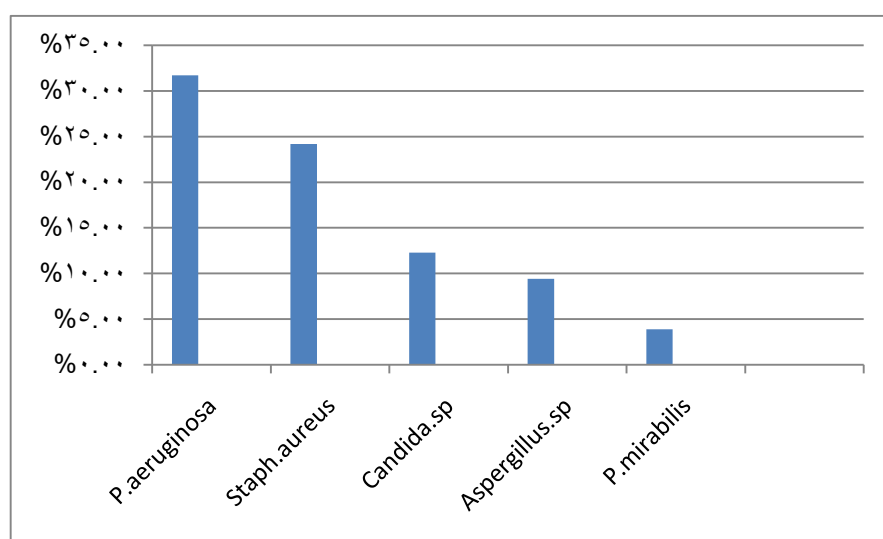


Fig 2: Frequency of most common pathogen isolated from patients with ear discharge.

Regarding the 59 Gram positive bacteria isolates; the most common one was 49 Staph.aureus (83%), five Strep.pneumoniae (8.5%), four Staph.epidermidis (6.7%) and one Enterococcus faecalis (1.8%) (Table II). MRSA constituted 44.9% of Staph.aureus isolates and Staph.epidermidis was considered as pathogenic because it was isolated purely from ear discharge of symptomatic patients. For 97 Gram negative bacteria, 13 different microorganisms were identified with the most frequent one being Pseudomonas aeruginosa (Table III). Among fungal isolates *Candida* species was most frequent, followed by *Aspergillus* species and *Penicillium* species. (Table IV).

Table II: Frequency of gram positive bacteria in ear discharge specimens. Total number of gram positive bacteria culture 59

Pathogen	Number	Percentage related to gram positive bacteria (59)	Percentage related to all isolates(202)
<i>Staph.aureus</i>	49	83%	24.3%
MRSA	22	37.3%	10.9%
<i>Strep. pneumoniae</i>	5	8.5%	2.5%
<i>Staph. Epidermidis</i>	4	6.7%	2.0%
<i>Enterococcus.faecalis</i>	1	1.8%	0.5%

Table III: Frequency of gram negative bacteria in ear discharge specimens. Total number of gram negative bacteria culture 97

Pathogen	Number	Percentage related to gram negative bacteria (97)	Percentage related to all isolates(202)
<i>P.aeruginosa</i>	64	65.9%	31.7%
<i>P.mirabilis</i>	8	8.2%	4.0%
<i>K.pneumoniae</i>	5	5.1%	2.5%
<i>E.cloacae</i>	5	5.1%	2.5%
<i>E.coli</i>	4	4.1%	2.0%
<i>A.baumannii</i>	3	3%	1.5%
<i>S.marcescense</i>	2	2%	0.99%
<i>C.freundii</i>	1	1%	0.5%
<i>M.morganii</i>	1	1%	0.5%
<i>H.influenzae</i>	1	1%	0.5%
<i>Providencia.sp</i>	1	1%	0.5%
<i>A.denitrificans</i>	1	1%	0.5%
<i>A.hydrophila</i>	1	1%	0.5%

Table IV: Frequency of fungi in ear discharge specimens. Total number of fungi positive specimens 46

Pathogen	Number	Percentage related to fungal growth culture (46)	Percentage related to all isolates (202)
<i>Candida</i> species	25	54.3%	12.3%
<i>Aspergillus</i> species	19	41.3%	9.4%
<i>Penicillium</i> species	2	4.4%	0.99%

Antibiotic susceptibility testing of Gram positive bacteria; Vancomycin, Teicoplanin and Linezolid had the highest susceptibility rate (TableV). Regarding Gram negative bacteria the highest sensitivity was for Imipenem, Ceftazidime and Cefotaxime (TableVI). *Pseudomonas aeruginosa* which was the most frequent pathogen, 98.75% were sensitive for Imipenem, 97.45% for Ceftazidime and 96.9% for Cefotaxime (TableVII). For *Staph.aureus* isolates, 100%, 98% and 93.95 were sensitive for Vancomycin (49 of 49 isolates), Teicoplanin (48 of 49) and Linezolid (46 of 49) respectively, (TableVIII).

Table V: Antibiotic susceptibility for gram positive bacteria. Total number 59.

Antimicrobial agent	Sensitive		Resistance	
	N	%	N	%
Ampicillin	50	84.7%	9	15.3%
Penicillin	46	77.9%	13	22.1%
Augmentin	49	83%	10	17%
Vancomycin	59	100%	0	0%
Clindamycin	49	83%	8	17%
Lincomycin	54	91.5%	5	8.5%

Erythromycin	48	81.3%	11	18.7%
Floxacin	49	83%	10	17%
Linezolid	55	93.2%	4	6.8%
Teicoplanin	56	94.9%	3	5.1%
Cephalothin	52	88.1%	7	11.9%
Cephazoline	50	84.7%	9	15.3%
Cefuroxime	51	86.4%	8	13.6%

Table VI: Antibiotic susceptibility for gram negative bacteria. Total number 97.

Antimicrobial agent	Sensitive		Resistance	
	N	%	N	%
Imipenem	93	95.9%	4	4.2%
Piperacillin/tazobactam	88	90.7%	9	9.3%
Gentamycin	78	80.4%	19	19.6%
Amikacin	90	92.7%	7	7.3%
Ciprofloxacin	87	89.7%	10	10.3%
Cefotaxime	92	94.8%	5	5.2%
Cefepime	91	93.8%	6	6.2%
Ceftazidime	93	95.9%	4	4.2%
Cefuroxime	83	85.5%	14	14.5%
Trimethoprim- Sulfamethoxazole	91	93.8%	6	6.2%

Table VII: Antibigram for the isolated *Pseudomonas aeruginosa* against routinely used antibiotics (% sensitive)

Organism	# of isolate	Pipercillin-Tazobactam	Cefepime	Cefotaxime	Ceftazidime	Cefuroxime	Imipenem	Amikacin	Gentamicin	Ciprofloxacin	Trimethoprim-Sulfamethoxazole
<i>P.aeruginosa</i>	64	90	96	97	98	78	99	94	82	85	20

Table VIII: Antibigram for the isolated *Staphylococcus aureus* against routinely used antibiotics (% sensitive)

Organism	# of isolates	Lincomycin	Clindamycin	Erythromycin	Trimethoprim-Sulfamethoxazole	Gentamicin	Linezolid	Teicoplanin	Vancomycin
<i>Staphylococcus aureus:</i>	49								
Methicillin- sensitive (MSSA)	27	82	78	67	86	87	95	97	100
Methicillin-resistant (MRSA)	22	65	68	31	75	81	92	98	100

Discussion

Our study was conducted to isolate and characterize the possible causative aerobic pathogens in patients with ear discharge visiting ENT clinic at King Hussein Medical center. In the present study the frequency of positive results was high (total =90.4%, single isolate=57.3%, mixed growth=33.1%), other studies showed some variation in the results and the high frequency of growth in different areas indicate that the otitis media is a

common disease ⁽¹¹⁾. Saba K et al reported single growth with 55.76% frequency.⁽¹²⁾ Our study showed 9.6% of specimens with absence of any type of growth, which is compatible with study conducted by Orji FT *et al.*⁽¹³⁾ We found that Gram negative bacteria were more frequent than Gram positive bacteria. This in agreement with other studies conducted in India, Gaza strip, Greece, Ethiopia, Turkey and South Korea.^(5,14)

The present study showed that the most common isolated pathogen was *Pseudomonas aeruginosa* followed by *Staph.aureus*. Similar results have been reported in Ireland, Greece, Pakistan and Palestine.⁽⁵⁾ *Staph.aureus* was the most common Gram positive bacteria (83%) with 37.3% (10.9% of all positive specimens) being methicillin resistant *Staph.aureus* (MRSA). In comparison with two studies conducted in India, the first study showed the frequency of MRSA was 7% and the second was 18% ⁽¹⁴⁾. Regarding Gram negative bacteria *Pseudomonas aeruginosa* was the most frequent one, which is in agreement with Derese H et al study.⁽¹¹⁾ Regarding antimicrobial susceptibility of Gram positive bacteria, the highest sensitivity was for Vancomycin, Teicoplanin and Linezolid with 100%, 94.9%, and 93.2% frequency rate respectively. The low sensitivity rate was found for Penicillin and Erythromycin, 77.9% and 81.3% respectively. Ramakrishna PJ et al reported that most *Staph.aureus* was resistant to Penicillin.⁽¹⁵⁾ For Gram negative bacteria, the highest sensitivity rate was for Imipenem, Ceftazidime and Cefotaxime with frequency rate 95.9%, 95.9% and 94.8% respectively.

Regarding fungal causative agent our study showed 13% of all samples were positive for fungal species, study conducted in Ghana reported 4% frequency rate of fungi ⁽¹⁾, Farahnaz B et al reported 11.4 %.⁽²⁾

Conclusion

The commonest microorganisms isolated from patients with ear discharge were *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with an increased frequency of methicillin resistant *Staph.aureus*. High level of antimicrobial sensitivity was found among most isolated microorganisms. Ear discharge culture should be requested routinely before starting empirical treatment and waiting for the microbiology report is recommended.

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