ANTIBIOTIC RESISTANCE PATTERN OF PSEUDOMONAS AERUGINOSA IN PATIENTS WITH OTITIS IN TEHRAN HOSPITALS

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ABSTRACT:
Background: Pseudomonas aeruginosa is one of the main agents of otitis. There are two types of otitis. Otitis media (OM) is acute, chronic or recurrent. There is high prevalence of OM in children. Inflammation or infection of pinea and ear canal called otitis externa (OE), can occur in human and some other creatures like dog. OE or swimmer’s ear is common in summer. The aim of this study is to identify outbreak of P. aeruginosa involved in OE and OM and drug sensitivity of this bacterium.

Materials and methods: All samples were taken from 186 patients who admitted in Emam Khomeini, Shahid shouride and Amiralam hospitals by otolaryngologists in Tehran, Iran, from October 2014 to July 2015. These Samples were taken from infected ears with cotton swabs and cultured in Muller Hinton, Citrimide and MacConkey Agar mediums, then incubated for 24- 48 hours in 37°C. Antibiotic testing was done using disk diffusion method.

Results: Twenty-two out of 186 samples were positive for P. aeruginosa (%12.3). Drug sensitivity pattern of P. aeruginosa showed that colistin was active against 95.6% of isolates followed by amikacin, imipenem, doripenem, cefepime (91.3%), ceftazidime (86.9%), ciprofloxacin, gentamicin, levofloxacin (78.2%) and aztreonam (73.9%).
Conclusion: Almost all the drugs that tested were effective against *P. aeruginosa* but the most effective drug was colistin. Especialists prescribe ciprofloxacin drobs more than other antibiotics in clinics, because of their effective advantages. Therefore more investigation with larger sample size is essential.

Keywords: Pseudomonas aeruginosa, otitis media, external otitis, Disk Diffusion Antimicrobial Tests.

INTRODUCTION

*Pseudomonas aeruginosa* is an epitome of important Gram-negative opportunistic pathogens that can cause various diseases, including otitis media (OM), otitis externa (OE), osteomyelitis, hot tub folliculitis, meningitis, endocarditis, pneumonia, urinary tract infection and septicemia (1-3).

OM is an inflammation of the middle ear associated with *P. aeruginosa* infection which can be seen in two forms of acute OM (AOM) and chronic suppurative OM (CSOM) (4). Accumulation of *P. aeruginosa* population in ear occur due to dermal edema, changes in the ear canal, narrowing of the lumen and formation of anaerobic environment in ear following reduction of ventilation, and increasing of humidity and temperature (5).

OE is an infection or inflammation of the external auditory canal (EAC) that most of people will be affected by it in their lives (6). OE is caused by a bacterial infection specially *P. aeruginosa*, although the condition can also be caused by irritation, fungal infections and allergies (7). In addition of *P. aeruginosa*, various microorganisms are involved in OE and OM that the most commonly found agents including *S. aureus*, *Coagulase-Negative Staphylococci* (CNS) and fungii such as *Aspergillus* and *C. albicans* (8, 9). There are many factors for creating these infections, but the most common is excessive moisture that enhance the pH and eliminate the cerumen. Hence keratin debris absorbs the water, which creates a nourishing medium for microbial growth (10).

Despite appropriate anibiotic therapy, the morbidity and mortality rate due to *P. aeruginosa* infections is high (11). Unfortunately, *P. aeruginosa* is intrinsically resistant to a wide range of antibiotics which could be mediated by several agents such as low permeability of *P. aeruginosa* outer membrane, production of hydrolyzing enzyme, efflux pumps and mutations in genes encoding the target sites of antibiotics (12, 13). On the other hand, the widespread use of antibiotics in recent years has been caused *P. aeruginosa* resistances to different classes of broad-spectrum antibiotics, thereby jeopardizing the selection of appropriate treatment (14).

The purpose of this study was to assess the current in vitro activity level of ten antimicrobial drugs from different antibiotic groups against *P. aeruginosa* in patients with OE and OM.

MATERIALS AND METHODS:

Samples collection

In this study, sampling was done by otolaryngologists from patients with suspected to OM and OE infections that referred to Imam Khomeini, Shahid shouride and Amiralam hospitals, Tehran, Iran, from October 2014 to July 2015. During the mentioned period, a total of 186 samples were collected. In order to prevent contamination of samples in clinic, cleaning the ear canal should be done before sampling, so an ear, nose and throat specialist (ENT) or his assistance cleaned ear canal from infected debris or cerumen before suctioning under clinical microscope. Then with striled cotton applicator throw striled ear spaculom and striled condition infected material was obtained.
P. aeruginosa isolation and identification
Ear secretions were cultured on Nutrient agar and MacConkey's media (Merk, Germany), and incubated in 37°C. Primary identification of P. aeruginosa and other organisms were done based on the conventional biochemical tests, including Gram staining, growth on cetrimide agar, growth at 42°C, Kligler iron agar, oxidase, catalase, pigment production, and oxidative-fermentative (OF) tests.

Antibiotic susceptibility test
Antibiotic susceptibility was done by Kirby-Bauer’s disk diffusion method on Muller-Hinton agar (Merk, Germany) according to the Clinical Laboratory Standard Institute (CLSI) recommendations. The antibiotic disks (MAST, UK) applied were ciprofloxacin (CIP, 5 μg), aztreonam (ATM 30μg), ceftazidime (CAZ 30μg), amikacin (AMK 30μg), imipenem (IPM 10μg), gentamicin (GM 10μg), cefepime (CPT 30μg), doripenem (Dor 10μg), levofloxacin (LVF 5μg) and colistin (CO 25μg). P. aeruginosa standard strain (ATCC 27853) was used as the quality control. They were incubated at 37°C for 18-24 hours. The diameter of the zone of inhibition was measured and compared to that of standard strain.

Statistical analysis
SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Both susceptibility and resistance were calculated as percentages with 95% confidence intervals and statistical significance was defined as a P-value <0.05.

RESULTS:
Out of 186 suspected patients to OM and OE infections, 23 were positive for P. aeruginosa (%12.3), 15 for S. aureus (8.06%), 11 for Coagulase Negative Staphylococci (CNS) (5.9%) and 22 for fungal organisms (11.8%) (Fig.1). Among isolates of P. aeruginosa, 9 isolates (39.1%) belonged to male patients and 14 (60.9%) belonged to female patients, with a mean age of 35.5 years (range, 6-65 years). The results of antibiotic sensitivity pattern of P. aeruginosa showed that colistin was active against in vitro with susceptibility 95.6% of isolates followed by amikacin, imipenem, doripenem, cefepime (91.3%), ceftazidime (86.9%), ciprofloxacin, gentamicin, levofloxacin (78.2%), and aztreonam (73.9%). These data are shown on Figure 2.

![Figure1. Distribution of OM and OE etiological agents.](image-url)
DISCUSSION:

OE and OM are the most commonly found diseases in otolaryngology wards. About 10% of the population suffer from this disease, regardless of gender and age (15). Several studies in Iran showed that P. aeruginosa which is an aerobic, nonfermenting, Gram-negative, and rod bacterium was responsible for 15-42% of OE and OM, 11%-32.4% of urinary tract infections, 17.2%-32.4% of pneumonia, 36.7% of blood stream infections, and 47% of wound infections (16-20).

According to the results of this study, from 186 suspected patients to OE and OM, 23 patients had positive culture for P. aeruginosa. The most common involved of P. aeruginosa was seen in women. Colistin (95.6%) was active against P. aeruginosa and significant resistance to aztreonam (73.9%) was observed. It seems that the use of colistin, amikacin, imipenem, doripenem, and cefepime antibiotics may be somewhat effective at Pseudomonas infections.

Noguerira et al. carried out a bacteriology study in 27 patients with OE and they observed that the culture of 10 patients were positive for S. aureus, 8 for P. aeruginosa, 5 for P. aeruginosa and S. aureus and 4 for fungal organisms. All bacteria tested exposed to gentamicin and quinolones proved to be sensitive (15).

Yang et al. showed that P. aeruginosa isolates had notable resistance (19.64%) against polymyxin B, piperacillin, piperacillin/tazobactam, ceftazidime, amikacin.

In study of Salahi et al, the minimum antibiotic resistance was seen through treatment with imipenem (40.91%), and the most antibiotic resistance pattern was observed through treatment with nalidixic acid (86.54%) (21).

The Korean National Survey of Antimicrobial Resistance (KNSAR) indicated that 21%, 22%, and 22% of P. aeruginosa strains were resistant to ceftazidim, cefepime, and imipenem, respectively (22).

Various reasons such as geographic diversification, accessibility to medical care and optimal use of antibiotics can cause differences in the antibiotic susceptibility patterns of the bacteria (23, 24). Although P. aeruginosa is one of the most troublesome microbiota which is innately resistant to several antibiotics such as macrolids, first, second and many third generation of cefalosporins. It was originally sensitive to the anti-pseudomonal beta-lactam class antibiotics (12).
One limitation of our study was the relatively low number of obtained strains of P. aeruginosa. Another restriction was in that we used only disk diffusion method and since the interpretation criteria of phosphomysin for P. aeruginosa are not yet, our study did not include phosphomycin susceptibility test results of isolates (25, 26).

CONCLUSION:
So far, many studies have been done on drug resistant of P. aeruginosa. The resistance rate of P. aeruginosa to the antibiotics classes is increasing significantly over time, whereas the rate of isolation of strains susceptible to all antibiotics is decreasing significantly. Increasing antibiotic resistance and the emergence of multidrug and extensively drug resistant (46.6% and 33%, respectively) of P. aeruginosa in Iran is worrisome and requires appropriate strategies to prevent the spread of this strain (28). Otolaryngologists are using ciprofloxacin drops as a first choice medication for treatment bacterial infection of the external and middle ear, because of its practical advantages and safety (non toxicity). In this study colistin is more sensitive than ciprofloxacin so we need more investigation in other studies.

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REFERENCES


